THE EFFECT OF ULTRASOUND ON THE ROOT MERISTEM OF ZEA MAYS.

by

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Lastly,but by no means least, I would like to express my gratitude to my mother for the care and patience with which she typed this thesis.

ERRATA

Page	Line	Should read
21	1.17	" is the fraction.... which is removed..."
21	3 lines up	" ... of cells is being lost due to ....."
24	6 lines up	" ... dying an intermitotic death ....."
45	3 lines up	" diminution "
46	8 lines up	" ... the dose of ionizing radiation alone..."
67	8 lines up	" rhythm "
74	1.11	" ... of root elongation were maintained ..."
77	1.17	" ... as a fraction of the average growth of control roots ..."
160	3 lines up	" for 10^3 cells, 24.6 $\pm$ 2.0 cells per hour.."
280	1.7	" lose "
295	last line	" ... which reaches .... "
309	--	For the lowest curve, P = 0
333	8 lines up	" boundary "

C O N T E N T S

SUMMARY.

SUMMARY.

The effects of pulsed and continuous beams of ultrasound on the roots of Zea mays.

The ultrasonic beam of the Impulsaphon M55 was calibrated using a radiation microbalance to measure the total acoustic energy across the beam at the position of the roots to be sonicated. These measurements were combined with relative intensity measurements within this cross section using a thermistor probe which was constructed for this purpose. Schlieren pictures of the beam revealed that it is unfocussed. For investigations of the pulse shape, frequency and duty cycle, a tantalum capacitor probe was used.

Roots of Zea mays seedlings were exposed to 1.0 MHz pulsed (2 ms pulses, 0.2 duty cycle) and continuous beams of ultrasound at average intensities in the range 0.02 - 0.82 W/cm^2 for periods of 1 to 180 minutes. The beam parameters used in these experiments are those that the Impulsaphon M55, a therapeutic instrument, is capable of producing.

No ultrasonic damage was observed for sonications at very low average intensities (less than 0.1 W/cm^2) and some of the short sonication times at the higher average intensities. The beam of a diagnostic instrument, the Diasonograph, also did not cause any observable damage to the roots.

The sonication effect increased with increasing

exposure time for a given intensity and with increasing intensity for a given exposure time. Such a response to ultrasonic irradiation has also been observed for the roots of the broad bean, Vicia faba (Bleaney and Oliver, 1972a). The ultrasonic exposure (intensity multiplied by duration) does not seem to be related to damage produced in the same way as dose (rads) and % survival for ionizing radiation.

The damage due to pulsed beams seems to be much greater than that for continuous beams of the same average power. According to Bleaney and Oliver (1972b) who have obtained similar results for the roots of Vicia faba, the pulses may be long enough for the higher power in the pulse to be the relevant parameter rather than the average power.

The curve of the fractional growth in the first day after sonication vs. the average ultrasonic intensity of pulsed as well as continuous beams of ultrasound seems to be sigmoid. Thus it may be possible to interpret the damage in terms of the target theory. The "hit" in the case of ultrasound may be a force of a certain magnitude required to produce an effect on a target.

In contrast to the response of the roots to treatment with ionizing radiation, the growth rate after exposure to ultrasound decreased to a minimum in the first day after exposure, recovering rapidly to the control value in the next few days. The results obtained are similar to those by Bleaney and Oliver (1972a) for the roots of Vicia, but the roots of Zea seem to be much more

sensitive to ultrasonic irradiation than those of Vicia which can possibly be explained in terms of the geometry of the root tip. As the minimum in the growth rate curve is not delayed (as was found for X-rays), it appears (Bleaney and Oliver, 1972a) that cells sterilized by ultrasound are not able to undergo any divisions, the radiation damage being expressed immediately (interphase death).

For the long sonication times at the average intensities of 0.62 W/cm^2 and 0.82 W/cm^2 (continuous beams) the recovery was very much slower and the roots did not recover to control values. This may be due to the meristem being reduced to such an extent that it is unable to recover completely (i.e. degeneration of cells may have been caused by the irradiation).

The dividing cells of the meristem seem to play a major role in the expression of the sonication damage to the roots. Evidence supporting this was obtained from sonications of different regions of the root tip as well as from surgical experiments.

Models for the cell kinetics in the X-irradiated root meristem of the broad bean as proposed by Oliver and Shepstone (1965) have been adapted for the sonicated meristem of Zea. Theoretical curves of growth-rate have been computed on the basis of the assumptions that cell death occurs in the first one tenth of the first cell cycle, that the cell cycle time (T) remains constant after exposure to ultrasound and that dying cells are removed from the population and do not contribute to root growth.

Curves have been computed for a complete recovery of the meristem (equilibrium growth rate as a fraction of controls, $G_{max} = 1$) or for the meristem being unable to recover completely so that the growth rate is reduced by a constant fraction and G_{max} is less than unity.

These theoretical growth curves have been compared with some of the experimental results for the roots of Zea. As well as having nearly the correct shape, the curves generated on the basis of the above assumptions seem to indicate that the maximum proportion of cells that can be removed before a reduction in the equilibrium size of the meristem occurs is of the order of 0.50.

The cell cycle time of 31.1 ± 1.9 hours for the meristematic cells in the root tip of Zea was determined using a colchicine metaphase accumulation technique. There was also no evidence of a tendency for diurnal rythm in root elongation throughout a 24-hour period.

Sonication of the dry seeds of Zea at the average intensity of 0.82 W/cm^2 (continuous beam) for one hour did not cause any change in the germination rate of the seeds and the subsequent growth of the roots.

Temperature effects.

A maximum temperature rise of $4^{\circ}C$ during sonication at the highest average intensity of 0.82 W/cm^2 was measured by inserting an iron-constantan thermojunction into the root tip. This rise in temperature is unlikely to be significant since the threshold for the onset of heat damage

was found to lie between 30 and 45°C and the sonication damage for the roots irradiated at 7°C was found to be equal to that for the roots sonicated at 19°C.

The progress of cells through the cell cycle during long sonications at 18-22°C does not seem to have any effect on sonication damage since roots grown for 48 hrs. at 7°C, sonicated at 7°C and then grown at 19°C were affected by ultrasound in the same way as roots grown and sonicated at the higher temperature.

The shapes of the growth curves obtained when roots were treated in hot water at 45°C for 15 minutes are similar to many of the growth curves for the sonicated roots. This may indicate an analogy (interphase death) between the damage caused by both modalities.

The effect of dissolved oxygen.

For various concentrations of dissolved oxygen, brought about by passing air, helium, oxygen and nitrogen through the water in the sonication tank, a marked reduction in damage to the roots was observed compared to the sonication effect in air-equilibrated water. Similar reductions in damage were found for all oxygen concentrations up to 22.2 ml/l, except for the lowest oxygen content of about 1.4 ml/l for which the damage was somewhat greater, which may possibly be ascribed to a "filtering effect" of the sound by the gas bubbles.

Dose-fractionation.

The split-dose experiments, similar to those by Bleaney and Oliver (1972a) for Vicia faba, revealed that any possible damage in surviving cells produced as a result of exposure to ultrasound at an average intensity of 0.82 W/cm^2 (continuous beam) is probably not repaired within a period of 24 hours.

Age-response to sonication.

No age-response was observed in meristems of Zea partially synchronized with hydroxyurea. A peak mitotic index of 20% at about 16 hours after removal from hydroxyurea may, however, not be sufficiently high to detect any possible variation of the sensitivity of the cells in relation to their position in the cycle.

Combined effects of ultrasound and X-rays.

Pulsed and continuous ultrasonic beams of high and low average intensities (see Table 4.8) were used in conjunction with a dose of 775 rads of X-rays which was given before, after or simultaneously with the sound. These experiments have shown that ultrasound and X-rays seem to act independently of each other, suggesting that the mechanism of damage due to X-rays is different from that due to ultrasound, possibly as an immediate type of death in the case of the latter, as opposed to a delayed (? reproductive)type of death in the case of the former (Bleaney and Oliver, 1972a).

An increase in the X-ray effectiveness under the action of ultrasound as reported by various authors (see Table 1.4 , Chapter I), has not been found.

Combined effects of ultrasound and drugs.

The expression of damage by vincristine was similar to that due to X-rays and other radiomimetic drugs, e.g. 8-ethoxycaffeine.

Pulsed and continuous beams of ultrasound of high and low average intensities (see Table 4.9) were used in conjunction with treatments in a 0.004% solution of vincristine for one or two hours before or after the sound. As for X-rays, ultrasound and vincristine seem to act independently of each other.

The growth curve for the roots treated with a 1.25 mM solution of hydroxyurea for 36 hours, however, shows an immediate decrease in growth rate after treatment and a subsequent rapid recovery similar to that observed for certain ultrasonic exposures. Hydroxyurea and ultrasound also seem to act independently of each other.

CHAPTER I.

INTRODUCTION.

EFFECTS OF ULTRASONIC WAVES ON LIVING MATTER.

The first recorded effect of ultrasound - acoustical waves at a frequency above the audible range - on living organisms was produced by accident over 50 years ago. Chilowsky and Langevin (1916) had been investigating the ability of ultrasonic signals to detect underwater obstacles and had noted that if fish swam into the ultrasonic beam they were instantly killed. The first deliberate attempt to affect biological material with ultrasound was also destructive; Wood and Loomis (1927) found that ultrasound could produce such diverse effects as the injury and death of small frogs and fish, the disruption of plant cells and protozoa, and the haemolysis of erythrocytes. In research that followed, it was noted that the sensitivities of various organisms are different and that some of the effect, such as partial paralysis, may be reversible. Some of the survival curves obtained in these early experiments were of the kind shown in Figure 1.1, which have the shape characteristic of the 'all or nothing' effect of ultrasound (Grabar, 1953), i.e. a certain minimum sonication time and intensity is necessary to produce an effect.

Perhaps the main value of this early work was that it stimulated research into the interaction between ultrasound and living matter, research which led eventually to the therapeutic use of ultrasound. Gradually it was realised that destruction was only one of the proper-

ties of ultrasonic radiation, and that by careful regulation of the treatment parameters (frequency, intensity, treatment time and pulsing of the radiation) beneficial results could also be achieved (Dyson, Pond, Joseph and Warwick, 1968).

Over the past ten years ultrasound has also become well established as the basis of a varied and expanding group of medical diagnostic techniques (Blitz, 1967; Brown and Gordon, 1967), and some misgivings over possible dangers, immediate or delayed, have arisen (Andrew, 1964; Connolly and Pond, 1967; Hill, 1968; Macintosh and Davey, 1970 and 1972). The clinical use of ultrasound has not been preceded by animal experimentation in which the model has been as sensitive as that in the clinical situation, as was the case when X-rays were first introduced as a diagnostic and therapeutic tool in medicine. For X-rays the possibility of any adverse effects was only realised when it was discovered that they were capable of sterilizing guinea pigs without any other obvious change in the well-being of the treated animals (Albers-Schoenberg,1903). However, recent results of animal experimentation, using diagnostic ultrasonic devices, have failed to disclose any deleterious effects (Woodward, Pond and Warwick, 1970; Taylor and Dyson, 1972). The nature and extent of the biological effects are, however, still uncertain (Hill, 1968). It therefore seems important to have a clear understanding of the factors that determine whether any potentially harmful forms of biological change may arise in

the course of any of the applications to which ultrasound is being put at present.

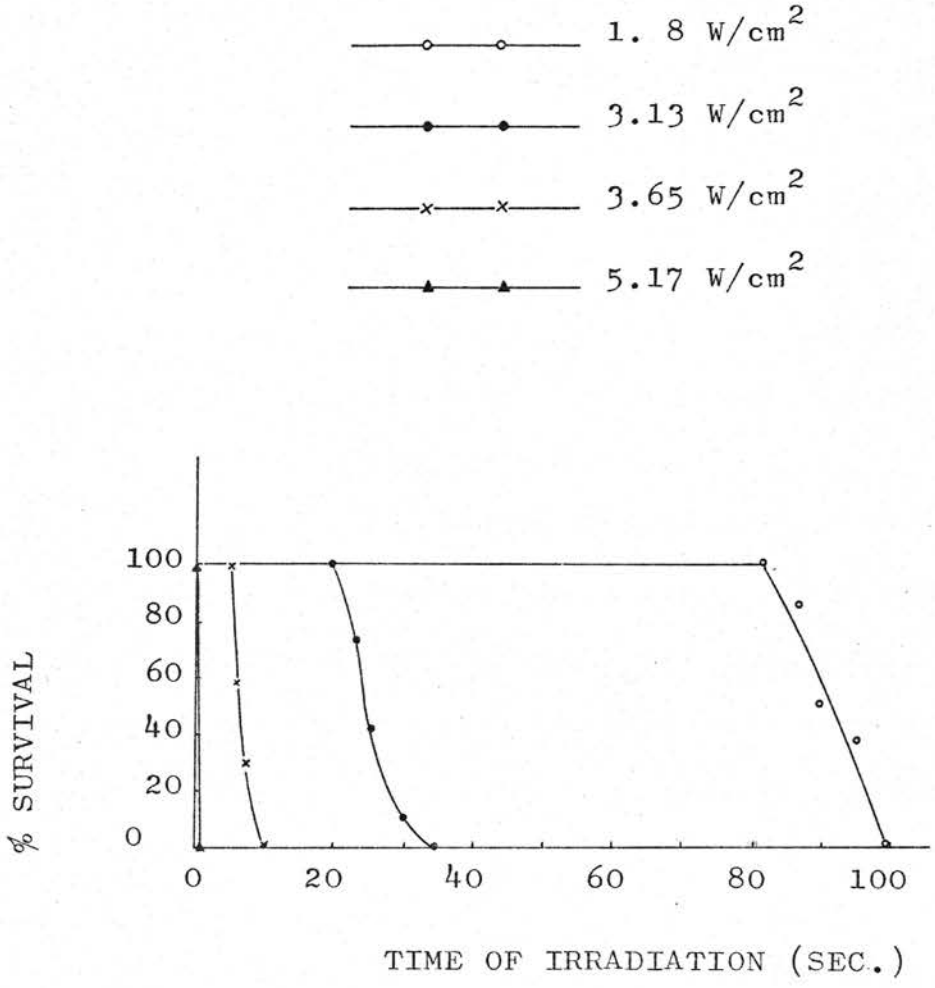
In parallel with this development a revival has occurred in studies of the mode of biological action of this form of radiation and, at the same time, "it has become apparent that the significance of much of the extensive pioneering work in this field is vitiated by the lack of an adequate system for measuring and reporting physical parameters of irradiation" (Hill, 1970).

Since the present studies are on the effects of ultrasound on the root meristem of the maize plant, the next few paragraphs will be devoted to this particular aspect of ultrasound biology.

FIGURE 1.1.

LETHAL EFFECT OF ULTRASOUND OF DIFFERENT INTENSITIES ON TADPOLES.

(FROM: BARTH, 1949)



SONICATION OF ROOT MERISTEMS.

As early as 1939 studies were carried out by Yamaha and Ueda on the effect of ultrasound on the root meristem of Vicia faba. They described the vacuolisation of nuclei and chromosome fragmentation. The immediate effect of ultrasound (400 kHz) has been studied in the meristematic cells of Narcissus by Newcomer and Wallace (1949). Induction of chromosomal aberrations have also been reported by Slotova, Karpfel and Hrazdira (1967). These authors irradiated meristematic cells of Vicia faba with ultrasound at an intensity in the range 0.2-3 W/cm^2 for times ranging from 1-20 min.

Watmough, Oliver and Cavanagh (1969) have reported the effect of protracted ultrasonic irradiation at 70 kHz on the growth of seedlings of Vicia faba. The pattern of reduction in root growth reported by these authors is similar to that previously observed under continuous gamma radiation exposure at some 2.5 rads/hr. (Oliver and Shepstone, 1965).

Recently, Bleaney and Oliver (1972a) have described experiments in which the roots of Vicia faba seedlings have been exposed to 1.5 MHz ultrasound at intensities in the range 1-4 W/cm^2 (continuous beams) for periods of 2 - 60 minutes. The subsequent average growth in eight days expressed as a fraction of the corresponding average growth for control roots, decreases with increasing exposure time and radiation intensity (Figure 1.2). Figure 1.3 shows the results obtained by Bleaney and Oliver (1972b) when

sonicating the roots of Vicia with a pulsed beam of ultrasound. They have found that for beams of the same average intensity, the use of a long pulse length (1ms) results in a larger reduction of root growth than when the short pulse length of 20 μs with the same duty cycle of 0.25 is used.

The response of the roots of Vicia to ultrasound can be compared to that following an X-radiation dose as shown in Figure 1.4 (Bleaney and Oliver, 1972a). The seedlings of Vicia have been widely used as a radiobiological test system (Read, 1959), and recently experiments using the roots of Zea mays have also been successful (Fenner, 1970; Hering, 1971). The score of radiation damage in these studies is based on gross inhibition of root growth. However, the results have a wider meaning and importance when it is appreciated that the reduction of root growth closely reflects the sterilizing of cells in the root meristem. Figure 1.5 illustrates the structure of the primary root of a Zea seedling; it is similar to that for Vicia which has been widely quoted in studies of cell population kinetics as a classical example of a stem cell compartment (Gilbert and Lajtha, 1965). The meristem of Zea (Vicia) contains actively dividing cells with a mitotic cycle of 22 - 29 hours (Table 1.3); cells produced by division in the meristem pass into the differentiating zone, and by elongation produce the macroscopic lengthening of the root. If the root tip is exposed to radiation, a proportion of the di-

viding cells are sterilized, and the subsequent growth of the root is thereby reduced below that of the controls, but returns to normal over a period of about ten days.

The X-ray curve in Fig. 1.4 is completely typical of the response to ionizing radiation. The growth rate remains unchanged for the first day, falls to a minimum at about five days, and then increases gradually back to the control value by some ten days post-irradiation. Growth in the first day is associated with elongation of cells which were already differentiated (and so had ceased to divide) at the time of exposure, and this process has been shown to be unaffected by low doses of X-radiation (Read, 1959). As the radiation-damaged cells in the meristem attempt division they may die, leading to a reduction in the meristem population and a consequent reduction in root growth rate. At these low dose levels such cells may, in fact, still be able to undergo one, two or three successful divisions. Thus, the expression of the full radiation damage is delayed, and the meristem population continues to fall over several cell cycles (of 24-30 hours for Vicia) to give the minimum growth rate at five days. Then, as the meristem is repopulated by division of surviving cells, the growth rate recovers to normal over a further period of about five days.

In contrast to this response, the growth rate falls immediately after exposure to ultrasound (Figure 1.4), the growth rate during the first day being lower for higher intensity and longer irradiation times (Figure 1.6).

This may indicate interference with the process of elongation of the differentiated cells (Bleaney and Oliver, 1972a). For Vicia the growth rate then recovers to the control value by some seven to eight days post-sonication. Thus, in view of the similarity of the growth pattern to that after X-irradiation, it may again be possible to interpret the inhibition of root growth in terms of damage to the dividing cells of the meristem (Bleaney and Oliver, 1972a). However, as the minimum in the growth rate curve is not delayed, it appears that cells sterilized by ultrasound are not able to undergo any divisions, the radiation damage being expressed immediately (Bleaney and Oliver, 1972a). Because of the similarity in the expression of the X-radiation and sonication damage, the mathematical models which have been used for explaining the behaviour of the root meristem of Vicia after X-irradiation will be modified (as described below) for the behaviour of the meristem of Zea after sonication.

Model systems for the meristem of Zea mays.

Theoretical models have been described for explaining the recovery of the root meristem of Vicia faba from single exposures of ionizing radiation at high dose rates (Hall, Lajtha and Oliver, 1962; Shepstone and Oliver, 1963). Two of these theoretical models (hereafter referred to as Model A and Model B) are to be adapted for the behaviour of the meristem of Zea mays after acute (short duration)

doses of ultrasound. They refer to the feedback-control relationships which are postulated to determine the reduction in the proportion of cells differentiating when the meristem is reduced below its normal size (in the present instance by a dose of ultrasound), thus providing an excess of cells produced by division over those lost by differentiation so that repopulation of the meristem can take place.

Model A.

This model considers the cells in the meristem to be in exponential growth with a uniform cell cycle time. Under normal equilibrium, production of new cells by division is assumed to be balanced by removal of an equal number of cells for differentiation. To provide for repopulation of a depleted meristem, it is postulated that the proportion of cells removed from the population for differentiation per unit time is itself proportional to the ratio of the meristem population at that time to the normal equilibrium population. This results in a corresponding increase in the proportion of cells dividing and a gradual increase in the total population back to the normal level.

Model B.

Here an attempt is made to provide the right type of feedback-control on the basis of possible biological response to a population change. It is suggested here that in the normal meristem all the cells present them-

selves for division but an equilibrium is maintained, because, for the meristem as a whole, only half of these cells are able to divide. The other half, failing to divide, differentiate. The proportion of cells able to divide varies through the meristem from virtually 100 per cent to zero at the edge of this region, due possibly to a variation in concentration of some substance which must be utilized for maintenance of reproductive integrity. In a depleted meristem, the given supply of this substance provides for more than half the cells reaching division to retain their reproductive integrity and so divide. This proportion increases as the meristem is reduced, thus enabling repopulation to occur.

The mathematical derivations of Model A and Model B are given in Appendix B.

Calculation of the model kinetics after a single acute dose of ultrasound.

The method of calculation adopted is similar to that which has been used for theoretical consideration of the kinetics of the bone-marrow stem cell system (Lajtha, Oliver and Gurney, 1962), and which has also been employed for obtaining theoretical growth - rate curves for Vicia faba and Zea mays roots under continuous radiation exposure at low dose-rate (Oliver and Shepstone, 1965; Hering, 1971).

It is assumed that the intermitotic cycle time is divided into ten equal sections or compartments (to be

denoted by the subscript n in any instance) and the appropriate proportion of the cell population occurring in each of these cell cycle compartments is calculated for the particular model which is assumed to apply. The progress of the group of cells in each cell cycle compartment to the next compartment at each time step can be calculated, considering the necessary changes in numbers as they are transferred from one compartment to the other.

In the case of the unirradiated meristem the change in the number of cells is due to the removal of cells from the population for differentiation.

In Model A, the population is regarded as being in exponential growth, a sufficient proportion of the cells throughout the cell cycle being removed for differentiation per hour to maintain a constant total population, and as a result the number of cells per compartment must vary exponentially through the cell cycle (Oliver, 1963). Then if there are say ten cells in the compartment before mitosis, the distribution of cells in each compartment will be initially as set out in Table 1.1 for t=0. The value of 'Q' in the above-mentioned table is found from the relation $Q^{10} = \frac{1}{2}$ (i.e. Q=0.933). It is the time constant for the exponential distribution of the cells throughout the cell cycle in accordance with the requirements of the model outlined earlier.

Thus a constant proportion, Q, of cells are being lost due to differentiation in each period of one-tenth of the cycle time, corresponding to transfer from one

compartment to the next, to leave a total of say 10 in the compartment prior to mitosis. A number 10 in this compartment will be doubled at division, to give 20 cells. This will be the state of affairs in the meristem at the onset of ultrasonic radiation.

After sonication, we proceed to find the number of cells being transferred from each compartment (n-1) to the next compartment (n) at the end of each interval of time (t) equalling one tenth of the cell cycle time T. The principle of calculation is then to set out a table as shown in Table 1.1, listing the number of cells in each of the ten cell cycle compartments. During each one tenth of a cell cycle, the cells in each cell cycle compartment will progress through this compartment to the next (adjacent) compartment.

(1-Q) is the fraction of the cells of the compartment which are removed by differentiation per one tenth of a cell cycle and this will be reduced in proportion to $(F)_t$, the total meristem population as a fraction of the steady state value at the time t after sonication. The value of F is changing continuously, but its value at the beginning of each time interval is assumed to apply throughout that short interval. Thus the fraction of cells differentiating per one tenth of the cell cycle will be (1-Q)F. Therefore at the time t, the number of cells proceeding from compartment (n-1) to compartment n is given by:

$$(A_n)_t = X_t(A_{n-1})_{t-1}$$

where $X_t = 1-(1-Q)F_t$.

There is, however, a further proportion (P) of cells lost due to sonication damage in the first one tenth of the first cell cycle and therefore the number of cells actually reaching $(A_n)_1$ is:

$$(A_n)_1 = X_1 \, k \, (A_{n-1})_0$$

where k (= 1-P) is the fraction of the cells of the compartment which are removed by sonication in the first one tenth of the first cell cycle.

Transfer of cells from compartment number 10 at the time t to the next, i.e. compartment number 1 at the time t=t+1 involves the special change at division. Cells must be removed for differentiation as before and the remainder is doubled to correspond to division.
Thus the number of cells proceeding from compartment number 1 at the time t to compartment number 2 at the time t=t+1 is given by:

$$(A_1)_{t+1} = 2X_{t+1} \, (A_{10})_t$$

Accounting again for the loss of cells due to sonication damage in the first one tenth of the first cell cycle, we have

$$(A_1)_1 = 2X_1 \, k \, (A_{10})_0$$

The fractional size of the meristem at any time t would be:

$$F_t = \frac{\sum_{n=1}^{10} (A_n)_t}{20\ (Q+Q^2+Q^3+.....+Q^{10})}$$

where $\sum_{n=1}^{10} (A_n)_t$ represents the sum of all the cells at the time t.

The growth rate G (at the time t) as a fraction of controls is therefore:

$$G_t = F_t^2 = \left[\frac{\sum_{n=1}^{10} (A_n)_t}{20\ (Q+Q^2+Q^3+.....+Q^{10})}\right]^2$$

This would be the growth rate if the population of cells in the meristem increases back to the normal level. For some of the ultrasonic doses used in the present experiments (Chapter IV) the growth curves level off at values of G less than 1, i.e. the meristem population at equilibrium is assumed to be reduced in size due to the radiation exposure. The value of G at which the growth curves (theoretical and experimental) level off will hereafter be referred to as G_{max}. Thus the growth rate as a fraction of controls is reduced by a constant fraction, and this has been accounted for in the calculation above by multiplying each calculated value of G_t by say m if the theoretical growth curve is to be compared with the experimental one levelling off at m.

A Computer program (Appendix C) has been written

in Fortran IV to calculate G for a large number of values of k and G_{max} on the Univac 1106 Digital Computer. The flow diagram for the calculation is also outlined in Appendix C.

For Model B, exactly similar considerations can be applied. In this case the cells are assumed to come up to division, when a certain proportion retain their reproductive integrity and divide; the remainder, unable to divide, differentiate. The dividing proportion is 0.5 in the normal equilibrium and higher in a meristem of reduced size, thus permitting recovery. It is again assumed that the intermitotic cycle time is divided into ten equal compartments and that there are ten cells in the last stage. In the case of this model there will also be ten cells in each of the other compartments. The initial situation will be as shown in Table 1.2 for t=0. This arrangement will then account for the fact that in this model there is a linear distribution of cells within the cell cycle.

After an acute dose of ultrasound a further proportion of the cells, which would be expected to divide in the first one tenth of the first cell cycle, fail to do so successfully, dying a mitotic death. This means that not only do these cells not produce new cells by division, but they themselves are also removed from the population. An equal proportion, P, of cells are removed by sonication from each of the ten compartments in the first one tenth of the first cell cycle.

If F is the fractional size of the meristem,

Model B states that only a fraction X_t of the possible $2\ (A_{10})_t$ will divide per unit time, where

$$X_t = \frac{1 - \exp(-1.595F_t)}{1.595F_t}$$

The equations describing the population kinetics of the meristem after an acute dose of ultrasound on the basis of Model B will therefore be as follows:

$$(A_n)_t = (A_{n-1})_{t-1}$$

$$(A_1)_t = 2X_t\ (A_{10})_{t-1}$$

Accounting for the loss of cells due to sonication damage in the first one tenth of the first cell cycle, we have

$$(A_n)_1 = k\ (A_{n-1})_0$$

and

$$(A_1)_1 = 2X_1\ k\ (A_{10})_0\ ,$$

where k is the fraction of the cells of the compartment which are removed by sonication in the first one tenth of the first cell cycle.

The fractional size of the meristem at any time t will be given by:

$$F_t = \frac{\sum_{n=1}^{10} (A_n)_t}{100}$$

where $\sum_{n=1}^{10} (A_n)_t$ is the sum of all the cells at the time t.

The growth rate G, as a fraction of controls is again derived from F:

$$G_t = \frac{1.595F_t + \exp(-1.595F_t) - 1}{0.7975}$$

Alternatively it can be shown that

$$G_t = \frac{(A_{10})_t (1 - X_t)}{5}$$

Again, for experimental growth curves levelling off at values of G less than 1, theoretical growth curves have been obtained for a large number of values of G_{max}.

A Computer program (Appendix C) has been written in Fortran IV to calculate G for a large number of values of k and G_{max} on the Univac 1106 Digital Computer. The flow diagram for the calculation is also outlined in Appendix C.

In the above formulae, no account has been taken of any possible effect of the ultrasonic irradiation on the length of the cell cycle. A lengthening of the cell cycle has been observed when root meristems were exposed to ionizing radiation (Clowes and Hall, 1962). The sensitivity of various mammalian cells to X-rays has also been found (using synchronized cell populations) to depend on the position of the irradiated cell in the cell cycle (Sinclair, 1968). As far as ultrasound is concerned, only a few investigations of this nature have been carried out (p. 39).

FIGURE 1.2.

G_8, THE AVERAGE GROWTH OF ROOTS DURING EIGHT DAYS POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH OF CONTROL ROOTS, PLOTTED AS A FUNCTION OF TOTAL EXPOSURE (INTENSITY IN W/cm^2 MULTIPLIED BY DURATION OF EXPOSURE IN HOURS) FOR DIFFERENT RADIATION INTENSITY LEVELS.

(FROM: BLEANEY AND OLIVER, 1972a)

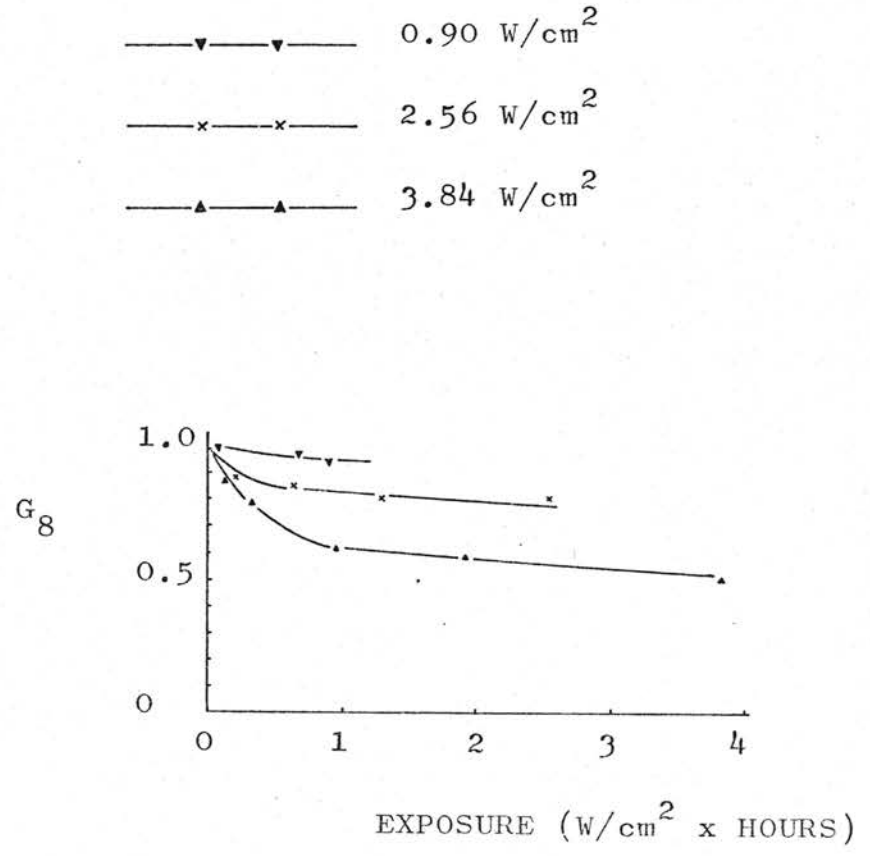


FIGURE 1.3.

AVERAGE GROWTH (G_8) OF *VICIA FABA* ROOTS IN EIGHT DAYS FOLLOWING EXPOSURE TO 1.5 MHz ULTRASONIC RADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, USING CONTINUOUS RADIATION (CIRCLES AND FULL LINE), 1 ms PULSES WITH 0.25 DUTY CYCLE (CROSSES AND DASHED LINE), AND 20 μs PULSES WITH 0.25 DUTY CYCLE (SOLID POINTS). ABSCISSA REFERS TO AVERAGE POWER IN ALL CASES.

(FROM: BLEANEY AND OLIVER, 1972b)

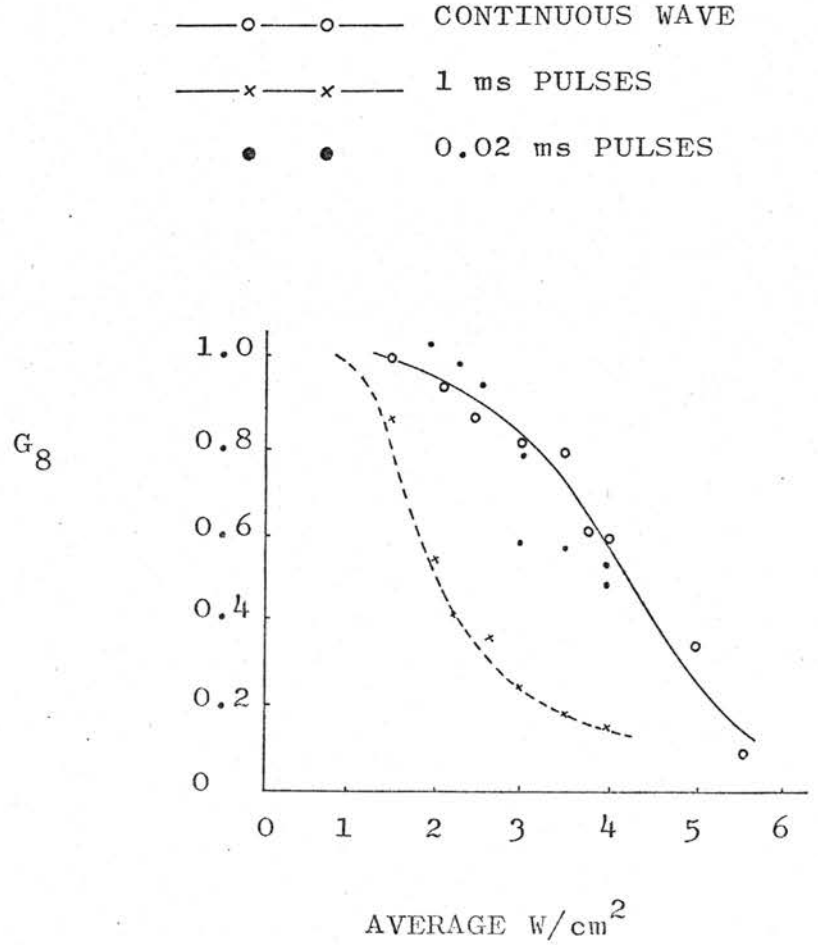


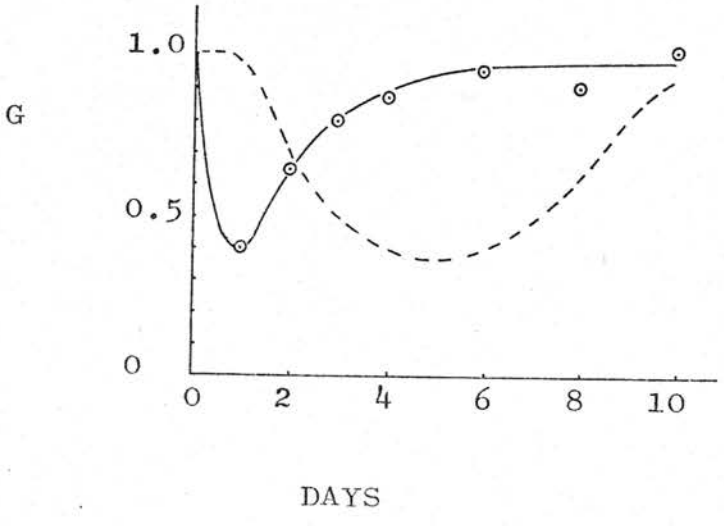
FIGURE 1.4.

VARIATION OF G, THE AVERAGE DAILY GROWTH OF IRRADIATED ROOTS AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS OF THE SAME AGE, WITH TIME AFTER EXPOSURE.

(FROM: BLEANEY AND OLIVER, 1972a)

_____ 1.5 MHz ULTRASONIC RADIATION AT 3 W/cm^2 FOR 15 MINUTES

--------- 125 RADS X-RADIATION (FROM HALL, LAJTHA AND OLIVER, 1962).



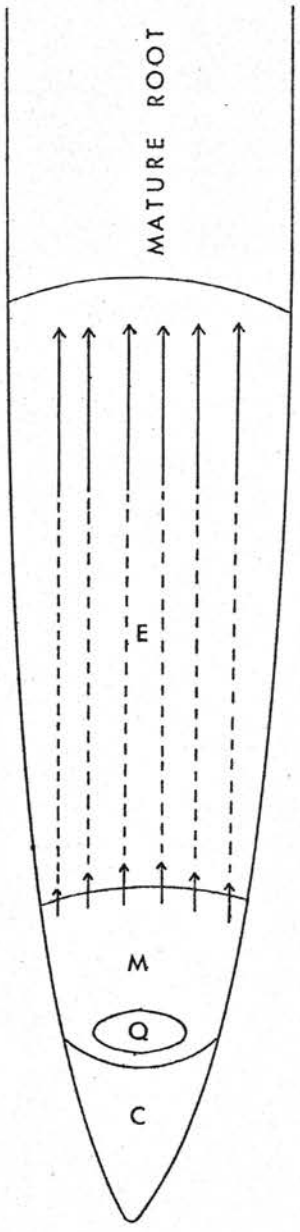


FIGURE 1.5.

THE STRUCTURE OF THE ROOT OF ZEA.

C. ROOT - CAP.

Q. QUIESCENT CENTRE.

M. DIVIDING MERISTEMATIC CELLS SURROUNDING THE QUIESCENT CENTRE.

E. ELONGATING ZONE.

FIGURE 1.6.

VARIATION OF G_1, THE AVERAGE GROWTH IN THE FIRST DAY POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, AS A FUNCTION OF IRRADIATED TIME AT DIFFERENT INTENSITY LEVELS.

(FROM: BLEANEY AND OLIVER, 1972a)

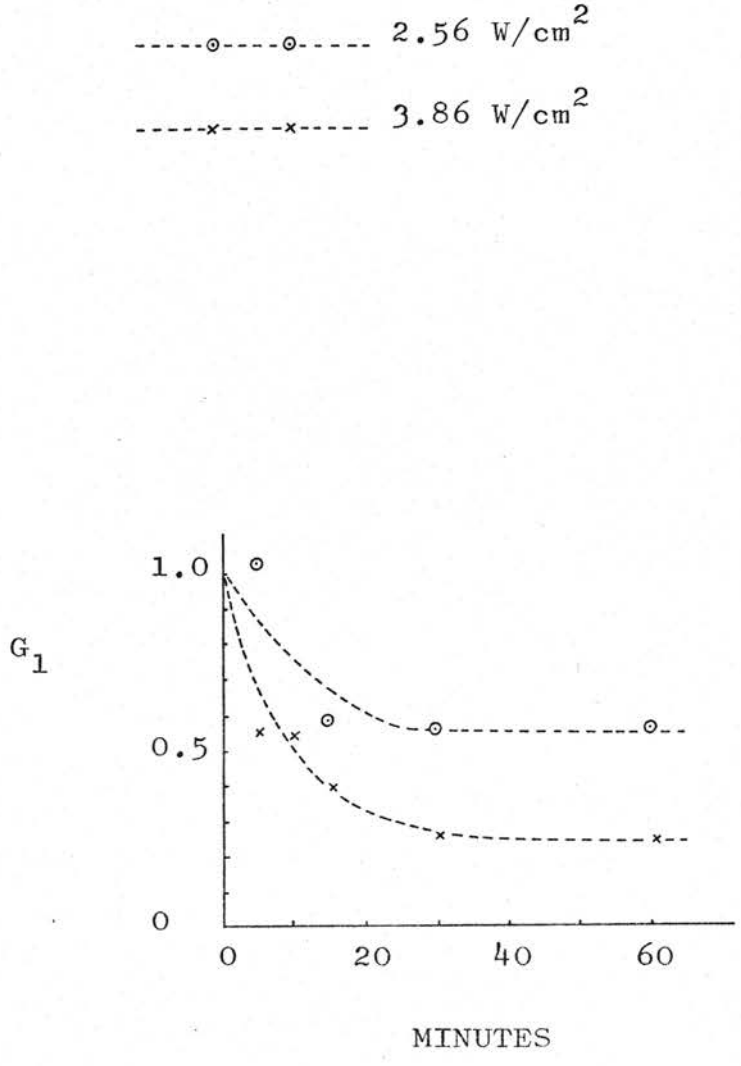


TABLE 1.1: SCHEME FOR THE CALCULATION OF G MODEL-A

Time t=0 Q=0.933

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_0=20Q$	$(A_2)_0=20Q^2$	-	-	-	-	-	-	-	$(A_{10})_0=20Q^{10}$

$$F_0 = \frac{\sum_1^{10}(A_n)_0}{20(Q+Q^2+\cdots+Q^{10})}$$

$$G_0 = F_0^2 = 1$$

Time t=1 (=1/10 of the cell cycle) $X_1=1-(1-Q)F_0$ $Y=X_1k$

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_1=2Y(A_{10})_0$	$(A_2)_1=Y(A_1)_0$	-	-	-	-	-	-	-	$(A_{10})_1=Y(A_9)_0$

$$F_1 = \frac{\sum_1^{10}(A_n)_1}{20(Q+Q^2+\cdots+Q^{10})}$$

$$G_1 = F_1^2$$

Time t=2 (=2/10 of the cell cycle) $X_2=1-(1-Q)F_1$

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_2=2X_2(A_{10})_1$	$(A_2)_2=X_2(A_1)_1$	-	-	-	-	-	-	-	$(A_{10})_2=X_2(A_9)_1$

$$F_2 = \frac{\sum_1^{10}(A_n)_2}{20(Q+Q^2+\cdots+Q^{10})}$$

$$G_2 = F_2^2$$

TABLE 1.2: SCHEME FOR THE CALCULATION OF G MODEL-B

Time t=0 $X_0 = \frac{1-\exp(-1.595F_0)}{1.595F_0} = \frac{1}{2}$

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_0=10$	$(A_2)_0=10$	-	-	-	-	-	-	-	$(A_{10})_0=10$

$F_0 = \frac{\sum_1^{10}(A_n)_0}{100}$

$G_0 = \frac{(A_0)_0(1-X_0)}{5}$

Time t=1 (=1/10 of the cell cycle) $X_1 = \frac{1-\exp(-1.595F_0)}{1.595F_0}$ $Y=kX_1$

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_1$ $=2Y(A_{10})_0$	$(A_2)_1$ $=k(A_1)_0$	-	-	-	-	-	-	-	$(A_{10})_1=k(A_9)_0$

$F_1 = \frac{\sum_1^{10}(A_n)_1}{100}$

$G_1 = \frac{(A_{10})_1(1-X_1)}{5}$

Time t=2 (=2/10 of the cell cycle) $X_2 = \frac{1-\exp(-1.595F_1)}{1.595F_1}$

Cell-cycle compartment (n)	1	2	3	4	5	6	7	8	9	10
No. of cells in each compartment (A_n)	$(A_1)_2$ $=2X_2(A_{10})_1$	$(A_2)_2$ $=(A_1)_1$	-	-	-	-	-	-	-	$(A_{10})_2=(A_9)_1$

$F_2 = \frac{\sum_1^{10}(A_n)_2}{100}$

$G_2 = \frac{(A_{10})_2(1-X_2)}{5}$

TABLE 1.3.

RATES OF MITOSIS IN REGIONS OF THE ROOT MERISTEM OF ZEA MAYS.

Quiescent Centre	Cap initials	Stele just above Q.C.	Stele 200-250 µm above Q.C.	Method	References
174	12	28	29	Metaphase Accumulation.	Clowes, 1961.
167	14	22	23	Pulse labelling	Clowes, 1965.

The figures are average durations of a mitotic cycle in hours.

From: Clowes, "Anatomical Aspects of Structure and Development." 1968.

The cell cycle time of the meristematic cells in the root tip of Zea.

A number of methods have been devised for determining the cell cycle time in asynchronous cell populations (Hoffman, 1953; Clowes, 1961; Clowes, 1965). According to Evans, Neary and Tonkinson (1957) a reliable approach is one which depends upon determining the number of new cells which are produced over given intervals of time, and information on the rate of production of new cells in the meristem can be obtained by a simple colchicine method. The principle of this method rests upon the fact that colchicine would not disturb the entry of cells into mitosis, but would prevent cells from passing out of mitosis by arresting the cells at the metaphase stage. With this technique the number of cells accumulated at metaphase, over a given period of colchicine treatment, can be easily determined. Data on metaphase accumulation by colchicine treatment of plant tissues have been published (Hawkes, 1942; Levine, 1951) but the results show an irregular accumulation of metaphases with time, particularly with the longer treatments. Evans, Neary and Tonkinson (1957) have shown that a steady metaphase accumulation rate can be obtained for the root tips of Vicia faba if treatments of short durations are used (Fig. 1.7). There is quantitative data about the rates of mitosis of the cells in the apex of Zea, both from continuous and pulse labelling of nuclei with thymidine and from the accumulation of phases of mitosis blocked

FIGURE 1.7.

METAPHASE SCORES FOR CONTROL AND COLCHICINE TREATED ROOTS OF VICIA FABA, FOR TREATMENTS OF UP TO 24 HOURS.

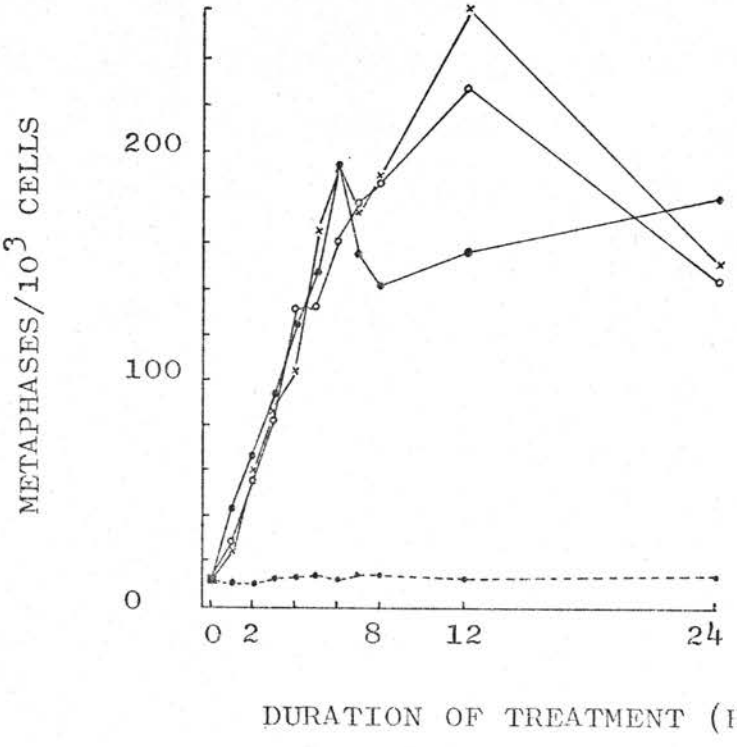
(Note the steady rate of metaphase accumulation with all concentrations of colchicine over the shorter periods of treatment).

----●----●---- DISTILLED WATER, CONTROL ROOTS

——×——×—— COLCHICINE 0.025%

——○——○—— COLCHICINE 0.05%

——●——●—— COLCHICINE 0.1%



by inhibitors (Table 1.3).

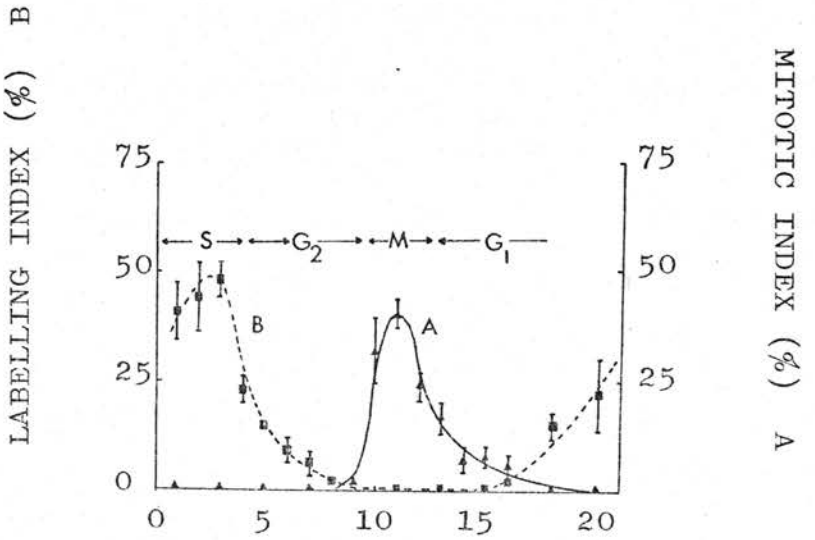
Synchronization of the cells in the meristem.

During the past few years a number of ingenious methods have been devised to produce synchronously dividing populations of mammalian cells cultured *in vitro*; these have been used to study the variation of the X-radiation response with the phase of the mitotic cycle (Terasima and Tolmach, 1963; Sinclair, 1965; Sinclair and Morton, 1966). Of the methods suggested for cells in culture, only those involving drugs would be suitable for *Zea* (Hall, personal communication). 5-Amino Uracil has been used successfully on the root meristem of *Zea* (Clowes, 1965a), and Hall, Brown and Cavanagh (1968) have synchronized the meristematic cells of *Vicia* with hydroxyurea. Figure 1.8 shows the labelling and mitotic indices measured at different times after removal of the seedlings of *Vicia* from hydroxyurea (Hall et al, 1968).

For Chinese hamster cells in culture, it has been shown that hydroxyurea kills cells that are synthesizing DNA but does not affect progression of cells through the non-DNA-synthesizing portion of the cycle; consequently, cells incubated in its presence accumulate at the end of the pre-DNA-synthetic phase (G_1). In this way hydroxyurea produces a synchronously dividing population of cells (Sinclair, 1965 and 1967).

FIGURE 1.8.

VARIATION OF THE MITOTIC INDEX AND PERCENTAGE OF CELLS LABELLED WITH TRITIATED THYMIDINE WITH TIME AFTER REMOVAL FROM A 24-HOUR EXPOSURE TO HYDROXYUREA.



The biological action of ultrasound in relation to the cell cycle.

Although a number of studies have been reported on the response of mammalian and plant cells to ultrasonic irradiation, there is very little in the literature on the age-response of sonicated cells.

Studies of the changes in the nucleus with regard to mitosis have been carried out by Selman (1952) on the root tips of Allium cepa. Mitotic figures observed for roots sonicated at intensities less than 2.5 W/cm^2 for periods of 5 to 10 minutes were found to be normal and no marked increase or decrease in the rate of mitosis was noted. A possible variation of the response of cells through the cell cycle has, however, only been investigated for mouse leukaemia cells in tissue culture (Clarke and Hill, 1969). They found that cells which are in mitosis during the sonication have a susceptibility to ultrasonic disintegration that is significantly above the average for the population. A fractional irradiation regime however, with ultrasound pulses sufficiently short (≤ 1 ms) so that the disintegration mechanism is inhibited, did not lead to detectable changes in the cell proliferation pattern.

So far, only the response of actively dividing populations of cells to ultrasonic treatment has been described. When metabolically inactive cells are exposed to ultrasound, however, the expression of sonication damage

in the subsequent growth may be different from that exhibited by a sonicated actively dividing population of cells. Such an investigation can readily be carried out using the dry seed and the growing root of Zea.

EFFECT OF ULTRASOUND ON SEEDS.

Interest in the effect of ultrasound on seeds has been aroused by various conflicting reports on the possible stimulation of the subsequent growth of the plant by treatment of its seed or roots with ultrasound, e.g. on peas (Istomina and Ostrovskij, 1936), on root tips of Narcissus following ultrasonic treatment at 400 kHz (Newcomer and Wallace, 1949), on seeds of cress (Tomberg, 1950), on Pisum (Spencer, 1952), on barley (Johnson and Obolensky, 1954), on grain (Busnel and Obolensky, 1955; Obolensky, 1957). The increase in germination has been questioned by Findley and Campbell (1953) who studied the effect of ultrasound on maize seeds. Haskell and Selman (1950) also observed a lower germination rate of the seeds of maize exposed to ultrasound at an intensity of 35 W/cm^2 (1MHz beam) for various times. The subsequent growth of the plants was normal. Gordon (1963) has suggested that the increase in germination rate can probably be accounted for by increased imbibition, thus forcing into action the basic processes of metabolism more quickly than in untreated seeds. The increase in growth rate is more complex, however, and would appear to be associated with a selective increase in anabolic activity (Dyson, Pond, Joseph and Warwick, 1968).

At certain intensities and frequencies, the action of ultrasound on biological material can be analogous to that of ionizing radiation (described later in this chapter). Cherry (1962) has made a study of the effect of ionizing radiation on the dry seeds of Zea mays and noted that a "delayed killing" of the roots occurred, i.e. no difference from controls is observed for several days, but later the irradiated group shows a decreased root length and a poor development of lateral roots. This effect has not been observed in any of the studies of the effects of ultrasound on seeds.

None of the processes which occur in the growing root, namely division, elongation and differentiation, take place in dry, ungerminated seeds. Seeds are more resistant than growing roots to environmental changes and survive ionizing radiation treatments that would kill roots (Shepstone, 1964). Furthermore, dry seeds are relatively inactive metabolically, so the effects of radiation can be modified experimentally without the immediate intervention of active cell metabolism.

Davidson (1960) has classified the factors producing modifications of the effects of ionizing radiations on seeds. Some of these factors may also apply when seeds are exposed to ultrasonic radiation.

These factors are as follows:-

(1) Factors operative before irradiation:

(a) Storage conditions: composition of the atmosphere, humidity, temperature, and degree of ploidy of the seed;

(b) Sensitivity of seeds varies with size, age and conditions of the season in which they were produced.

(2) Factors operative after irradiation but before germination, when irradiation and germination are separated in time:

(a) Water content and changes in water content;

(b) Temperature and

(c) the composition of the atmosphere.

As soon as germination begins, all treatments are related to cell metabolism and the reaction of the seed root approaches that of the growing root.

Several effects of radiation can be measured. These include (1) inhibition of germination, (2) chromosome aberrations at first mitosis in roots and shoots of seedlings and at meiosis in mature plants, (3) inhibition of mitosis, (4) reduction in the growth of coleoptiles and of roots and shoots, (5) decrease in the number of plants surviving to maturity, (6) lower fertility, as indicated by pollen fertility or seed set and (7) increased mutation frequencies in the progeny of irradiated plants.

Structure of the seed of Zea mays.

The seed of Zea mays is represented diagramatically in Figure 1.9. It is relatively simple in structure, consisting essentially of an embryo and a reserve food supply (endosperm) surrounded by an envelope or testa. The embryo consists of a radicle, a plumule, a single cotyledon

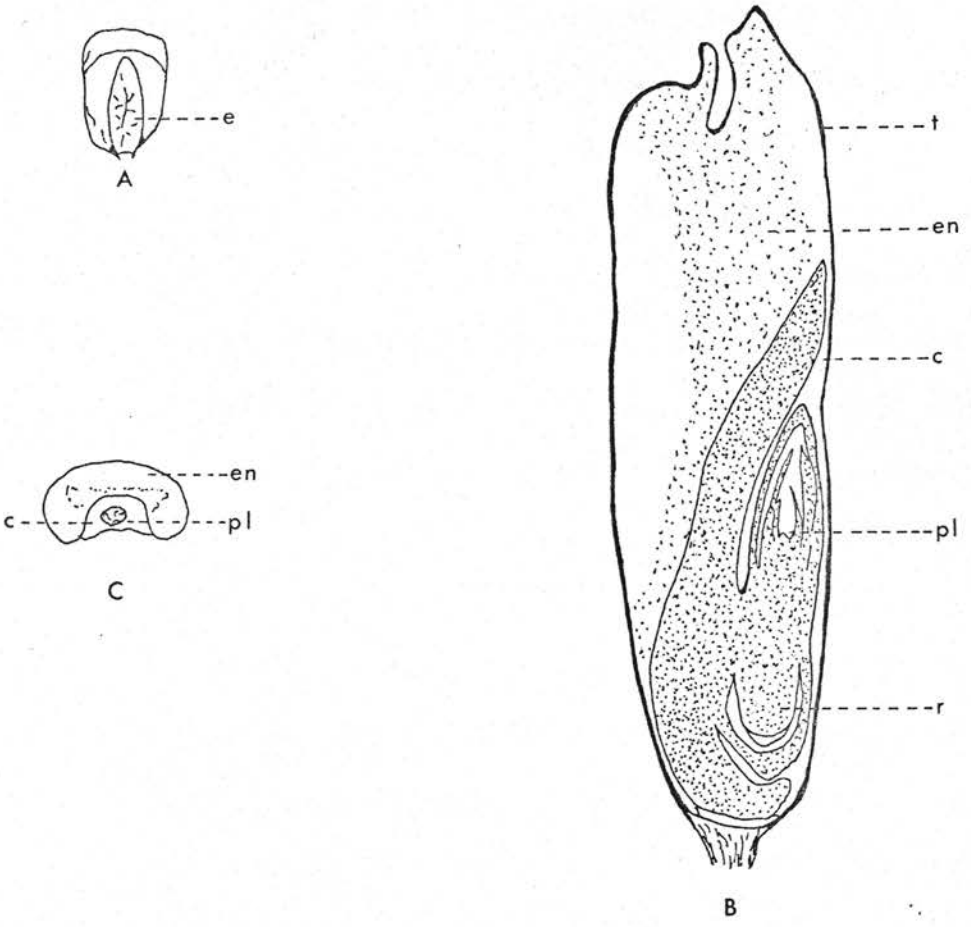


FIGURE 1.9.

STRUCTURE OF THE GRAIN OF MAIZE.

A: ENTIRE GRAIN SHOWING THE OUTLINE OF THE EMBRYO.

B: LONGITUDINAL SECTION OF THE COMPLETE GRAIN (MUCH ENLARGED).

C: CROSS-SECTION OF GRAIN WITH ENVELOPE REMOVED.

r =	radicle	t =	testa
pl =	plumule	en =	endosperm
c =	cotyledon	e =	embryo

From: F.E. Fritsch and E.T. Salisbury:

"Botany for students of medicine and pharmacology," p.32.

P. Weatherwax:

"The story of the maize plant," p.32.

and a hypocotyl which connects the radicle and the plumule. The process of germination leads eventually to the development of the embryo into a seedling.

In the early embryonic stages of development cell division occurs throughout the young organism, but as the embryo enlarges the addition of new cells is confined to the meristems. Thus at the time of sonication one may assume that the embryonic radicle has a well-defined, although metabolically inactive meristem.

As mentioned earlier, there is a similarity in the expression of the radiation damage observed when roots are exposed to ultrasound and X-rays respectively. A study of the combined action of both modalities may, therefore, throw some light on the actions of ultrasound.

INTERACTION BETWEEN ULTRASOUND AND X-RAYS.

Although the direct use of ultrasound in tumour therapy, as advocated by Hovarth (1944; 1946) is now considered to be of very limited value (Herrick, 1953; Lehman and Krusen, 1955), it has been reported by Woeber (1959; 1965) that the use of ultrasound in conjunction with X-ray therapy can increase the effectiveness of the latter. In his study Woeber reported complete regression of Walker carcinomas in rats following 350 rads of X-rays accompanied by ultrasonic irradiation at 1 W/cm^2. Equivalent results were reported following 600 rads of X-rays alone, implying that the ultrasonic irradiation increased the X-ray effectiveness by a factor of about 1.7. Woeber's results suggested that synergism between X-irradiation and ultrasound would occur with the use of an ultrasonic intensity which, by itself, had no observable effect on the tumour cells.

Experience of a similar nature has been reported by Pydorich (1966). In his treatment of eyelid epithelioma with ultrasound (0.8 MHz, 0.8 W/cm^2) in combination with 40-50 kV X-rays, the X-ray doses could be reduced by 40-42 per cent.

By application of 2 MHz ultrasound at an intensity less than 0.001 W/cm^2 simultaneously with ^{60}Co gamma radiation, Spring (1969, 1972) was able to report a demunition from 90 krads in the 37 per cent dose in observations of the germinative capacity of grass seeds (*Lolium italicum*).

Detailed combined irradiation studies were also made by Clarke, Hill and Adams (1970) on mouse lymphoma cells in suspension as well as on transplanted tumours in Marshall rats. The effects observed by Woeber were, however, not detected and the authors attribute this discrepancy to possible temperature effects in the in vivo system.

M.H. Repacholi, Woodcock, Newman and Taylor (1971) in their studies on the electrophoretic mobility of Ehrlich ascites-tumour cells have found that the irradiation with ultrasound at 1 MHz frequency, with a peak intensity of 10 W/cm^2, pulsed 1:10 for 5 minutes, gave 15% reduction in mobility; 1000 rads of 220 kV X-rays gave the same reduction, while combined treatments gave 30%. This suggests that the individual effects of the X-rays and ultrasound on the cell membrane may be additive.

A compilation of all the related studies is given in Table 1.4. The figures in the column entitled "Increased X-ray Effectiveness" are the ratio of the dose of ionizing radiation to the dose of ionizing radiation given in conjunction with ultrasound to obtain the same result or reaction.

The effects of certain drugs on tissues have been described as being similar to those of X-rays (Read,1959). Some of these drugs, e.g. vincristine, have also been successfully used in cancer chemotherapy. It has been shown, as described above, that the sensitivity of cells to X-rays can be increased by simultaneous treatment with ultrasound

and thus a possible interaction between ultrasound and vincristine may produce similar effects (which are to be investigated).

TABLE 1.4.

EXPERIMENTS IN WHICH VARIOUS BIOL. MATER. WAS IRRADIATED BY SIMULTANEOUS ULTRASONIC AND IONIZING RADIATION.

AUTHORS	BIOLOGICAL MATERIAL USED	IONIZING RADIATION AND DOSES	ULTRASONIC RADIATION FREQUENCY & INTENSITY	INCREASED X-RAY EFFECTIVENESS (SEE TEXT)
Conger, (1948)	Inflorescence of Tradescantia	250 kV X-rays 250 rads	9100 Hz (sonic)	30% increase in chromosome aberrations
Lehman and Krusen, (1955)	Solid Ehrlich ascites carcinomas in vivo	135 kV X-rays	1 MHz 8.4 Wcm^{-2}	2.0
Woeber, (1959)	Walker carcinomas in rats	60 kV X-rays 350 R	1 Wcm^{-2}	1.7
Rydorich, (1966)	Eyelid epithelioma	40-50 kV X-rays 3470-4950 rads	0.8 MHz 0.8 Wcm^{-2}	1.7
Spring, (1969)	Germinative capacity of grass seeds	^{60}Co γ-rays 2500-15000 rads	2 MHz 0.001 Wcm^{-2}	2.6
Clarke, Hill & Adams, (1970)	L5178Y lymphoma cells in vitro	250 kV X-rays 100-400 rads	1 MHz 5 Wcm^{-2}	1 ± 0.2
	Tumour BICR/M1 transplanted in rats	250 kV X-rays 2000 R	1 MHz 0.5 Wcm^{-2}	1 ± 0.3
Spring, Rytilä & Blomqvist, (1970)	HeLa cells in vitro	^{60}Co γ -rays 50-400 rads	2 MHz 0.001 Wcm^{-2}	1.7 at 50 rads 1.0 at 200 rads 0.7 at 400 rads
Repacholi, (1970)	Ehrlich ascites tumour cells in vitro	220 kV X-rays	1 MHz 10 Wcm^{-2}	Reduction in electrophoretic mobility
Repacholi, Woodcock, Newman & Taylor, (1971)	Ehrlich ascites tumour cells in vitro	220 kV X-rays	1 MHz 10 Wcm^{-2}	Reduction in electrophoretic mobility
Laviniitty, Arvaranta and Otkänen (1971)	Effect on barley seeds	^{60}Co γ-rays 0 - 20 krads	20 kHz or 80 kHz ? ?	Increase in radioresistance

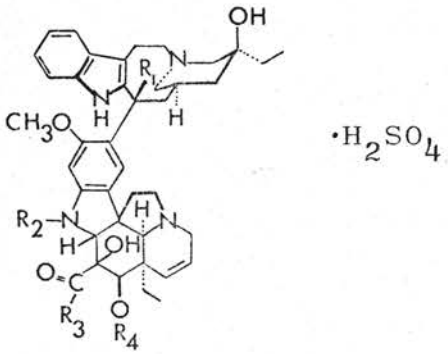
EFFECT OF THE SPINDLE-POISON VINCRISTINE ON CELLS.

A number of alkaloidal substances with anti-tumour activity have been obtained from the plant Vinca rosea Linn. (periwinkle). These alkaloids (e.g. vincristine, the molecular structure of which is shown in Fig. 1.10), produce an increase in cells in metaphase (Fig. 1.11) both in tissue culture and in vivo (Cutts, Beer and Noble, 1960; Palmer, Livengood, Warren, Simpson and Johnson, 1960; Cardinali, 1963; Frei, Whang, Scoggins, Van Scott, Rall and Ben, 1964). The mechanism of this stathmokinetic effect is unknown, and its relationship to the toxicity and anti-tumour properties of these alkaloids is obscure (Frei et al, 1964).

The age response (i.e. the relative lethality of the agent as a function of cell position in the mitotic division cycle) of synchronized lymphoma cells exposed in vivo to vincristine has been studied by Madoc-Jones and Mauro (1970). These authors found that cells in the S-phase of the mitotic cycle were most sensitive (Fig. 1.12). Similar results were also obtained by these authors when synchronized HeLa cells in tissue culture were exposed to vincristine (Madoc-Jones and Mauro, 1968). Lower drug concentrations or shorter exposure times yielded age-response curves identical in shape to the ones obtained with high concentrations.

FIGURE 1.10.

VINCRISTINE SULFATE IS THE SALT OF AN ALKALOID OBTAINED FROM THE FLOWERING HERB, THE PERIWINKLE PLANT (VINCA ROSEA LINN.). ORIGINALLY KNOWN AS LEUROCRISTINE, IT HAS ALSO BEEN REFERRED TO AS LCR AND VCR. THE EMPIRICAL FORMULA IS $C_{46}H_{56}N_4O_{110} \cdot H_2SO_4$. THE PROPOSED STRUCTURAL FORMULA IS SHOWN BELOW (NEUSS, 1964a; NEUSS, 1964b).



$\cdot H_2SO_4$

$R_1 = COOCH_3$

$R_2 = CHO$

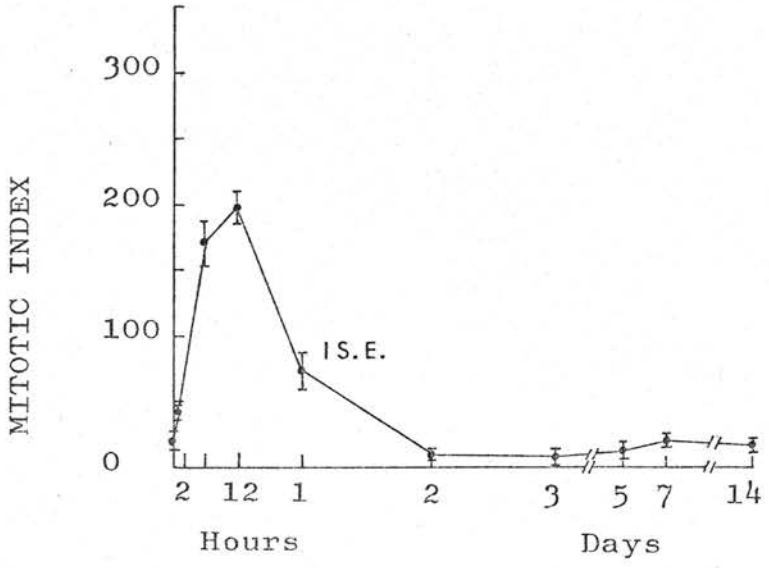
$R_3 = OCH_3$

$R_4 = COCH_3$

FIGURE 1.11.

CHANGES IN THE RAT MARROW MITOTIC INDEX AFTER ADMINISTRATION OF VINCRISTINE (0.9 mg/kg).

(FROM: FREI, WHANG, SCOGGINS, VAN SCOTT, RALL AND BEN, 1964).

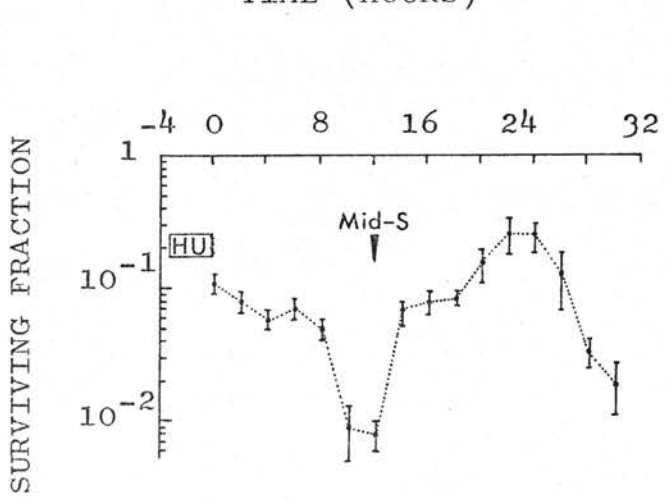


TIME AFTER VINCRISTINE

FIGURE 1.12.

AGE RESPONSE OF MURINE LYMPHOMA CELLS TO VINCRISTINE, 0.33 mg/mouse, 3 - HOUR EXPOSURE. THE BOX MARKED HU DENOTES THE TIME OF SYNCHRONIZING PRETREATMENT WITH HYDROXYUREA (0.5 mg/mouse); THE LOWER LIMITS DENOTE SURVIVING FRACTION AFTER SYNCHRONIZATION TREATMENT ONLY.

(FROM: MADOC-JONES AND MAURO, 1970).



Comparison of the effects on cells of X-rays and nucleotoxic drugs.

Considerable work has been done on the effects of various spindle poisons (also described as "radiomimetic" or "nucleotoxic") on the cells of the broad bean meristem (e.g. Revell, 1953; Kihlman, 1955; Evans, Neary and Tonkinson, 1957; Read, 1959).

Read's experiments with 8 - ethoxycaffeine (to be represented by E O C), to investigate the analogy between the mechanism of action of such drugs and the mechanism of damage produced by ionizing radiation, arose from the discovery by Fries and Kihlman (1948) that caffeine and theophylline could produce mutations in the fungal cells of an ascomycete. Kihlman and Levan (1949) then found that various purine derivatives could induce chromosome changes in the root-tip cells of the onion, Allium cepa, if the roots were immersed in a solution for a few hours. These changes appeared to be of the same kind as those produced by X-rays, i.e. a stickiness of the chromosomes of cells which were in division at the time of treatment leading to clumping, anaphase bridges, and structural chromosome changes in cells which were in interphase when treated. A further similarity arose when Kihlman (1955) showed that the production of chromosome aberrations by E O C in Vicia cells was influenced by the concentration of dissolved oxygen. Read (1959) found a close similarity between the effect on the roots of Vicia produced by X-rays and by immersion in oxygenated E O C.

Read (1954) also found that the shape of the growth curves for the roots of Vicia treated with nitrogen mustard were similar to those for the X-rayed roots (Fig.1.13). The growth rate of the X-rayed roots, however, began to diminish about one day earlier than that of the roots treated with nitrogen mustard. Read attributed this result to a difference in age-response since Revell (1953) showed that the peak of the sensitivity of chromosomes to breakage by nitrogen mustard is at the beginning of the resting phase, while that to X-rays is at the end, the two peaks being separated by about 20 hours.

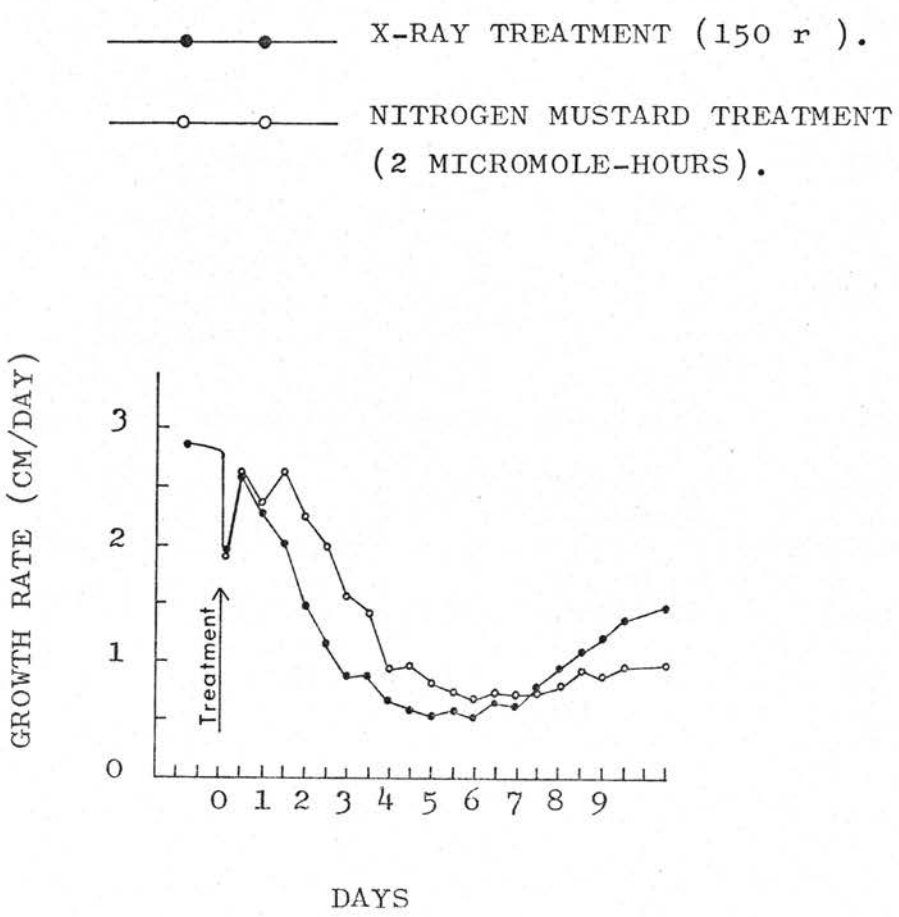
The minimum growth rate of Vicia after exposure to X-radiation occurred about 6 days later, but after E O C appreciable recovery had been made by this time (Read,1959).

Finally, returning to the effects of ultrasound, it can be said that since the original work by Langevin in 1916, an extensive literature on the subject of ultrasound has grown up (Bergmann, 1954; Elpiner, 1964). Unfortunately, however, it is only comparatively recently that there has been anything approaching a proper appreciation of the physical complexity of the subject, and that it has become possible even to formulate useful hypotheses as to the various mechanisms of action involved (Hill, 1968).

FIGURE 1.13.

COMPARISON OF THE GROWTH CURVES FOR THE ROOTS OF VICIA FABA WHEN TREATED WITH X-RAYS OR NITROGEN MUSTARD.

(FROM: READ, 1954).



BIOPHYSICAL MODES OF ACTION OF ULTRASONIC WAVES.

In spite of the efforts of many investigators and the multiplicity of publications on the subject of the mode of biological action of ultrasound, this problem is far from being solved even in the cases where there seems to be rather good support for the theory in question.

The various mechanisms of action of the ultrasound which can induce effects on biological material can be classified as follows: thermal action, "cavitational" and "direct" mechanisms of action.

Thermal effects.

The mechanisms leading to absorption of a sound wave in a medium are not fully understood and sound is absorbed to very different degrees by different media (Hill, 1968). Total absorption coefficients quoted in the literature, are usually calculated from the reduction of intensity observed on introduction of the medium into the path of the sound beam, and are the sum of at least two components, due to true absorption and scattering respectively. It is only the true absorption component that corresponds to energy deposition (and hence, temperature rise) directly in the path of the beam, although scattered radiation will, of course, be subject to subsequent absorption in the vicinity of the primary beam.

Temperature rise may in itself be a significant factor in a biological system and its magnitude in a

particular situation will depend both on the beam parameters, in time and space, and on the absorbing and scattering properties of the medium and its properties as a heat sink (thermal conductivity and heat capacity).

In fluids and quasi-fluids (such as soft tissue) only longitudinal waves are propagated, transverse waves being almost completely absorbed within the first half-wavelength distance from their point of introduction. Such absorption may occur in the vicinity of any acoustic impedance boundary involving fluid or soft tissue, as a result of the partial conversion of energy from longitudinal to transverse mode that accompanies refraction.

"Cavitation".

A group of phenomena that is characteristic of the acoustic frequency in the range 16 kHz - 10 MHz (which includes the frequency range used in medical therapeutic and diagnostic beams as well as biological research) is that covered by the general term "cavitation" (Webster, 1963; Flynn, 1964). Most ordinary liquids contain stable microbubbles, or other minute nuclei, around which bubbles of dissolved gas are found to grow under the action of moderate ultrasonic fields. On reaching a certain size that is characteristic of the sound frequency, such bubbles exhibit mechanical resonance, with vibration amplitude that may be several orders of magnitude greater than that of the particles of the liquid in the absence of the bubble (Nyborg, 1965a; 1965b). This enhancement of vibration amplitude, together with small-scale patterns of fluid

movement or "microstreaming" that it induces (Kolb and Nyborg, 1956), can lead to high shear and tensile stresses being set up in the liquid, sufficient to break structures such as macromolecules and cell membranes (Hughes and Nyborg, 1962).

The above phenomenon is commonly termined stable cavitation to distinguish it from a more violent form, known as collapse cavitation (or transient cavitation) which occurs when the acoustic intensity is sufficiently great to cause the cavitation bubbles to collapse completely during a compression phase of the vibration. In the collapse phase Noltingk and Neppiras (1950,1951) predict instantaneous temperatures of the order of 10^4 ^oK and pressures of the order of 10^6 atmospheres. In aqueous media one consequence of this collapse phenomenon (not fully explained theoretically although well established experimentally) is the production of a variety of short-lived chemical free-radical species (Grabar and Prudhomme, 1949; Jarman, 1960; Elpiner, 1964). In this respect ultrasound exhibits an analogy with ionizing radiation. Cell breakage, however, is independent of free radical formation (Hughes and Rogers, 1960).

The products of degradation of tri-peptides exposed to ultrasound are found to be dependent on the nature of the ambient gas and it is a general observation that the nature of the gases dissolved in a solution irradiated under conditions of transient cavitation influences the effect of ultrasonic treatment (Elpiner and Sokolskaya, 1962). Solutions of serum albumin were not

degraded under an atmosphere of helium, but did degrade in the presence of oxygen, hydrogen and nitrogen (Stefanovic, Kostic, Bresjanac and Ziranovic, 1959). Such observations suggest that degradation in an ultrasonic field proceeds, at least partly, through the interaction of the macromolecule with an activated species, such as a free radical, produced from the solvent.

Esche (1952) characterized the "cavitation threshold" as the onset of detectable wide-band noise outside the frequency band of the applied sound field. It appears, however, that the generation of such noise results from the shock waves associated only with the extreme collapse form of cavitation, and the occurrence of cavitation-like phenomena in the megahertz region at intensities much lower than Esche's thresholds has in fact been recognized (Figure 1.14), particularly in connection with the ultrasonic cleaning process (Neppiras, 1965). The methods used by Hill (1972) for determining the dependence on frequency of cavitation in air-equilibrated water include the degradation of DNA molecules in aqueous solution, sonochemical changes of potassium iodide and observations of the first sub-harmonic of the driving frequency. The evidence of sub-harmonic signal measurements indicate that the cavitational mode responsible cannot readily be stimulated in mammalian tissues (Hill, 1972). Hill, Clarke, Crowe and Hammick (1969) as well as Hill (1972) have shown that, in a 1 MHz beam, pulsing conditions may have a marked influence on the cavitation

FIGURE 1.14.

FREQUENCY DEPENDENCE OF CAVITATION IN AIR-EQUILIBRATED WATER, AS DETERMINED BY VARIOUS METHODS (CONTINUOUS-WAVE ULTRASOUND). (FROM: HILL, 1972)

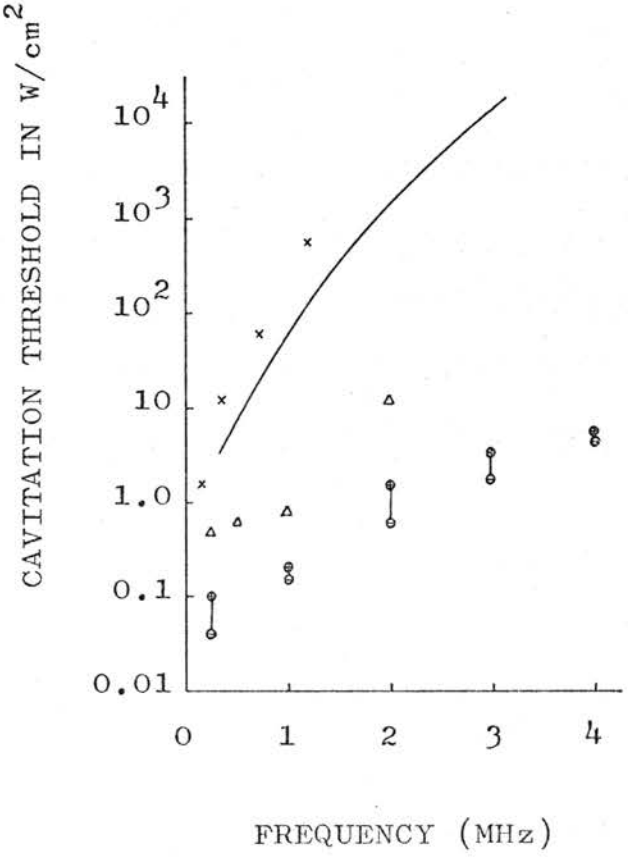
⊕ POSITIVE INDICATION OF CAVITATION (HILL, 1972).

⊖ NEGATIVE INDICATION OF CAVITATION (HILL, 1972).

Δ NEPPIRAS (1965)

× BARGER (QUOTED BY APFEL, 1970).

—— ESCHE (1952).



activity in that a maximum effectiveness of DNA degradation occurs at a pulse duration of about 30 ms, with a rapid decrease as the pulse length is reduced towards 1 ms (Figure 1.15). Clarke and Hill (1970) in their studies on the survival of mouse leukemia cells grown in vitro have shown that cell death induced by a 1 MHz ultrasonic beam at an average intensity of 1-5 W/cm^2 arises mainly due to the vibrating bubble mechanism, chemical effects playing a relatively minor role and delayed effects, of the kind known to follow ionizing radiation (i.e. inhibition of mitosis, Gray and Scholes, 1951), being absent.

"Direct" mechanism of action.

Most of the very large number of types of change that have been reported as having been produced by ultrasound in both physical and biological systems (Elpiner, 1964) can be explained on the basis of one or other of the above general classes of mechanism (thermal and cavitational). A third and possibly a more "direct" class of mechanism may, however, be involved.

Evidence for a "direct" class of mechanism comes from a variety of types of experiment, all of which have been carried out in such a way that both cavitation and significant temperature rise were considered to be either prevented or adequately allowed for. In biological systems, inactivation of the spinal cord of hypothermic mice has been reported by Dunn and Fry (1957), and temporary paralysis of activity in small, unicellular organisms has

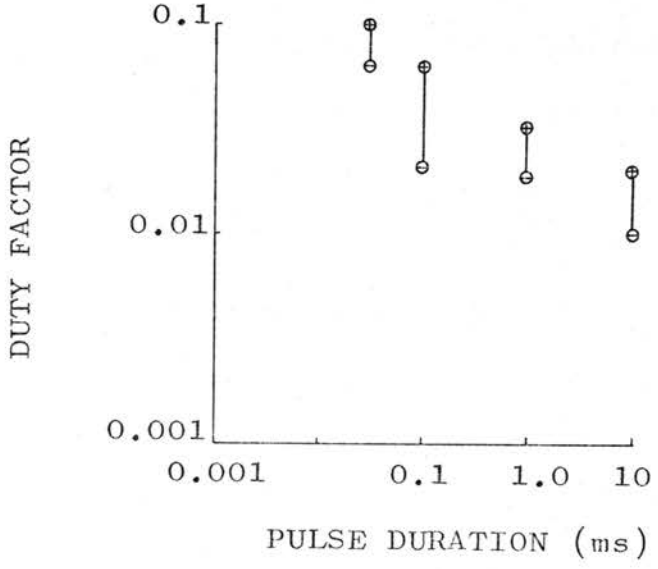
FIGURE 1.15.

DEPENDENCE OF CAVITATION ON PULSING PARAMETERS (2-MHz BEAM). INTENSITY IN THE PULSE=4.7 W/cm^2 (SPACIAL AVERAGE).

(FROM: HILL, 1972)

⊕ POSITIVE INDICATION OF CAVITATION

⊖ NEGATIVE INDICATION OF CAVITATION



been produced by the action of frequencies of 250 and 500 MHz (Dunn and Hawley, 1965). Considerable acceleration of wound healing under the action of ultrasound has been reported by Dyson, Pond, Joseph and Warwick (1968) and this also appears to be a manifestation of a "direct" mechanism of action.

Another result which appears to lend support to the hypothesis of a direct mechanism of ultrasound, is the demonstration by Woeber (1959, 1965) of synergism between X-rays and ultrasound in tumour therapy. The magnitude of the effect observed here was appreciably greater than that to be expected on the basis of temperature dependence of radiosensitivity.

Further evidence for the existence of a direct mechanism of action comes from work with physical systems. Degradation of DNA has been reported to occur at intensities of the order of 30 W/cm^2 at 1 MHz in the absence of cavitation (Hawley, MacLeod and Dunn, 1963) while certain changes such as the breakdown of structural viscosity (Freundlich and Gillings, 1938) and variations in acoustic absorption (Fedorova, 1965) have been reported in concentrated solutions of certain proteins and other polymers, at considerably lower intensities. Such changes may be the result of direct interactions of the sound field with the polymer bond structure, and a further observed effect that may be related to this is an increase in the rate of diffusion for certain ionic species diffusing through a protein (gelatin) gel (Arkhangelskii, 1962). Another effect in this class

that has been reported recently is the acceleration of hydrolysis of sucrose, which occurs at intensities of the order of 3 W/cm^2 at 3 MHz (Woodcock, 1967).

The phenomena described in the preceding paragraphs appear to be due in some way to stressing the molecular structure under the action of the sound field. Another physical means by which ultrasound might, in principle, exert an influence on biological structures is through the fluid microstreaming that is produced in the vicinity of interfaces within acoustically inhomogeneous media. It has been shown to occur in living cells to which ultrasound at 20 kHz is applied locally from the tip of a vibrating needle (Wilson, Wiercinski, Nyborg, Schnitzler and Sichel, 1966). Under these conditions the cell contents, sometimes including major structures such as molecules, can be seen to undergo rapid vortical motion. However, the irradiation conditions here are physically very different from those in a uniform beam and the extent, if any, to which such microstreaming may tend to occur in tissue exposed to therapeutic or diagnostic beams has not yet been determined (Connolly and Pond,1967).

CHAPTER II.

THE PROBLEMS STATED.

THE PROBLEMS STATED.

In view of the expanding use of ultrasound in medicine a better understanding of the mechanisms by which ultrasonic irradiations produce their effects on living organisms is very desirable. For this purpose the following experiments will be carried out.

(a) The roots of Zea will be exposed to the beam of the Impulsaphon M55 under various conditions for times in the range 1-180 minutes. The above-mentioned instrument is capable of producing pulsed (2ms pulses, 0.2 duty cycle) and continuous beams of ultrasound at a frequency of 1 MHz and average intensities in the range 0.02 - 0.82 W/cm^2. Where possible, the results on Zea will be compared with those by Bleaney and Oliver (1972 a and b) on Vicia.

(b) The beam of the generator will be calibrated using radiation balance measurements in combination with beam profile measurements, for which a thermistor probe will be built. A tantalum capacitor probe will be used to determine the frequency, pulse shape and duty cycle of the ultrasonic beam.

(c) Hall, Lajtha and Oliver (1962) have considered the growth pattern of the bean root in terms of the proportion of cells maintaining reproductive integrity and two theoretical kinetic models allowing recovery after X-irradiation (acute doses) have been formulated. Adaptations of these two models (termed Model A and Model B) were used by Oliver and Shepstone (1965) to

explain the behaviour of the root meristem under conditions of protracted gamma-irradiation. These adapted models will be modified and computer programs will be written to calculate theoretical growth curves for conditions of acute ultrasonic irradiation.

Acute sonications of Zea will be carried out in order to provide experimental curves for comparison with the computer calculated results.

(d) The characteristic shape of the survival curve for the cells in the root meristem of Zea mays exposed to X-rays is sigmoid (Shepstone, 1964; Fenner, 1970). This implies a threshold type of response in which damage must be accumulated before its effect becomes apparent. A search will be made for such a threshold type of response in the present experiments on ultrasound.

(e) The intermitotic cycle time of the meristematic cells in the root of Zea mays will be determined using a metaphase accumulation technique described by Evans, Neary and Tonkinson (1957) for the root meristem of Vicia faba. It will also be necessary to determine whether there is a tendancy for diurnal rythm in root elongation throughout a 24-hour period.

(f) It has been found possible to alter the X-radiation sensitivity by certain changes in the environment of the roots during, or immediately before irradiation (e.g. change in the amount of oxygen present), which are not in themselves harmful. The sensitivity was also found to depend on the nature (LET) of the radiation (gamma rays, alpha rays, etc.) and the manner of delivery

(dose rate, fractionated doses). Bearing this in mind, experiments will be carried out to investigate the effect on sonication damage of the temperature at which the roots are kept before or during sonication, the concentration of dissolved oxygen and the position of the meristematic cells in the cell cycle (so-called "age response"). The doses of ultrasound will also be fractionated to determine whether there is any recovery of the sonicated cells between doses.

(g) The sensitivity to X-rays has been found to be different for the elongating and meristematic cells in the root tip. These cells may also respond differently to ultrasonic irradiation and the meristematic and elongating regions of the root tip will thus be individually exposed to ultrasound. Surgical experiments will be used to determine to what extent the elongating zone contributes to the length of the root once the whole or part of the meristematic zone has been removed.

(h) The roots of Zea will also be exposed to ultrasound in combination with X-rays and the anti-cancer agent vincristine, since there have been reports (outlined in Chapter I) that the use of ultrasound (continuous wave) in conjunction with X-ray therapy can increase the effectiveness of the latter in the treatment of tumours. The effects of spindle poisons on tissues have been described as being similar to those of X-rays (e.g.Berry, 1969) and thus a combined treatment with ultrasound and vincristine may produce results similar to those observed for ultrasound and X-rays, which is to be

investigated. In experiments (Chapter I) in which ultrasound and X-rays were used simultaneously, the dose of ultrasound was in itself not sufficiently large to produce an effect on the tissue involved. In similar experiments on Zea the roots will be exposed to high and low doses of ultrasound (pulsed as well as continuous beams) which, when given alone, will either affect the roots to a considerable extent or will hardly affect the growth of the roots at all.

(i) An attempt will be made to determine whether ultrasonic radiation at a very much lower average intensity than that (see Chapter I) used by Haskell and Selman (1950) will affect the dry seeds of Zea to such an extent that a change will be observed in the germination rate and in the growth of the roots after germination of the sonicated seeds.

(j) If the threshold at which ultrasonic damage to the roots becomes apparent is low enough, the roots will be used to determine whether certain diagnostic equipment in common clinical use is capable of causing a change in the growth of the roots.

CHAPTER III.

MATERIALS AND METHODS.

cont.

PAGE

CULTURE METHODS.

Seed of Zea mays (Variety "Kalahari Blitz"), were germinated, and roots grown as described in detail previously (Hering, 1971). The method of culture employed was based on that used by Hall, Lajtha and Oliver (1962) for Vicia faba.

After 2-3 days in moist Vermiculite the seedlings, now having a primary root of length of 2-3 cm, were transferred to the culture tank and grown for two to three days at 19°C ± 0.2°C. This temperature was maintained using a Braun thermostatically controlled heater in conjunction with a Grant cooling unit. The Braun heater also comprised a vigorous stirrer which effectively aerated the water. It was found unnecessary to cover the cotyledons during the experiment with moist surgical gauze, a procedure which was adopted in previous experiments (Hering, 1971) to prevent dehydration. The dehydration of the cotyledons was found not to influence the growth rate of the roots.

Observations of Mottram (1913) and Jungling & Langendorff (1930) showed that there is a tendancy for diurnal rythm in root elongation throughout a 24 hr. period. In order to eliminate this diurnal fluctuation many investigators remove the plumules and culture their seedlings under conditions of darkness (Gray & Scholes,1951). Under the present conditions of culture the growth rate of the primary root of Zea mays (as measured by root elongation per day) decreases from about the third day after

germination (Fig. 3.1). In the sonication experiments measurements were to be made at different times of the day, so that it was necessary to investigate growth rate throughout a 24 hr. period within the period of gradual decrease in growth rate.

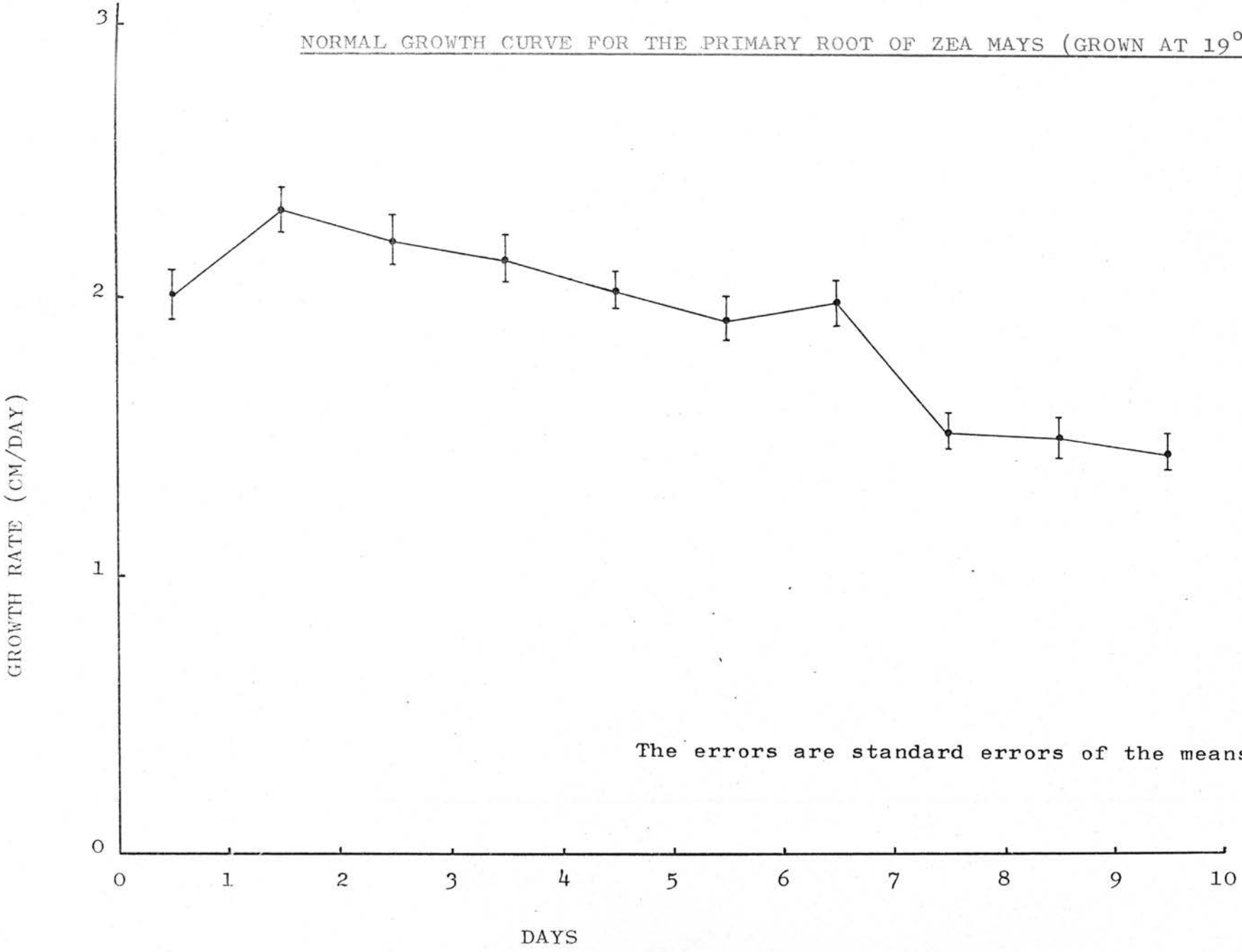
For this purpose measurements of root length were made at 2 hr. intervals throughout a day and night. Root elongation measurements were then continued for a further 3 days, but over periods of 12 and 24 hrs. only. For this experiment the roots were selected as follows. Three days after transferring the roots from the Vermiculite to the culture tank, the roots were remeasured at daily intervals and their growth increments over 2 days were determined. A homogeneous sample of seedlings was then selected on the basis of growth increment, and this sample was randomized into groups required for the experiment. The latter commenced on the day of randomization. For these measurements two groups of seedlings were used, each group consisting of twenty seedlings. In one of the groups measurements were made at 2 hr. intervals throughout a period of 24 hrs. Measurement of the second group did not commence until measurement of the first group had already been conducted for 10 hrs. In this way it was possible to check whether root elongation was affected through handling the maize roots, by comparing the measurements made on the first group with the measurements made on the second group. Data for root elongation over a 24 hr. period are given in Fig. 3.2 . The

curve presented was obtained for root elongation measurements on the first group of seedlings; each point thus represents a mean value for twenty roots. Standard errors are shown so that tabular data are not given. No difference was found between the two groups, both sets of data giving fits to regression lines of virtually zero slope (slope= -0.0013 for the first group and 0.0011 for the second group). Analysis of the additional "daytime" data over the three days following and the two days preceding the experiment, showed that the uniformity and the rate of decrease of root elongation was maintained.

Under the conditions of culture described it is clear that there is no rythmical fluctuation in root elongation over a period of a couple of days, and it would appear that the effect of light on the growth processes in the root is eliminated by the removal of the plumules. Consequently in all the experiments to be described, no attempt was made to regulate light conditions, but shoots were removed daily at the same time as the adventitious roots.

FIGURE 3.1.

NORMAL GROWTH CURVE FOR THE PRIMARY ROOT OF ZEA MAYS (GROWN AT 19°C).

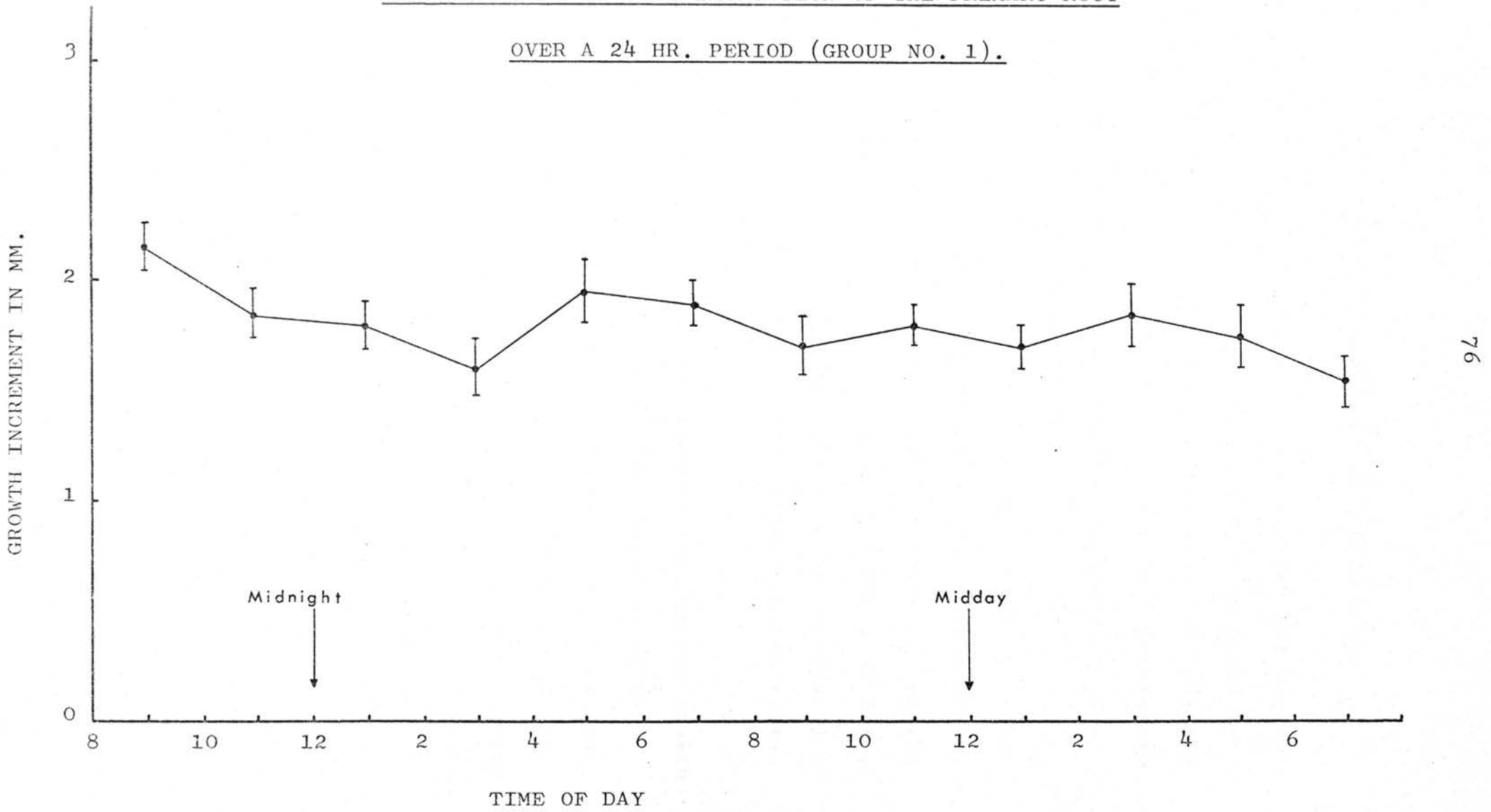


The errors are standard errors of the means.

FIGURE 3.2 .

THE VARIATION IN ROOT ELONGATION OF THE PRIMARY ROOT

OVER A 24 HR. PERIOD (GROUP NO. 1).



SCORING RADIATION AND VINCRISTINE DAMAGE.

Unless stated otherwise, the treated and control roots in all the present experiments were measured immediately after treatment and then at approximately the same time ($\pm$ 1 hour) every day following the treatment for at least 10 days. The measuring technique is described elsewhere (Hering, 1971).

Gray & Scholes (1951) reported that in the case of Vicia this handling did not affect the growth of the root. The growth rate of two control groups of Zea, the one measured every 12 hrs. and the other every 48 hrs., showed no significant differences, thereby establishing that the process of measuring did not affect the roots to any detectable extent.

After each set of measurements the average growth increment for the corresponding time interval of the treated roots was evaluated and expressed as a fraction of the growth of control roots for the same period (G). Each increment was regarded as that pertaining to a time halfway between the times at which the measurements were made. It was found that the growth rate of the controls was not constant, but decreased steadily during the course of the experiment (Fig. 3.1). It was for this reason that the growth increments were expressed in terms of control growth increments over the same period, i.e. with respect to controls of the same age.

For all treatments, curves were drawn showing the

variation of the growth rate as a fraction of controls of the same age (G) with time. The minimum value of G, referred to as G_{min}, was one of the parameters used to assess treatment damage. This parameter has been employed by various authors (e.g. Gray and Scholes, 1951; Hall, Lajtha and Oliver, 1962; Hering, 1971) in their studies on the effect of X-radiation on the root meristem of Vicia faba and Zea mays. This entity is marked G_{min} in Figure 3.3 .

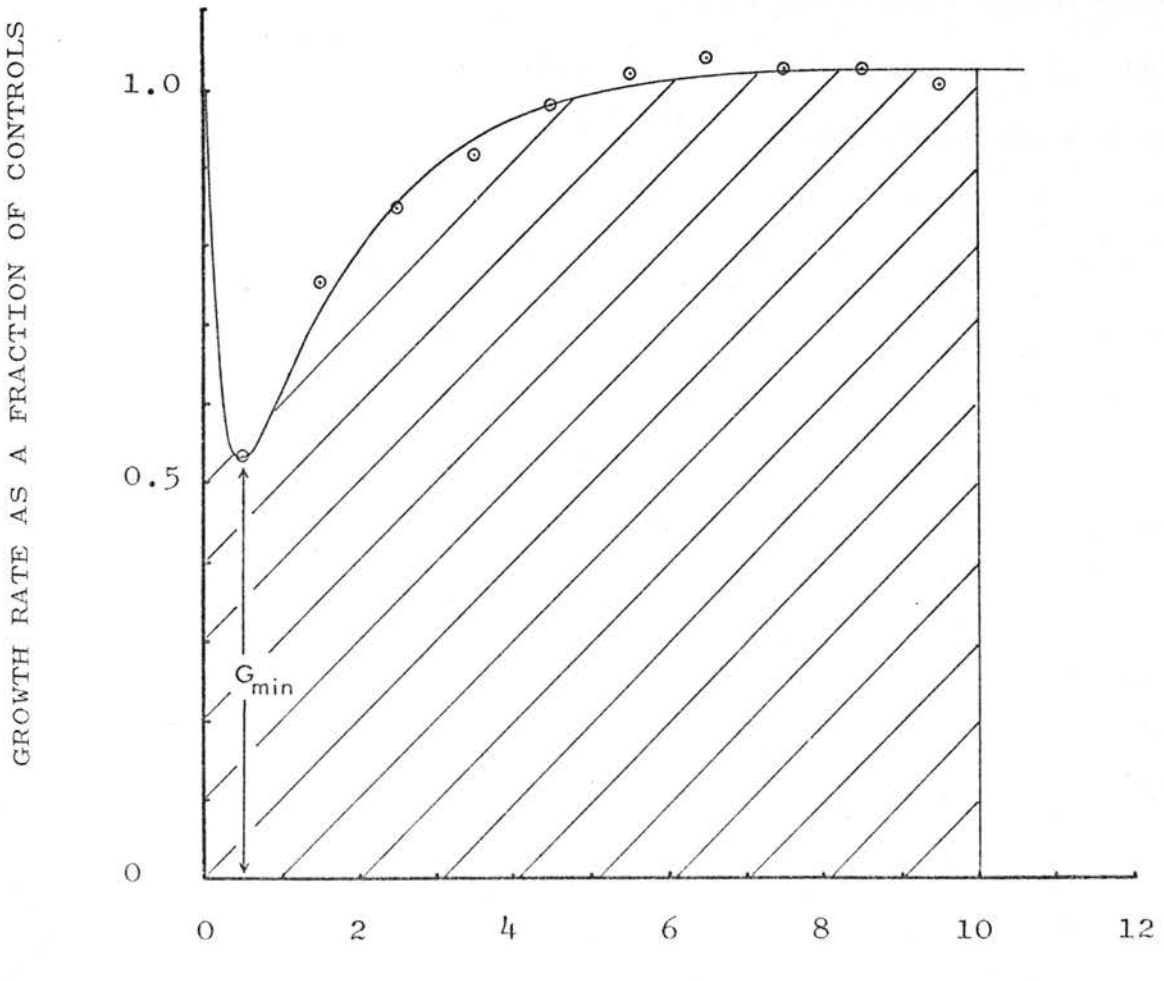
The response to ionizing radiations has also been considered in terms of the average growth in ten days following exposure for each group of irradiated roots, expressed as a fraction (G_{10}) of the average growth in ten days for the corresponding group of control roots (Read, 1952). It is, in effect, the area under the curve up to the tenth day in Figure 3.3 . This parameter, however, has been used for X-irradiated roots whose growth rate had recovered to normal by ten days after exposure. In the present experiments, various treatments caused a reduction in the growth rate which did not recover to normal by ten days after treatment since some of the treated roots had died and some were growing only slowly. Although G_{10} seems to be a rather doubtful entity in this case, it was calculated for these groups as well.

Since sonication damage is expressed immediately (Chapter IV), G_1, the average growth in the first day post-sonication as a fraction of the corresponding average

growth for control roots, has been used in the assessment of sonication damage. Errors in measurement, however, are more significant in the growth increment over one day than over ten days (Bleany and Oliver, 1972 a).

FIGURE 3.3 .

VARIATION IN THE GROWTH RATE OF THE ROOTS OF ZEA AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR 90 MIN. G_{min} IS THE 'MINIMUM GROWTH RATE', AND THE AREA UNDER THE CURVE UP TO THE TENTH DAY IS THE 'GROWTH IN TEN DAYS'.



METHOD OF SONICATION OF THE ROOTS OF ZEA MAYS.

The roots of Zea were exposed to ultrasonic radiation at 1 MHz supplied by the Impulsaphon M55, a therapeutic instrument capable of either continuous or pulsed operation and giving pulse durations of 2 ms, with a duty factor of 0.2 (Fig. 3.4). The ultrasound intensity could be controlled stepwise from 0.1 to 0.8 W/cm^2 for the continuous beam and from 0.02 to 0.2 W/cm^2 for the pulsed beam. The calibration of the ultrasonic beam is discussed elsewhere in this chapter.

The piezo-electric transducer of 3.2 cm diameter was mounted on a jig which is shown in Fig. 3.5 . This perspex jig was constructed to hold 5 Zea mays seedlings at one time so that the root tips were positioned exactly in the centre of the ultrasonic beam (Fig. 3.6). This was accomplished by inserting the five roots into a glass tube with a narrow opening at one end through which the root tips protruded by 0.5 - 0.7 cm. This glass tube plus seedlings was positioned on the jig, the roots then being at a distance of 7 cm from the crystal face. The jig was immersed in a Perspex tank (68cm x 25cm x 30cm deep) filled with air-equilibrated distilled water at 18 - 22°C (Fig. 3.7). The jig was supported inside the tank by a Perspex stand, the slight angulation of which was introduced to keep the probe out of the water at the far end since the latter was not water-tight here when it was first used in these experiments.

Roots of length between 6.0 and 7.5 cm were selected for sonication (and for controls), because this was found to be the optium range of the length of the roots for the latter to be easily mounted on the sonication jig as described above.

The absorber (consisting of glass wool mounted on an indented neoprene rubber sheet) at the end of the tank eliminated any reflected radiation, thus ensuring that the roots were subjected to a simple travelling wave pattern.

The root tips were free in the water but confined within a volume of about 0.02 ml in the centre of the field. Thus it was considered unnecessary to rotate the roots during the exposures to equalise the dose received.

For the experiment in which the roots of Zea were sonicated at a low temperature, another Grant cooling unit was used to reduce the temperature of the water in the sonication tank to $7^{o}C$. The roots were then positioned on the jig and left for 15 minutes to equilibriate before irradiation.

To investigate the effect on sonication damage of dissolved gases oxygen, nitrogen, air and helium were bubbled (using a diffuser stone) through the water in the sonication tank for a few hours respectively (the supply of the gas was not turned off during the irradiation of the roots). The seedlings were then positioned on the jig and left for 15 minutes to equilibriate before sonication. At this stage measurement of the oxygen content

of the water commenced using the YSI Oxygen Meter (Model 51A) and was continued throughout the sonication period.

For the sonication of different regions of the root tip, a hollow cylinder was constructed to achieve partial shielding of the root tip. The cylinder (open at both ends) had a double wall with an air space in between, the metal walls (brass) being 0.05 mm thick. The overall length of the cylinder was 1.4 cm with an internal and external diameter of 2 mm and 3 mm respectively.

The elongating zone was sonicated as follows. The roots were positioned on the jig so that the tips extended beyond the glass tube by 0.8 cm. The metal cylinder was then used to cover the root tips so that only 0.5 cm of the roots between the cylinder and the glass tube was exposed to ultrasound, the lower 3 mm of the root being shielded.

For the sonication of the meristematic zone, the roots were positioned on the jig without using the glass tube and the lower 2 - 3 mm of the roots were sonicated, the elongating zone being shielded by the metal cylinder.

In all the sonication experiments two groups of five roots each were sonicated in turn (at the same average intensity and for the same time) and the length of the primary root was measured immediately following the sonication of the second group (or following the second sonication of the second group for the fractionated irradiations). The ten roots of the control group were also measured at this time. The seedlings used as

controls were always handled in the same manner and at the same times as the roots treated with ultrasound. Subsequent measurements were made at 6, 12 and 24 hrs. following the first measurement and thereafter at daily intervals for at least ten days. The growth rate of the ten sonicated roots was compared with that of the ten control roots as described in " Scoring radiation and vincristine damage."

FIGURE 3.4 .

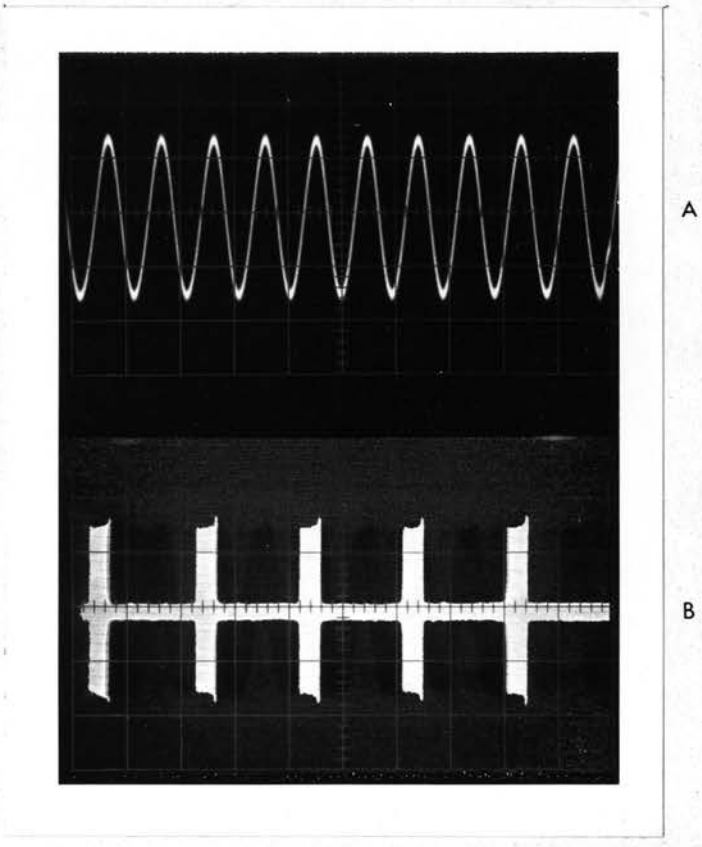
PULSE SHAPE OF THE ULTRASONIC BEAM USED

IN THE PRESENT EXPERIMENTS.

(OBTAINED WITH THE TANTALUM CAPACITOR PROBE)

TOP TRACE: $0.82 W/cm^2$ (AVERAGE INTENSITY) CONT. BEAM

BOTTOM TRACE: $0.21 W/cm^2$ (AVERAGE INTENSITY) PULSED BEAM



A: 1 μs/cm; 0.1V/cm

B: 5 ms/cm; 0.1V/cm

FIGURE 3.5 .

PERSPEX JIG TO HOLD TRANSDUCER AND MAIZE SEEDLINGS

(OR TANTALUM CAPACITOR PROBE)

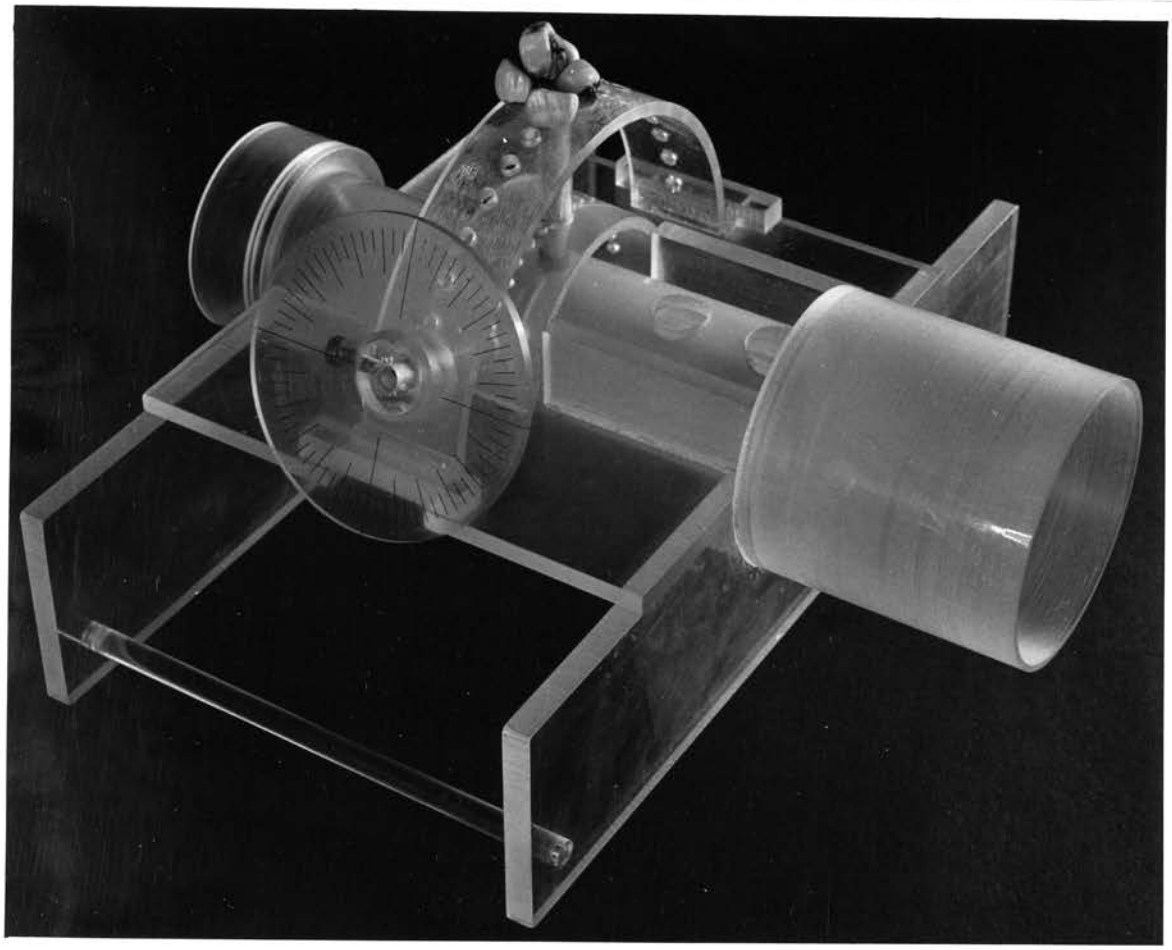


FIGURE 3.6 .

POSITION OF SEEDLINGS ON SONICATION JIG.

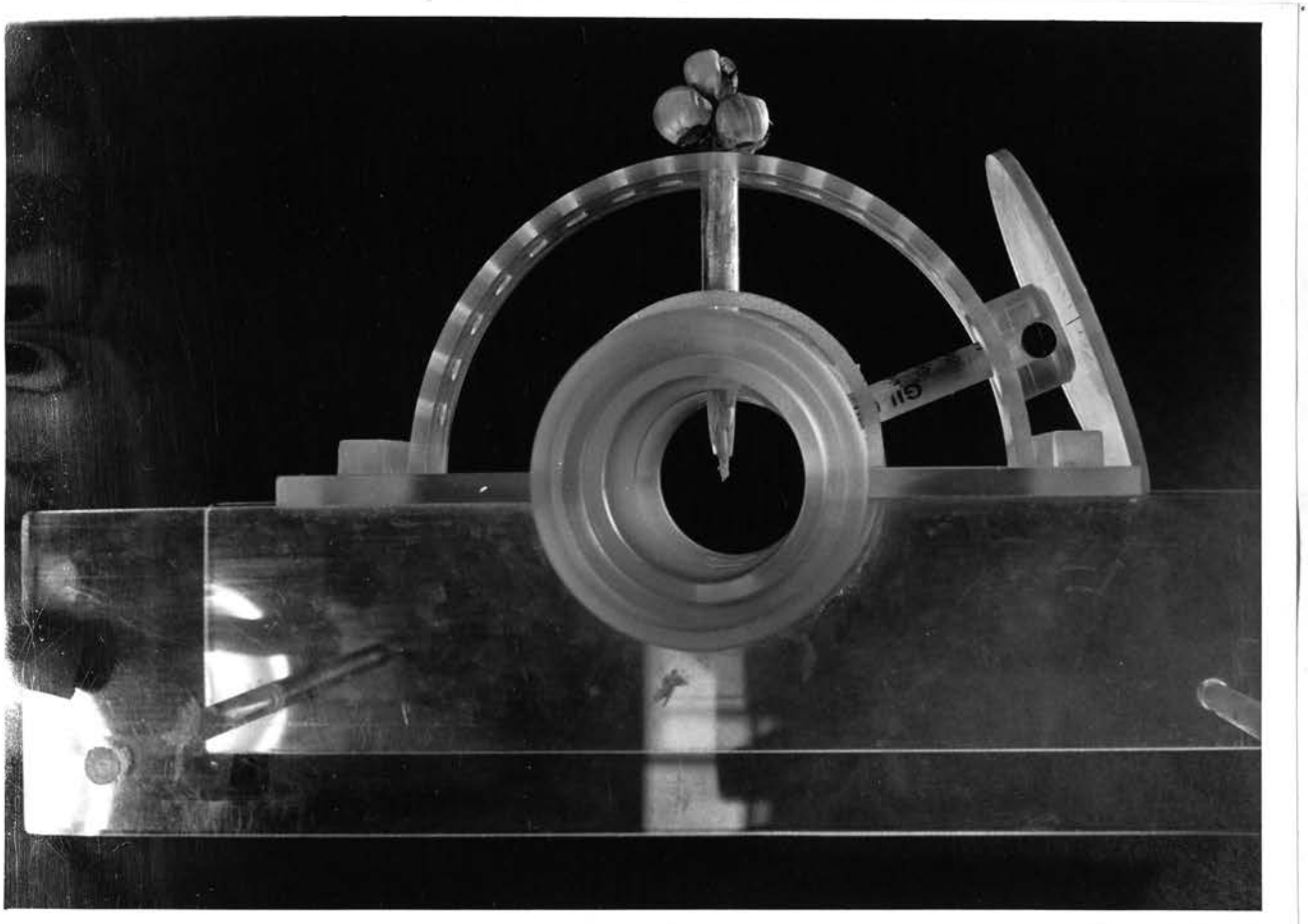
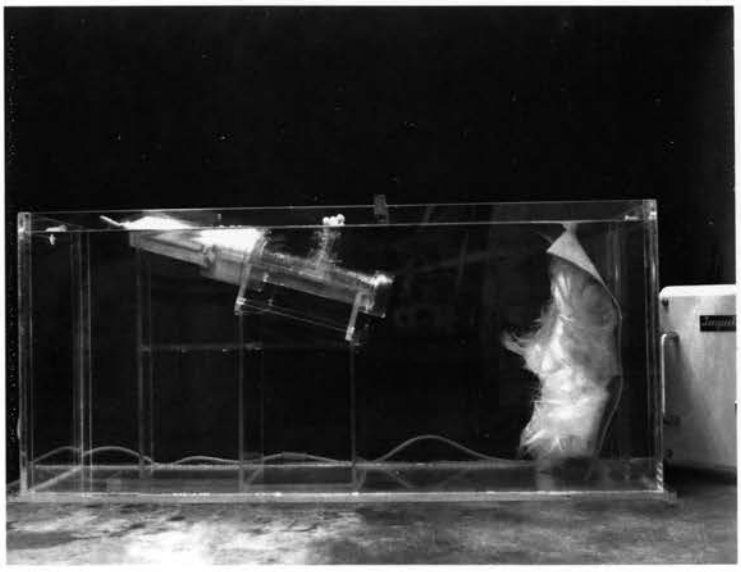


FIGURE 3.7 .

JIG WITH SEEDLINGS AND TRANSDUCER IMMERSED IN WATER FOR SONICATION OF THE ROOTS.



CALIBRATION OF THE ULTRASONIC BEAM.

Measurement of the total energy flux.

Two approaches are available for the absolute measurement of energy flux in ultrasonic beams; calorimetry and the measurement of radiation force.

Calorimetry has been used successfully (Wells, Bullen, Follet, Freundlich and Angell James, 1963) but is generally less convenient than is a measurement of radiation force (Hill, 1970).

The force exerted on a surface by any form of radiation energy corresponds to the rate of momentum transfer from the beam to the surface and, for a perfectly absorbing surface (or for one causing 90^{o} reflection of the beam) the radiation force F (Newtons) along the beam axis is related to the power intercepted W (Watts) by the relation $W = FV$, where V is the wave velocity (ms^{-1}) of the radiation in the propagation medium. Inserting the propagation velocity of sound in water at $20^{o}C$, we find the practical calibration constant: 69 mg/W (for total reflection the corresponding constant would be double this value).

A modified version of the radiation balance designed by Kossoff (1965) has been used for the calibration of the ultrasonic beam used in the present experiments (Fig. 3.8). In this balance a circular aluminium plate (60 mm diam.) was suspended in water by means of two thin copper wires (0.18mm diam.) so as to make an angle of 45^{o}

to the vertical. The frame from which the threads were suspended was designed for attachment to the hook of a chemical microbalance. A potential source of instability in an arrangement of this type is the effect of temperature variations on the apparent weight in water of the reflector plate. Thus the temperature of the air-equilibrated distilled water in the tank was kept at $20^{o}C$ using a thermostatically-controlled immersion heater if the temperature had to be increased, or using a Grant cooling unit if the temperature had to be decreased.

The transducer whose beam was to be calibrated was mounted vertically immediately above the reflector plate with the crystal face 7 cm from the centre of the latter. The critical angle for acoustic reflection at a water/aluminium interface is 14^{o}, and the beam is thus totally reflected into a horizontal orientation and terminates in a scattering and absorbing structure consisting of glass wool backed by an indented neoprene rubber sheet with which the whole tank was lined.

Any errors arising from variations in the angle of contact between the water and the suspension fibres can readily be eliminated by use of an appropiate measuring sequence in relation to connecting the ultrasonic generator and resetting the balance. Under these conditions, and apart from the small returned signal from the absorber arrangement, the method is free of systematic error and it is normally possible to achieve a precision of $\pm$ 1mW (the variance found for ten measurements).

Although it may not represent the ultimate in sensitivity (Wells, Bullen and Freundlich, 1964; Kossoff, 1965; Wemlen, 1968) its performance appears to be fully adequate in the light of present knowledge of ultrasound biology.

From the beam profile measurements (p. 123) the diameter of the ultrasonic beam 7cm from the crystal face was found to be 3.0 cm. This figure represents the average value of the diameter of some of the profile curves drawn using the thermistor probe. Thus the average intensity in W/cm^2 was calculated by dividing the total beam power (Watts) as measured with the radiation balance by 7.07 cm^2, the cross-sectional area of the beam 7cm from the transducer. Crossfield measurements confirmed the circular symmetry of the beam (p. 123), which was assumed at first.

The output of the Impulsaphon M55 was found to be reasonably consistent as shown in Table 3.1 . Nevertheless, the output of the generator was checked between experiments.

In some experiments (p. 82) oxygen, nitrogen, helium and air were bubbled through the water during the sonication of the roots of Zea. It was thought possible that the different amounts of dissolved gases could affect the transmission of the ultrasound through the water. The above measurements of the average ultrasonic intensities (pulsed and continuous beams) were thus repeated with various amounts of dissolved oxygen

in the water. Oxygen, nitrogen, helium and air were passed through the water, so that the oxygen content of the latter was the same as in the experiments in which the roots were sonicated. The flow of gas was maintained during measurements of the average ultrasonic intensity and the streaming of the water in the tank (caused by the rising gas bubbles) was reduced considerably when the diffuser stone was placed between the absorber and the wall of the tank. "Equal density" spheres were used to detect the presence, magnitude and direction of any currents in the water. The small amount of streaming present when the measurements were made did not cause any variation in the zero reading of the balance, i.e. the reading of the balance when no ultrasound was present. There was hardly any fluctuation in the oxygen content of the water (measured with the YSI Oxygen Meter, Model 51A) during measurements of ultrasonic intensity. When air, nitrogen and helium were used the oxygen content during measurements did not change by more than 1%, whereas when oxygen was used the oxygen content dropped by about 2% during measurements. The results of these measurements are listed in Table 3.2 and 3.3 . A comparison of the results in Tables 3.1 , 3.2 and 3.3 indicates that there is only an appreciable reduction in the higher average ultrasonic intensities measured when various gases are passed through the water. For the different values of the oxygen content, however, the reductions in the average intensity were found to be similar.

FIGURE 3.8 .

ULTRASONIC RADIATION MICROBALANCE.

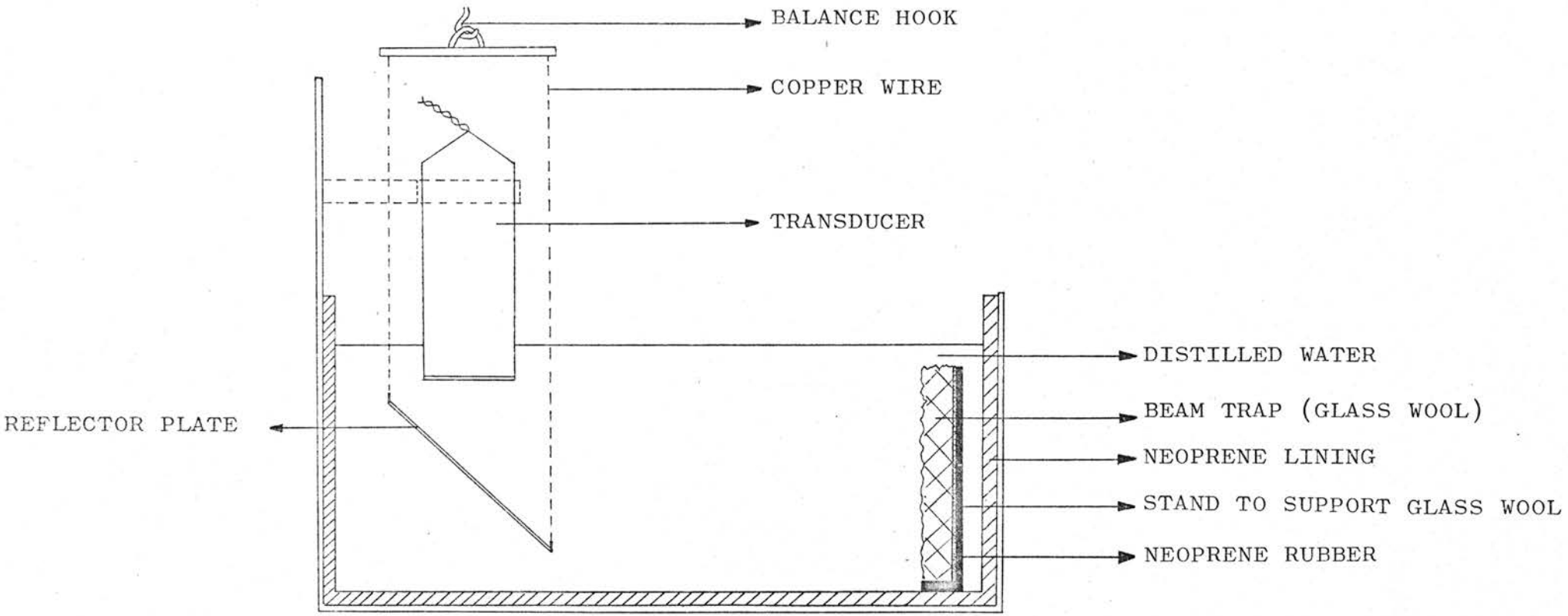


TABLE 3.1

AVERAGE ULTRASONIC INTENSITIES OBTAINABLE WITH THE IMPULSAPHON M55.

AVERAGE ULTRASONIC INTENSITY(W/cm^2)	MODE
0.819 ± 0.010	CONTINUOUS
0.618 ± 0.007	"
0.384 ± 0.008	"
0.264 ± 0.005	"
0.103 ± 0.005	"
0.205 ± 0.005	PULSED
0.164 ± 0.007	"
0.097 ± 0.003	"
0.050 ± 0.002	"
0.022 ± 0.002	"

Each value represents the mean of 20 measurements with the radiation balance. The errors are standard errors of the means.

Measurements 7cm from transducer face.

TABLE 3.2

THE EFFECT OF DISSOLVED GASES ON THE AVERAGE ULTRASONIC INTENSITY MEASURED WITH THE RADIATION BALANCE.

AVERAGE ULTRASONIC INTENSITY

W/cm^2

CONTINUOUS BEAM

AIR	OXYGEN	HELIUM	NITROGEN	
6.9ml/l O_2	22.2ml/l O_2	2.0ml/l O_2	2.5ml/l O_2	1.4ml/l O_2
0.676 ± 0.035	0.645 ± 0.037	0.646 ± 0.024	0.695 ± 0.024	0.653 ± 0.041
0.536 ± 0.024	0.505 ± 0.022	0.542 ± 0.027	0.543 ± 0.020	0.518 ± 0.026
0.331 ± 0.010	0.317 ± 0.011	0.320 ± 0.013	0.345 ± 0.018	0.322 ± 0.019
0.237 ± 0.015	0.238 ± 0.013	0.251 ± 0.018	0.226 ± 0.013	0.231 ± 0.010
0.091 ± 0.013	0.091 ± 0.013	0.096 ± 0.010	0.092 ± 0.011	0.095 ± 0.008

Each value represents the mean of 4 measurements with the radiation balance. The errors are standard errors of the means.

Measurements 7cm from transducer face.

TABLE 3.3

THE EFFECT OF DISSOLVED GASES ON THE AVERAGE ULTRASONIC INTENSITY MEASURED WITH THE RADIATION BALANCE.

AVERAGE ULTRASONIC INTENSITY

W/cm^2

PULSED BEAM

AIR	OXYGEN	HELIUM	NITROGEN	
6.9ml/l O_2	22.2ml/l O_2	2.0ml/l O_2	2.5ml/l O_2	1.4ml/l O_2
0.191 ± 0.009	0.180 ± 0.008	0.180 ± 0.012	0.197 ± 0.008	0.192 ± 0.010
0.160 ± 0.010	0.148 ± 0.011	0.153 ± 0.013	0.142 ± 0.009	0.158 ± 0.010
0.097 ± 0.003	0.091 ± 0.003	0.092 ± 0.005	0.092 ± 0.003	0.096 ± 0.002
0.053 ± 0.006	0.048 ± 0.006	0.049 ± 0.003	0.051 ± 0.002	0.051 ± 0.003
0.021 ± 0.001	0.022 ± 0.002	0.022 ± 0.003	0.020 ± 0.002	0.019 ± 0.003

Each value represents the mean of 4 measurements with the radiation balance. The errors are standard errors of the means.

Measurements 7cm from transducer face.

Beam profile measurements.

A number of approaches are possible to the problem of ultrasonic beam profile measurement and factors determining the choice of method include the intensity range to be covered, requirements for resolution in both time and space, calibration accuracy and convenience.

An ultrasonic field may be plotted by measuring its intensity distribution by means of a non-directional microphone. In addition to being non-directional, a microphone used for investigating pulsed beams must possess non-selective frequency characteristics; in the case of a continuous wave beam, the microphone must be small in size to avoid the establishment of standing waves. It is not possible to achieve this last condition at frequencies above about 1 MHz (Wells, 1969).

Schmitt (1961) has described how ceramic capacitors may be used as sound probes for plotting ultrasonic field distributions. Such capacitors are inexpensive, easily obtainable and quite small sizes are available.

Measurements using a tantalum capacitor probe.

In order to investigate the beam profile at a distance of 7cm from the crystal face, a sound probe was constructed using a tantalum capacitor of 1000 pF as a sound-sensitive device (Fig. 3.9). The clipped leads were connected directly to a flexible shielded cable.

The shielded cable was supported by a thin glass tube of 3mm diameter (external). The sound sensitive tip had a cross-sectional dimension in the order of 3mm and was used with its output directly coupled to a Tektronix storage oscilloscope (Type 549) with a high-gain differential amplifier, Type 1A7A. The capacitor was polarized by applying a bias voltage of 1.5 volts.

The glass tube could be moved inside a guide on the sonication jig (Fig. 3.10) so that the microphone could probe the sound field across the beam 7cm from the crystal face. As shown in Fig. 3.11 the probe could be turned on its own axis and be fixed at any desired angle on the jig using a circular protractor and the pointer that could be clamped in any position on the stem of the probe. At the same time it was also possible to move the probe across the beam whilst maintaining a particular angle of incidence of the ultrasound to the probe. This was achieved using a graduated scale that could be fixed at right angles onto the circular protractor.

Figure 3.12 shows the variation of the output of the probe with angle for a pulsed beam with an average intensity of 0.21 W/cm^2 as measured with the radiation balance. The receiving pattern for a continuous beam was found to be similar to that for the pulsed beam.

The output at an angle of 0^o was measured with the probe at various positions (1mm apart) on a line perpendicular to the beam axis (Fig. 3.13). This profile curve for the 0.82 W/cm^2 continuous beam was found to be

similar in shape to the one obtained for the 0.21 W/cm^2 pulsed beam as shown in Figure 3.14 . Figure 3.15 shows the profile curve obtained for the very low intensity of 0.02 W/cm^2 (pulsed beam). The values of the nominal powers quoted represent average values measured by pressure balance in the absence of the probe and jig. The profile curves drawn for all other intensities were similar to the ones depicted in Figures 3.13 , 3.14 and 3.15 .

These profiles with the probe at 0^o were drawn for various average intensities of the pulsed and continuous beam. The average ultrasonic intensities were then plotted against the corresponding areas under the profile curves as shown in Figures 3.16 and 3.17 . From the latter it can be seen that the output of the tantalum capacitor probe is directly proportional to the average ultrasonic intensity for the continuous beam but not for the pulsed beam. A straight line was fitted to the points in Fig.3.16 employing a method of least squares and the correlation coefficient was found to be 0.9977. A polynomial of the 2nd. degree was fitted to the data in Fig.3.17 again employing a method of least squares.

For the probe at an angle of 225^o a variation of the average ultrasonic intensity with the area under the corresponding profile curve was obtained as shown in Fig. 3.18 for the continuous beams. At this angle the curve was not a straight line. For the pulsed beams (Fig. 3.19) the relationship between the area under the

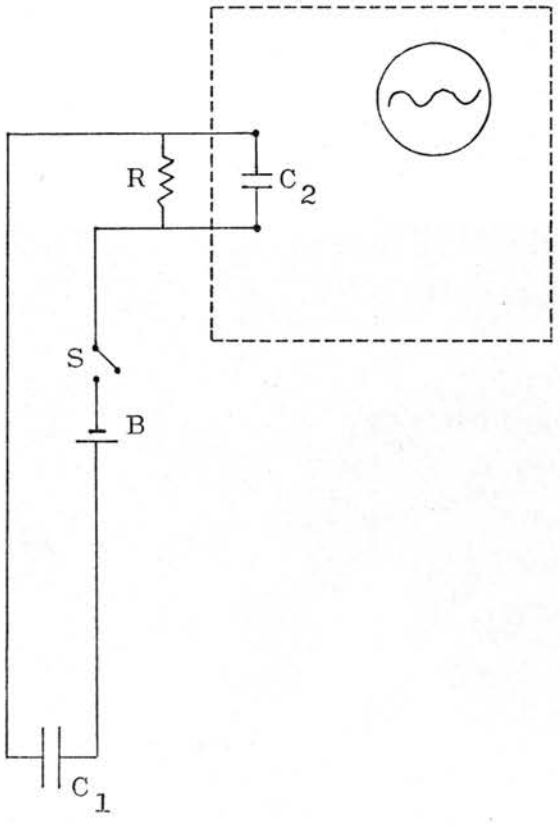
profile curve and the average intensity was also found to be non-linear. A polynomial of 2nd. degree was also fitted to these results. The shapes of the profile curves obtained with the probe at 225^{o} were found to be similar to those obtained with the probe at 0^{o}.

Because of the non-linearity of the tantalum capacitor probe as well as the change in the output of the probe with the angle of incidence of the ultrasonic beam, a thermistor probe was built for the beam profile measurements. Apart from being non-directional this probe has much smaller dimensions than the tantalum capacitor probe and the formation of standing waves will be reduced even further when a continuous beam is used. Since the thermistor probe has much smaller dimensions than the tantalum capacitor probe, the former is much more suitable than the latter for observing a change in ultrasonic intensity for a small displacement of the probe, especially for measurements near the periphery of the ultrasonic beam.

The thermistor probe is, however, only useful for intensity measurements and the tantalum capacitor probe will have to be used for obtaining information involving pulse shape, duty factor and frequency of the ultrasonic beam as shown in Figure 3.4 for the beam used in the present experiments.

FIGURE 3.9 .

CIRCUIT DIAGRAM FOR THE TANTALUM CAPACITOR PROBE.



R .	2.2	MEGOHM RESISTOR
B .	1.5	VOLT BATTERY
C_1.	10^3	pF CAPACITOR
C_2.	47	pF CAPACITOR
S .	ON - OFF SWITCH	

FIGURE 3.10.

POSITION OF THE TANTALUM CAPACITOR PROBE

ON THE SONICATION JIG.

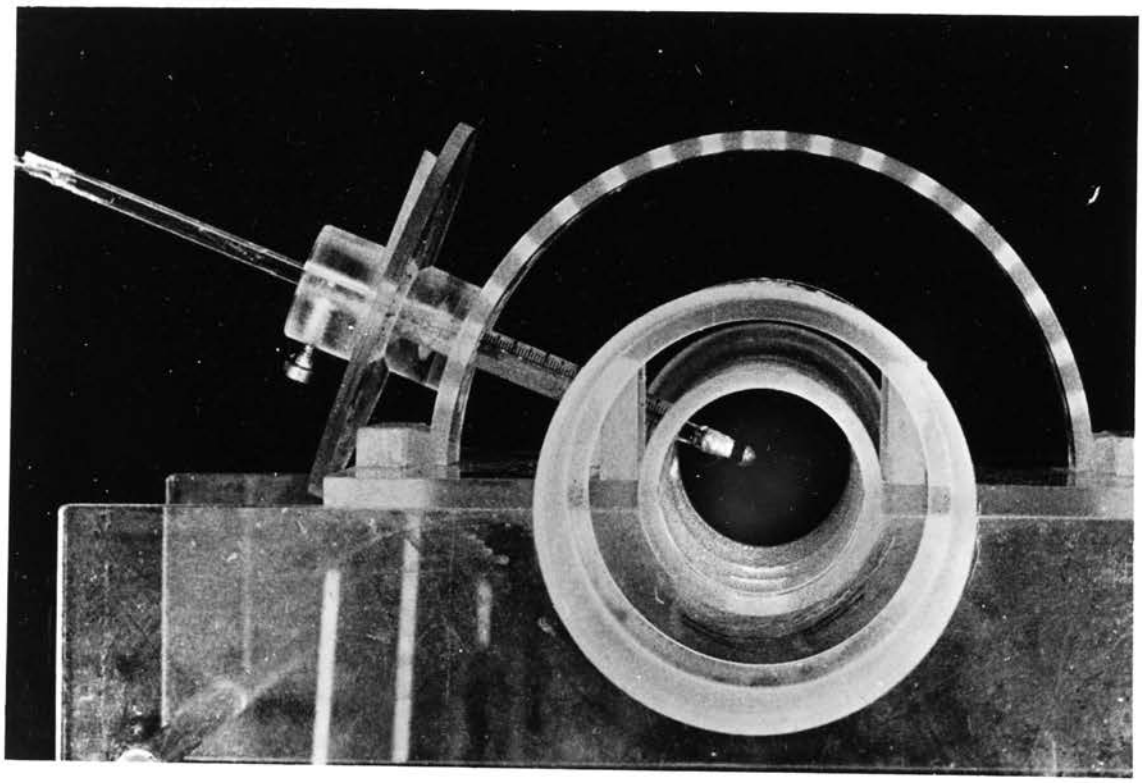


FIGURE 3.11.

PICTURE INDICATING HOW THE POSITION OF THE TANTALUM CAPACITOR IN THE ULTRASONIC BEAM WAS DETERMINED.

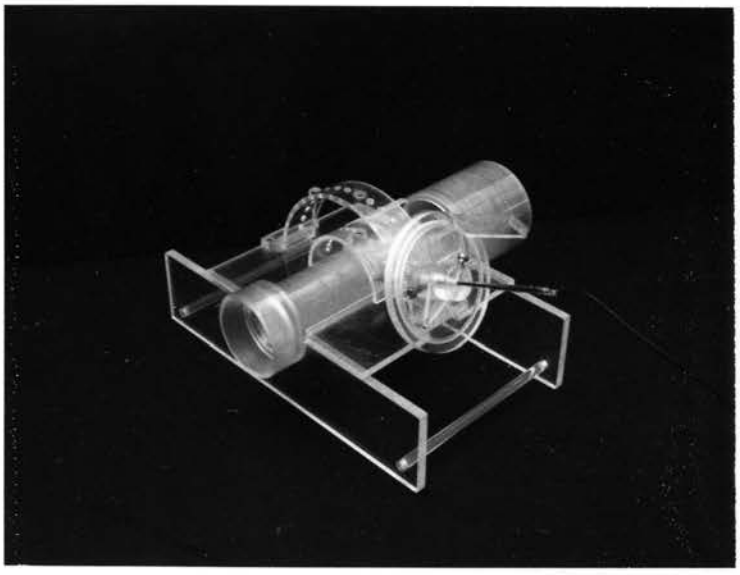
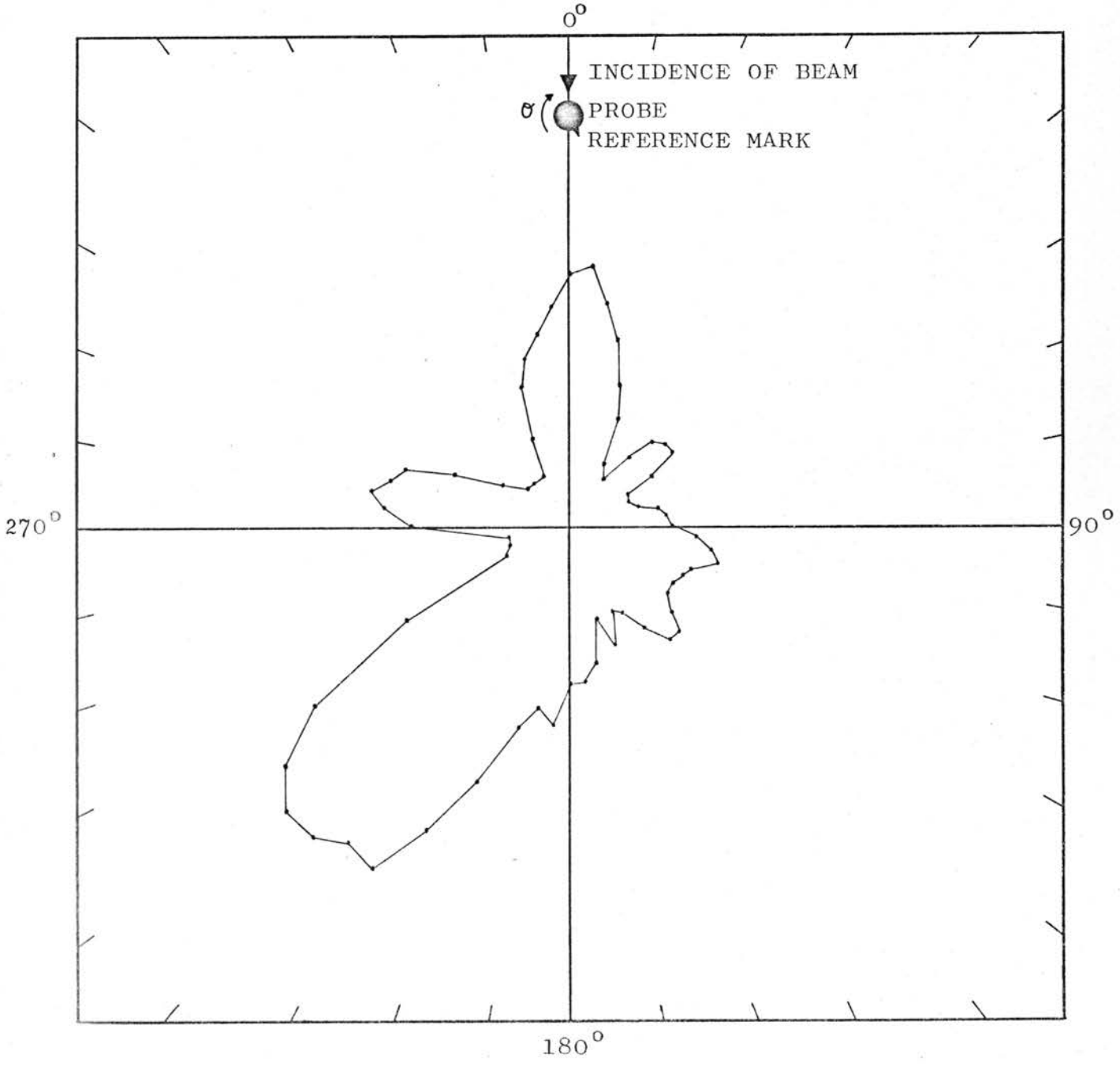


FIGURE 3.12 .

VARIATION OF THE OUTPUT OF THE TANTALUM CAPACITOR PROBE WHEN THE LATTER IS TURNED THROUGH AN ANGLE IN THE ULTRASONIC BEAM.

(AVERAGE INTENSITY = 0.21W/cm^2, PULSED BEAM).



(These measurements were made with the capacitor in the centre of the ultrasonic beam. Similar results were obtained with the capacitor 1 cm away from the centre).

FIGURE 3.13 .

BEAM PATTERN TAKEN WITH THE TANTALUM CAPACITOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.82W/cm^2, CONTINUOUS BEAM). PROBE AT 0°.

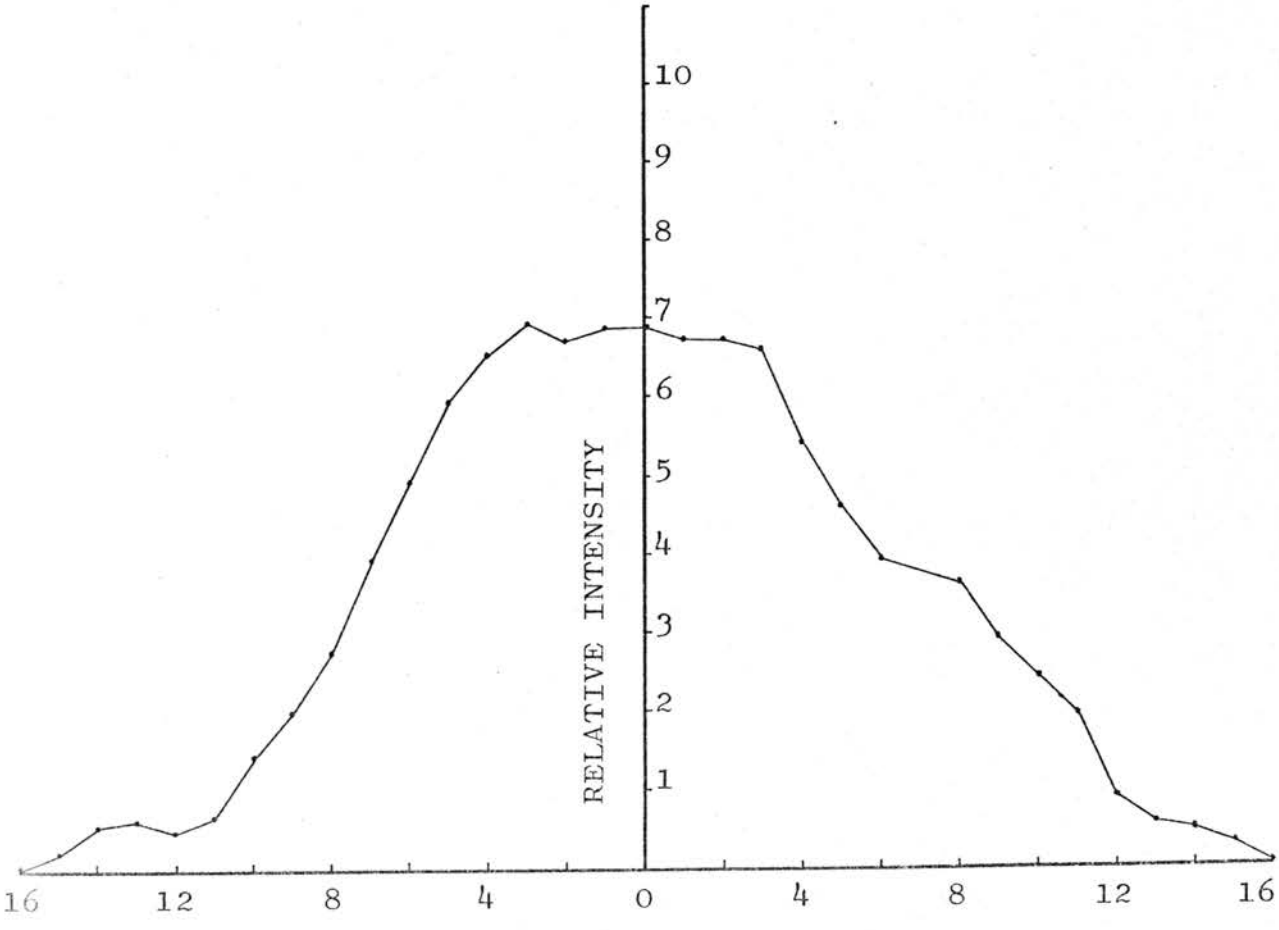


FIGURE 3.14 .

BEAM PATTERN TAKEN WITH THE TANTALUM CAPACITOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.21W/cm^2, PULSED BEAM). PROBE AT 0°.

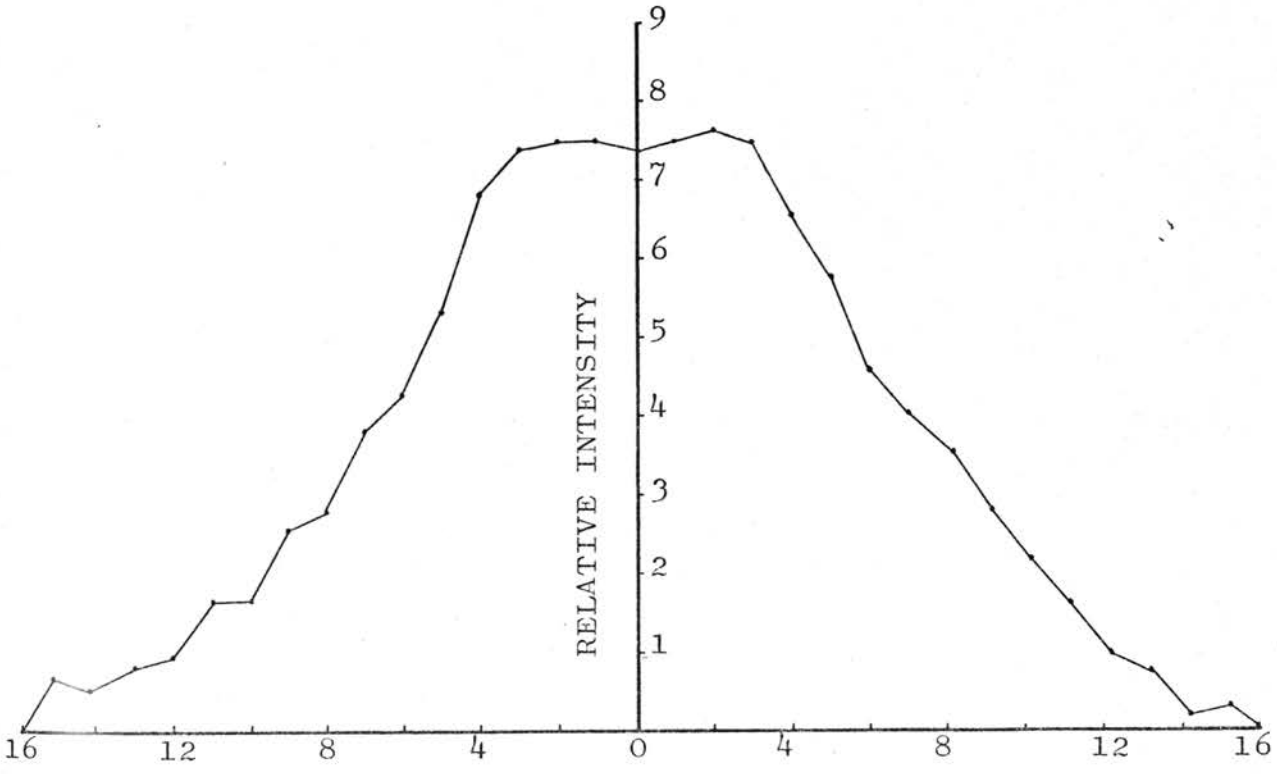


FIGURE 3.15.

BEAM PATTERN TAKEN WITH THE TANTALUM CAPACITOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.02W/cm^2, PULSED BEAM). PROBE AT 0°.

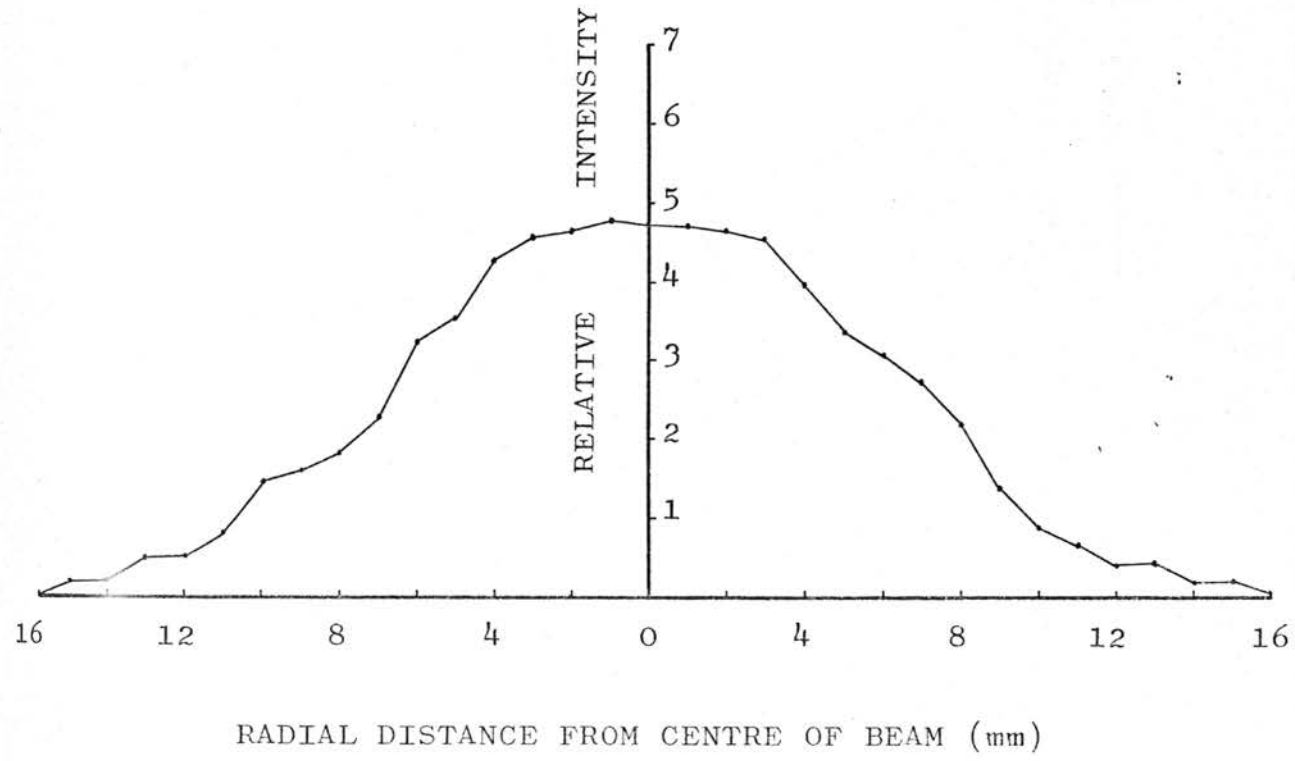


FIGURE 3.16 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY (CONTINUOUS BEAMS). PROBE AT 0°.

Each point represents the mean obtained for 2-3 profile curves.

The errors are standard errors of the means.

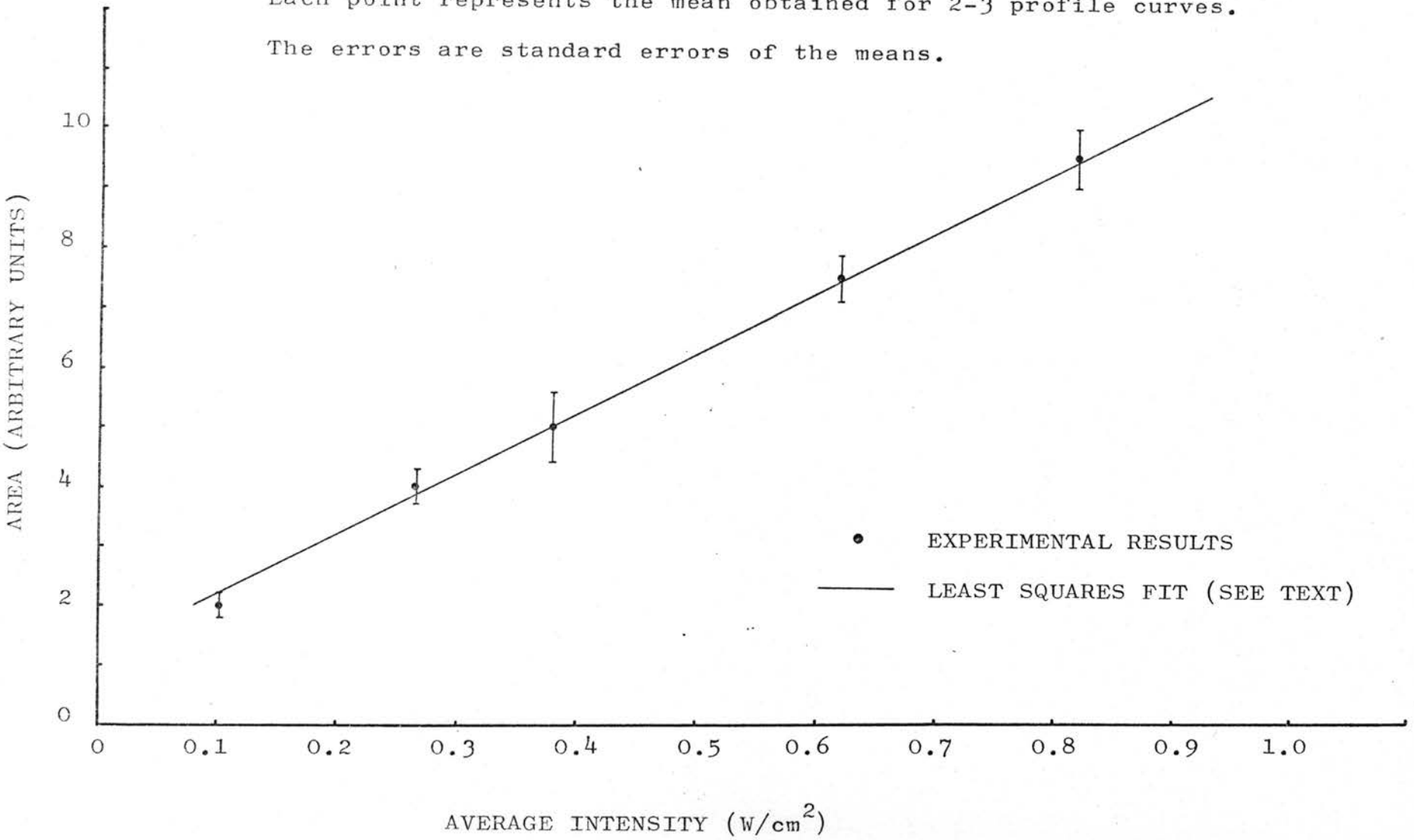


FIGURE 3.17.

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY (PULSED BEAMS). PROBE AT 0^{o}.

Each point represents the mean obtained for 2-3 profile curves. The errors are standard errors of the means.

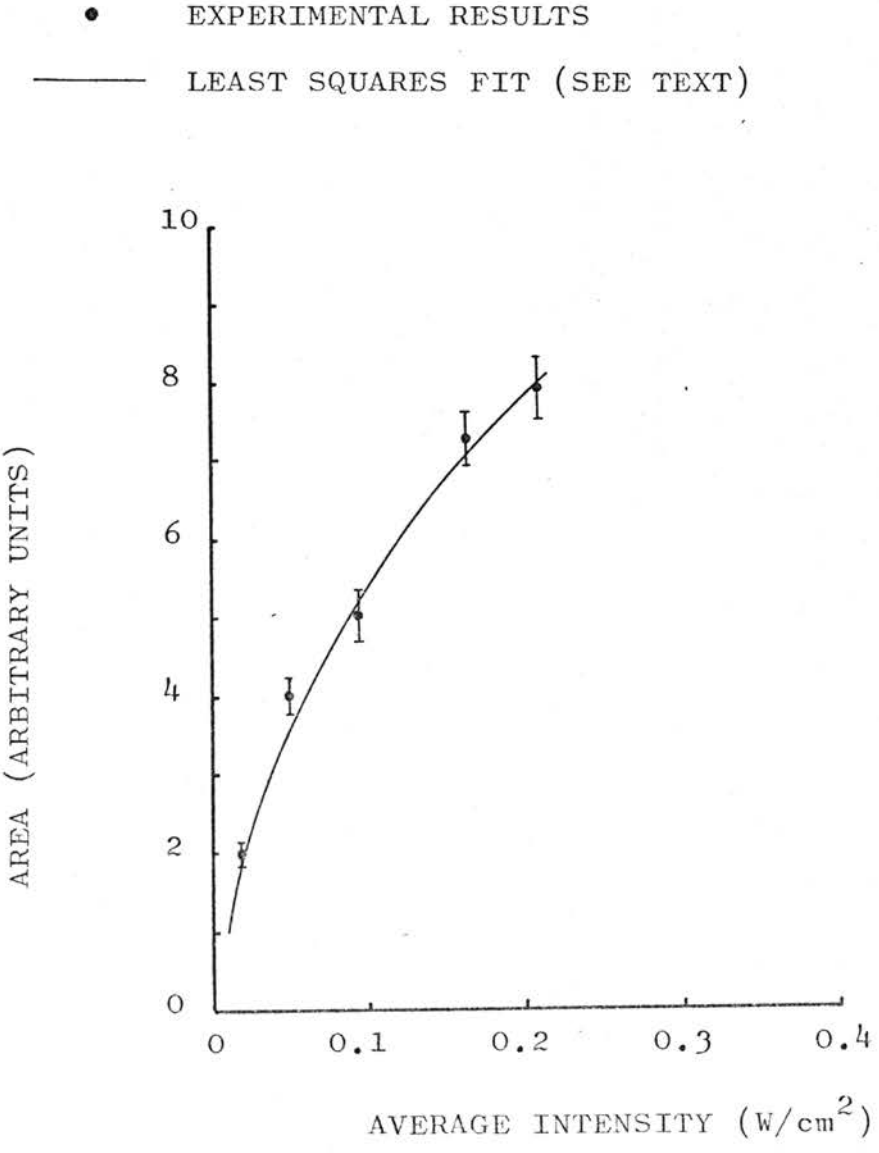


FIGURE 3.18.

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY (CONTINUOUS BEAMS). PROBE AT 225°.

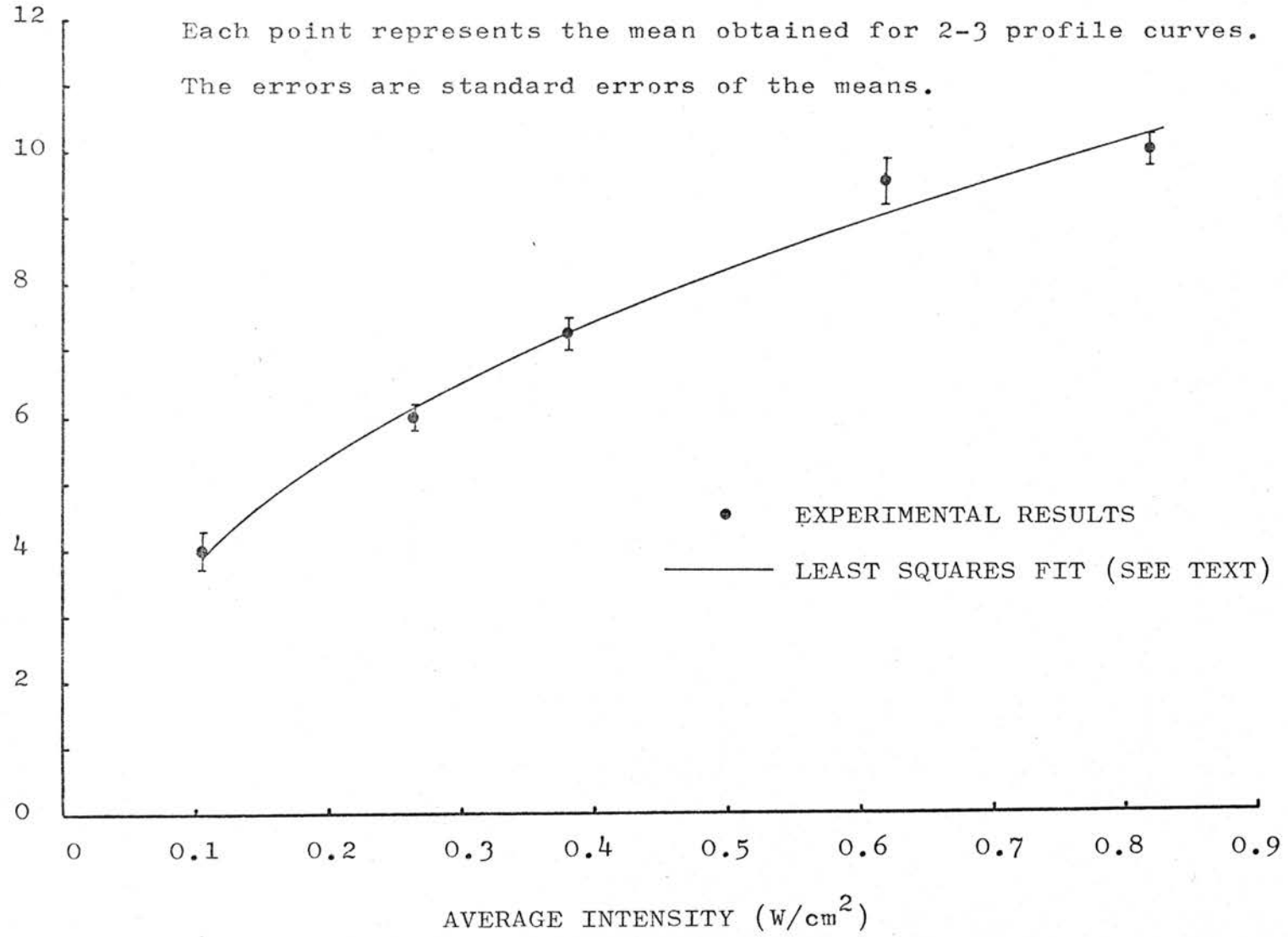
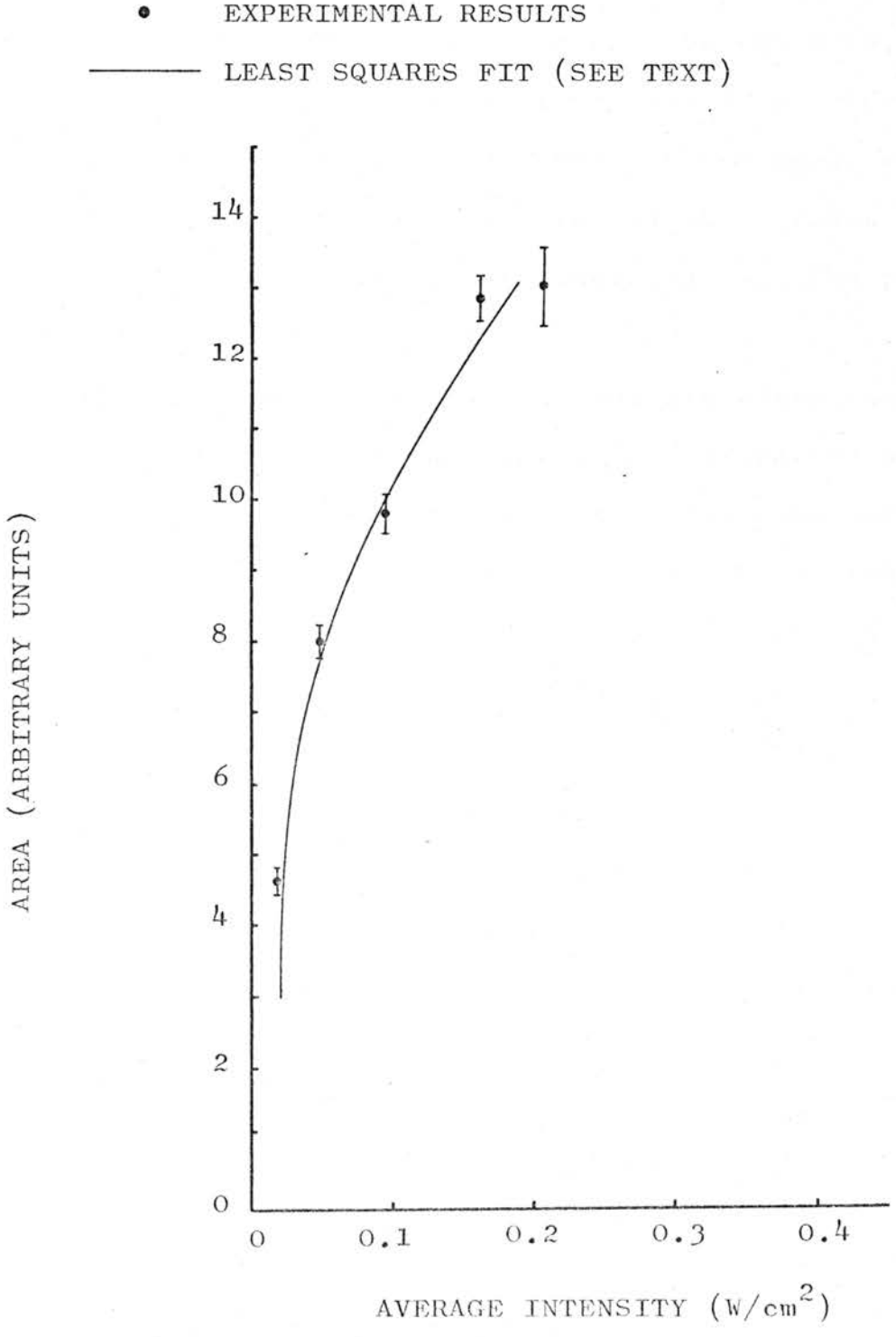


FIGURE 3.19 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY (PULSED BEAMS). PROBE AT 225°.

Each point represents the mean obtained for 2-3 profile curves. The errors are standard errors of the means.



Intensity measurements using a thermistor probe.

The thermistor probe used consisted of an ITT Thermistor (Type U23, US) of very small dimensions (0.4mm diam.) glued to the tip of a Pasteur pipette (external diameter = 1.2mm) as shown schematically in Fig. 3.20 .

The output of the probe was amplified using a Wheatstone bridge, the circuit diagram of which is shown in Fig. 3.21 and this amplified signal provided the Y-input to an X-Y Recorder (Omnigraphic 2000). For all the intensity measurements care had to be taken that the water in the sonication tank was earthed properly.

The probe was attached to a movable clamp mounted on a stand above the tank (Fig. 3.22) and any displacement of the probe perpendicular to the beam axis could be read off from a vernier scale to the nearest 0.1mm. The correct distance between the crystal face and the probe was obtained by moving the stand accordingly. The displacement of the probe was recorded using the potential difference across a sliding resistor (10kΩ max.) coupled to the movable clamp as the X-input to the abovementioned recorder. The pen of the recorder moved 4.31 ± 0.01cm along the X-axis for a displacement of 1cm of the probe. The error is the standard error of the mean of 12 displacements measured.

Using the probe as described above, profile curves

were drawn, indicating the variation of ultrasonic intensity across the beam at 7cm from the crystal face. These measurements were done for various average beam intensities, with the transducer either

(a) fixed to the wall of the sonication tank so that only the crystal face is in contact with the water in the tank as shown in Fig. 3.22 , or

(b) mounted on the sonication jig, but the probe was kept in a horizontal position rather than at an angle as described previously, or

(c) suspended freely in a horizontal position in the water (i.e. the entire probe is immersed in the water but not mounted on the jig).

Between two and four profile curves were drawn for each average intensity of the ultrasonic beam,pulsed and continuous. The areas under these profile curves were measured using a Hewlett-Packard Digitizer (Model 9107A) coupled to the Hewlett-Packard Calculator/Printer (Model 9120A). The percentage standard error of the mean for these areas was found to be in the range 1 - 10%.

Measurements to check the circular symmetry of the beam.

The circular symmetry of the ultrasonic beam was checked at three different average ultrasonic intensities, viz. 0.82 W/cm^2 (cont. beam), 0.21 W/cm^2 (pulsed beam) and 0.02 W/cm^2 (pulsed beam).

Cross-field measurements were made 7cm from the

transducer face, at each of these intensities, as follows. The displacement of the probe perpendicular to the beam axis (up and down) could be read off from the vernier as before (Y-displacement). The stand holding the probe as shown in Figure 3.22 could be moved "sideways" across the width of the tank perpendicular to the beam axis (X-displacement), and this displacement was measured on a scale attached to one of the supports. The position of the stand could, however, only be determined to the nearest millimetre. For every displacement (2mm) of the stand the distribution of intensity across the beam was measured as before.

The above measurements were made with the transducer fixed to the wall of the sonication tank. Similar measurements were also made with the transducer mounted on the sonication jig which was kept in a horizontal position. In this case profile curves were, however, only drawn for every displacement of 0.5cm of the stand because additional holes had to be drilled into the Perspex tube of the sonication jig to make these measurements possible. In the case of the latter, cross-field measurements were also made at the same average intensities of 0.82 W/cm^2 (cont. beam), 0.21 W/cm^2 (pulsed beam) and 0.02 W/cm^2 (pulsed beam) as measured on the radiation balance in the absence of the probe and jig.

FIG. 3.20 : THERMISTOR PROBE.

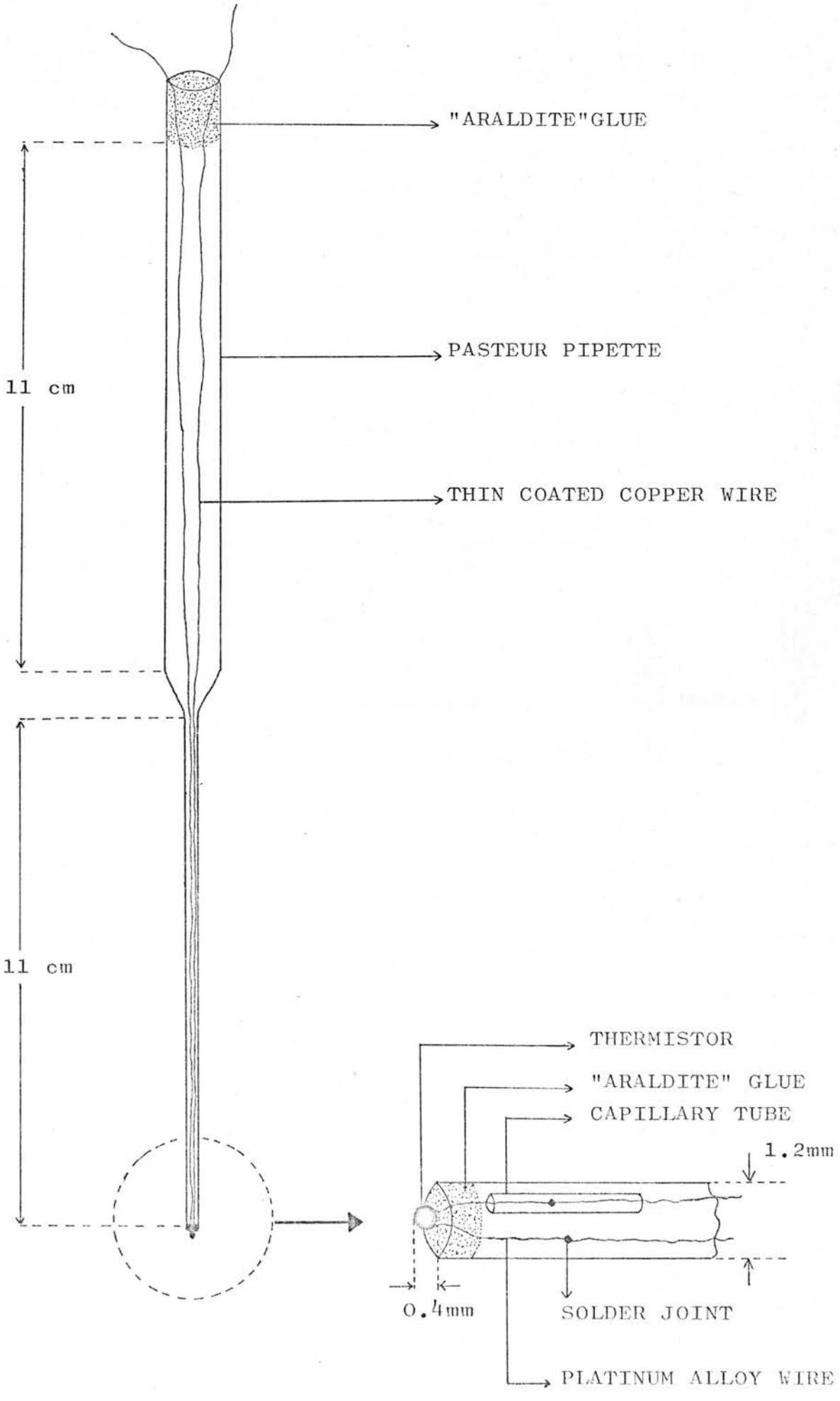
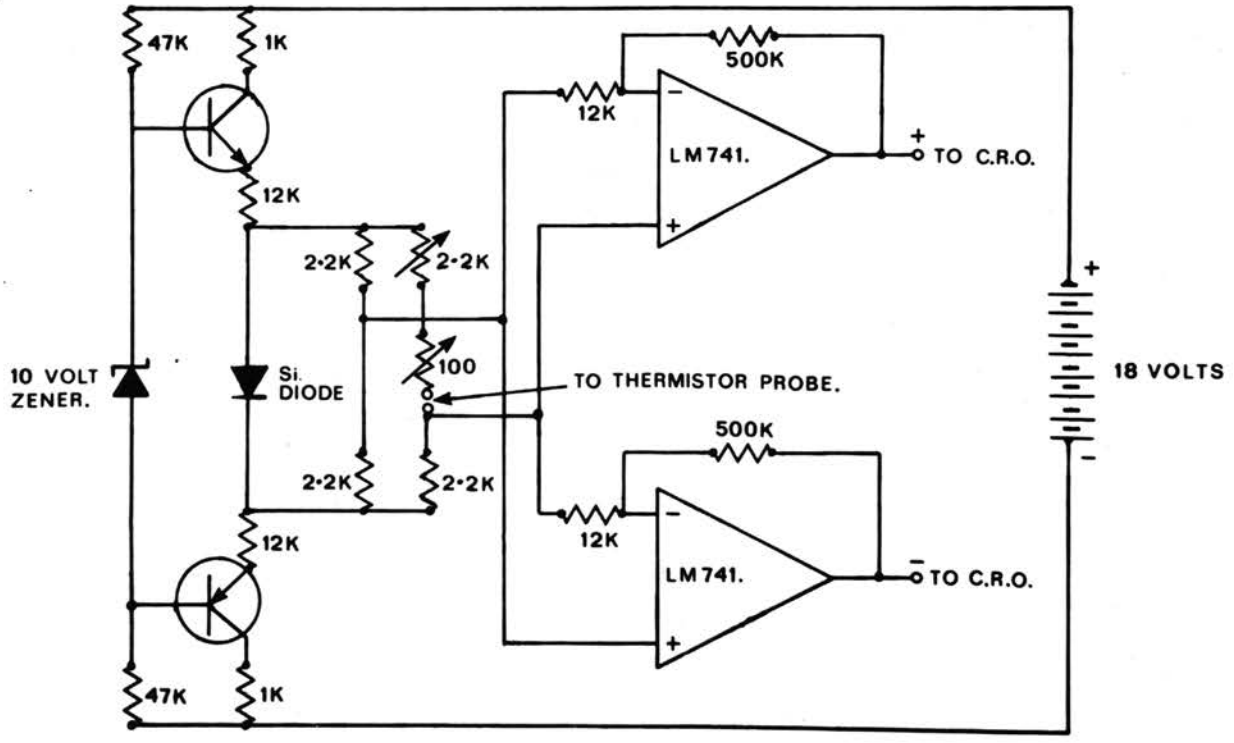


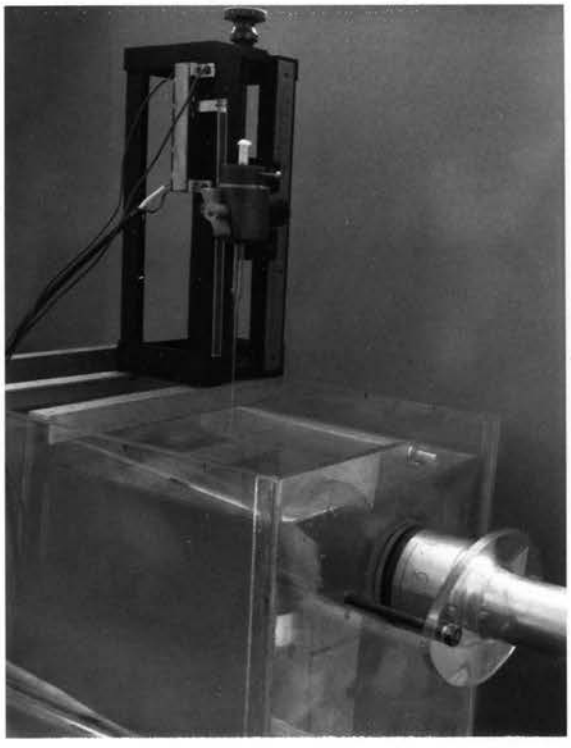
FIGURE 3.21 .



WHEATSTONE BRIDGE

FIGURE 3.22 .

POSITION OF THE THERMISTOR PROBE (NOT CONNECTED TO THE WHEATSTONE BRIDGE) ON THE SONICATION TANK.

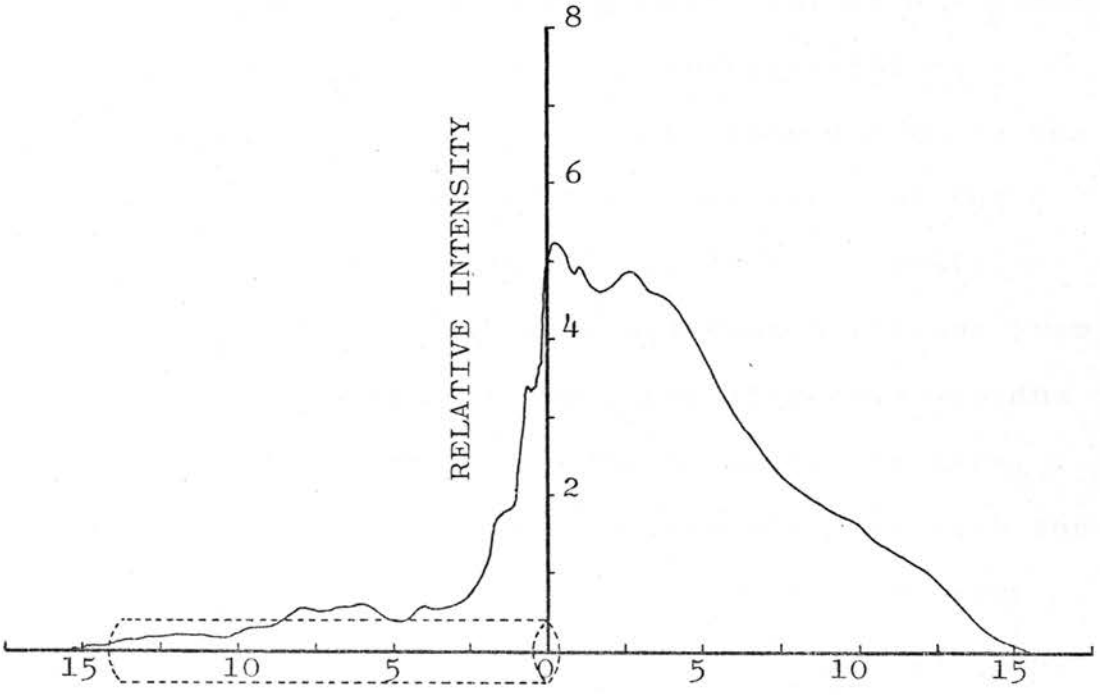


Measurement of the variation in ultrasonic intensity near the orifice of the double-walled metal cylinder used for the sonication of the different regions of the root tip (p. 83).

To make this measurement possible, the metal cylinder (described on p. 83) was glued onto the end of a thin Perspex rod which in turn was fixed to the wall of the sonication tank. It was thus possible to position the cylinder vertically in the ultrasonic beam so that an orifice was exactly in the centre of the beam and 7cm from the transducer fixed to the wall of the sonication tank as described earlier. In the experiments using the cylinder to shield parts of the root tip during sonication an average intensity of 0.82 W/cm^2 (cont. beam) was used, and for this reason the same average intensity was chosen for the present experiment. The thermistor probe was used to measure the variation in ultrasonic intensity inside and outside the cylinder along a vertical line perpendicular to the beam axis. The variation in ultrasonic intensity along this path is shown in Figure 3.23.

FIGURE 3.23 .

VARIATION IN THE ULTRASONIC INTENSITY NEAR THE ORIFICE AND INSIDE THE DOUBLE-WALLED METAL CYLINDER USED IN THE PRESENT EXPERIMENTS. (AVERAGE ULTRASONIC INTENSITY = 0.82 W/cm^2, CONTINUOUS BEAM, 7cm FROM THE TRANSDUCER FACE).



RADIAL DISTANCE FROM THE CENTRE OF THE BEAM (mm)

--------- POSITION OF METAL CYLINDER

———————— PROFILE CURVE

Results of the intensity measurements with the thermistor probe.

The profile curves obtained with the transducer positioned as described in (a), (b) and (c) above were found to be similar in shape. The profile curves were also found to be similar for all the average intensities used, for the continuous as well as the pulsed beams. Some examples of these profile curves are given in Figures 3.24, 3.25 and 3.26 . The peak intensity was found to be in the centre of the beam and there was an equal decrease of intensity on both sides of the maximum. In some of the initial experiments profile curves were obtained which were somewhat different from those described above. It was found that this was due to a fault in the earthing of the water in the tank. The shapes of the beam profile curves obtained with the thermistor probe were similar to those obtained with the tantalum capacitor probe (Figures 3.13 , 3.14 and 3.15).

Figures 3.27, 3.28 , 3.29 and 3.30 show the variation of the areas under the profile curves with the average ultrasonic intensity of the beam as measured on the radiation balance for both the pulsed and the continuous beam. The areas in Figures 3.27 and 3.28 are for the transducer mounted on the wall of the tank, whereas those for Figures 3.29 and 3.30 were obtained with the transducer mounted on the sonication jig. The

variation of the areas under the profile curves with the average intensities of the pulsed and continuous beams used, when the transducer was suspended freely in a horizontal position in the water, were similar to those shown in Figures 3.29 and 3.30 .

A polynomial of 2nd degree was fitted (using a method of least squares) to each of the set of values plotted in Figures 3.27 , 3.28 , 3.29 and 3.30 respectively. Except for the very low intensities, the relationship between intensity and area seems to be linear. This is confirmed by the values of the correlation coefficients (of 0.9636, 0.9979, 0.9675 and 0.9913 for the data depicted in Figures 3.27 , 3.28 , 3.29 and 3.30 respectively) obtained from the least squares fit of straight lines to the respective experimental values. Since the output of the thermistor probe is linear (except for very low intensities) it seems to be suitable for measuring the variation of intensity across the continuous and pulsed beams used in the present experiments.

The ultrasonic intensity distribution as measured with the transducer mounted on the sonication jig seemed to be the same as that measured with the probe fitted to the side of the tank or suspended freely inside the tank. This was found by the measurements described in (a), (b) and (c) above. The average area under the profile curves obtained with the probe mounted on the wall of the tank equalled 3.77 ± 0.05 in^2 *, whereas the average area with

* Calculator computes areas in square inches.

the probe on the jig equalled 4.06 ± 0.11 in^2. The area with the probe suspended in the tank, viz. 3.73 ± 0.10 in^2, was found to be similar to that with the probe fixed to the wall of the tank. The errors are standard errors of the means. Four areas were obtained in each case and the average intensity (as measured on the radiation balance) of 0.82 W/cm^2 (cont.beam) was used to obtain these areas. Similar results were obtained with various other average intensities (pulsed and continuous beams).

With the transducer mounted on the jig, it was found that there was no significant variation in the profile curves when the thermistor probe was moved across the beam at 7 ± 0.3cm from the crystal face, 0.7cm being the radius of the hole in the jig through which the probe was passed for intensity measurements. (The same hole was used when positioning the roots for sonication as described earlier). The areas obtained were 4.06 ± 0.11 in^2 (Probe in the centre of the holes), 4.12 ± 0.08 in^2 (Probe passing through the holes at 6.7cm) and 4.10 ± 0.10 in^2 (Probe passing through the holes at 7.3cm). For these measurements the average intensity of 0.82 W/cm^2 (cont. beam) 7cm from the crystal face was used.

From the profile curves plotted in the experiments above a mean was obtained for the diameter of the ultrasonic beam 7cm from the crystal face. The diameter of the pulsed beams was found to be similar to the diameter of the continuous beams. Also, the beam diameters were found to be similar for all the different average

intensities used, irrespective of whether the transducer was fixed to the wall of the tank, positioned on the sonication jig or suspended freely in the sonication tank. Thus the diameters obtained from 20 profile curves were pooled, giving a mean diameter of 3.0 ± 0.05cm. The error is the standard error of the mean.

Results on the circular symmetry of the beam.

The measurements (with the transducer fixed to the wall of the sonication tank) of the cross-field distribution with X- as well as Y-displacements of the probe (p. 113), indicated that there was virtually no deviation from circular symmetry for the beams at the three average intensities used. The profile curve in Figure 3.31 (which is similar to the profile curve in Figure 3.24), shows the variation of ultrasonic intensity along a line in the X-direction through the centre of, and perpendicular to, the ultrasonic beam. The points on this curve are the central peak values of intensity obtained from the set of profile curves plotted as described earlier (p. 113). Also, from the set of profile curves drawn, the points at which the ultrasonic intensity reached zero were obtained and plotted as shown in Figure 3.32. Similar results were obtained for all the other average intensities used.

From the measurements with the transducer on the sonication jig, curves similar to those in Figures 3.31 and 3.32 were obtained.

FIGURE 3.24 .

BEAM PROFILE OBTAINED WITH THE THERMISTOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.82 W/cm^2, CONTINUOUS BEAM).

THE ULTRASONIC TRANSDUCER WAS MOUNTED ON THE SONICATION JIG.

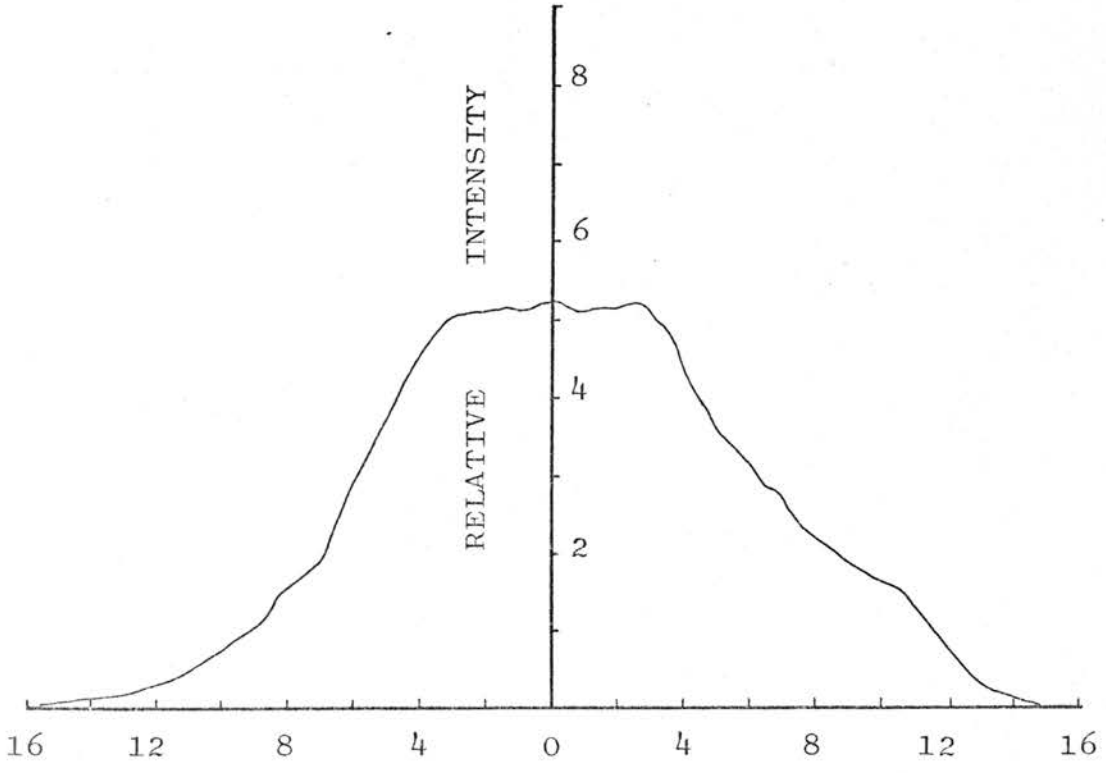


FIGURE 3.25 .

BEAM PROFILE OBTAINED WITH THE THERMISTOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.21 W/cm^2, PULSED BEAM).

THE ULTRASONIC TRANSDUCER WAS MOUNTED ON THE SONICATION JIG.

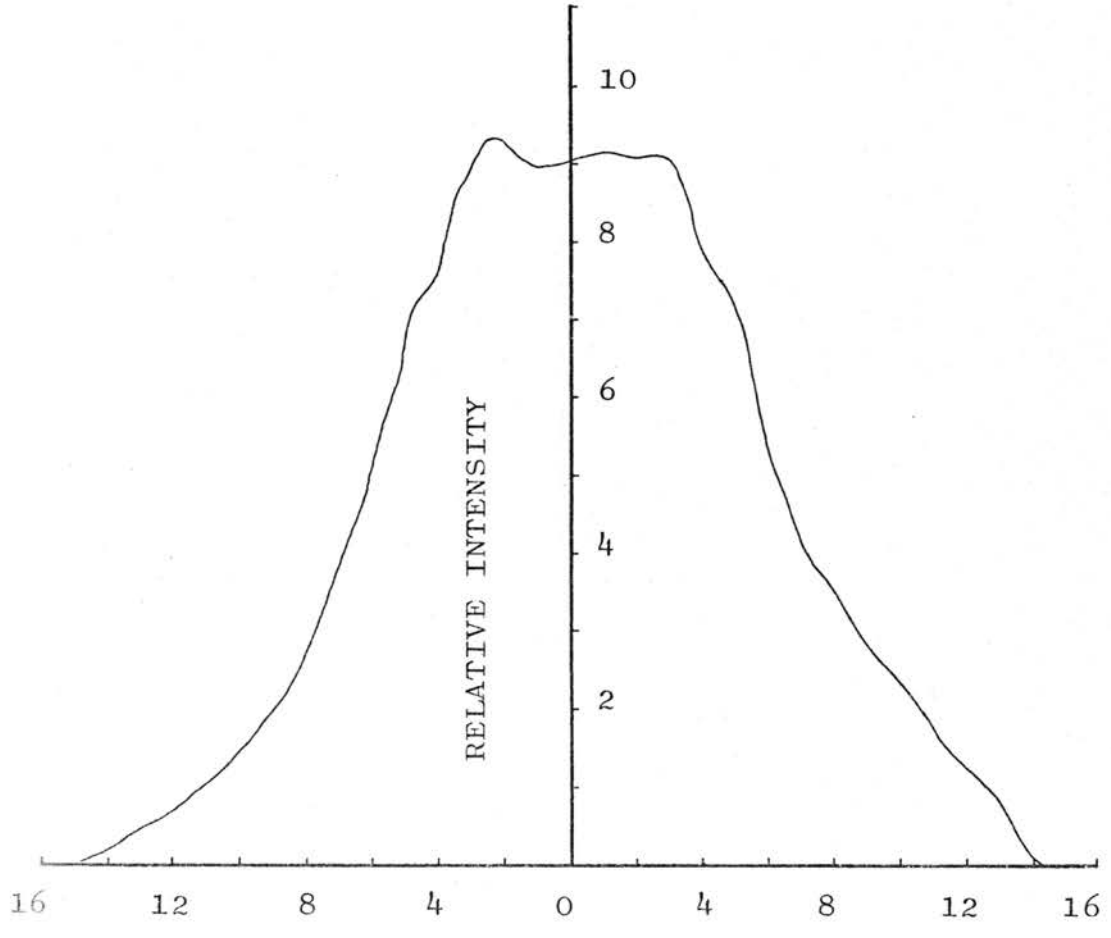


FIGURE 3.26 .

BEAM PROFILE OBTAINED WITH THE THERMISTOR PROBE 7 cm FROM THE CRYSTAL FACE (AT AN AVERAGE INTENSITY OF 0.02 W/cm^2, PULSED BEAM).

THE ULTRASONIC TRANSDUCER WAS MOUNTED ON THE SONICATION JIG.

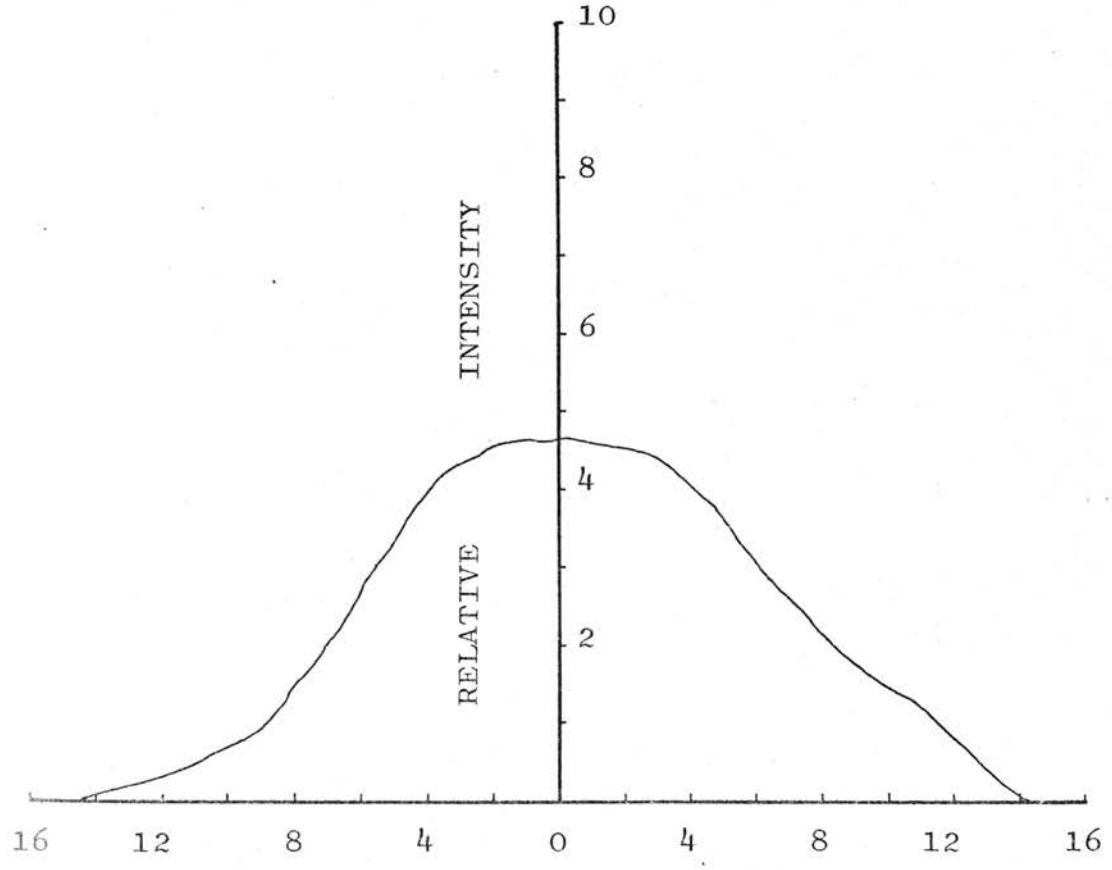


FIGURE 3.27 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY, PULSED BEAM. TRANSDUCER FIXED TO THE WALL OF THE SONICATION TANK.

Each point represents the mean obtained for 2-4 profile curves. The errors are standard errors of the means.

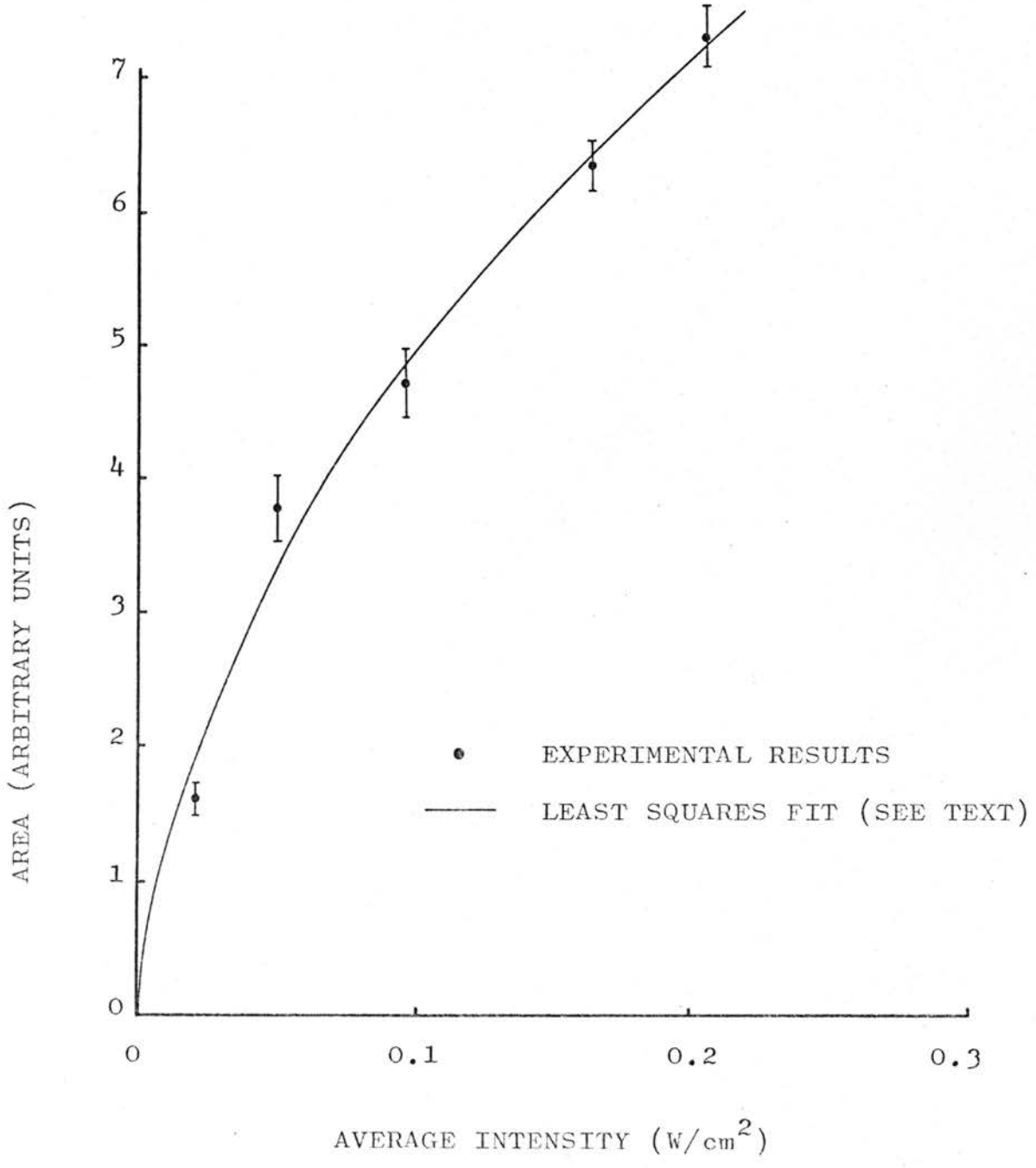


FIGURE 3.28 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY, CONTINUOUS BEAM. TRANSDUCER FIXED TO THE WALL OF THE SONICATION TANK.

Each point represents the mean obtained for 2-4 profile curves. The errors are standard errors of the means.

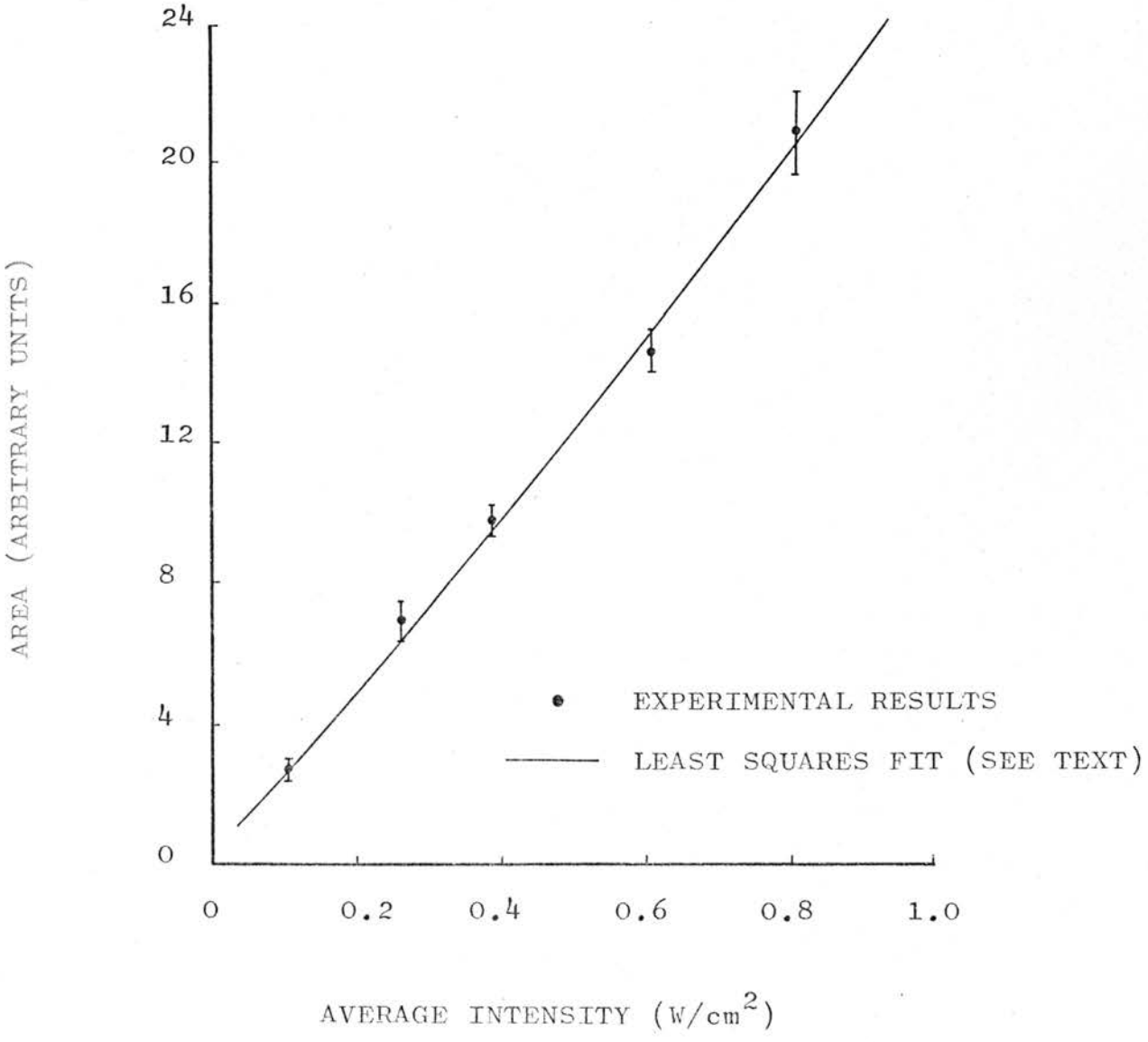


FIGURE 3.29 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY, PULSED BEAM. TRANSDUCER MOUNTED ON SONICATION JIG.

Each point represents the mean obtained for 2-4 profile curves. The errors are standard errors of the means.

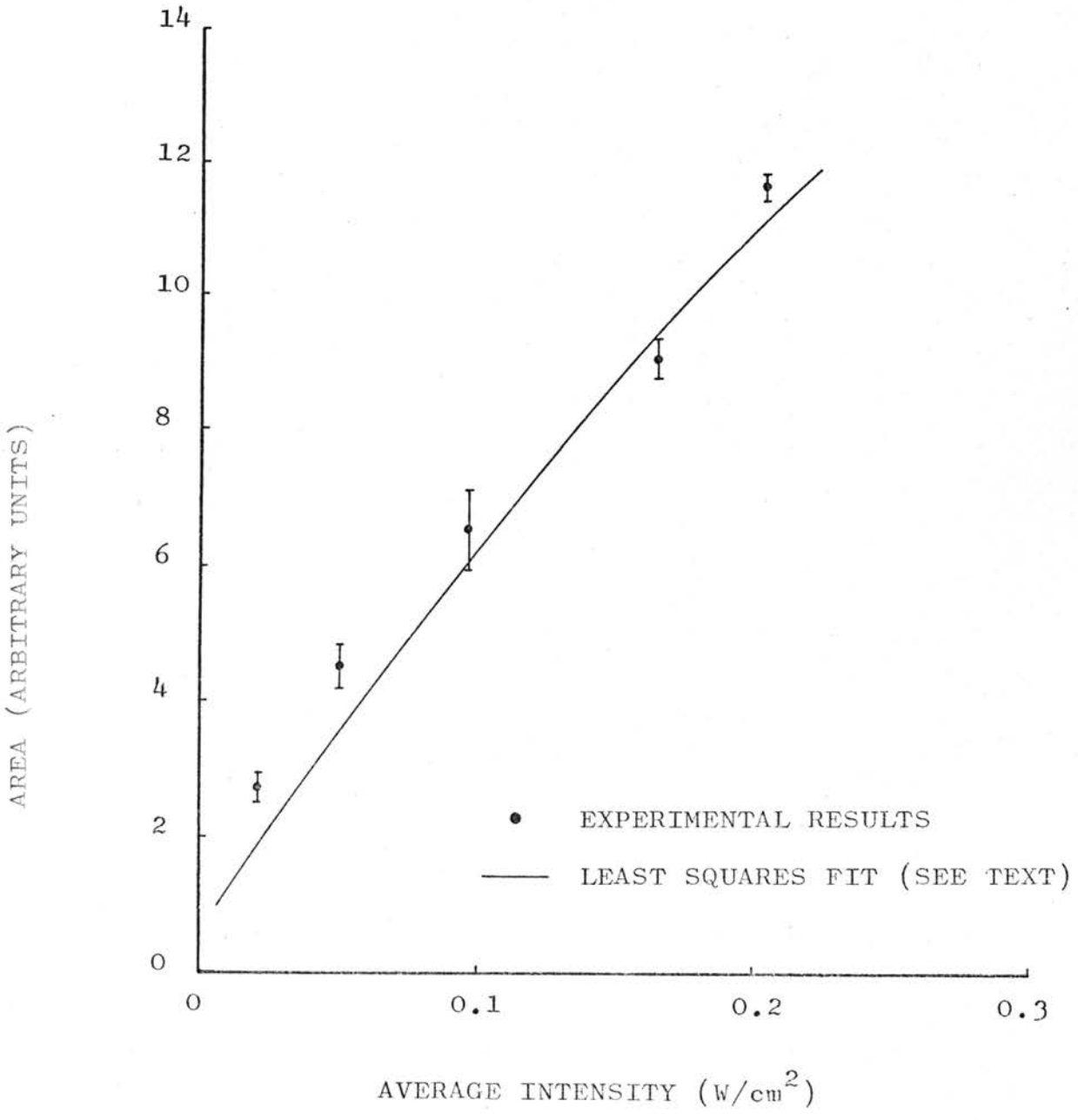


FIGURE 3.30 .

VARIATION OF THE AREA UNDER THE PROFILE CURVES (TOTAL WATTS) WITH THE AVERAGE ULTRASONIC INTENSITY, CONTINUOUS BEAM. TRANSDUCER MOUNTED ON SONICATION JIG.

Each point represents the mean obtained for 2-4 profile curves. The errors are standard errors of the means.

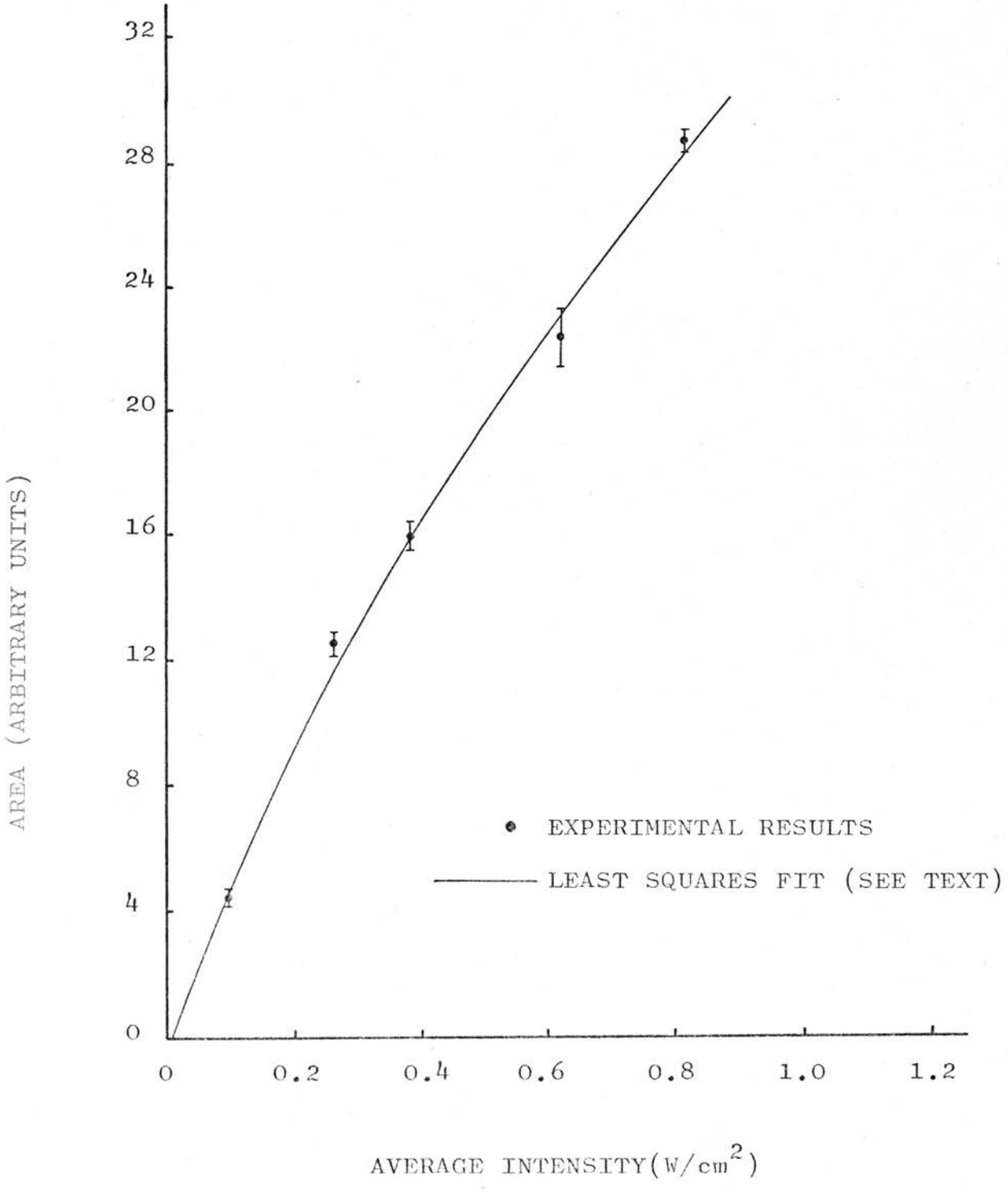


FIGURE 3.31 .

VARIATION OF THE ULTRASONIC INTENSITY ALONG A LINE IN THE X-DIRECTION THROUGH THE CENTRE OF, AND PERPENDICULAR TO, THE ULTRASONIC BEAM (SEE TEXT).
(AVERAGE ULTRASONIC INTENSITY = 0.82 W/cm^2, CONTINUOUS BEAM, 7cm FROM THE TRANSDUCER FACE).

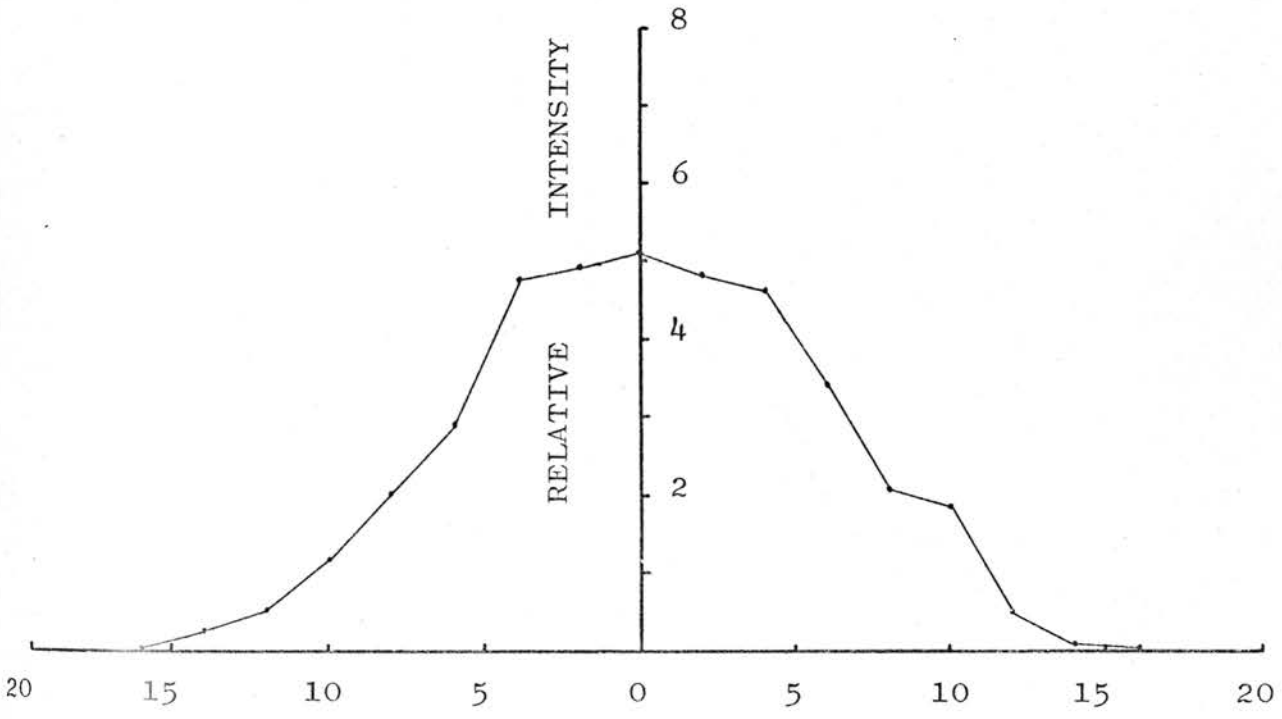
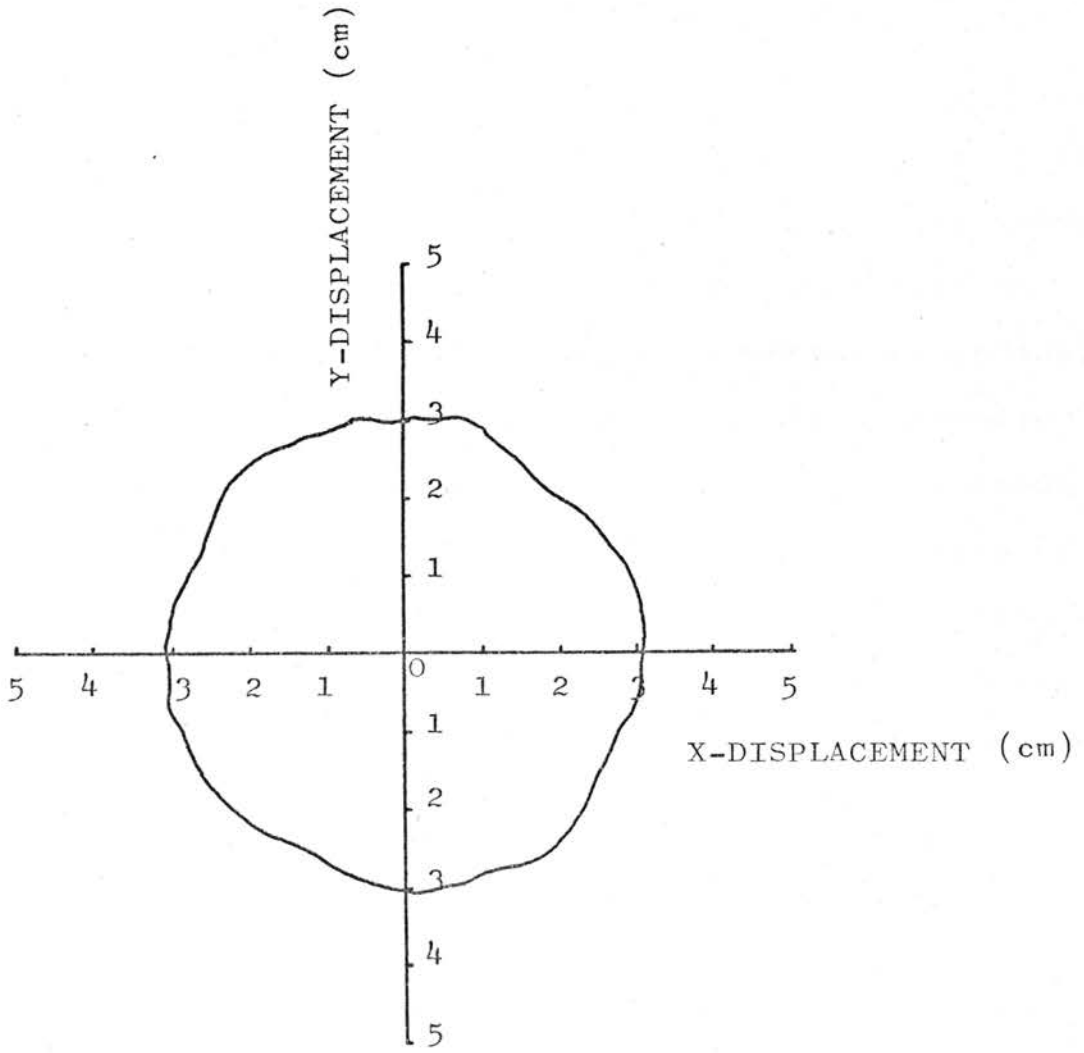


FIGURE 3.32 .

DIAGRAM SHOWING THE CIRCULAR SYMMETRY OF THE ULTRASONIC BEAM USED IN THE PRESENT EXPERIMENTS.
(AVERAGE ULTRASONIC INTENSITY = 0.82 W/cm^2, CONTINUOUS BEAM, 7cm FROM THE TRANSDUCER FACE).



SCHLIEREN PICTURES.

The propagation of an ultrasonic wave is associated with changes in the density of the medium through which it travels. Working independently Debye and Sears (1932) and Lucas & Biquard (1932) demonstrated that a transparent medium supporting an ultrasonic wave diffracts light. This diffraction occurs because the density of the medium varies in sympathy with the ultrasonic wave, and the refractive index of the medium depends upon its density.

Several Schlieren systems have been described in the literature for the visualization of ultrasonic waves. They all share the same basic principles. A beam of light is arranged to pass through a transparent medium, usually water, in which is established the ultrasonic field to be investigated. The light is then focussed on an obstruction (e.g. razor blade), near the edge of the latter so that none reaches the observer when the ultrasonic field is zero. However, when ultrasound is present and changes the refractive index of the medium, the light which passes through the disturbed areas no longer falls at the original focus, but it is deviated and falls on a screen behind the obstruction.

The Schlieren system employed in the present experiments was based on that constructed by Willard (1947) and Barnes & Burton (1949). In this system (Fig. 3.33) a slit source of light was used which was

brought into parallel beam by means of a lens. This beam was arranged to travel through a water tank in which the ultrasonic disturbance was occurring, and was then focussed by means of a second lens on a knife-edge obstruction (razor blade).

A Schlieren picture of the beam used in the present experiments is shown in Fig. 3.34 . The Schlieren pictures taken at various distances from the crystal face and with all the possible average intensities of the pulsed and continuous beams that the Impulsaphon is capable of producing (p. 94), revealed that the beam is unfocussed.

FIGURE 3.33 .

SCHLIEREN SYSTEM USED IN THE PRESENT EXPERIMENTS.

U. ULTRASONIC DISTURBANCE
L. LENS
G. GLASS-WALLED TANK
T. SCREEN (TRANSLUCENT FOR PHOTOGRAPHY)
W. WATER
S. SLIT LIGHT SOURCE
A. ABSORBER (GLASS WOOL ON NEOPRENE RUBBER)
K. KNIFE-EDGE OBSTRUCTION

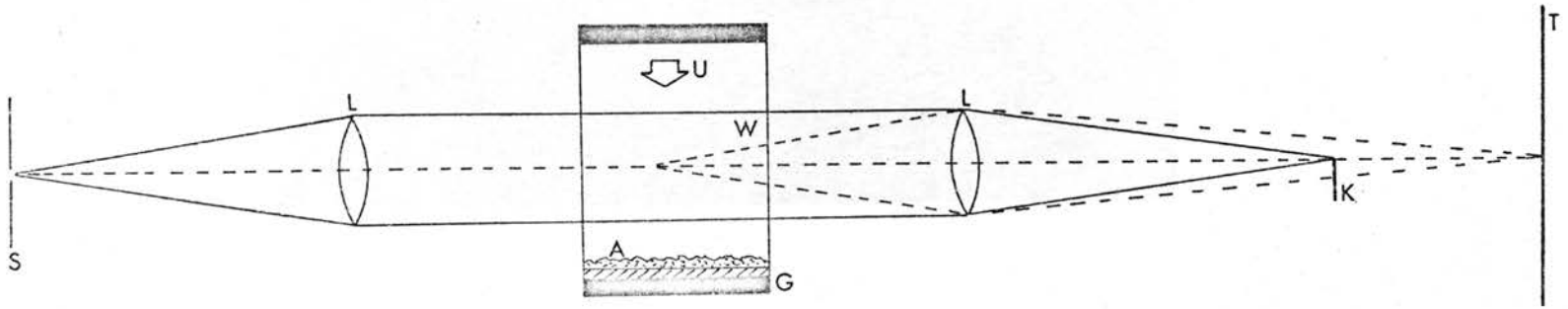
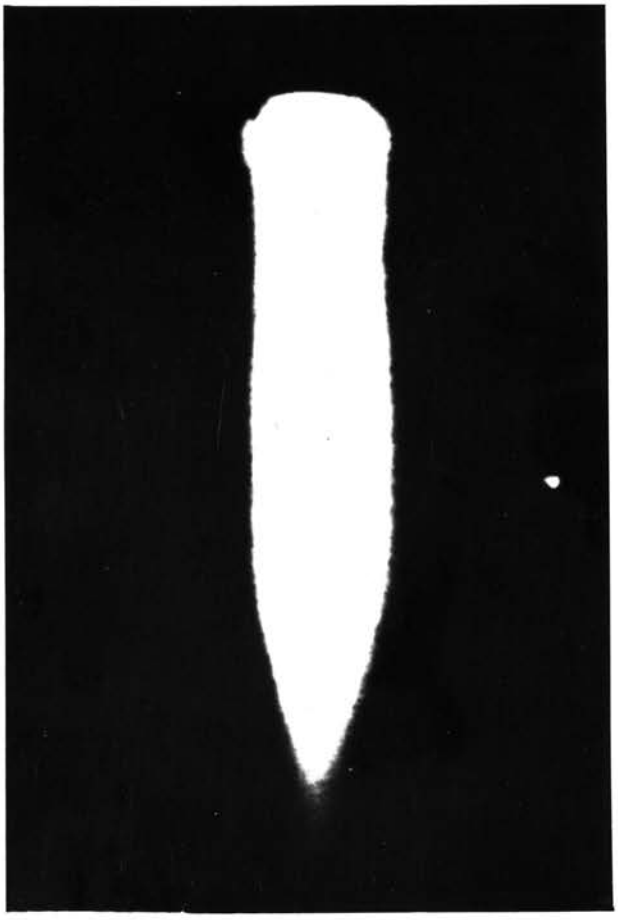


FIGURE 3.34.

SCHLIEREN PHOTOGRAPH OF THE 0.38 W/cm^2 ULTRASONIC BEAM USED IN THE CURRENT EXPERIMENTS (ACTUAL SIZE). THE CONVERGING BEAM AT THE BOTTOM OF THE PICTURE IS DUE TO THE FAULTY IMAGE FORMED AT THE EDGE OF THE LENS.



METHOD OF SONICATION OF THE DRY SEEDS OF ZEA.

From the introductory discussion in Chapter I it will be realised that it is imperative that the irradiated and the control seeds must, in such an investigation, all be subjected to exactly the same storage and growing conditions, and must also have been obtained from the same crop. This requirement was meticulously adhered to in the present experiments.

In each of three individual experiments, twenty dry seeds of Zea were sonicated and twenty seeds were used as controls. The ultrasonic generator was again the Impulsaphon M 55. For each exposure to ultrasound, ten seeds were placed in a single layer on some glass wool covering the floor of a Perspex tank (30cm x 30cm and 15cm deep) filled with distilled water. Thus for each experiment two groups of ten seeds were sonicated. The area covered by the 10 seeds was made to correspond to the area of the crystal face, i.e. approximately 7 cm^2. The latter was positioned vertically above the seeds to be sonicated as shown in Figure 3.35, so that the distance between the top of the seeds and the face of the transducer was equal to 7 cm. The seeds were placed on the glass wool with their embryos facing the transducer because the embryo is obviously the part of the seed which the ultrasound must affect to cause any change in the normal development of the seedling. Although some of the ultrasonic radiation is expected to be reflected from the surface of the seeds,

the overall reflection of ultrasound will be reduced by having glass wool as an absorber of any sound reaching it through the layer of seeds, particularly the open spaces between them. The walls of the tank were also lined on the inside with indented neoprene rubber sheeting to absorb any stray ultrasonic radiation.

In all three experiments the seeds were exposed for 60 min. at an average intensity of 0.82 W/cm^2 (cont. beam). The quoted intensity of 0.82 W/cm^2 represents the average value measured by pressure balance as described previously.

The thermistor probe was used to measure the variation of the ultrasonic intensity at the position of the seeds. The values of the output of this probe at various points across the beam at seed-level were compared with the corresponding values obtained from the beam profile curve which was plotted for the 0.82 W/cm^2 cont. beam (with no obstacle in the path of the beam) as described earlier (p. 112). The values of the output of the probe in the present experiment did not differ by more than $\pm$ 15% from those obtained for the unimpeded beam.

The temperature of the water in the sonication tank was kept at $19^{\circ}C \pm 0.2^{\circ}C$ using a Braun thermostatically controlled heater and a Grant cooling unit. The temperature did not rise during sonication. The control seeds were also kept in water at $19^{\circ}C \pm 0.2^{\circ}C$ for 60 min.

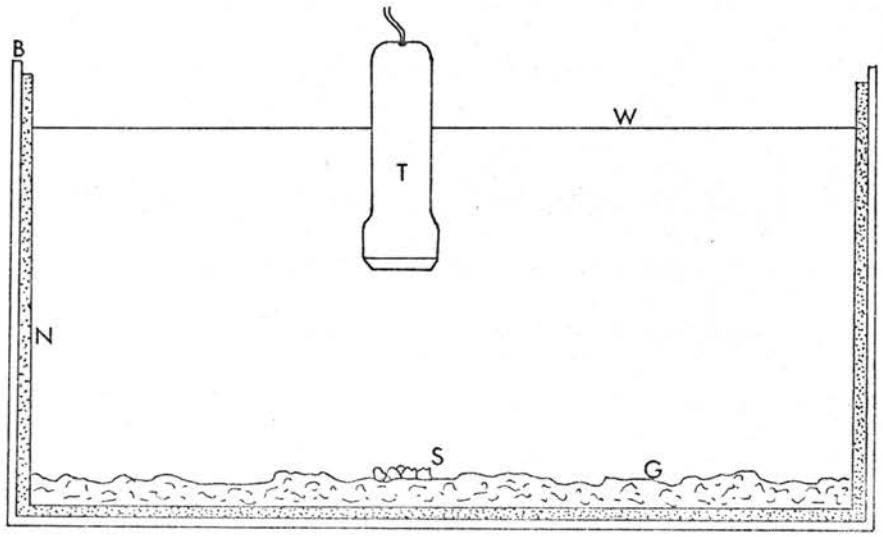
After sonication, the seeds were subjected to the same process of soaking and planting in Vermiculite as

described earlier in this chapter. After soaking for three days, all the seeds were transferred to the Vermiculite, irrespective of whether they had germinated or not. After three days in the tank of Vermiculite, all the seedlings were dug up and the germination rate scored. Seeds which had not germinated were discarded, and the remainder were transferred to the culture tank maintained at $19^{o}C \pm 0.2^{o}C$ after plumule and adventitious roots had been removed as before. The control groups were treated in exactly the same way.

After transfer to the culture tank the lengths of the roots were measured immediately and thereafter at daily intervals for fifteen days.

FIGURE 3.35 .

DIAGRAM SHOWING HOW THE DRY SEEDS WERE SONICATED.



S. SEEDS

W. WATER LEVEL

G. GLASS WOOL

N. NEOPRENE RUBBER

T. TRANSDUCER

B. PERSPEX TANK

METHODS OF EXPOSURE OF THE ROOTS OF ZEA TO X-RAYS IN CONJUNCTION WITH ULTRASOUND.

In the synergistic experiments on Zea mays, the roots of the latter were exposed to continuous and pulsed beams of ultrasound of various average intensities as listed in Table 4.9 . The roots were exposed to ultrasound in combination with an X-ray dose of 775 rads and the latter was applied before, after or simultaneously with the sound. Ultrasonic intensities (pulsed and continuous beams) were chosen such that, on their own, they either reduced the growth rate of the roots to a considerable extent or hardly affected the growth at all.

In the experimental methods to be described, the ultrasonic and X-ray beams were mutually independent, so that the dose delivered by one system was in no way affected by the presence of the other.

The methods of irradiation used in the experiments in which the X-rays and the ultrasonic beams were applied separately are described elsewhere (Hering, 1971; this Chapter, p. 81). The X-radiation source was a Philips 250/25 X-ray Therapy Unit operated at 250 kVp, 15 mA with an added Thoraeus filter (3.5 mm Cu H.V.L.). A 10x10 applicator was used. This beam was calibrated by means of a Baldwin Farmer Dosemeter at the site of irradiation as described previously (Hering, 1971).

For the experiments in which the roots were simultaneously exposed to X-rays and ultrasound, it was

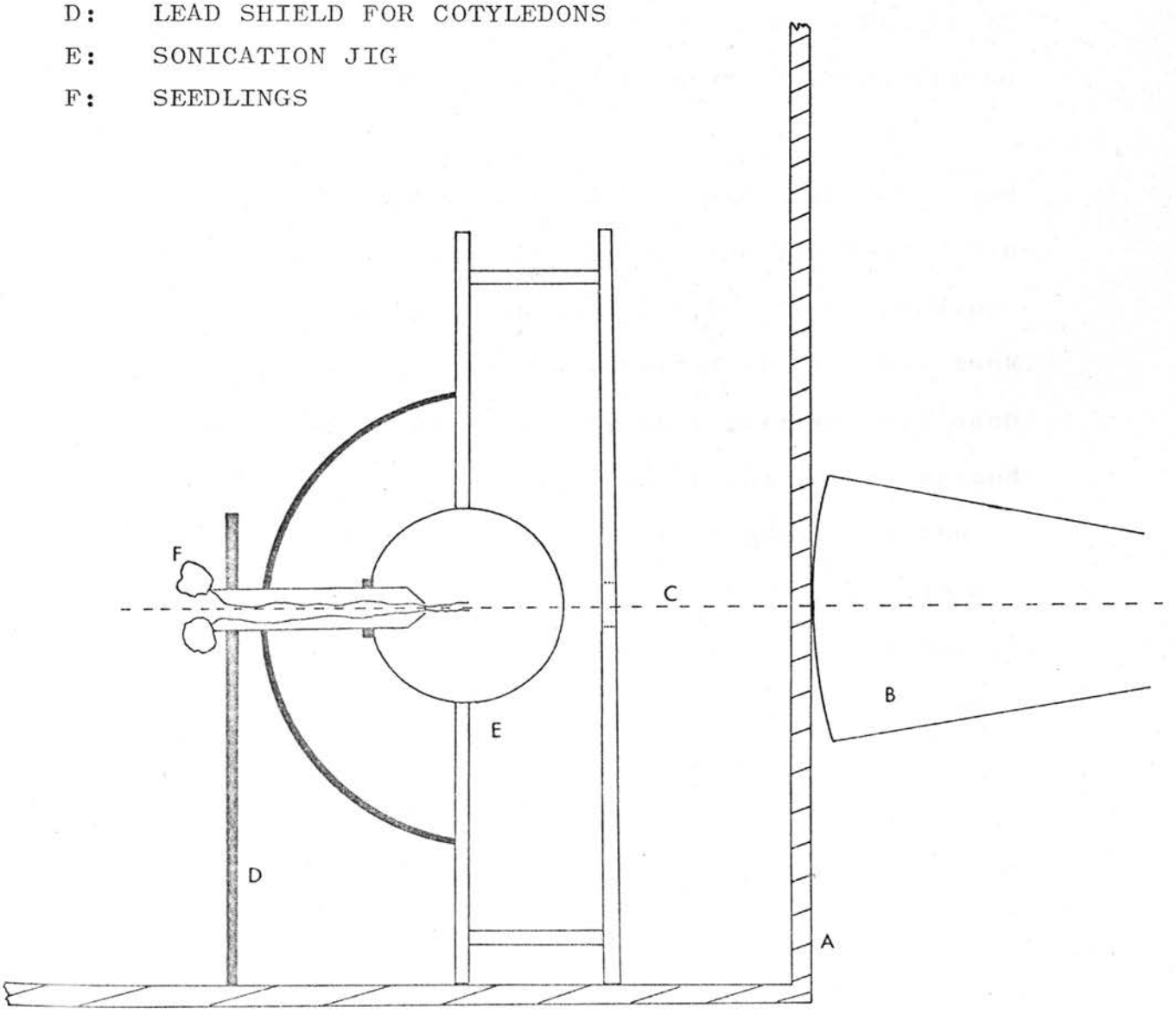
found necessary to immerse the sonication jig vertically in the sonication tank so that the former could be moved closer to the wall of the latter as shown in Figure 3.36. The jig could now be placed close enough to the applicator of the X-ray unit so that it was possible to expose the roots to 775 rads of X-rays in a time equalling the sonication times used in the experiments in which the roots were exposed to X-rays and ultrasound separately. This was, however, not possible for the short sonication times of 2 and 5 minutes used. In this case beams of very much lower average intensities, viz. 0.05 W/cm^2 (pulsed beam) and 0.10 W/cm^2 (continuous beam) respectively, were used which, on their own, hardly changed the growth rate of the roots if applied to the latter for 40 min. (Figures 4.39 and 4.40).

The roots, now being parallel to the axis of the X-ray beam (instead of being perpendicular to the beam axis as was the case when the roots were exposed to ultrasound and X-rays separately), were again exposed to 775 rads on their own in order to obtain the growth curve for the roots when irradiated in this position. The X-ray dose rate was determined using a Baldwin Farmer Dosemeter at the site of irradiation in the absence of the roots and jig. There is no change in the average intensity of the ultrasonic beam at the position of the roots if the jig is turned through an angle (p. 113). No air was passed through the water in the sonication tank when the roots were simultaneously exposed to X-rays and ultrasound.

FIGURE 3.36 .

DIAGRAM SHOWING THE POSITION OF THE SEEDLINGS AND THE JIG FOR THE EXPERIMENTS IN WHICH ULTRASOUND AND X-RAYS WERE APPLIED TO THE ROOTS SIMULTANEOUSLY.

A: TANK WALL
B: APPLICATOR OF X-RAY UNIT
C: X-RAY BEAM AXIS
D: LEAD SHIELD FOR COTYLEDONS
E: SONICATION JIG
F: SEEDLINGS



METHODS OF TREATMENT OF THE ROOTS OF ZEA WITH VINCRISTINE IN COMBINATION WITH ULTRASOUND.

The vincristine treatments were carried out by suspending the seedling roots in a Perspex container (10 cm x 0.5 cm and 5 cm deep) containing 25 ml of a 0.004% vincristine solution (Fig. 3.37). The vincristine solution was made up by dissolving 1 mg of lyophilized vincristine sulfate (purchased from Eli Lilly and Company,Indianapolis,U.S.A.) in 25 ml of distilled water.

Ten seedlings were used for each treatment, and these were treated simultaneously in a solution of vincristine which was kept at $19^{o}C \pm 0.2^{o}C$ by suspending the Perspex container in the water of the culture tank. The vincristine solution was freshly prepared for each treatment since for a solution which was used a second time, the reduction in the subsequent growth of the roots was found to be less than that obtained with a freshly prepared solution (Figure 3.38). A similar effect was observed when the number of roots per treatment was increased from ten to thirty (Figure 3.38).

The presence of 10 mg of lactose with each 1 mg of vincristine sulfate supplied had no adverse effect on the growth of the vincristine treated roots (Fig.3.39). This was checked by treating roots in a solution of lactose (10 mg of lactose in 25 ml of distilled water) in the same way and for the same time as for the vincristine treatments.

In all treatments the solutions were continuously aerated. Under these conditions of continuous aeration the complicating factor of a possible mitotic inhibition due to anoxia was removed. The groth curves obtained with the roots treated in an 0.004% vincristine solution for 1 and 2 hours respectively, but with nitrogen being passed through the water, were found to be similar to those obtained under aerated conditions (Figures 3.40and 3.41). The oxygen content of the vincristine solution was measured during treatment with the YSI Oxygen Meter (Model 51A) and found to be a steady 2.2ml/litre when nitrogen was used and 6.8 ml/litre for the aerated water. The roots were always transferred from the culture tank into the vincristine solution after air (or nitrogen for the experiment mentioned above), had been bubbled through the container for 15 minutes. The temperature of the water in the container was found to be equal to that of the water in the culture tank at this stage.

Care was taken that the mucilaginous substance covering the root tips was removed from each root before treatment. This was achieved by gently wiping the root tips with filter paper. The removal of this substance did not cause any change in the growth rate of the untreated roots (Figure 3.39). When this substance was not removed, however, widely differing (and inconsistent) results were obtained for the same vincristine treatment times (Figure 3.38).

The seedlings used as controls (10 for each

experiment) were handled in the same manner and at the same time as the vincristine treated roots, but were suspended with the roots in a similar vessel containing distilled water. The mucilaginous layer was also removed from the root tips of the controls. It was considered possible that this change from tap water to distilled water could give rise to a change in the subsequent growth rate of the roots. Thus twenty roots were kept in distilled water (as described above) for 2 hrs. and the growth as a fraction of controls compared with a group of twenty seedlings kept in the culture tank (Figure 3.39). No difference in growth was observed between treated and untreated roots.

The curves in Figures 3.38, 3.39, 3.40 and 3.41 represent the means of at least two experiments (For errors, see p. 173).

Two different vincristine treatment times were used in the current experiments, viz. 1 hr. and 2 hrs. After vincristine treatment the roots were rinsed in distilled water and then transferred back to the culture tank or were positioned on the sonication jig.

The roots were sonicated (as described earlier on p. 81) before or after vincristine treatment and as before, ten roots were used for each exposure.

FIGURE 3.37 .

VINCRISTINE TREATMENT. THE PERSPEX CONTAINER WITH SEEDLINGS AND VINCRISTINE SOLUTION IS SUSPENDED IN THE WATER OF THE CULTURE TANK.

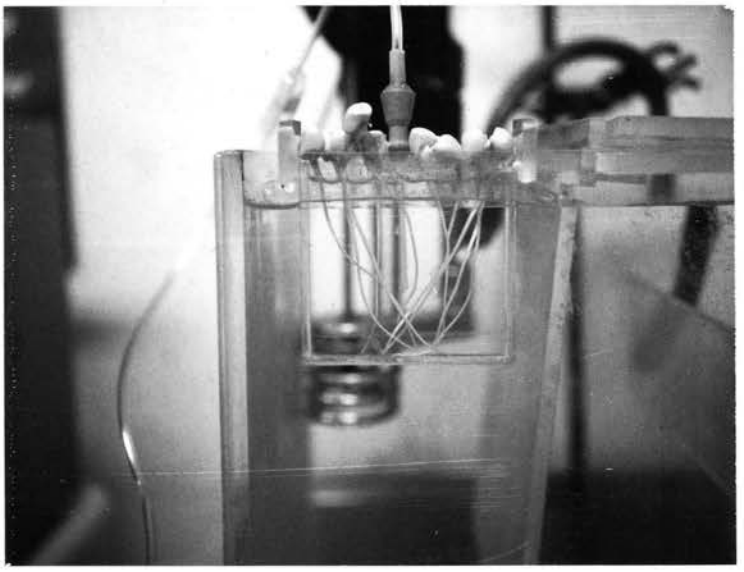


FIGURE 3.38 .

VARIATION OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) WITH TIME AFTER TREATING THE ROOTS IN A 0.004% SOLUTION OF VINCRISTINE FOR 2 HOURS IN VARIOUS WAYS. A SMALL AIR-PUMP WAS USED TO BUBBLE A STEADY STREAM OF AIR THROUGH THE VINCRISTINE SOLUTION. FOR A,B AND C BELOW THE MUCILAGINOUS LAYER WAS REMOVED FROM THE ROOT TIPS.

A — 10 ROOTS TREATED IN A FRESHLY PREPARED SOLUTION OF VINCRISTINE.

B — 10 ROOTS TREATED IN THE SOLUTION THAT WAS USED TO TREAT THE ROOTS IN A.

C — 30 ROOTS TREATED IN A FRESHLY PREPARED SOLUTION OF VINCRISTINE.

D — 10 ROOTS TREATED IN A FRESHLY PREPARED SOLUTION OF VINCRISTINE. THE MUCILAGINOUS SUBSTANCE WAS NOT REMOVED.

E — AS FOR D (REPEAT EXPERIMENT).

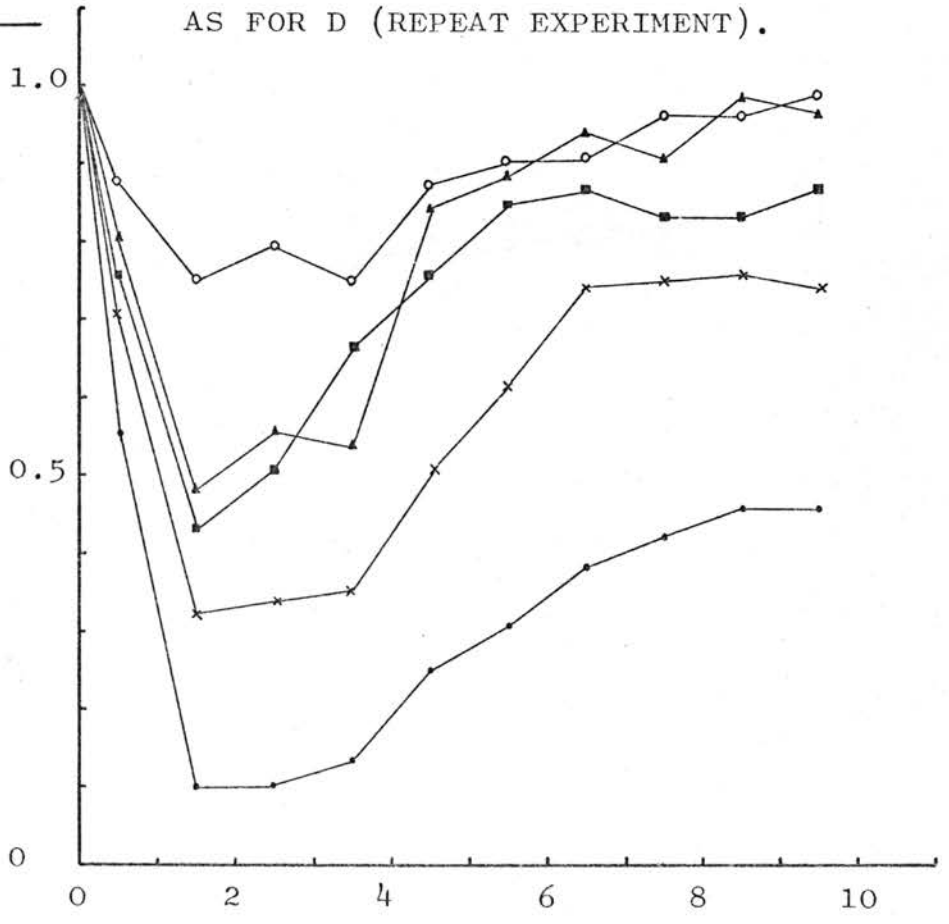


FIGURE 3.39.

GROWTH CURVES INDICATING THAT THERE IS NO ADVERSE EFFECT ON THE ROOTS WHEN TRANSFERRED FROM THE TAP WATER IN THE CULTURE TANK TO DISTILLED WATER OR A LACTOSE SOLUTION. FOR A AND B THE MUCILAGINOUS LAYER WAS REMOVED.

A —●— ROOTS TRANSFERRED TO DISTILLED WATER FOR 2 HRS.

B —×— ROOTS TRANSFERRED TO A LACTOSE SOLUTION (10mg/25 ml) FOR 2 HRS.

C —○— ROOTS TRANSFERRED TO DISTILLED WATER FOR 2 HRS. (MUCILAGINOUS LAYER NOT REMOVED).

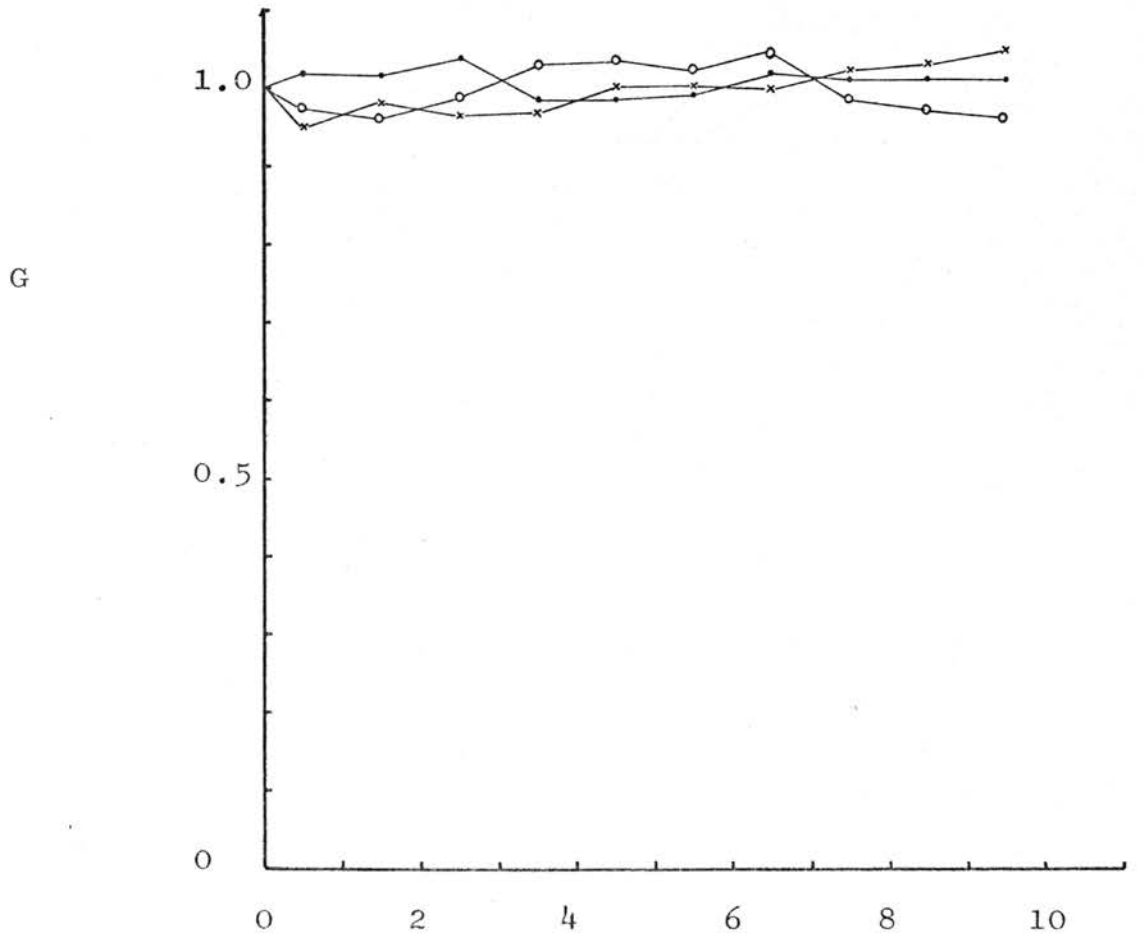


FIGURE 3.40 .

EFFECT OF TREATING THE ROOTS FOR 1 HOUR IN AN 0.004% VINCRISTINE SOLUTION WITH EITHER AIR OR NITROGEN BEING PASSED THROUGH THE WATER IN THE TREATMENT VESSEL. THE MUCILAGINOUS LAYER WAS REMOVED.

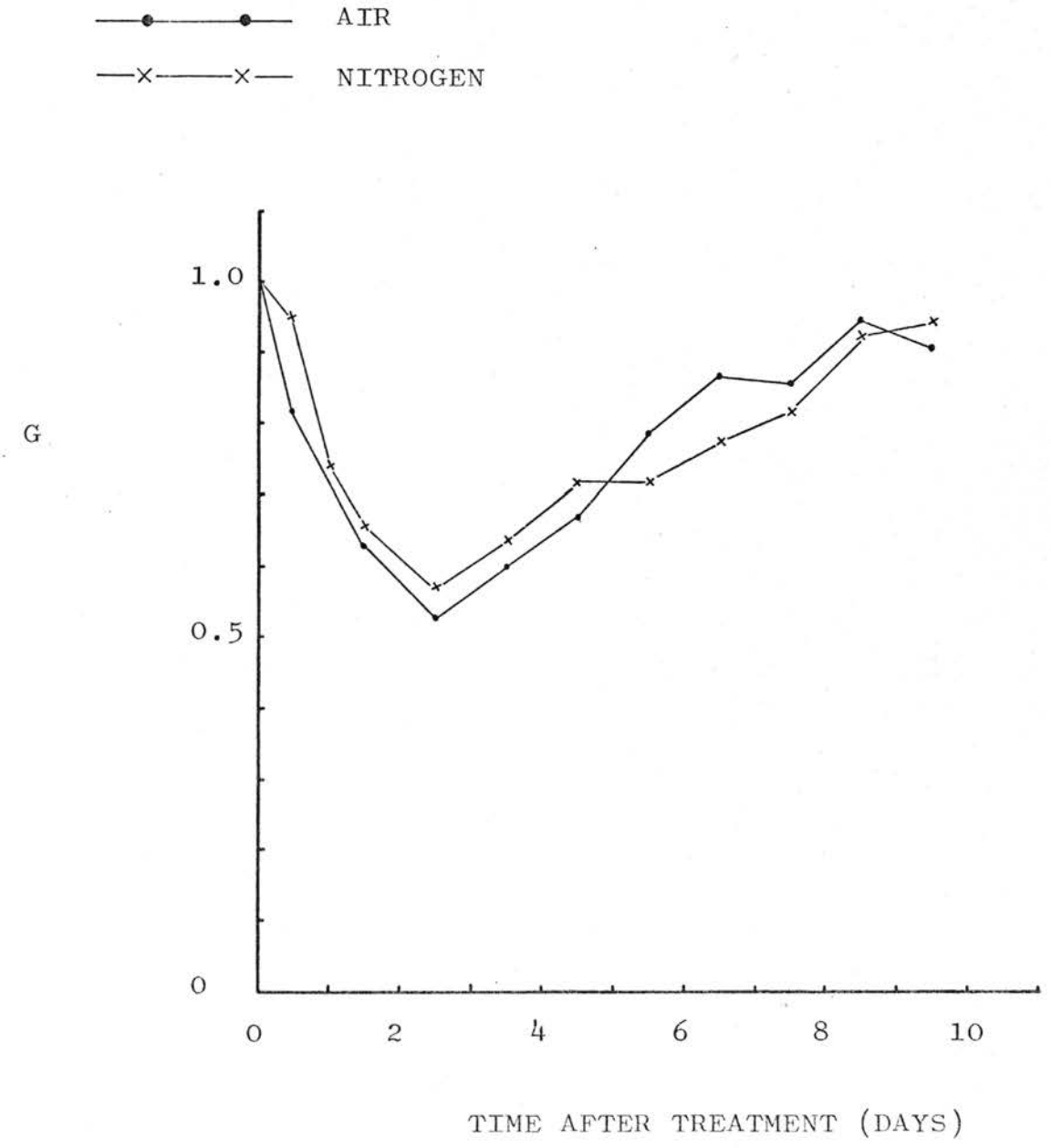
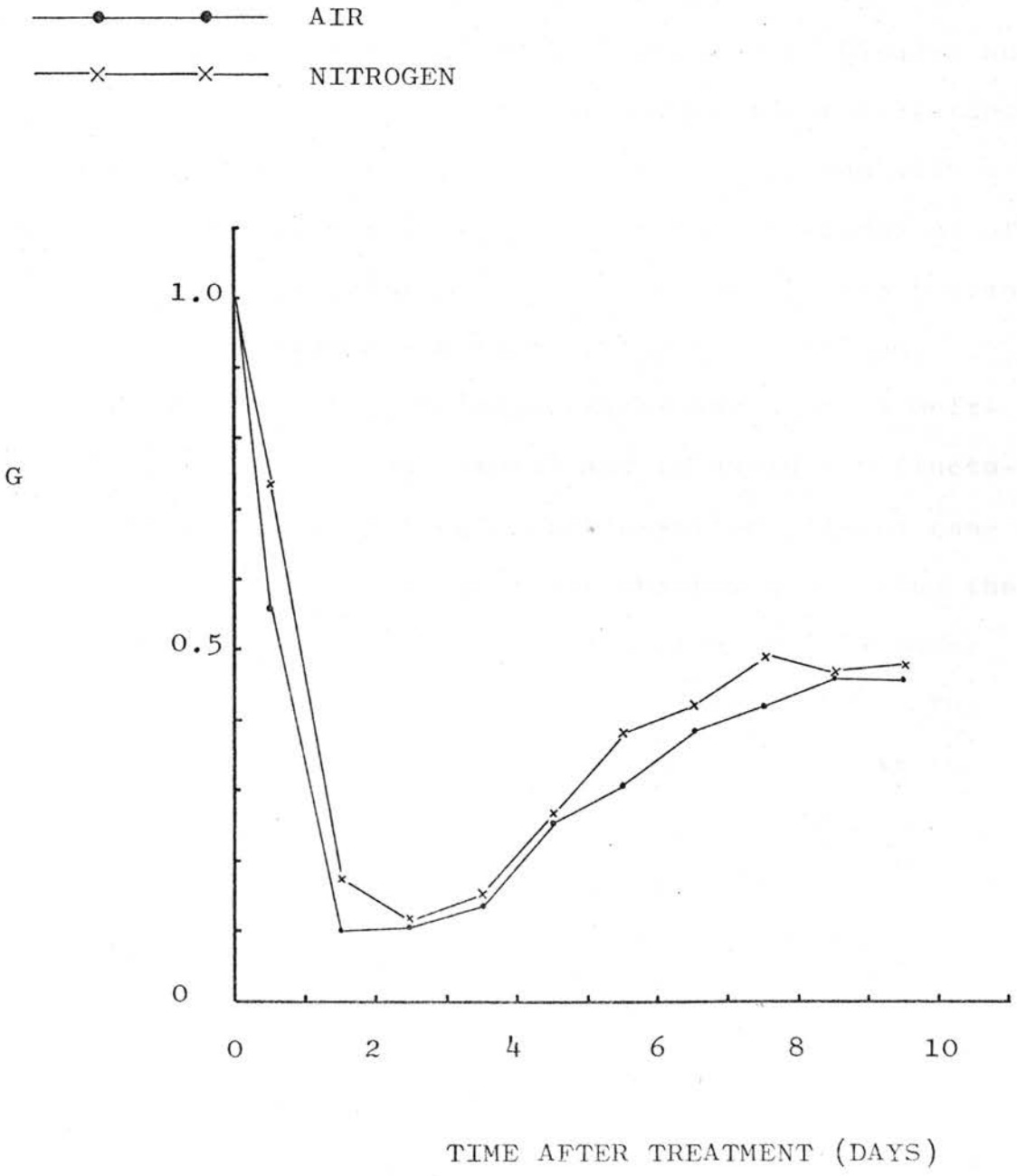


FIGURE 3.41 .

EFFECT OF TREATING THE ROOTS FOR 2 HOURS IN AN 0.004% VINCRISTINE SOLUTION WITH EITHER AIR OR NITROGEN BEING PASSED THROUGH THE WATER IN THE TREATMENT VESSEL. THE MUCILAGINOUS LAYER WAS REMOVED.



MEASUREMENT OF THE TEMPERATURE RISE INSIDE A ROOT DURING SONICATION OF THE LATTER.

For the measurement of the temperature rise inside a root of Zea an iron-constantan thermocouple probe was constructed. The thinnest iron and constantan wires obtainable in this country had a diameter of about 200 µm and a junction as small as 100 µm (as used by Bleaney and Oliver, 1972 a) could thus not be made. After soldering the two wires together, the junction was covered with a thin layer of lacquer to avoid any possible corrosion of the metals at the junction. The diameter of this thermojunction was measured and found to be about 600 µm.

One of the two thermojunctions was kept in melting ice (reference temperature) and to avoid any fluctuations in this temperature a double-walled Perspex container was constructed, the inner chamber containing the thermojunction surrounded by melting ice and the outer chamber also containing melting ice. (Fig. 3.42). The water formed was drained away through an outlet at the bottom of the container. The other thermojunction was inserted in a single root. The root with the inserted thermojunction was then placed in the ultrasonic beam, 7 cm from the crystal face of the transducer which was mounted on the sonication jig.

The output of this thermocouple was measured using a Tektronix storage oscilloscope (Type 549) with a high-gain differential amplifier, Type 1A7A. It was found

necessary to earth the water in the sonication tank. Table 3.4 shows the variation of the electromotive force with temperature for the iron-constantan thermocouple used in the present experiments.

The temperature of the water in the sonication tank was found to be 19.8°C at the time when the above measurements were made.

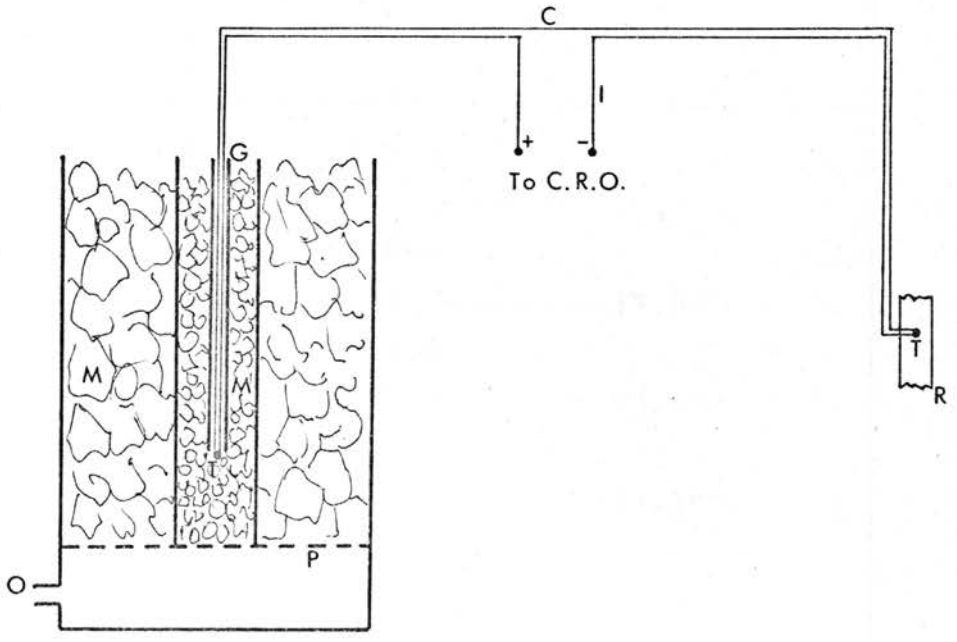
Method of exposure of the roots to various high temperatures.

In this experiment the terminal 0.5 cm portions of the root tips were immersed in water at 30°C, 45°C and 60°C for 15 minutes respectively. A Braun thermostatically controlled heater was used to keep the water at the required temperature (± 0.2°C). Ten roots were were simultaneously treated at each of the abovementioned temperatures.

After treatment, the roots were returned to the culture tank and their daily growth was measured and compared with that of the ten control roots as described previously. The length of the roots at the time of treatment was again in the range 6.0 to 7.5 cm.

FIGURE 3.42 .

THE USE OF THE IRON-CONSTANTAN THERMOCOUPLE TO MEASURE THE TEMPERATURE RISE INSIDE A ROOT OF ZEA DURING SONICATION.



M. MELTING ICE

G. THIN GLASS TUBE

T. THERMOJUNCTION

O. OUTLET

R. ROOT OF ZEA

I. IRON WIRE

C. CONSTANTAN WIRE

P. PERFORATED BOTTOM

TABLE 3.4.

ELECTROMOTIVE FORCE FOR THE IRON-CONSTANTAN THERMOCOUPLE USED IN THE PRESENT EXPERIMENTS.

mV	^{o}F	$^{o}F/mV$
0.5	49.43	
		34.108
1.0	66.49	
		33.963
1.5	83.47	
		33.632
2.0	100.28	
		33.360
2.5	116.96	
		33.338
3.0	113.63	

From R.A. Louw, 1971. "Velocity and temperature distributions for mercury in turbulent flow." M.Sc. Thesis (University of Cape Town).

DETERMINATION OF THE CELL CYCLE TIME OF THE MERISTEMATIC CELLS IN THE ROOT TIP OF ZEA MAYS USING A METAPHASE ACCUMULATION TECHNIQUE.

The method employed was that used by Evans, Neary and Tonkinson (1957) for Vicia faba.

Maize seed were germinated and roots grown as described previously (p. 72). After 2-3 days in moist Vermiculite the seedlings, now having a primary root of length 2-3 cm, were transferred to the culture tank and grown for 2 days at 19°C ± 0.2°C. Cytological studies commenced at this stage.

Squash preparations were made using the Feulgen squash method (Heitz, 1936; Darlington and La Cour, 1938; Hillary, 1940). The root tips were fixed in acetic - alcohol (1:3) for 10 minutes at room temperature, rinsed in distilled water and macerated by hydrolysis in 1N HCl at 60°C for 6 mins. After rinsing, the root tips were stained in leuco-basic fuchsin for 2-3 hrs. For comparative mitotic counts between roots it was thought advisable to use root tips of equal length, and this was achieved by first excising the root cap of each root, and then cutting off the terminal 0.6 millimetre of the root under a dissection microscope. A Neubauer ruling was used to provide a measure of length. Each 0.6 mm root tip was then used to make one slide, the tip being squashed in a drop of 45% acetic acid. Scoring was aided by the use of a Polaroid photomicrographic attach-

ment to a Nikon microscope (Model L-Ke). Pictures were taken of meristematic cells along randomly selected traverses (Fig.3.43). In each slide two samples of 500 cells were scored so that 1000 cells were used from each root tip. The criteria used to distinguish between the mitotic stages of these cells were those adopted by Gray and Scholes (1951) for Vicia faba as shown in Fig.3.44 . In the colchicine treated roots no distinction was made between the various types of metaphase configurations (Barber and Callan, 1913; Eigsti and Dustin, 1955), or between the early restitution nuclei and the true anaphases and telophases which are found in the shortest treatments. For convenience the anaphases, telophases and early restitution nuclei were all classified as ana-telophases (Guttman, 1952).

Colchicine treatment:

The colchicine treatments were carried out by suspending the seedling roots in a Perspex container (10 cm x 0.5 cm and 5 cm deep) containing 20 ml of an 0.05% colchicine solution (p. 147 Fig.3.37). For each treatment-time ten seedlings were used, and these were treated simultaneously in a solution of colchicine which was discarded after being used once. The latter was kept at $19^{o}C \pm 0.2^{o}C$ by suspending the Perspex container in the water of the culture tank. In all treatments the solutions were continuously aerated. Under these conditions of continuous aeration the complicating factor of mitotic

inhibition due to anoxia was removed. Care was taken that the mucilaginous substance covering the root tips was removed from each root before treatment (see p.145). The seedlings used as controls were handled in the same manner and at the same times as the colchicine treated roots, but were suspended with the roots in a similar vessel containing distilled water.

Fixations were made after 0,1,2,3,4,5,6,12 and 24 hr. treatments. Five slides were made for each treatment time and the first 4 slides were scored. The remaining five roots were discarded. The mean values obtained from each group of four slides are given in Table 3.5 .

In the control experiments the seedlings were transferred from the tap water in the culture tank into distilled water. It was considered possible that this change could give rise to a change in the M.I. (Mitotic Index being defined as the number of cells in mitosis expressed as a percentage of the total number of cells scored), but an analysis of variance on the data did not reveal any significant change either in M.I. ($P>0.1$ for 6 and 27 d.f.) or in metaphase counts ($P>0.1$). Similarly, it was found that the fixation made at 0 hr. in the colchicine series did not differ from the fixations made in the control series, so that the total results were pooled. The pooled data gave a mean value of 18.4 ± 0.7 metaphase cells per 10^3 cells scored (Table 3.5), and this mean was taken as the metaphase score at time 0. For the analysis of variance, a program was written to perform the calculation

on the Wang 370 Desk Calculator.

The effect of the duration of colchicine treatment on metaphase accumulation.

The number of metaphases accumulated was found to be regular over the period from 1 to 6 hrs. (Fig.3.45). This suggests that colchicine treatment does not inhibit the entry of cells into mitosis during this period. Metaphase accumulation was found to become irregular after the 6 hr. treatment, the rate of accumulation from 6 to 12 hrs. being very much lower than that found in the first 6 hrs. of treatment. After 24 hrs. of treatment the number of metaphases decreased considerably. These results are similar to those obtained by Evans, Neary and Tonkinson for Vicia faba (Fig. 1.7). These authors also found that the rate of accumulation of metaphases from 1 to 6 hrs. was the same for different concentrations of colchicine. In view of the different efficiencies of various cencentrations of colchicine for disrupting already formed spindles (Inoué, 1952), the rate of accumulation of metaphases over the first hour of treatment was neglected.

The presence of a few ana-telophase cells in roots treated from 2-6 hrs. suggests that the number of metaphases which are accumulated per hour is not quite the same as the number of metaphases which enter mitosis in 1 hr. Evidently some cells which are held up at the metaphase stage must sometimes pass on into interphase.

If we assume that the time taken for these cells to escape from c - metaphase to interphase is roughly equivalent to the time an untreated cell takes to complete telophase, i.e. about 30 min., then Evans, Neary and Tonkinson (1957) deduce that about 2 ± 1 cells escape from metaphase each hour. Allowing for this small correction, the mean number of cells which pass into metaphase between 1 and 6 hrs. in colchicine-treated roots is 24.6 ± 2.0 per hour.

From these results, the value for the cell cycle time of 31.1 ± 1.9 hrs. was calculated as indicated in Appendix A.

FIGURE 3.43 .

SQUASHES PREPARED FROM THE ROOT TIPS OF ZEA MAYS

SHOWING CELLS IN VARIOUS PHASES OF DIVISION (x450).

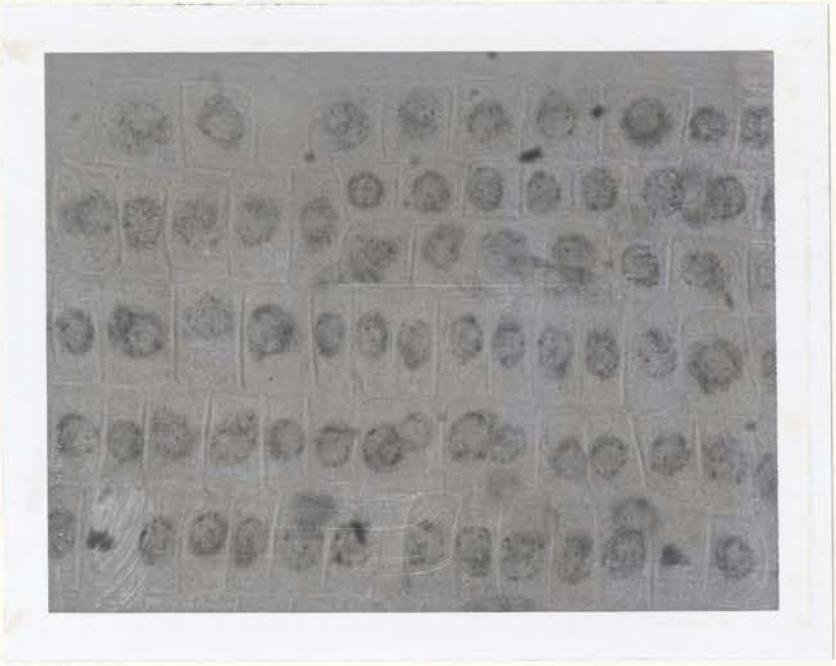


FIGURE 3.44.

DRAWINGS TO ILLUSTRATE THE DIFFERENT PHASES OF DIVISION OF A VICIA FABA MERISTEMATIC CELL BASED ON THOSE OF GRAY AND SCHOLES (1951).

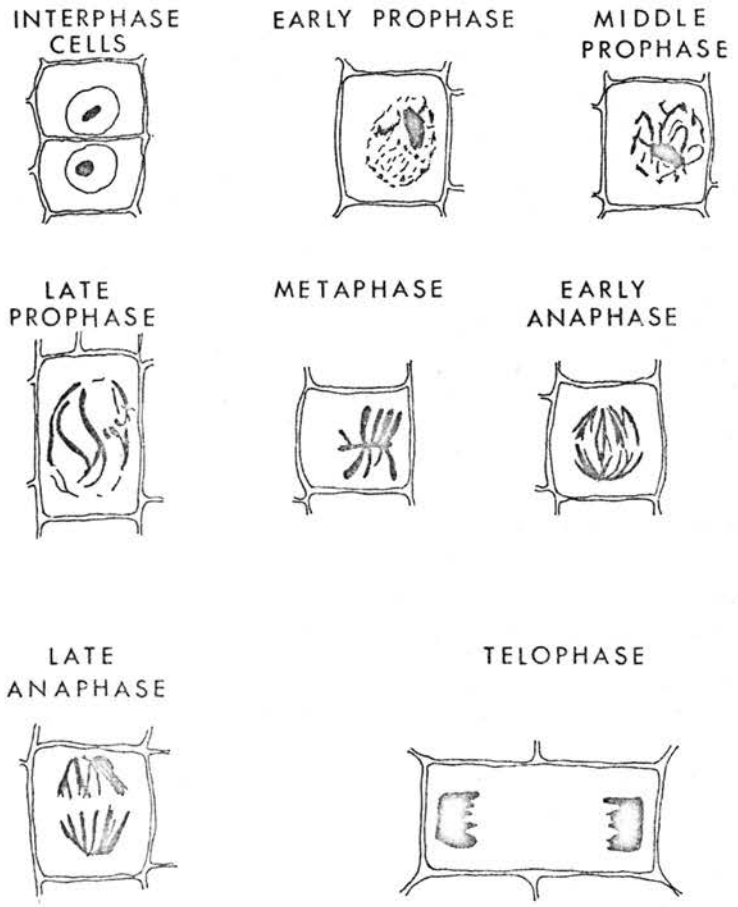
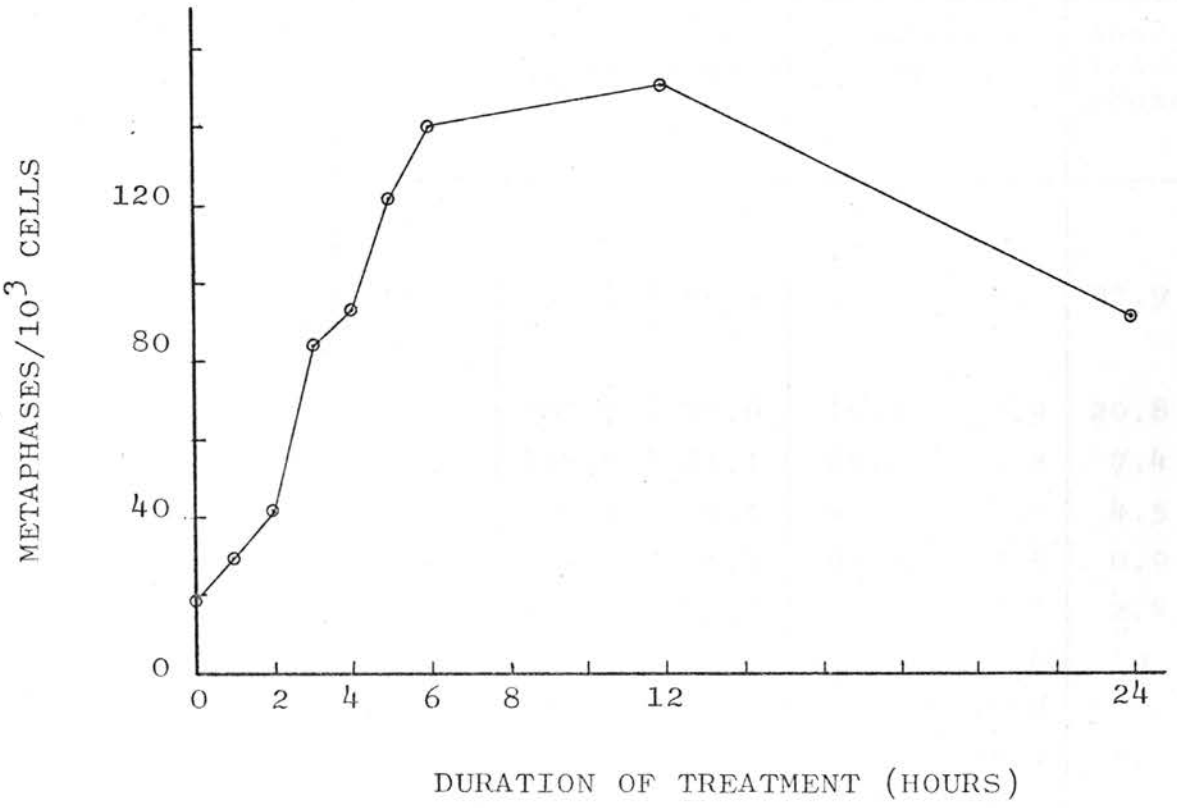


FIGURE 3.45.

METAPHASE SCORES FOR THE COLCHICINE SERIES, FOR TREATMENTS OF UP TO 24 HRS. EACH POINT IS A MEAN VALUE FOR FOUR SLIDES (4000 CELLS). FOR STANDARD ERRORS SEE TABLE 3.5 .



The slope of the regression line fitted to the points in the figure above was found to be 22.6 $\pm$ 1.7. The error is the standard error in the value of the slope (Topping, p. 106). The correlation coefficient was found to be 0.9888. The metaphases/10^3 cells for the treatments of 0,12 and 24 hrs. were not included in the calculations (see text).

TABLE 3.5.

EFFECT OF COLCHICINE ON THE MITOTIC INDEX.

Treatment	Duration of treatment(hrs.)	No. of slides scored	Mean No. of cells/1000 scored				M.I.
			Interphase	Prophase	Metaphase	Ana-Telophase	
Total control + 0 hr.	-	40	892.4	56.3	18.4 ± 0.7	32.9	10.8
Colchicine 0.05%	0	4	905.7	49.0	16.5 ± 1.9	20.8	9.4
	1	4	898.0	65.1	29.6 ± 3.2	7.4	10.2
	2	4	898.1	55.5	42.0 ± 4.0	4.5	10.2
	3	4	865.2	50.4	83.6 ± 7.5	0.9	13.5
	4	4	854.2	50.9	92.5 ± 10.1	2.4	14.6
	5	4	816.6	62.6	120.7 ± 11.6	0	18.3
	6	4	806.2	54.7	139.1 ± 12.2	0	19.4
	12	4	793.6	56.1	149.0 ± 11.1	1.3	20.6
	24	4	871.3	38.6	90.2 ± 8.6	0	12.9

The error in the number of cells in metaphase is the standard error of the mean.

SYNCHRONIZATION OF THE MERISTEMATIC CELLS IN THE ROOT TIP OF ZEA AND SONICATION OF THE CELLS IN DIFFERENT PHASES OF THE MITOTIC CYCLE.

Hydroxyurea was used to synchronize the cells since 5-Amino-Uracil was not available at the time when these experiments were carried out. The minimum time necessary to leave the roots in the hydroxyurea solution is equal to the period $G_2 + M + G_1$ (Hall, personal communication), but to be on the safe side a time period equal to 36 hours was chosen, which is slightly longer than the cell cycle time of 31.1 hours determined previously (p. 160).

The method of the treatment of the roots in a 1.25 mM solution of hydroxyurea and the subsequent sonication was similar to that used for the combined vincristine and ultrasonic treatments (p. 144). The concentration of the solution of hydroxyurea was the same as that used by Hall, Brown and Cavanagh (1968) for the root meristem of Vicia faba.

Assessment of the degree of synchrony.

At various times after removal from the hydroxyurea the terminal 1 cm of each of 4 roots was cut off, fixed in acetic alcohol, and squash preparations were made and the slides scored for the proportion of cells in mitosis (mitotic index) as described elsewhere (p. 156).

CHAPTER IV.

RESULTS.

cont.

PAGE

THE EFFECTS OF PULSED AND CONTINUOUS BEAMS OF ULTRASOUND ON THE ROOTS OF ZEA.

Figures 4.1 , 4.2 , and 4.3 show the variation of the fractional growth rate (G) with time after exposure to continuous beams of ultrasound at average intensities of 0.82, 0.62 and 0.38 W/cm^2 respectively and for sonication times ranging from 1 to 150 minutes. The curves for the pulsed beams at average intensities of 0.21 and 0.16 W/cm^2 and for sonication times ranging from 2 to 180 minutes, are shown in Figures 4.4 , 4.5 , 4.6 and 4.7 .

For the sonications at the average intensities of 0.38 W/cm^2 (cont. beam) for 15 min. (Fig. 4.3) and 0.16 W/cm^2 (pulsed beam) for 2 and 10 min. respectively (Fig. 4.6), no effect of irradiation is apparent in the growth curves. A similar result was also obtained with the lowest possible average intensity (7cm from transducer) that the Impulsaphon is capable of producing, viz. 0.02 W/cm^2 (pulsed beam), when the roots were sonicated for 180 min. (Fig. 4.8). For all the other sonication times and average intensities used, the growth curves are found to reach a minimum immediately after sonication as shown in Figures 4.1-4.7. The curves in Figures 4.1-4.7 indicate that this minimum is reached during the first day post-sonication. Measurement of root length at 6hrs. and 12hrs. after sonication respectively, indicated that the minimum in growth rate was already reached in the

first 6 hours after treatment. Errors in measurement as well as variations between repeat experiments, were found to be very much more significant in the growth increment over 6hrs. (or 12hrs.) than over 1 day, and it was thus thought that the growth increment over the first day would provide the best estimate of the minimum in the growth rate (Bleaney and Oliver, 1972a).

The initial decrease in growth was followed by a recovery, which was found to be more rapid for the short sonication times and lower average intensities (Figures 4.1-4.7). Also, there was a complete recovery of all the roots in these situations and the growth curves levelled off at about unity ten days post-sonication. For the longer sonication times at the average intensities of 0.82 and 0.62 W/cm^2 (cont.beams) respectively, the recovery was not complete and the growth curves levelled off at values of G less than unity by about the tenth day after sonication. These values decreased with increasing exposure time for a given average intensity and with increasing average intensity for a given exposure time as shown in Figure 4.9. In this figure, the average growth in the tenth day post-irradiation as a fraction of the corresponding growth for the control roots, was taken as an approximation of the value at which the growth curves level off.

The shapes of the growth curves are found to be similar for all irradiation times and intensities, except for the very long sonication times ($\geqslant$ 60 min.) at the

average intensity of 0.82 W/cm^2, where there was hardly any recovery of the roots after sonication (Fig. 4.1). An appreciable proportion of the roots in these groups died and the remainder were growing slowly after receiving the dose of ultrasound.

The number of roots in a sonicated group that died or were growing more slowly than the corresponding control roots 10 days after sonication, are listed in Table 4.1 for the continuous beams. For the pulsed beams used in these experiments, none of the sonicated roots were either dead or growing slowly ten days after irradiation. For all the intensities and sonication times for which the growth curves levelled off at values less than unity, some of the roots in the group had died and/or some were growing slowly (Table 4.1). In this table the figures in the column entitled '% roots with reduced growth' were obtained from the number of roots with a length less than half of the average length of the roots in the corresponding control group ten days after sonication. The growth rate of some of the roots of length less than half of that of the controls, was equal to the average growth rate of the latter at this time. These roots were not included in the figures given in Table 4.1 . A root was considered dead when its total growth in ten days after sonication was less than 1 cm. Measurement of root length up to 18 days after sonication indicated that the irradiation with ultrasound at the intensity levels used in the present

experiments, did not cause any "delayed death" of the roots (see p. 41). On the other hand there was also no "delayed recovery" of any of the roots after ten days post-sonication.

The mean diameter of the roots that recovered completely from sonication damage was found to be equal to that of the control roots ten days after irradiation. For the sonication times and average intensities at which the growth rate of the roots did not return to normal, the mean diameter of the roots ten days post-sonication was found to be slightly less than that of the control roots (Table 4.2). For the measurements of the diameter of the sonicated roots, no distinction was made between roots that had died or were growing slowly and roots that had recovered. It was sometimes impossible to measure the diameter of the dead roots since these had started to necrose by ten days after sonication.

The fractional growth in ten days (G_{10}) for the various sonication times and intensities are listed in Table 4.3 for the pulsed as well as the continuous beams. Although G_{10} is a rather doubtful quantity when an appreciable proportion of the roots in a group die and/or grow slowly as a result of sonication, the values of G_{10} have been calculated for these groups as well. The value of this parameter decreased with increasing exposure time for a given intensity and with increasing intensity for a given exposure time. In Figures 4.10

and 4.11 the average values of G_{10} from Table 4.3 for the pulsed and continuous beams, are plotted against total exposure (W/cm^2 multiplied by hours) indicating that for a given exposure, higher intensity always produced a greater reduction in root growth.

Figures 4.12 and 4.13 show the variation observed in the value of G_1, the average growth in the first day post-irradiation as a fraction of the corresponding average growth for the control roots, with the duration of exposure at the average intensities of the continuous and pulsed beams used in the present experiments.

For sonication times less than or equal to 30 min., the values of G_{10} in Table 4.3 and the graphs in Figures 4.1 - 4.8 represent the mean obtained for three experiments. For sonication times longer than 30 min., the values of G_{10} and the graphs in Figures 4.1 - 4.8 represent the mean obtained for two experiments. The three graphs in Figure 4.14 were obtained for the roots of Zea treated with ultrasound at an average intensity of 0.82 W/cm^2 (cont. beam) for 30 min. The curves indicate the consistency in the results obtained when experiments were repeated. The mean of these three curves is also shown in Figure 4.14. The consistency in all the other experiments was of the same order as that depicted. The values of G_{10} in Figure 4.14 give an indication of the variation of this parameter between experiments.

In Figure 4.15 the values of G_1 (for an exposure time of 60 minutes) are plotted against the average

ultrasonic intensities of the pulsed as well as the continuous beams, showing that continuous irradiation is less damaging than exposure to a pulsed ultrasonic beam at the same average power.

The errors in these experiments are expressed as the standard error of the mean for the ratio of the means of the irradiated and control groups. Error bars are not included on all the graphs in order to preserve clarity of presentation, but are always of the same order as those depicted in Figure 4.1 . Errors were calculated for each individual experiment and the standard errors of the means for two or three repeat experiments were calculated according to the method given by Topping (p.63). For all experiments in which the sonication times are less than or equal to 30 minutes, the errors in Fig. 4.1 and Table 4.3 are standard errors of the means for three experiments. For all experiments in which the sonication times are greater than 30 minutes, the errors are the standard errors of the means for two experiments. Programs were written to perform these calculations on the Wang 370 and Hewlett-Packard 9120A Desk Calculators.

In a control experiment two groups of roots (five seedlings in each group) were mounted in turn (as described on p. 81) on the jig in the sonication tank and left in this position for three hours. The variation in the growth rate of the ten treated roots as a fraction of controls (ten seedlings kept in the culture tank) with time after treatment, indicates (Fig. 4.16)

that such handling of the roots does not affect the growth of the latter.

Results of the fractionation experiments using equal divided doses.

To investigate the possibility of recoverable damage, the radiation effects were compared for a single 30-minute exposure (average of 3 experiments) and for two exposures of 15 minutes at the same average intensity of 0.82 W/cm^2 (cont. beam) separated by 1, $2\frac{1}{2}$, 5 and 24 hours respectively. These results are shown in Figure 4.17 and Table 4.4 . The sonication damage was found to increase with an increasing time interval between the doses.

FIGURE 4.1 .

PATTERNS OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONTINUOUS BEAM) FOR VARIOUS TIMES AS INDICATED.

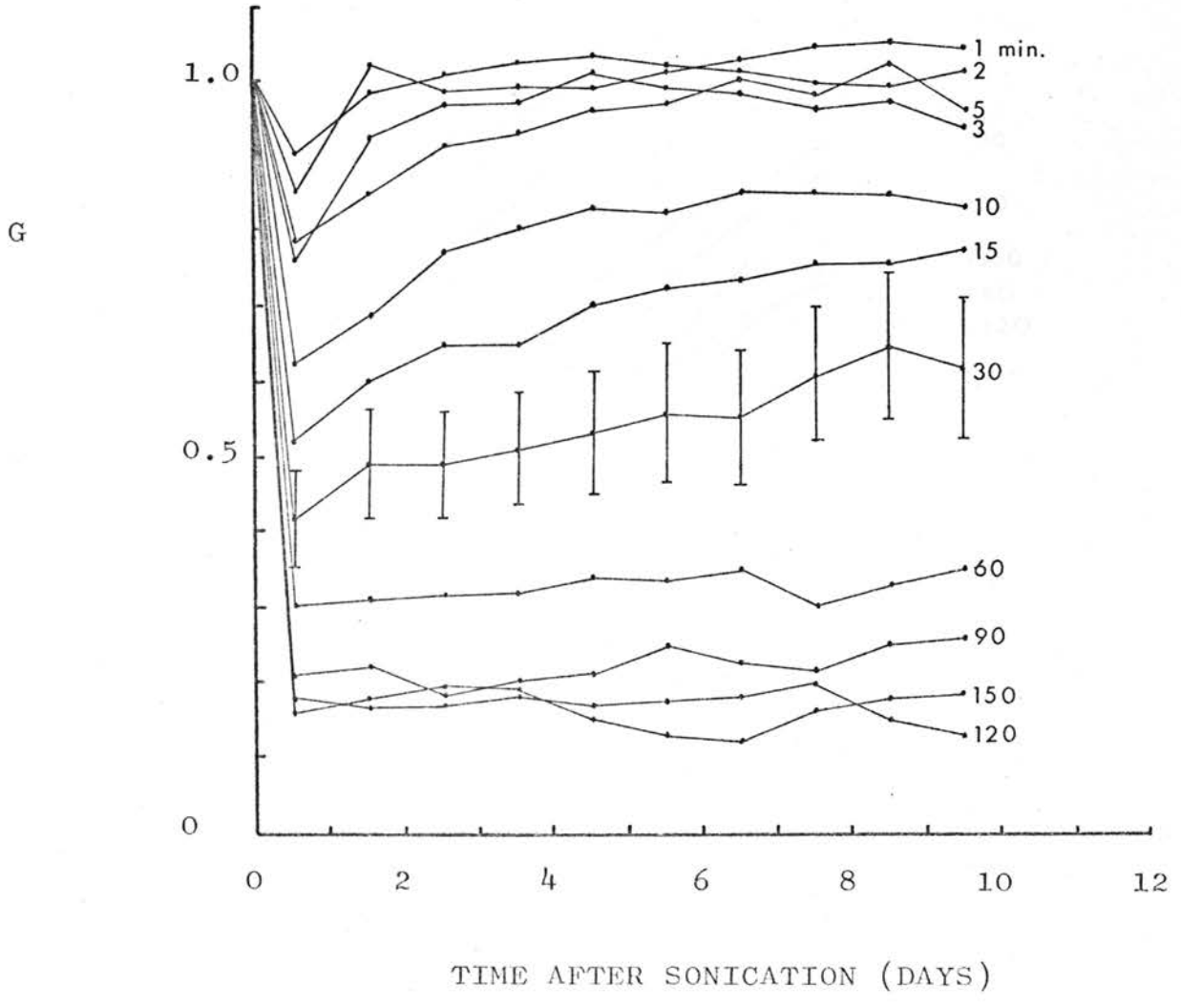


FIGURE 4.2 .

AS FOR FIGURE 4.1, BUT AT AN AVERAGE INTENSITY OF 0.62 W/cm^2 (CONT. BEAM) FOR VARIOUS TIMES AS INDICATED.

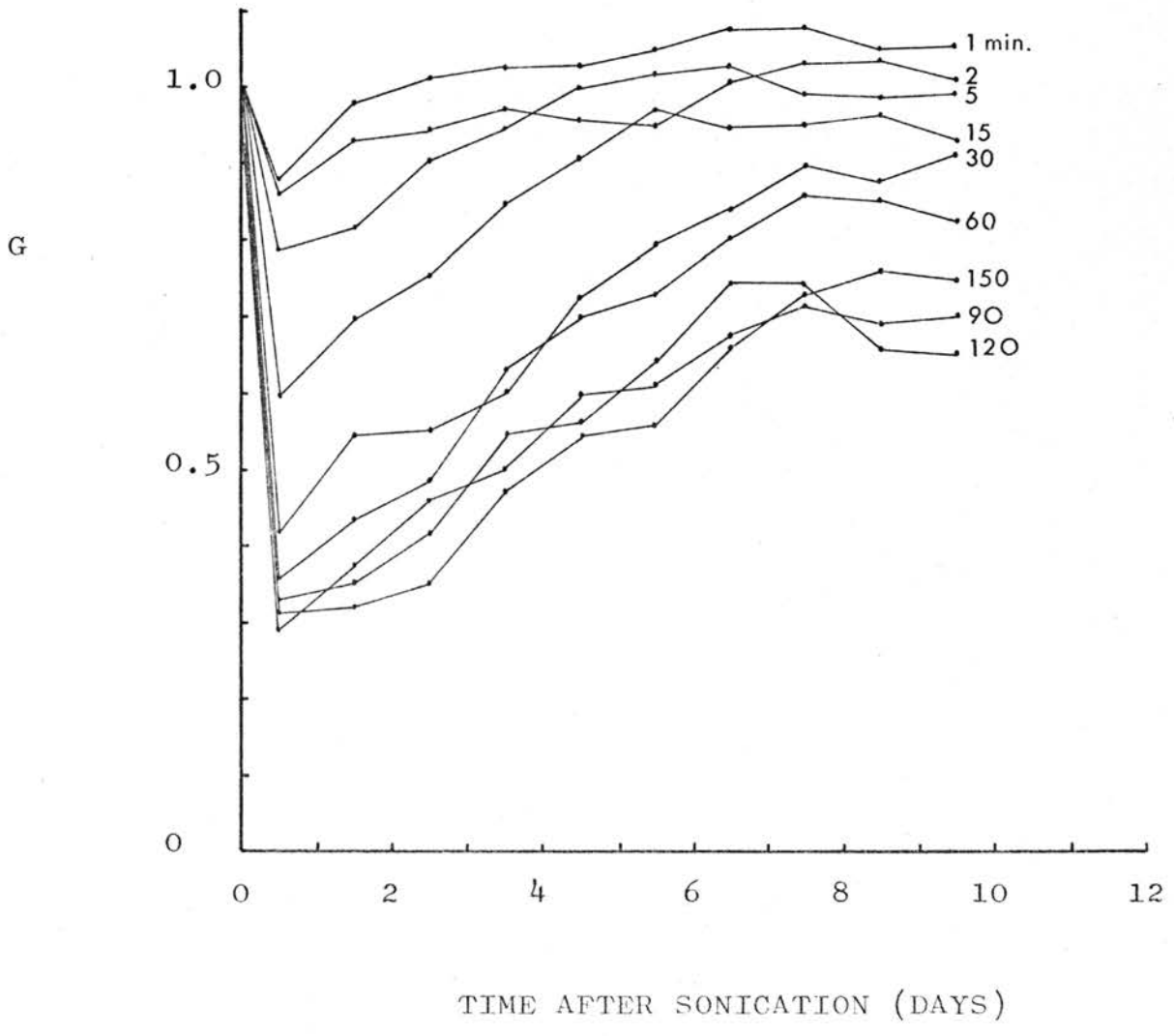


FIGURE 4.3 .

AS FOR FIGURE 4.1, BUT AT AN AVERAGE INTENSITY OF 0.38 W/cm^2 (CONT. BEAM) FOR VARIOUS TIMES AS INDICATED.

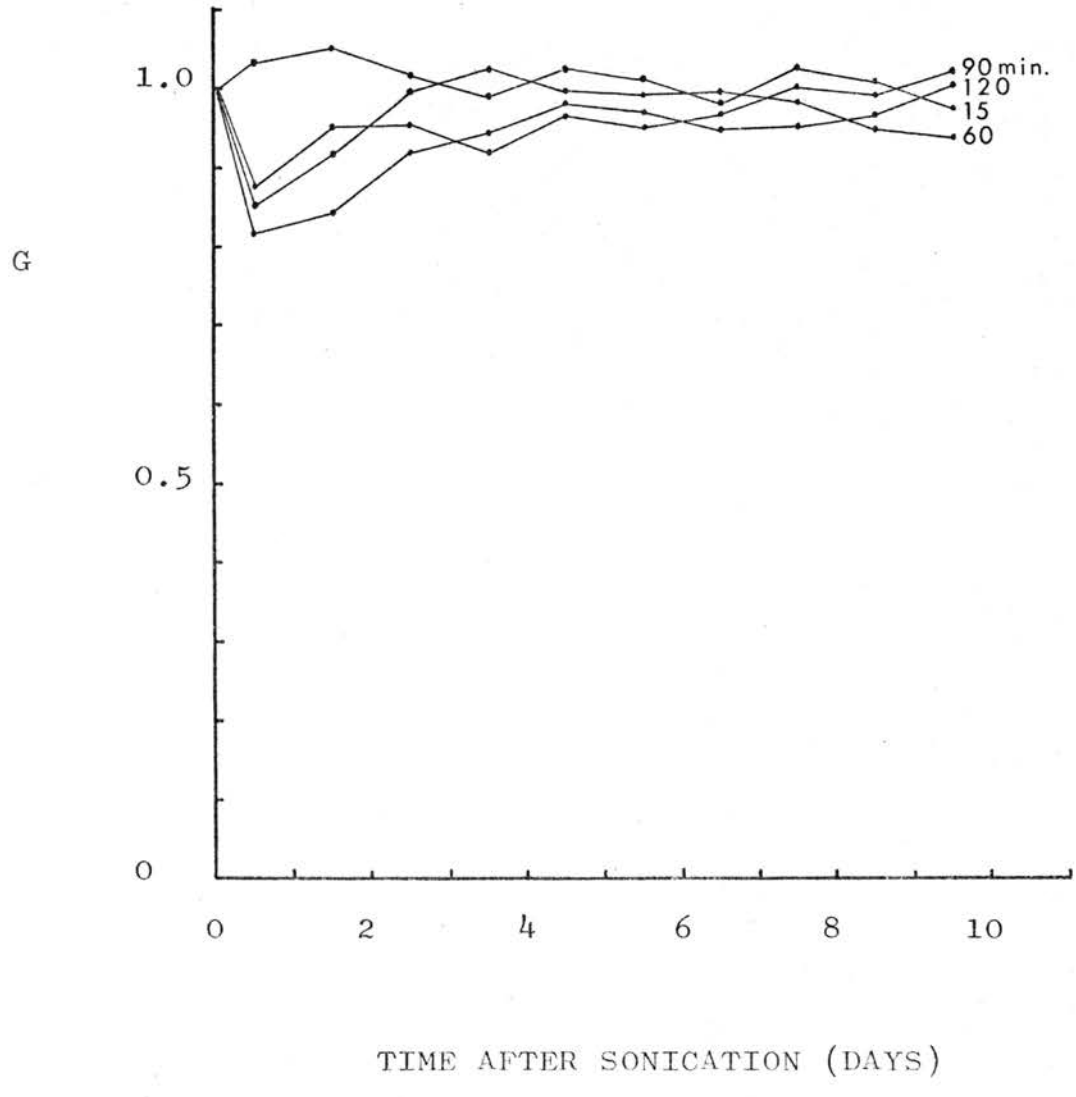


FIGURE 4.4 .

AS FOR FIGURE 4.1, BUT AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR VARIOUS TIMES AS INDICATED.

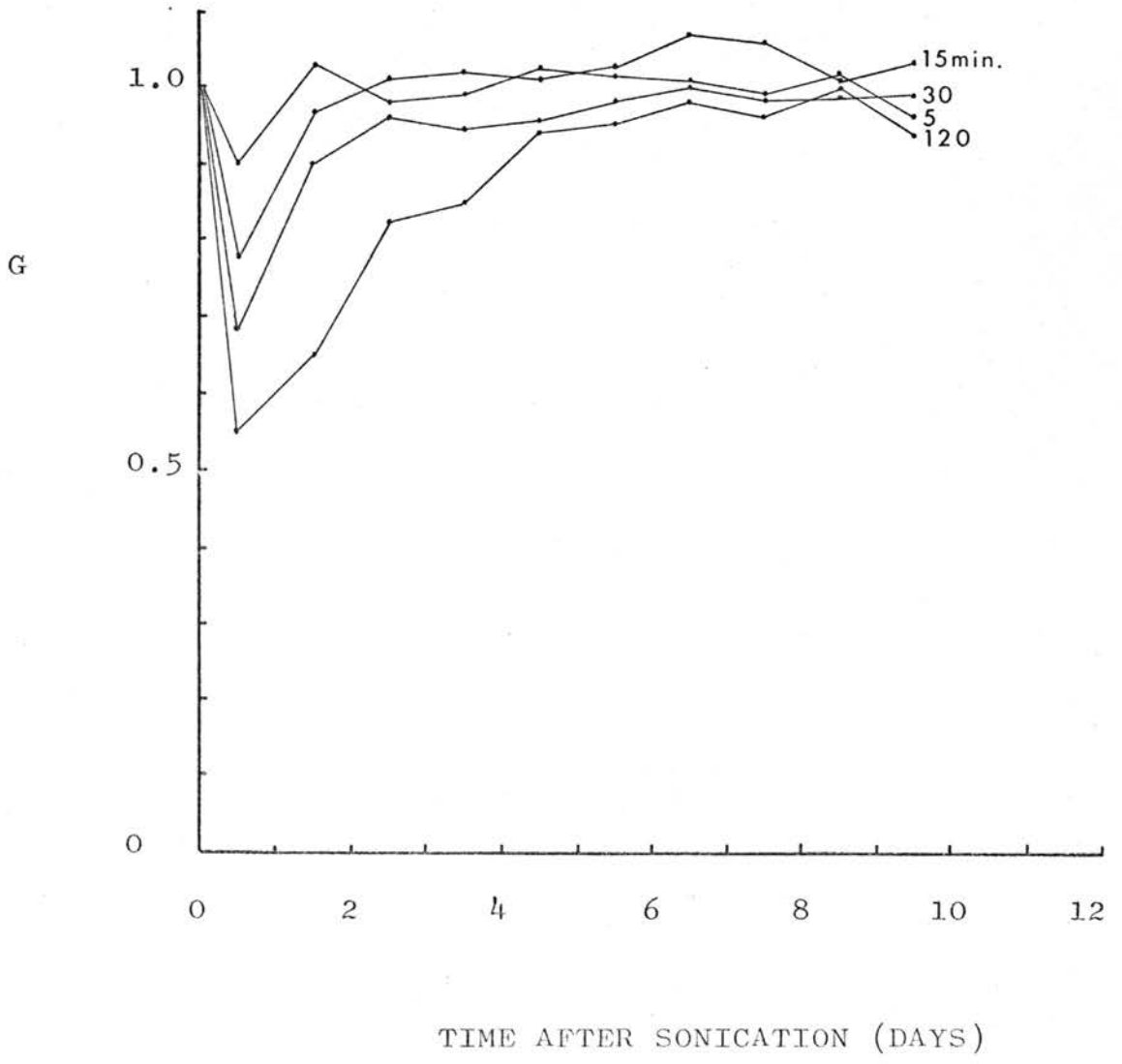


FIGURE 4.5 .

AS FOR FIGURE 4.4 , FOR VARIOUS TIMES AS INDICATED.

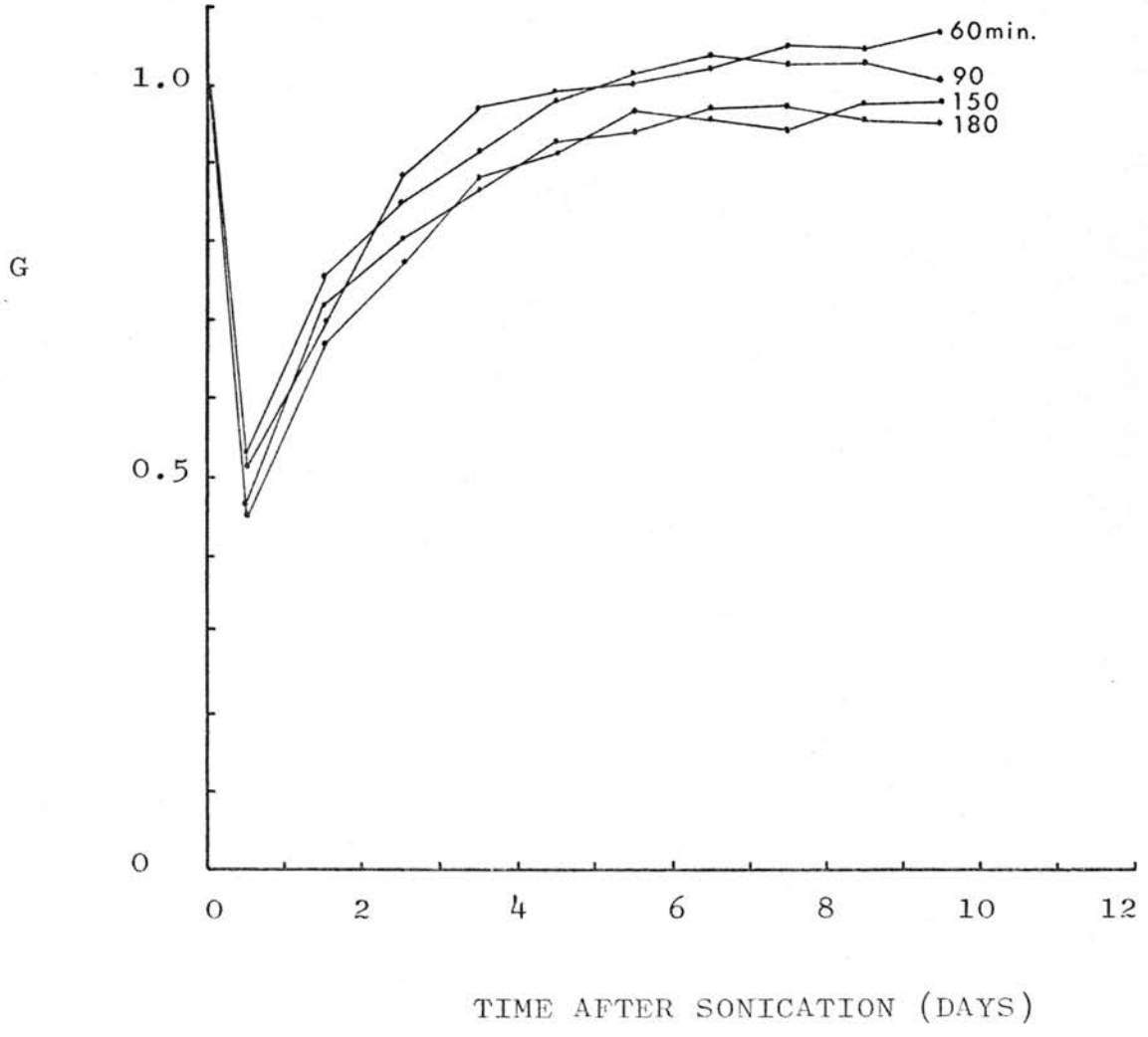


FIGURE 4.6 .

AS FOR FIGURE 4.1 , BUT AT AN AVERAGE INTENSITY OF 0.16 W/cm^2 (PULSED BEAM) FOR VARIOUS TIMES AS INDICATED.

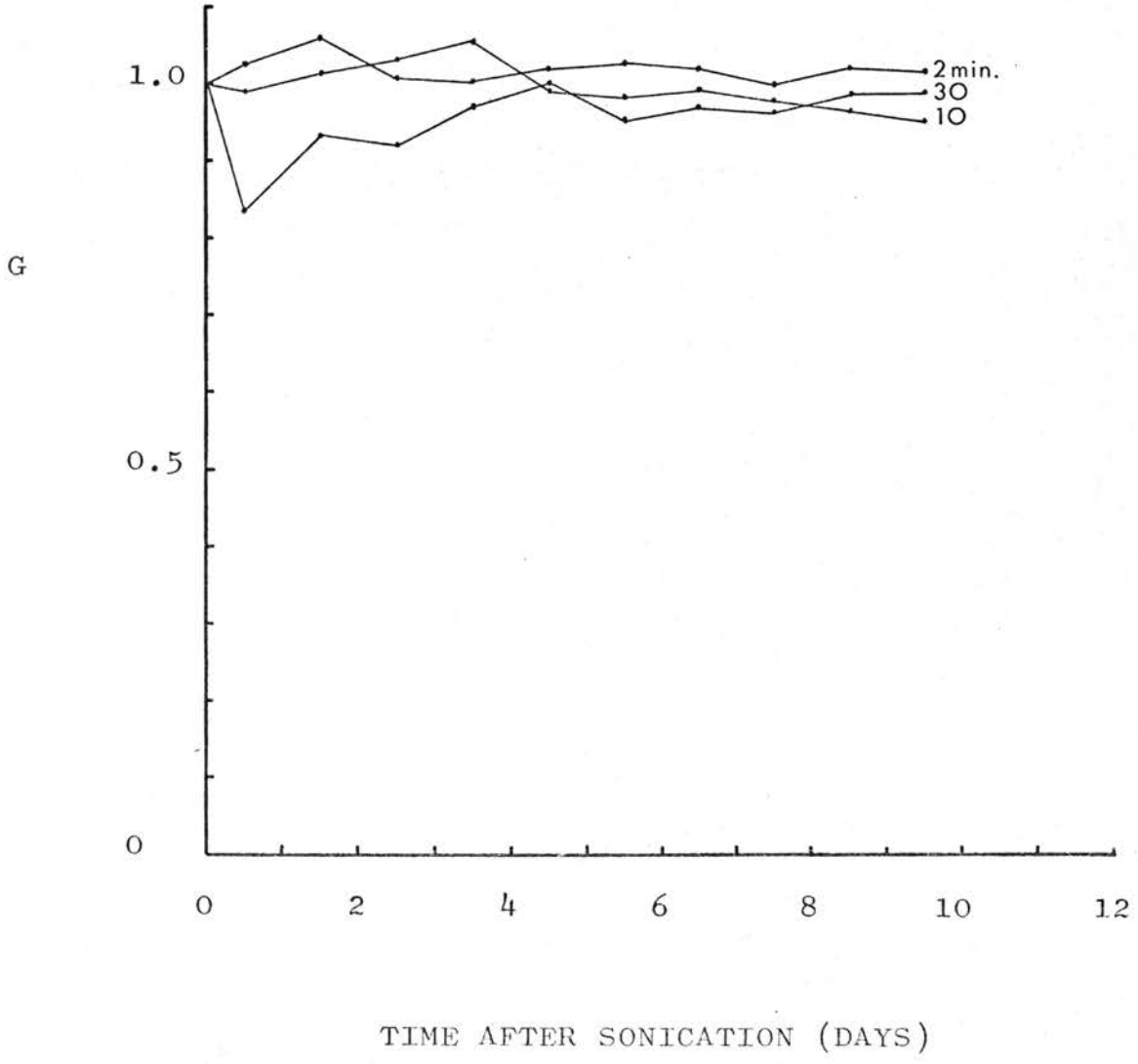


FIGURE 4.7 .

AS FOR FIGURE 4.6, FOR VARIOUS TIMES AS INDICATED.

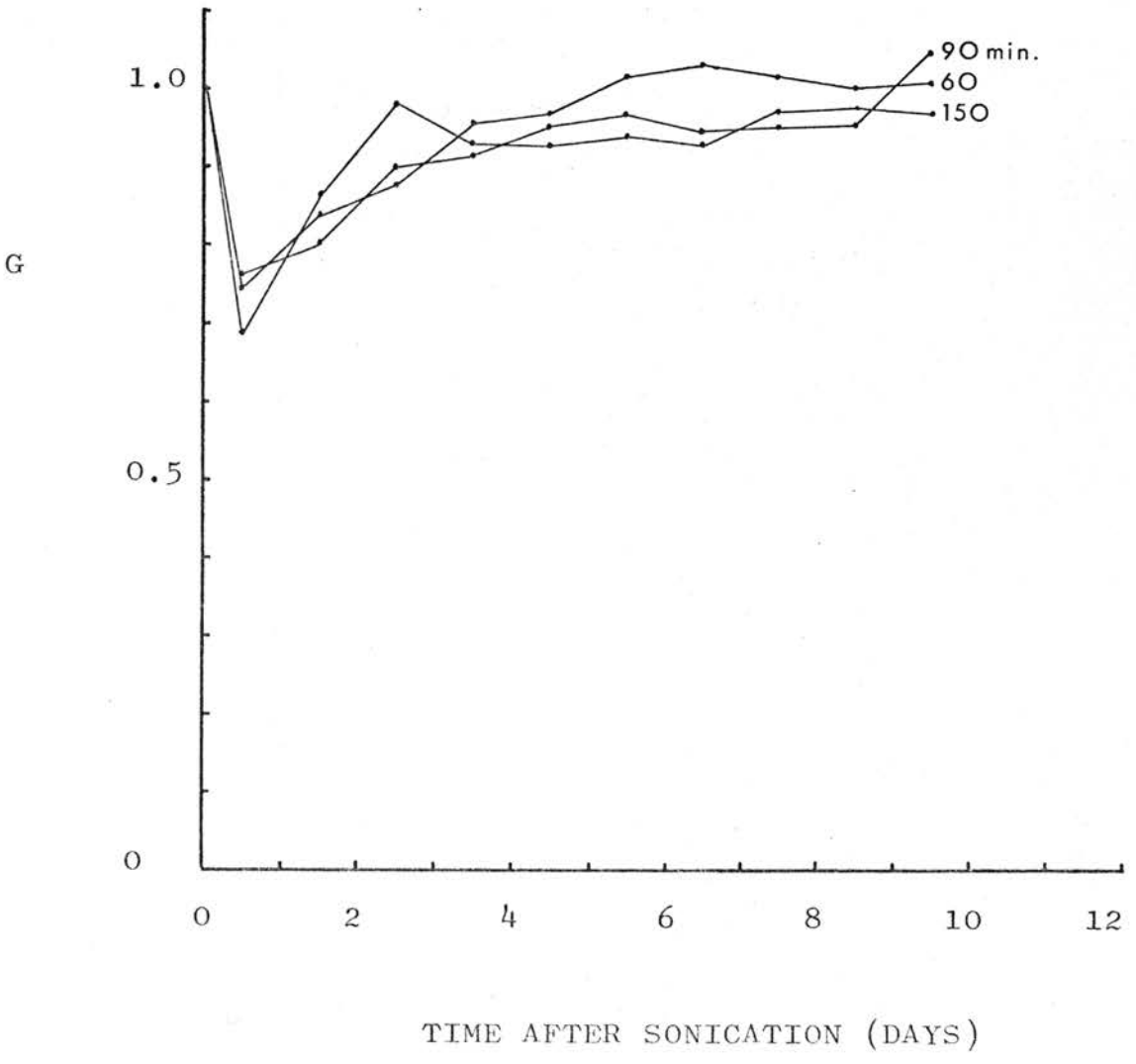


FIGURE 4.8 .

GROWTH CURVE FOR THE ROOTS OF ZEA SONICATED AT THE VERY LOW AVERAGE INTENSITY OF 0.02 W/cm^2 (PULSED BEAM) FOR 180 MIN.

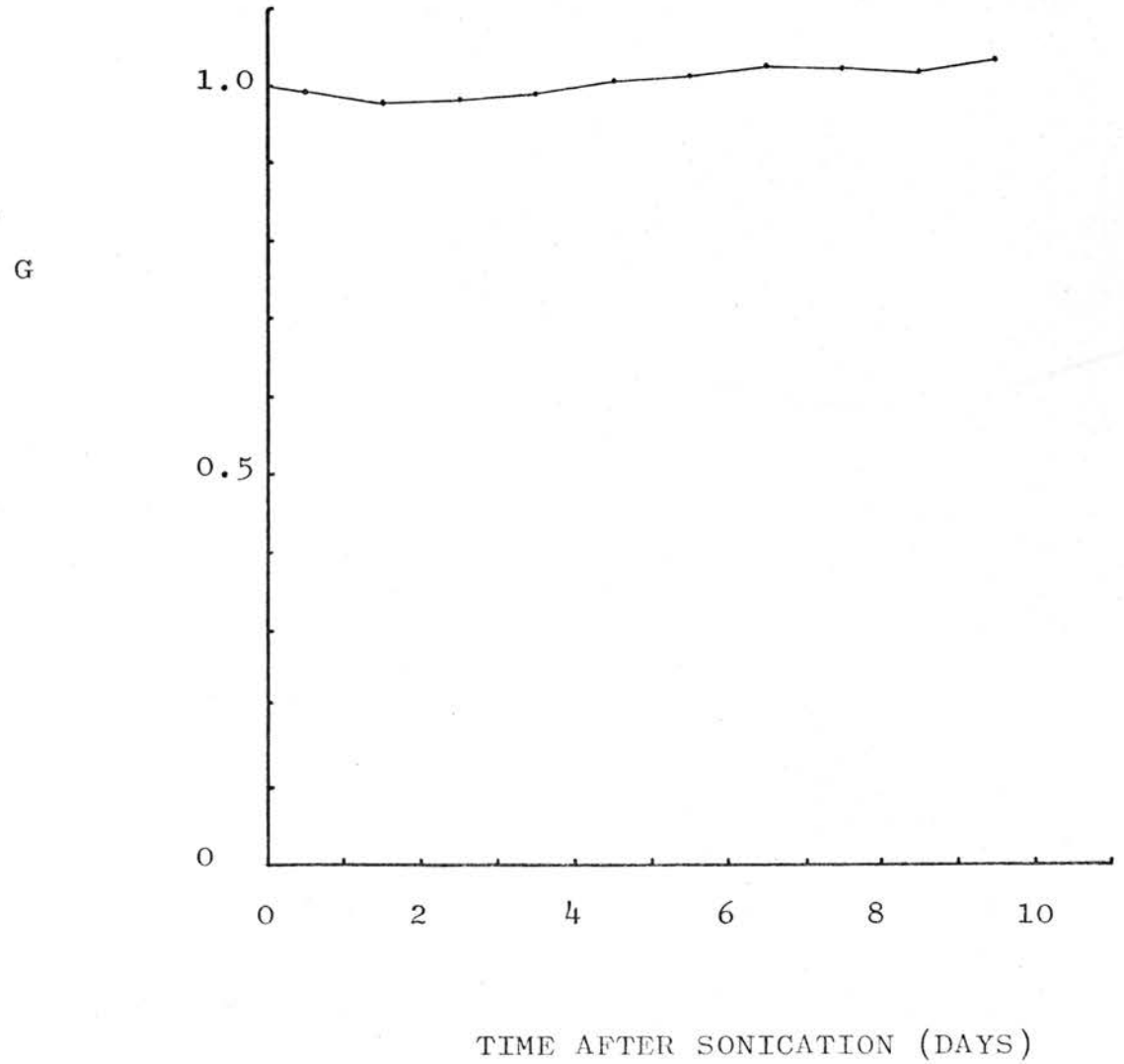


FIGURE 4.9 .

THE VARIATION OF THE AVERAGE GROWTH IN THE TENTH DAY POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING GROWTH FOR THE CONTROL ROOTS, WITH THE DURATION OF EXPOSURE AT THE AVERAGE INTENSITIES OF 0.82 AND 0.62 W/cm^2 RESPECTIVELY.

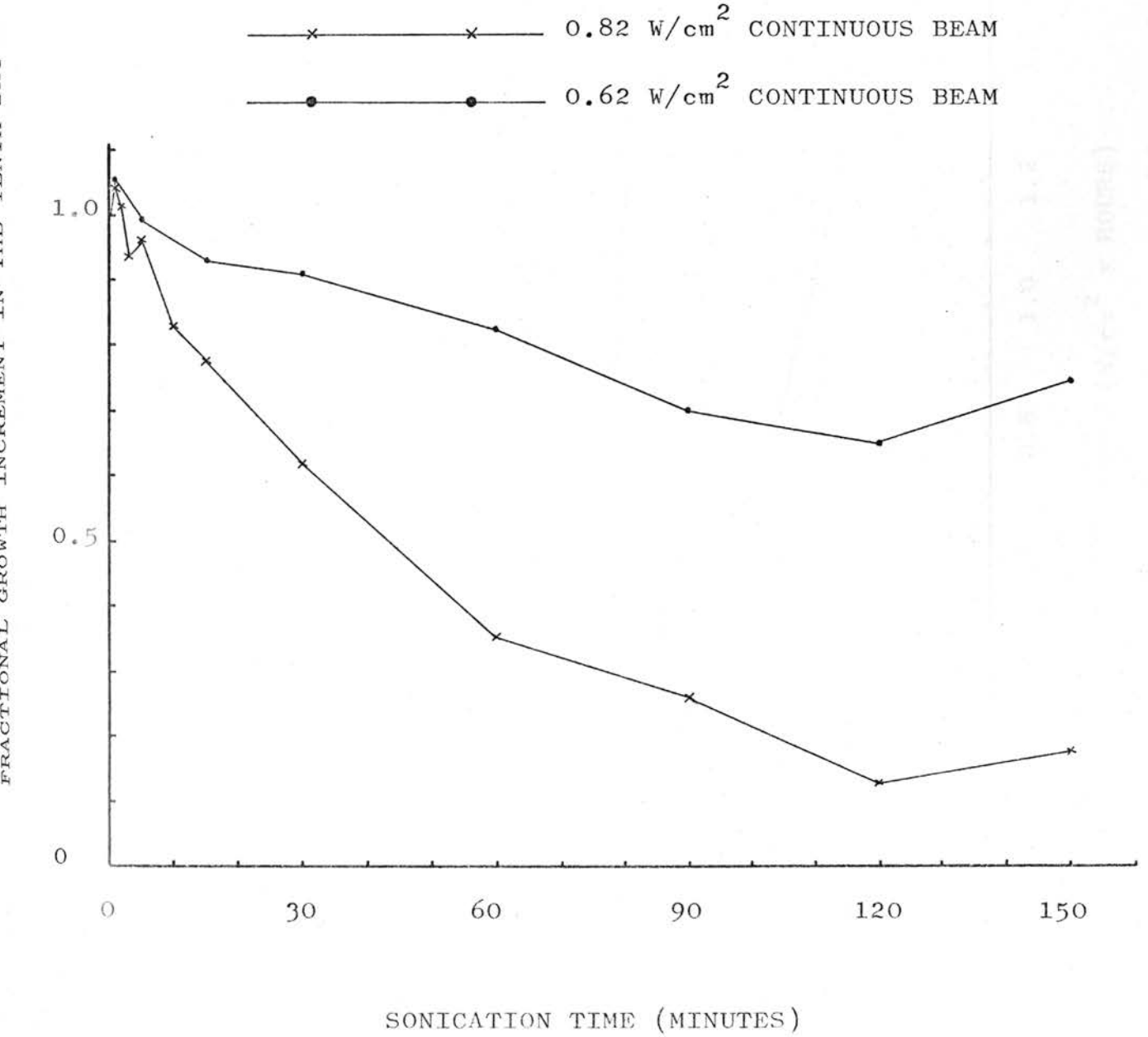


FIGURE 4.10 .

G_{10}, THE AVERAGE GROWTH OF ROOTS DURING TEN DAYS POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH OF CONTROL ROOTS, PLOTTED AS A FUNCTION OF TOTAL EXPOSURE (INTENSITY IN W/cm^2 MULTIPLIED BY DURATION OF EXPOSURE IN HOURS) FOR THE AVERAGE RADIATION INTENSITIES AS INDICATED.

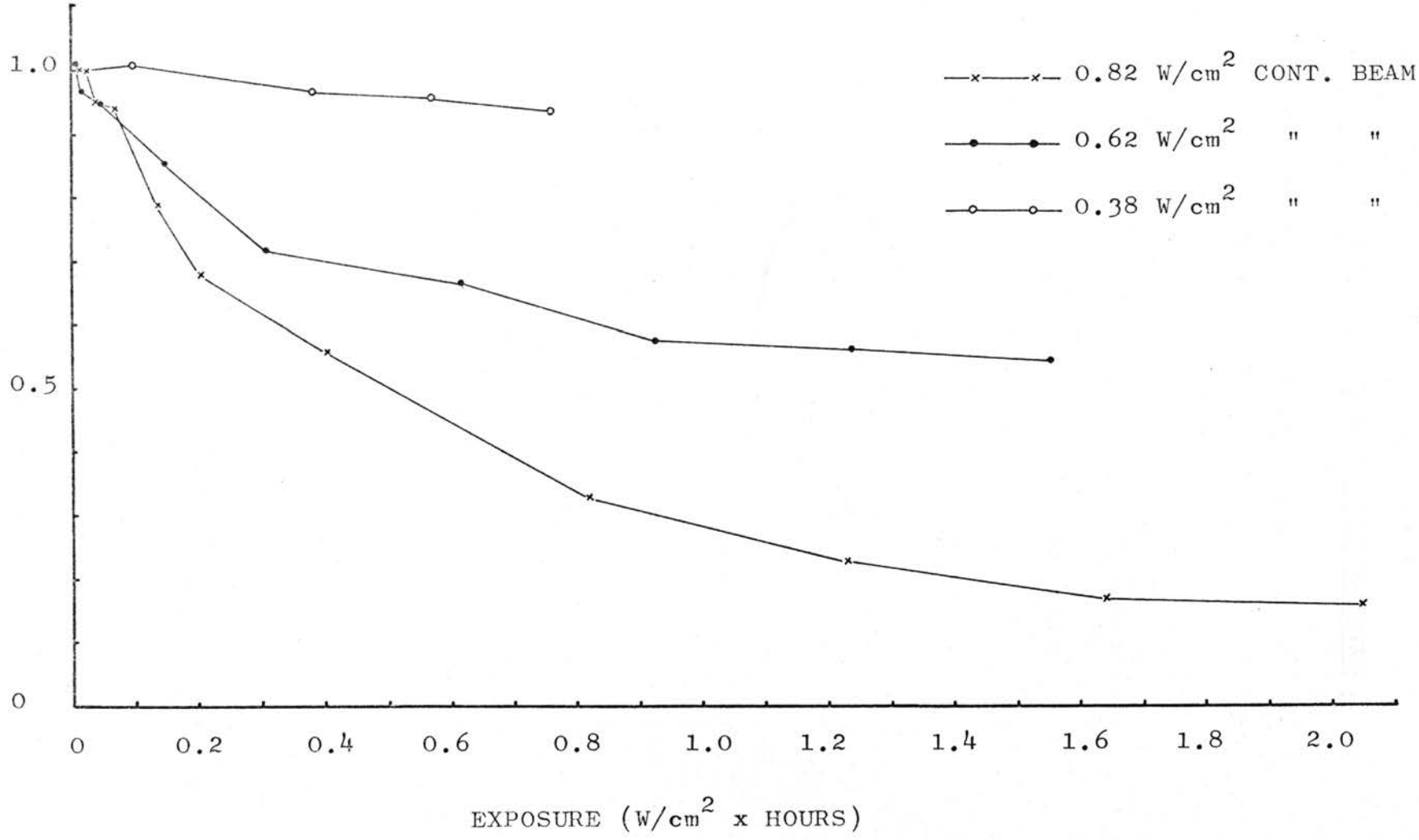


FIGURE 4.11 .

AS FOR FIGURE 4.10, BUT FOR THE AVERAGE INTENSITIES OF 0.21 W/cm^2 AND 0.16 W/cm^2 (PULSED BEAMS).

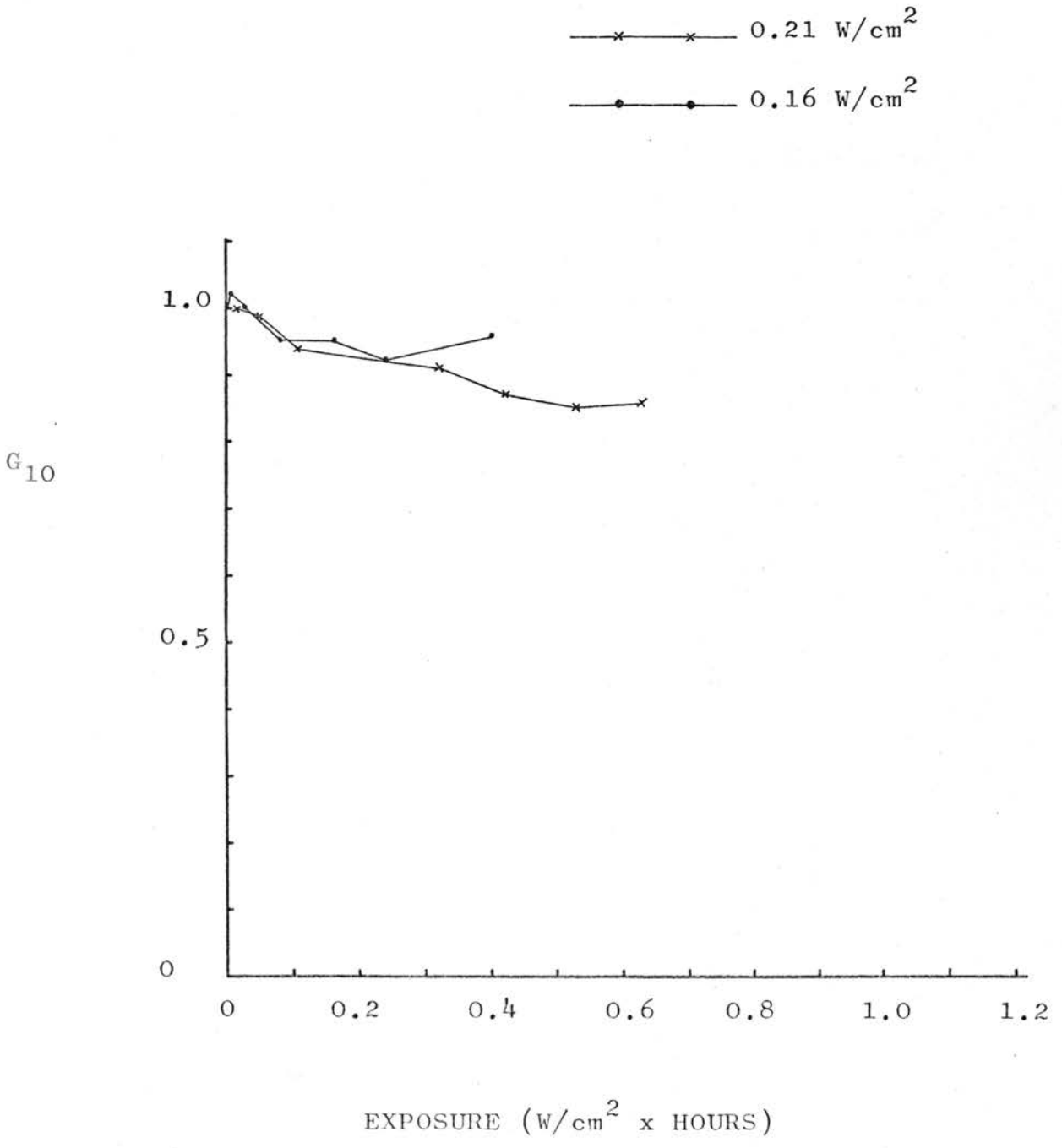


FIGURE 4.12 .

VARIATION OF G_1, THE AVERAGE GROWTH IN THE FIRST DAY POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, AS A FUNCTION OF SONICATION TIME FOR DIFFERENT AVERAGE INTENSITIES OF A CONTINUOUS BEAM AS INDICATED.

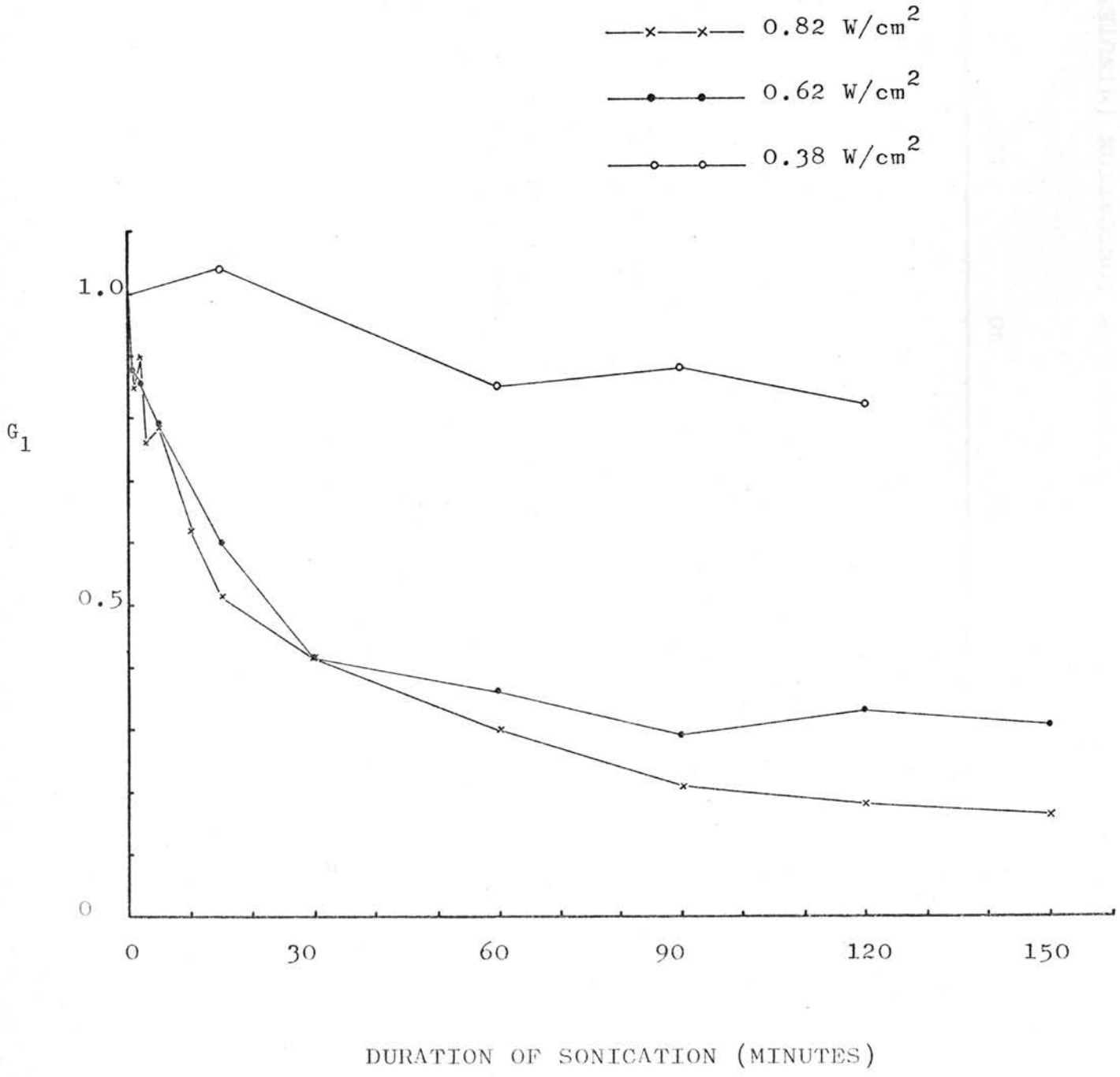


FIGURE 4.13.

AS FOR FIGURE 4.12 BUT FOR DIFFERENT AVERAGE INTENSITIES OF A PULSED BEAM.

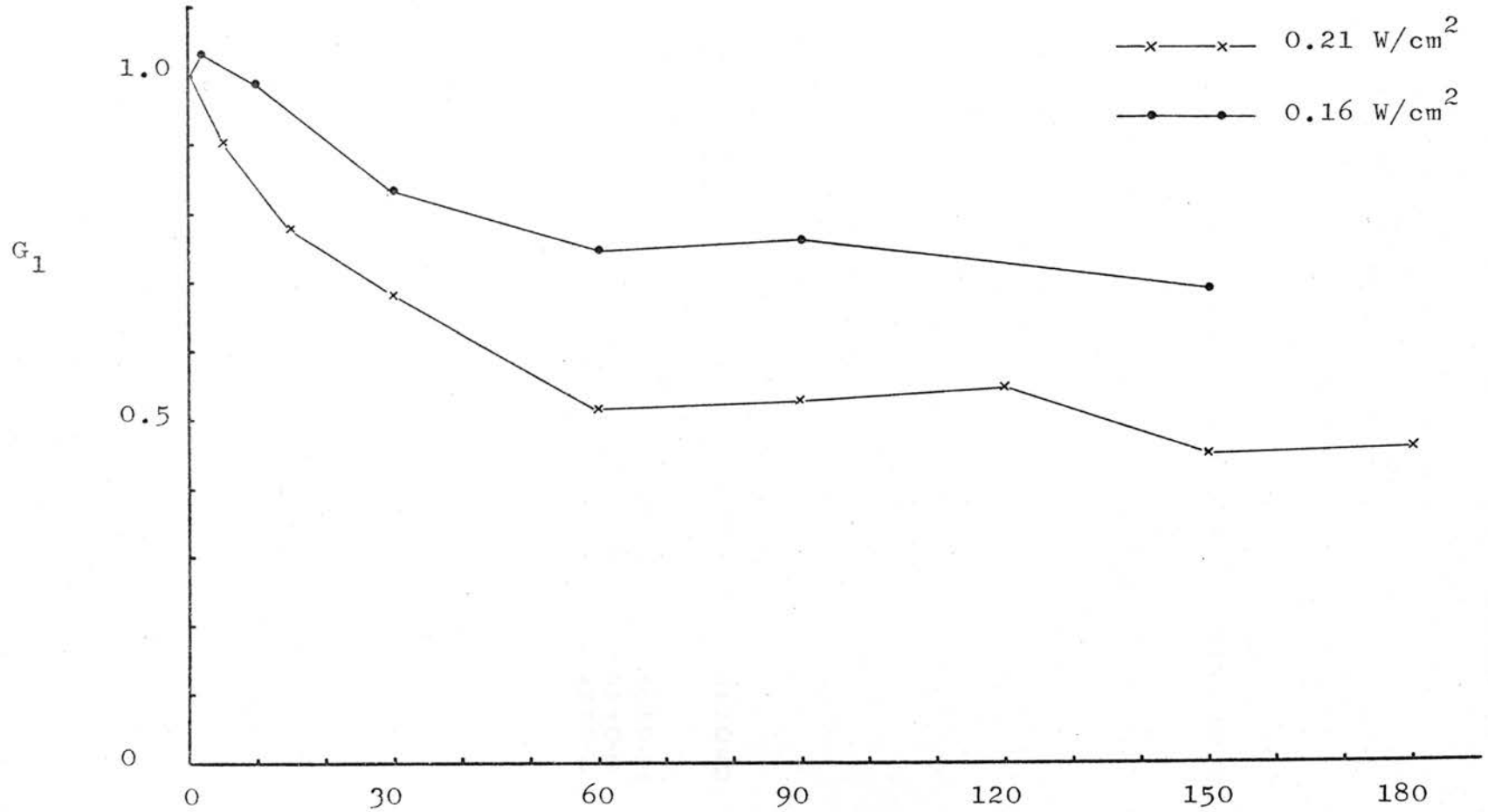


FIGURE 4.14.

VARIATION OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER SONICATIONS AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONTINUOUS BEAM) TO SHOW THE VARIATION OBTAINED BETWEEN EXPERIMENTS.

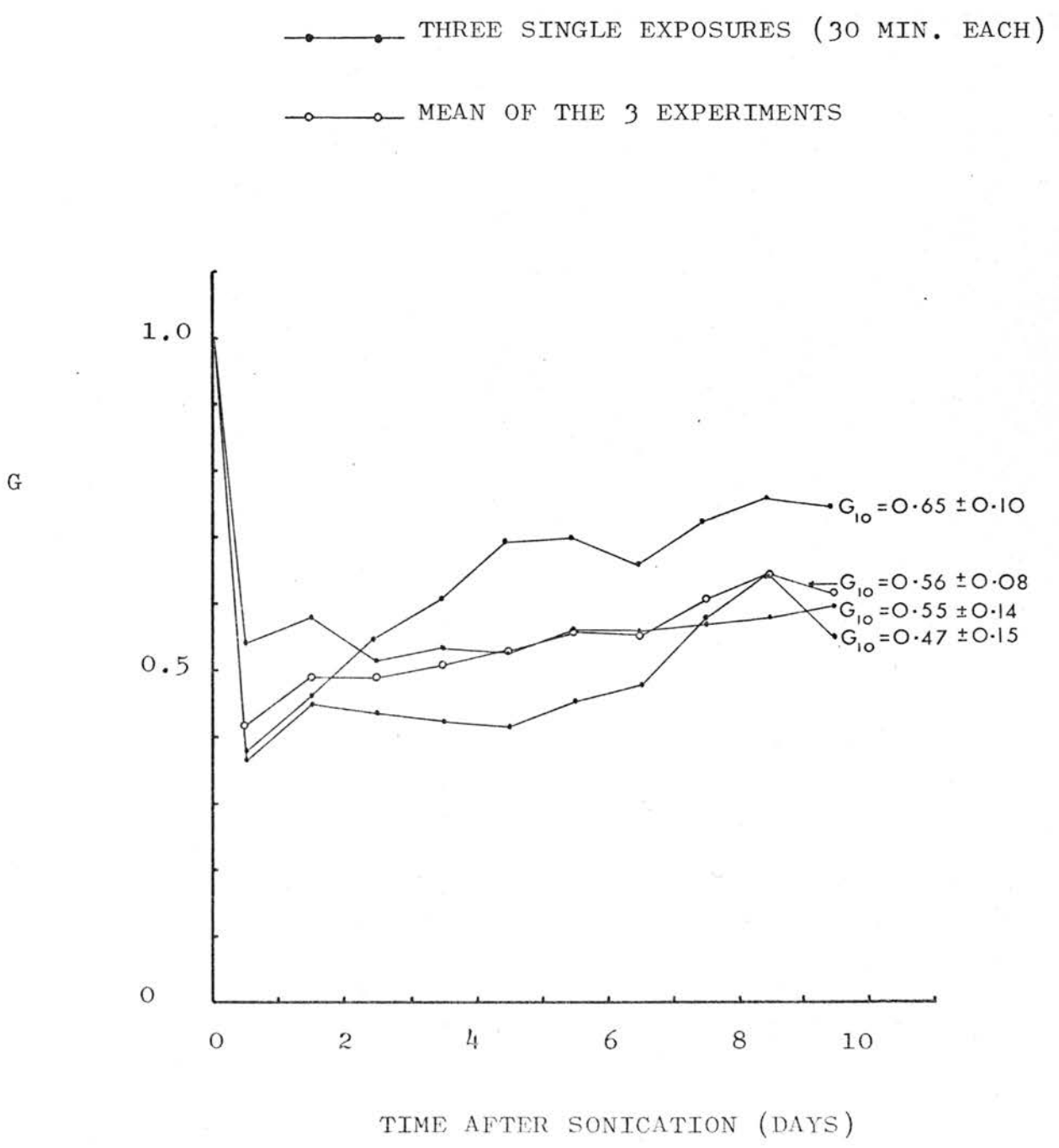


FIGURE 4.15.

VARIATION IN THE AVERAGE GROWTH OF THE ROOTS OF ZEA IN THE FIRST DAY FOLLOWING SONICATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS (G_1), WITH THE AVERAGE INTENSITIES OF THE PULSED AND CONTINUOUS BEAMS OF ULTRASOUND (APPLIED FOR 60 MINUTES). (Each point represents the mean of at least 2 experiments).

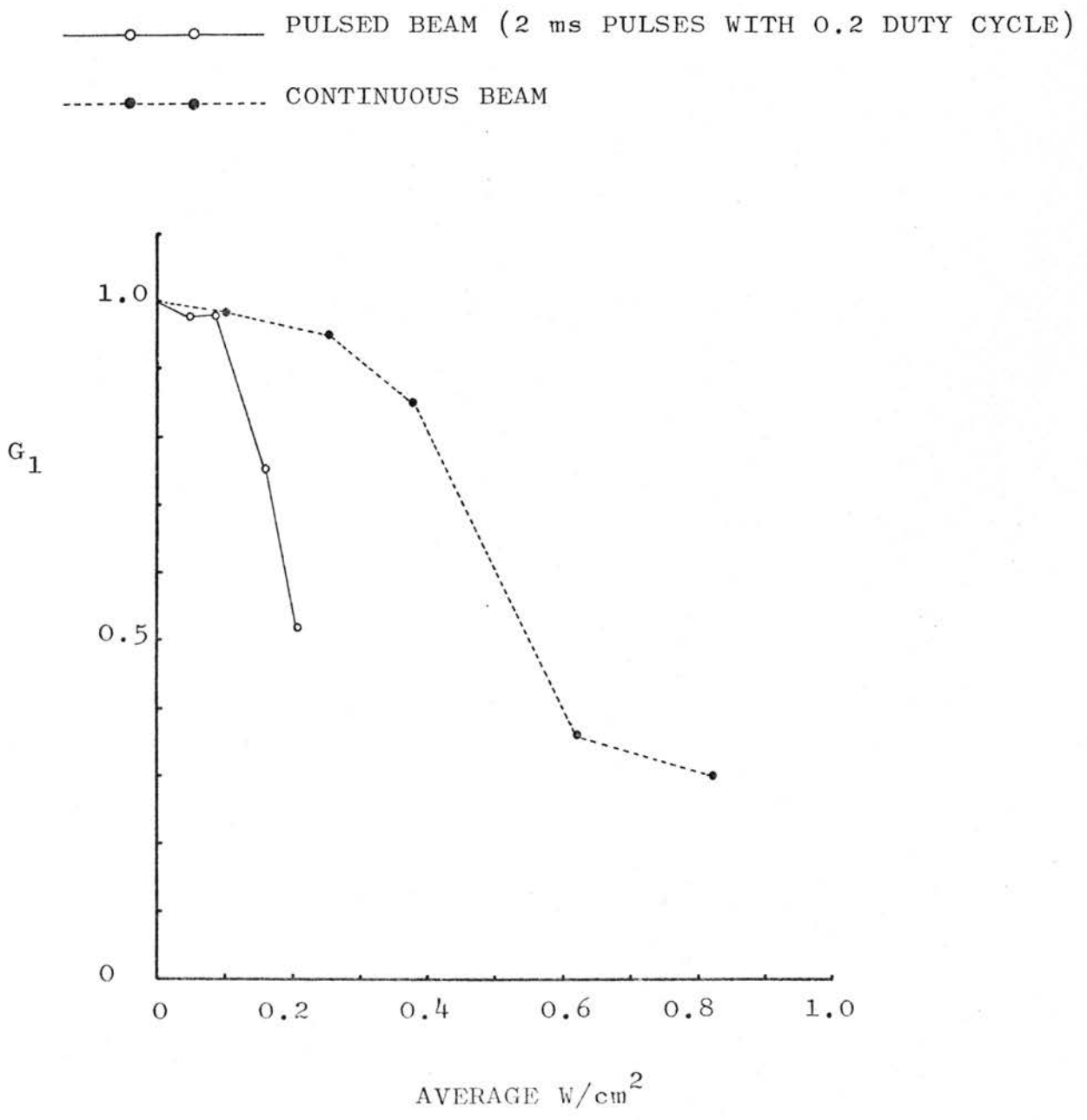


FIGURE 4.16.

GROWTH CURVE SHOWING THAT THERE IS NO ADVERSE EFFECT ON THE ROOTS WHEN THEY ARE POSITIONED ON THE SONICATION JIG (SEE TEXT).

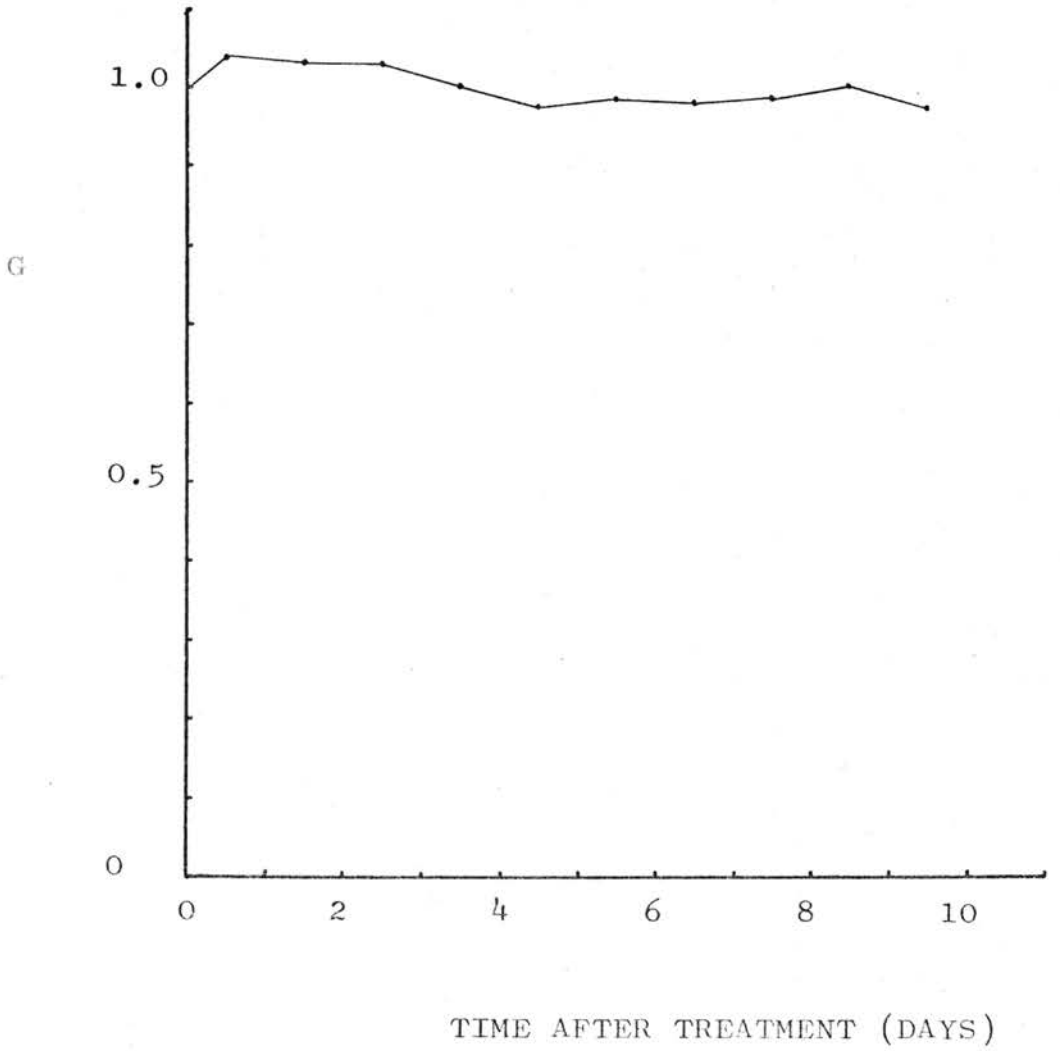


FIGURE 4.17.

PATTERNS OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) FOLLOWING TWO SONICATIONS OF 15 MIN. EACH, SEPARATED BY TIME INTERVALS IN THE RANGE 0 TO 24 HRS. AS INDICATED. (AVERAGE INTENSITY = 0.82 W/cm^2, CONT. BEAM).

(Each curve represents the mean of at least 2 experiments).

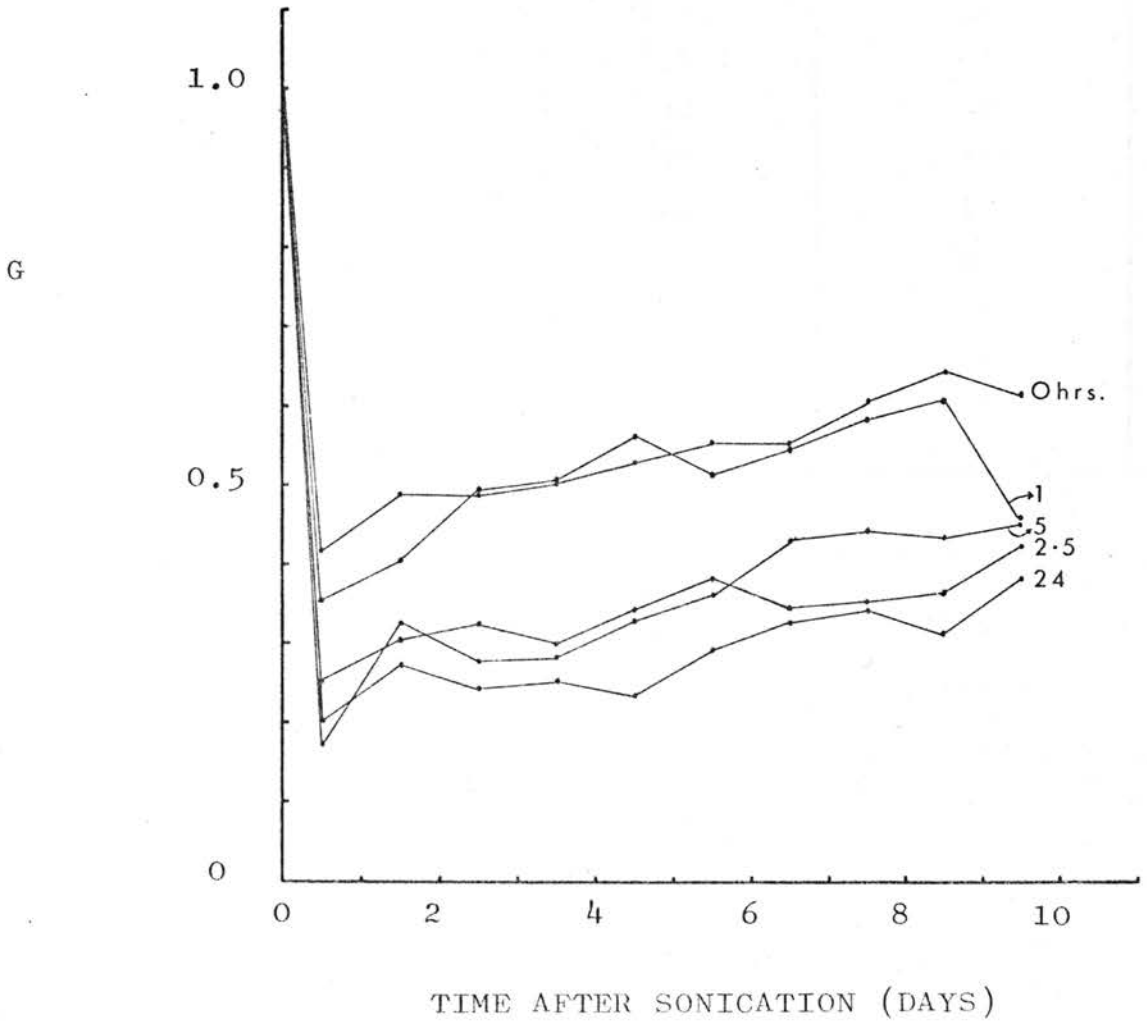


TABLE 4.1 .

PERCENTAGE OF THE ROOTS THAT HAD DIED OR WERE GROWING "SLOWLY" AT TEN DAYS POST-SONICATION.

AVERAGE ULTRASONIC INTENSITY W/cm^2	MODE	SONICATION TIME MIN.	% ROOTS WITH REDUCED GROWTH	% OF DEAD ROOTS
0.82	CONT.	1,2,3,5	0	0
"	"	10	1	8
"	"	15	2	10
"	"	30	15	25
"	"	60	20	40
"	"	90	12	53
"	"	120	15	59
"	"	150	18	56
0.62	CONT.	1,2,5,15	0	0
"	"	30	8	0
"	"	60	10	0
"	"	90	15	3
"	"	120	11	4
"	"	150	16	2
0.38	CONT.	15,60,90	0	0
"	"	120	1	0

For sonication times of up to and including 30 min., the figures in the last two columns represent the mean of three experiments. For the sonication times greater than 30 min., the figures in the last two columns represent the mean of two experiments. The percentages are given to the nearest 1%.

TABLE 4.2 .

CHANGE IN THE DIAMETER (MEAN OF TEN ROOTS) OF THE ROOT OF ZEA WITH DISTANCE FROM THE ROOT TIP, FOR SONICATED (0.82W/cm^2 CONT. BEAM FOR 30 MIN.) AND CONTROL ROOTS TEN DAYS AFTER EXPOSURE.

DISTANCE FROM TIP (mm)	MEAN DIAMETER (mm)	
	SONICATED ROOTS	CONTROL ROOTS
0.1	0.28 ± 0.04	0.37 ± 0.06
0.2	0.44 ± 0.03	0.49 ± 0.06
0.3	0.49 ± 0.02	0.58 ± 0.06
0.4	0.51 ± 0.02	0.61 ± 0.07
0.5	0.49 ± 0.04	0.62 ± 0.07
0.6	0.50 ± 0.03	0.62 ± 0.06
0.8	0.51 ± 0.04	0.63 ± 0.05
1.0	0.50 ± 0.04	0.63 ± 0.06
1.2	0.52 ± 0.03	0.62 ± 0.06
1.4	0.53 ± 0.04	0.65 ± 0.06
1.6	0.54 ± 0.03	0.65 ± 0.06
1.8	0.55 ± 0.04	0.64 ± 0.07
2.0	0.55 ± 0.04	0.64 ± 0.07

Each value represents the mean of ten measurements with a dissection microscope. A Neubauer ruling was used to provide a measure of length.

The errors are standard errors of the means.

TABLE 4.3 .

AVERAGE GROWTH OF THE ROOTS IN TEN DAYS POST IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS (G_{10}).

AVERAGE ULTRASONIC INTENSITY W/cm^2	MODE	EXPOSURE TIME (MINUTES)											
		1	2	3	5	10	15	30	60	90	120	150	180
0.82	CONT.	1.00 ±0.06	1.00 ±0.06	0.95 ±0.07	0.94 ±0.06	0.79 ±0.06	0.68 ±0.08	0.56 ±0.08	0.33 ±0.09	0.23 ±0.07	0.17 ±0.08	0.16 ±0.07	-
0.62	CONT.	1.02 ±0.07	0.97 ±0.06	-	0.95 ±0.05	-	0.86 ±0.07	0.72 ±0.07	0.67 ±0.07	0.58 ±0.06	0.57 ±0.08	0.55 ±0.07	-
0.38	CONT.	-	-	-	-	-	1.01 ±0.08	-	0.97 ±0.07	0.96 ±0.07	0.94 ±0.08	-	-
0.21	PULSED	-	-	-	0.99 ±0.05	-	1.00 ±0.06	0.94 ±0.06	0.92 ±0.06	0.91 ±0.07	0.87 ±0.07	0.85 ±0.06	0.86 ±0.06
0.16	PULSED	-	1.02 ±0.07	-	-	1.00 ±0.05	-	0.95 ±0.07	0.95 ±0.05	0.92 ±0.06	-	0.96 ±0.07	-
0.02	PULSED	-	-	-	-	-	-	-	-	-	-	-	0.97 ±0.06

TABLE 4.4 .

COMPARISON OF THE EFFECT OF A GIVEN TOTAL EXPOSURE DELIVERED AS A SINGLE DOSE OR AS TWO EQUAL FRACTIONS.

AVERAGE ULTRASONIC INTENSITY W/cm^2	SONICATION TIME (MIN)	TIME INTERVAL BETWEEN EXPOSURES (HRS.)	G_{10}
0.82 CONT.	30	0	0.56 ± 0.08
"	15 + 15	1	0.48 ± 0.13
"	15 + 15	$2\frac{1}{2}$	0.33 ± 0.09
"	15 + 15	5	0.35 ± 0.11
"	15 + 15	24	0.28 ± 0.09

The errors are standard errors of the means (see p. 173).

Morphological changes after sonication.

Some of the sonicated root tips were found to turn sideways after sonication (i.e. at right angles to the normal downward growth) but turned back into the original direction with subsequent growth (Figure 4.18). This "kinking" of the roots was not observed for the short sonication times and/or low average ultrasonic intensities. For the sonications lasting for 1-3 hours, the root tips turned slightly towards the transducer face during sonication.

Some of the roots sonicated for more than15 minutes at the high average ultrasonic intensities of the pulsed and continuous beams, viz. 0.21 W/cm^2 and 0.82 W/cm^2 respectively, were found to have translucent tips after sonication. With subsequent growth, however, the appearance of the newly added length of the root was similar to that before sonication.

Squash preparations were made of sonicated roots using the Feulgen squash method as described elsewhere. The squashes were made immediately after sonication, one day later and finally on the fifth day post-irradiation. A squash of an untreated root is shown in Figure 4.19. Immediately after sonication at an average intensity of 0.82 W/cm^2 (continuous beam) for 30 minutes, a large number of lysed cells seem to be present as shown in Figure 4.20 . Characteristic is also the appearance of small densely stained areas in some of the cells of the sonicated tissue. Since Feulgen stains nucleic acid (Darlington

and La Cour, 1938) the constituents of these areas may have originated from the nuclei. These areas are also sometimes fragmented (Figure 4.20). There are some cells which seem to be intact but have an abnormal shape. These abnormalities are still present on the first day after sonication, although to a lesser extent (Figure 4.21) and there seem to be fewer disrupted cells. Five days after sonication the tissue looks virtually normal and the small densely stained areas have disappeared (Figure 4.22). Similar damage was observed for all the other intensities of the pulsed and continuous beams used in the present experiments although the degree of damage varied with the average ultrasonic intensity used and the sonication time. At the very low average intensities no damage was noted.

FIGURE 4.18.

PHOTOGRAPH OF A ROOT OF ZEA TWO DAYS AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR 60 MINUTES, SHOWING THE "KINK" CAUSED BY SONICATION.

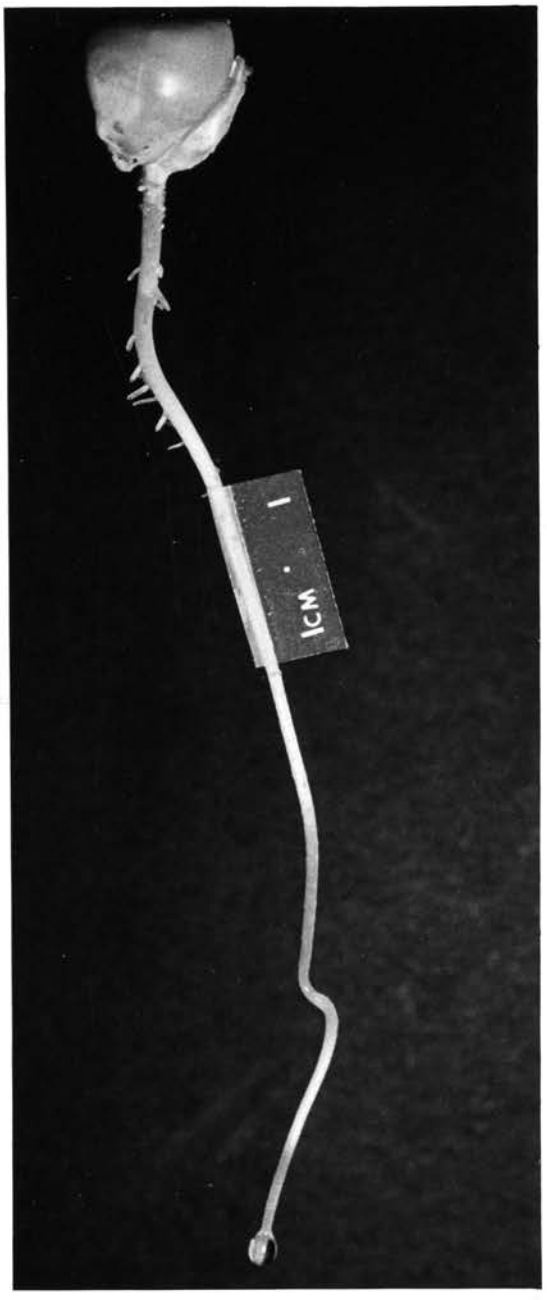


FIGURE 4.19.

SQUASH PREPARATION OF AN UNTREATED ROOT (x 450).

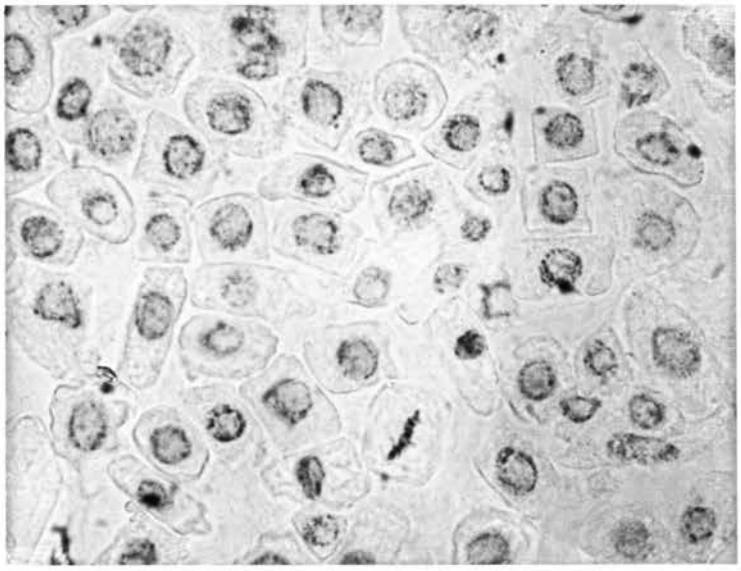


FIGURE 4.20.

SQUASH PREPARATION OF A ROOT OF ZEA IMMEDIATELY AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) FOR 30 MINUTES (x 450).

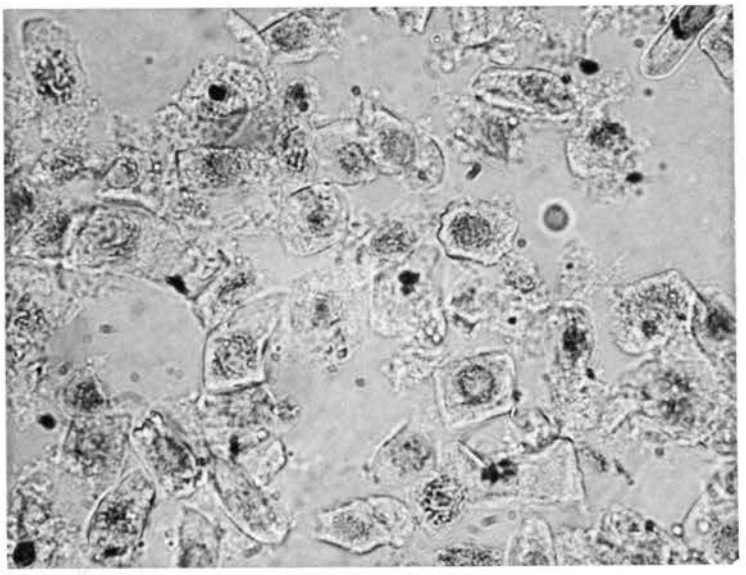


FIGURE 4.21.

SQUASH PREPARATION OF A ROOT OF ZEA ONE DAY AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) FOR 30 MINUTES (x 450).

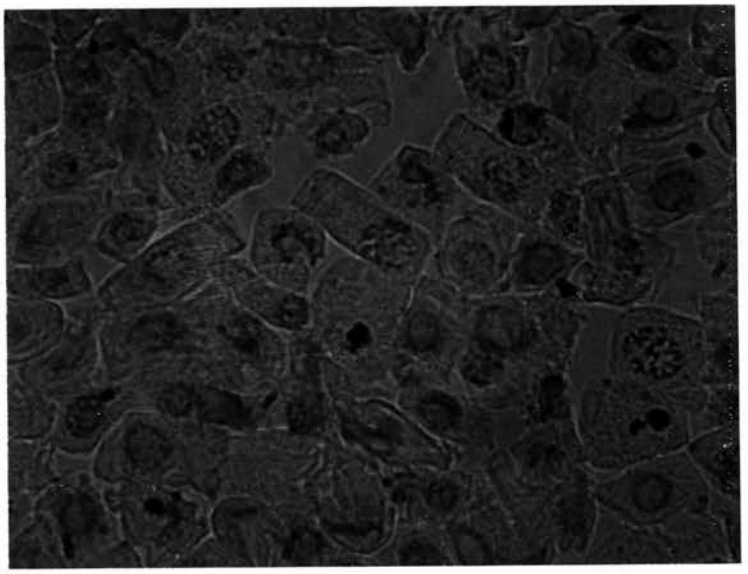
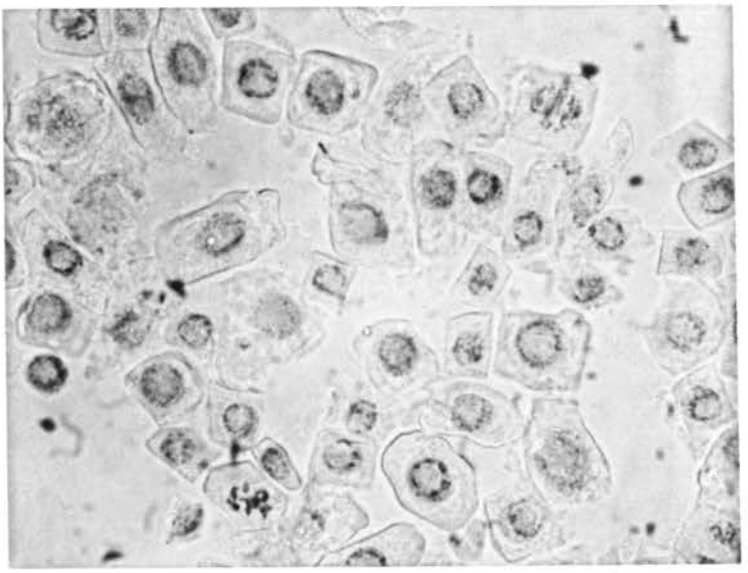


FIGURE 4.22 .

SQUASH PREPARATION OF A ROOT OF ZEA 5 DAYS AFTER SONICATION AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) FOR 30 MINUTES (x 450).



Results of the sonication of different regions of the root tip.

In order to determine which part of the root tip (i.e. the meristematic region or the elongating zone) is primarily responsible for the immediate drop in growth rate after sonication, the regions mentioned above were individually exposed to ultrasound at an average intensity of 0.82 W/cm^2 (cont. beam) as described previously (p. 83).

In Figures 4.23 and 4.24 the resulting growth curves for the sonication times of 15 and 30 minutes respectively, are compared to those obtained for the roots irradiated in the normal way (p. 81). These figures indicate that the simultaneous sonication of the meristem and elongating zone causes an immediate drop in the growth rate after sonication which is greater than that observed when the elongating zone is exposed to ultrasound and the meristem is shielded. When the meristem is exposed and the elongating zone is shielded from ultrasound, the immediate drop in growth rate is also larger than that observed for the shielded meristem.

The values of G_{10} (Table 4.5) also indicate that if the meristem only is sonicated, the growth of the roots is less than if the meristem is shielded and the elongating zone is sonicated on its own.

The elongating zone is considered to start at about 2 mm from the root tip since the division of meristematic cells occurs up to this point (Cook, 1959).

According to Cook the region of great extension is between 2 mm and 8 mm.

In a surgical experiment various lengths of the root tip were removed in order to find to what extent the elongating zone contributes to root growth subsequent to the removal or complete damage of the whole meristematic region or part of the meristematic region.

Root lengths ranging from 0.2 to 3 mm were removed under a dissection microscope and a Neubauer ruling was used to provide a measure of length.

The subsequent growth of the roots treated in this way (Fig. 4.25) indicates that if the first 3 mm is completely removed, the growth as a fraction of controls decreases to about 0.25 within the first three hours after removal of the tip and growth ceases altogether within the first day. If 2, 1, 0.8 and 0.6 mm of the root tip are removed, growth ceases within $1\frac{1}{2}$, 2, 4 and 6 days respectively.

When 0.5 or 0.4 mm of the tip is removed, the root does not cease to grow altogether but continues to grow at a reduced rate. The growth rate as a fraction of controls decreases for the first 5 days and then levels off at about 0.5.

It is perhaps of interest to note here that if only part of the root cap is removed (say 0.2 mm) the growth rate as a fraction of controls levels off at about 0.9 instead of the expected 1.0. Repetitions of this experiment always resulted in a fractional growth rate less than 1.0.

FIGURE 4.23.

EFFECT OF SHIELDING THE ROOT TIPS.

(Each curve represents the mean of at least 2 experiments.

For errors, see p. 173).

×——× 0.82 W/cm^2 CONTINUOUS BEAM FOR 15 MIN., MERISTEM SHIELDED

●——● " " " " " " " NO SHIELDING

○——○ " " " " " " " MERISTEM EXPOSED ONLY

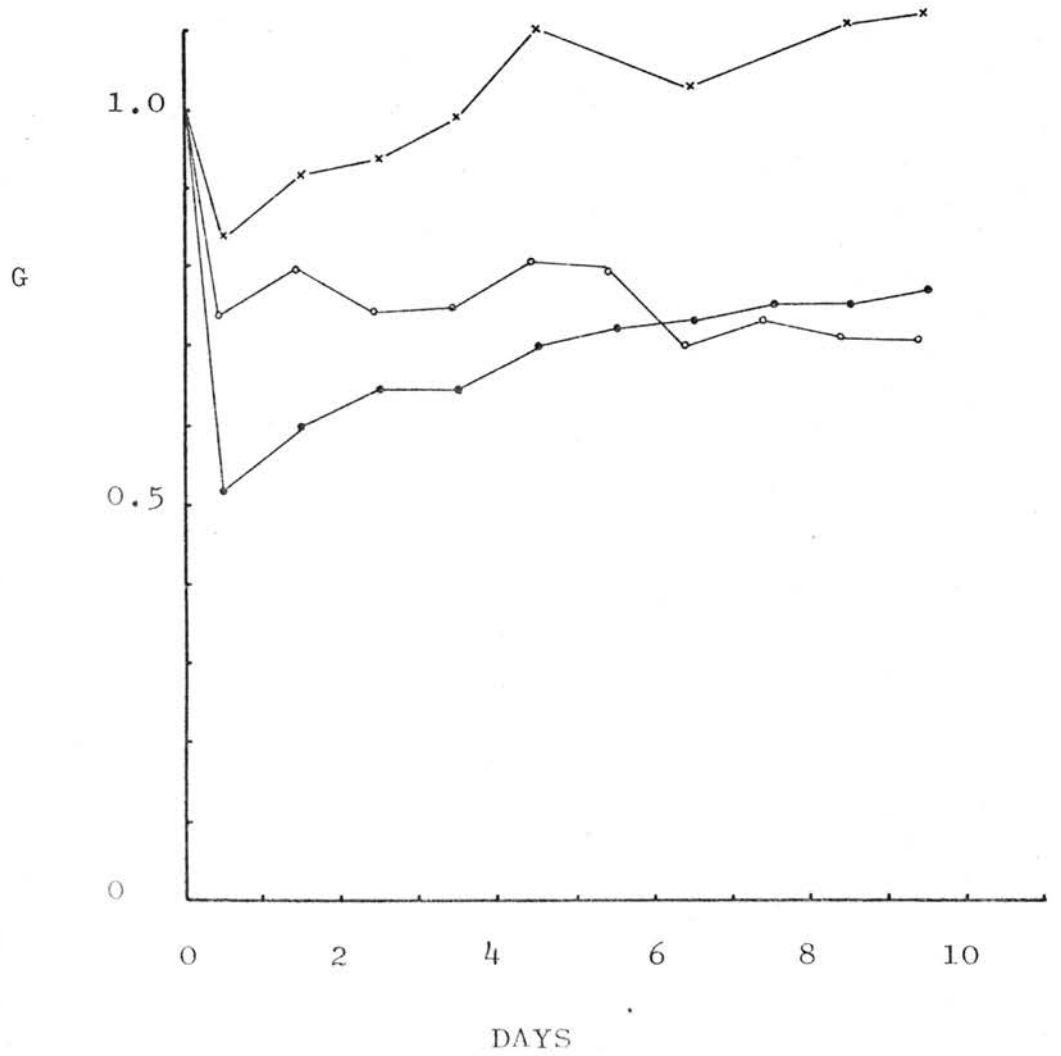


FIGURE 4.24.

EFFECT OF SHIELDING THE ROOT TIPS.

(Each curve represents the mean of at least 2 experiments.

For errors, see p. 173).

×——× 0.82 W/cm^2 CONTINUOUS BEAM FOR 30 MIN., MERISTEM SHIELDED

●——● " " " " " " " NO SHIELDING

○——○ " " " " " " " MERISTEM EXPOSED ONLY

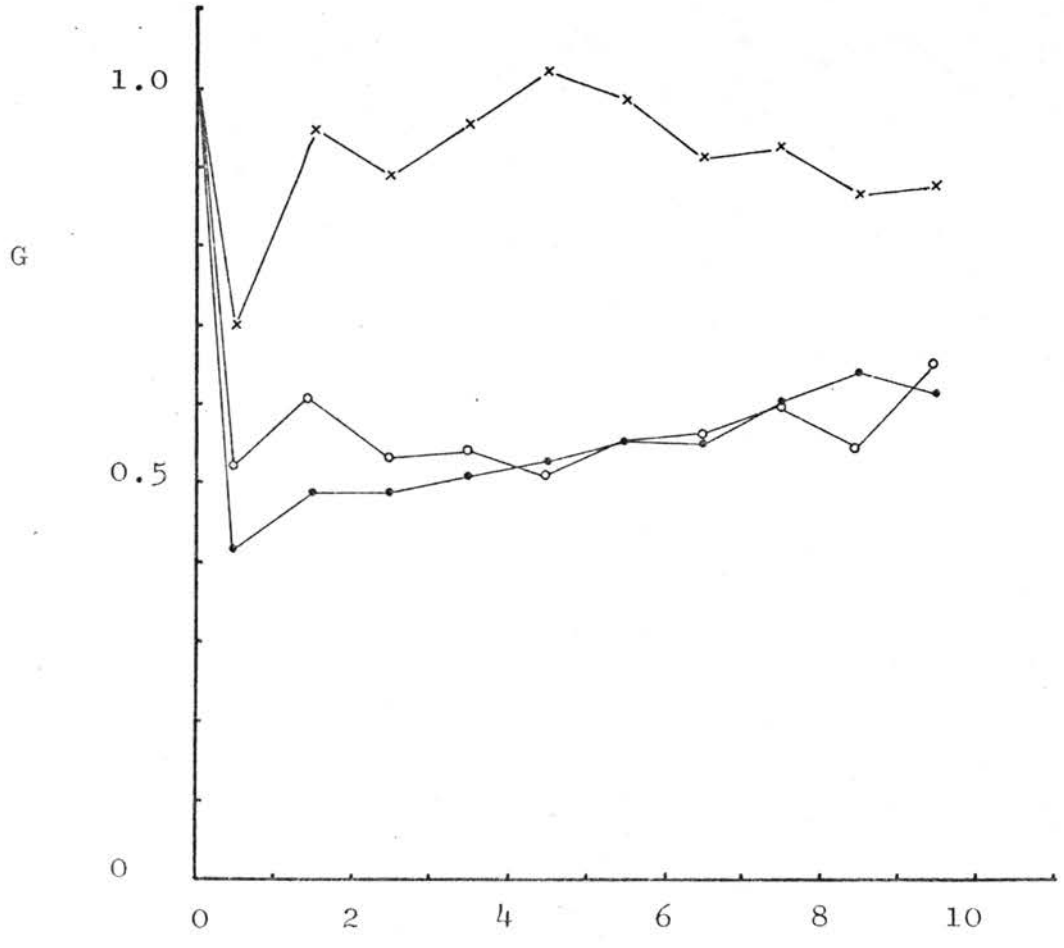
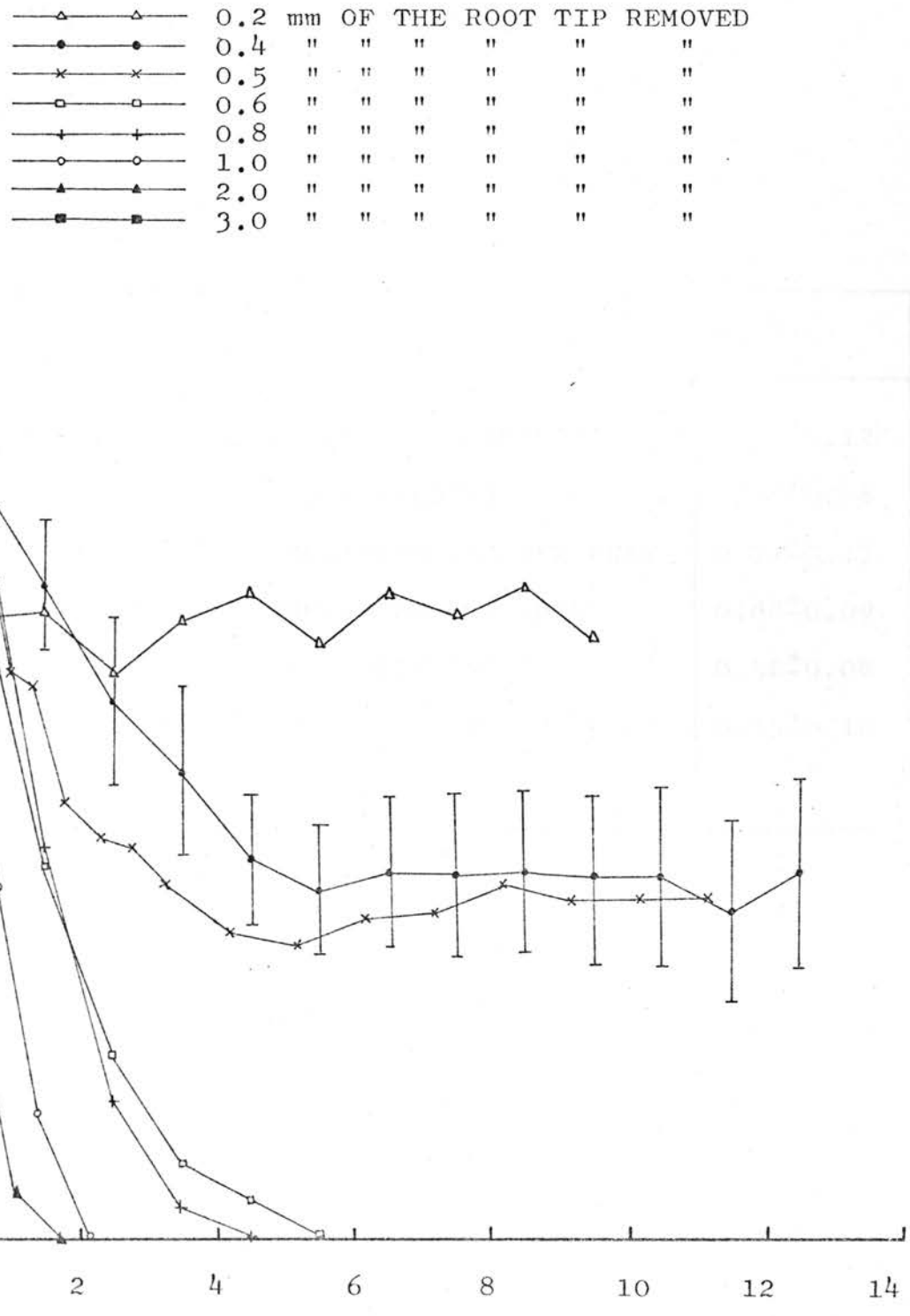


FIGURE 4.25.

GROWTH RATE AS A FRACTION OF CONTROLS AFTER REMOVAL OF VARIOUS LENGTHS OF THE ROOT TIP.



The errors are standard errors of the means. Each point represents the mean for 15 roots.

TABLE 4.5 .

VALUES OF G_{10} SHOWING THE EFFECT OF SHIELDING VARIOUS PARTS OF THE ROOT TIP.

	G_{10}
0.82 W/cm^2 CONT. BEAM FOR 15 MIN., MERISTEM SHIELDED	0.97 ± 0.12
" " " " " " " NO SHIELDING	0.68 ± 0.08
" " " " " " " MERISTEM EXPOSED ONLY	0.74 ± 0.17
" " " " " 30 " MERISTEM SHIELDED	0.88 ± 0.09
" " " " " " " NO SHIELDING	0.56 ± 0.08
" " " " " " " MERISTEM EXPOSED ONLY	0.55 ± 0.10

Influence of dissolved oxygen on the sensitivity of Zea mays to ultrasound.

The oxygen content of the water in the sonication tank could be changed from that in air-equilibrated (i.e. non-aerated) water by passing oxygen, nitrogen, helium and air through the latter. Nitrogen was used to displace the oxygen in the water, but it was thought possible that nitrogen could also influence the sonication damage since the effect of ultrasound on certain organic compounds was found to depend on the nature of the ambient gas (p. 58). Thus the totally non-reactive gas, helium, was also used instead of nitrogen.

The method of sonication, the measurement of the oxygen content of the water and the measurement of the average ultrasonic intensities used in these experiments, are described elsewhere.

The growth curves and the values of the fractional growth in ten days (G_{10}) for the roots sonicated with various concentrations of dissolved oxygen in the water are shown in Figure 4.26 and Table 4.6 respectively.

For these experiments the highest possible output of the ultrasonic generator was used since an appreciable reduction in the average intensity was only observed for the higher intensities of the continuous beam (p. 95). The reduction in the highest average intensity obtainable in air-equilibrated water 7 cm from the crystal face, viz. 0.82 W/cm^2 (cont. beam), was found to be similar for all the oxygen concentrations

achieved by passing various gases through the water (Table 4.6). When gases were bubbled through the water the average intensity of 0.82 W/cm^2 was reduced to values which were approximately equal to the average intensity of 0.62 W/cm^2 (cont. beam) in air-equilibrated water. Thus the growth curve for the roots irradiated at this intensity (mean for three experiments in air-equilibrated water) is compared with those obtained when the roots were sonicated for the same times but with the various gases being bubbled through the water (Fig. 4.26).

There was a significant difference in the growth of the roots when sonicated in air-equilibrated and aerated water respectively (Fig. 4.26). The oxygen content, however, did not change appreciably, viz. 6.9 ml/l for the aerated water as compared to 6.7 ml/l (mean for three experiments, viz. 6.70, 6.80 and 6.65 ml/l) for the non-aerated water.

In the presence of large amounts of oxygen (22.2ml/l) the sonication damage was found to be not significantly different from the damage produced under aerated conditions (Fig. 4.26).

When the oxygen content was reduced to 2.5 ml/l and 2.0 ml/l by passing nitrogen and helium through the water respectively, there was again a similar amount of damage as for the aerated conditions. When the amount of oxygen was reduced to 1.4 ml/l there seemed to be a larger amount of sonication damage (Fig. 4.26).

Control experiments revealed that in the absence

of ultrasound the changes in the concentration of dissolved oxygen as mentioned above had no adverse effect on the subsequent growth of the roots (Fig. 4.27).

FIGURE 4.26.

THE EFFECT OF DISSOLVED GASES ON THE ULTRASONIC DAMAGE TO THE ROOTS.

- 0.70 W/cm^2 CONT. BEAM FOR 30 MIN. IN N_2 (2.5 ml/l O_2)
- 0.65 " " " " " " " N_2 (1.4 ml/l O_2)
- 0.65 " " " " " " " He_2 (2.0 ml/l O_2)
- 0.65 " " " " " " " O_2 (22.2 ml/l O_2)
- 0.68 " " " " " " " AERATED WATER (6.9 ml/l O_2)
- 0.62 " " " " " " " AIR-EQUILIBRATED WATER (6.7 ml/l O_2)

(Each curve represents the mean of at least two experiments).

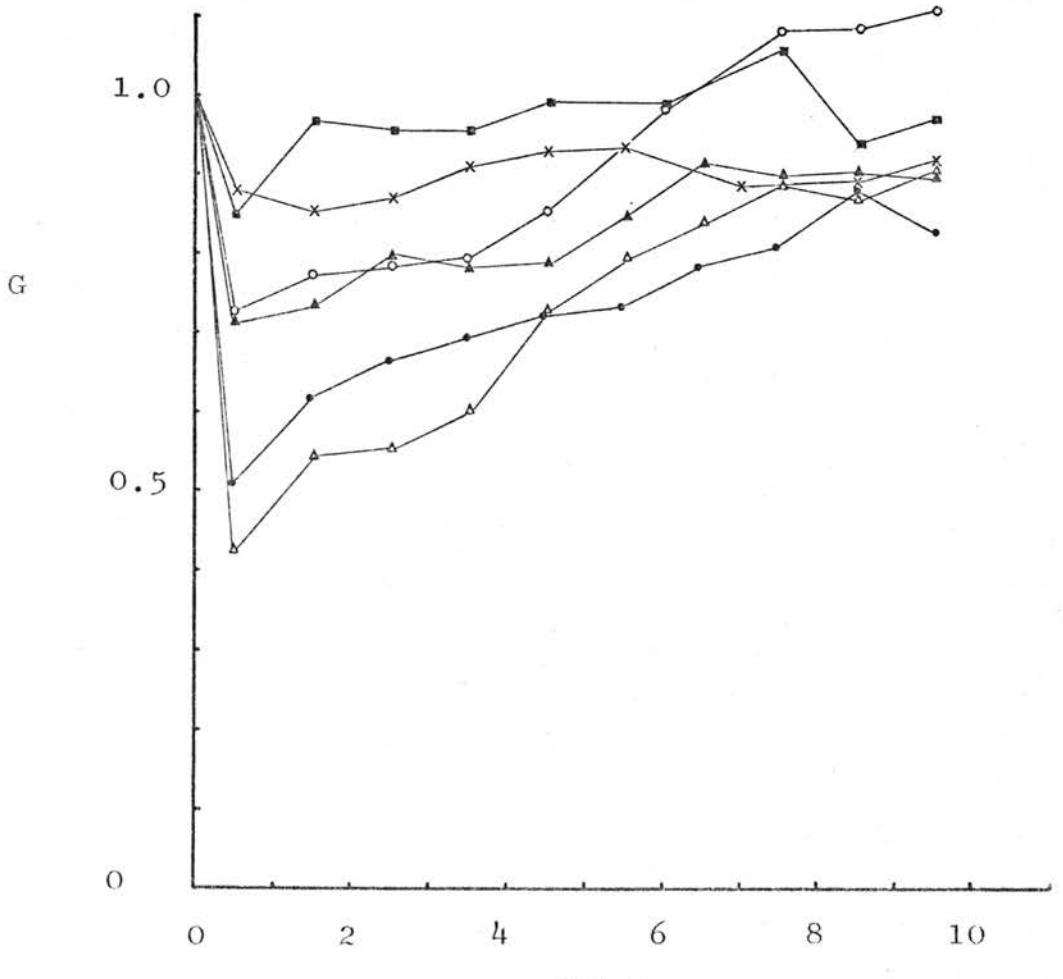
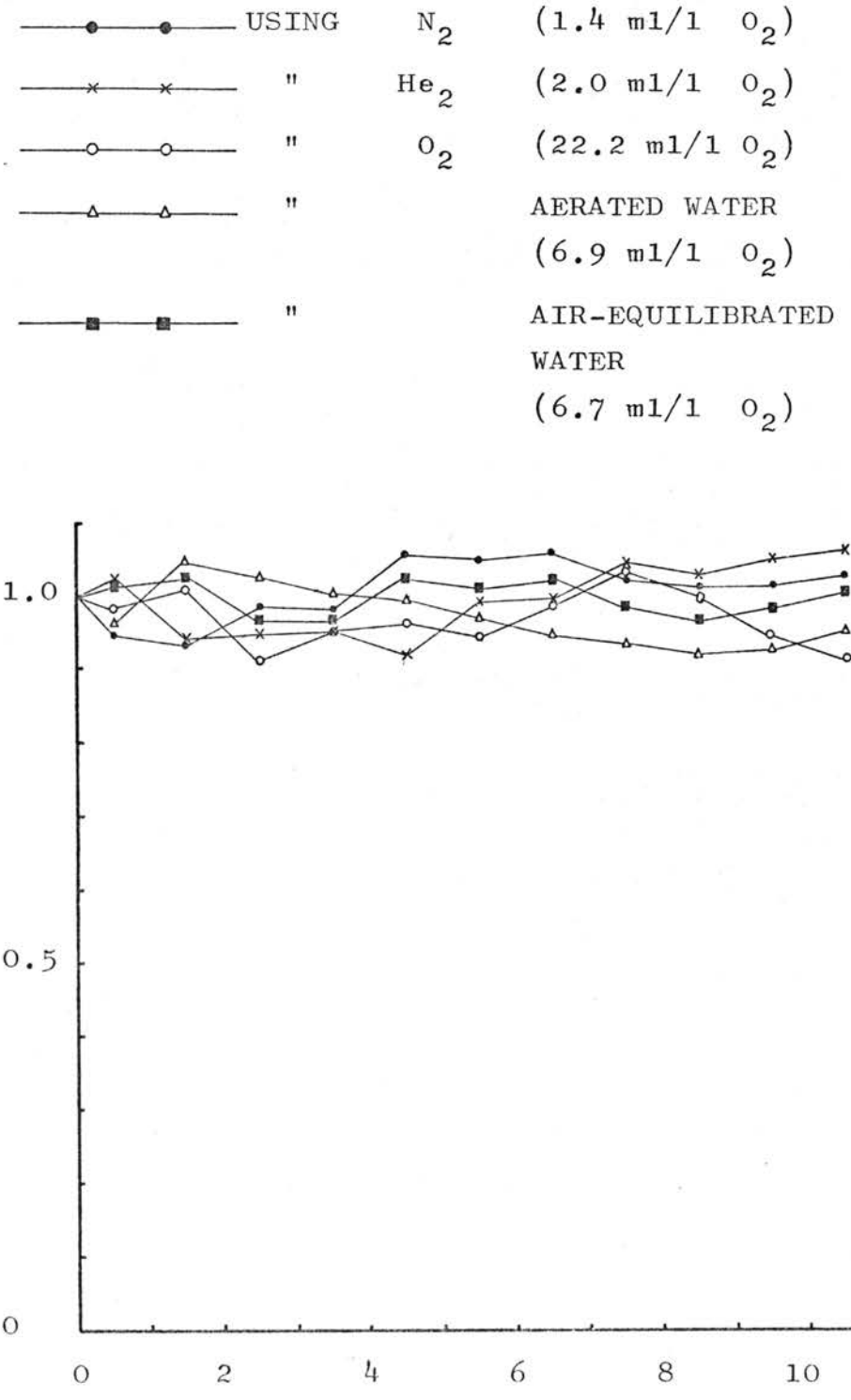


FIGURE 4.27.

EFFECT OF A CHANGE IN THE CONCENTRATION OF DISSOLVED OXYGEN ON THE SUBSEQUENT GROWTH OF THE ROOTS. (30 MINUTE TREATMENTS).



(Each curve represents the mean of at least two experiments).

TABLE 4.6 .

VALUES OF G_{10} TO SHOW THE EFFECT OF DISSOLVED GASES ON THE ULTRASONIC DAMAGE TO THE ROOTS.

	G_{10}
0.70 W/cm^2 CONT. BEAM FOR 30 MIN. IN N_2 (2.5 ml/l O_2)	0.91 ± 0.12
0.65 " " " " " " " N_2 (1.4 ml/l O_2)	0.72 ± 0.09
0.65 " " " " " " " He_2 (2.0 ml/l O_2)	0.83 ± 0.14
0.65 " " " " " " " O_2 (22.2 ml/l O_2)	0.89 ± 0.06
0.68 " " " " " " " AERATED WATER (6.9 ml/l O_2)	1.03 ± 0.17
0.62 " " " " " " " AIR-EQUILIBRATED WATER (6.7 ml/l O_2)	0.72 ± 0.07

Temperature effects.

Bleaney and Oliver (1972 a) have used a 100 μm thermojunction inserted in a single root of Vicia to measure the temperature rise during ultrasonic irradiation. This was found to be only about 2°C for the maximum intensity of 4 W/cm^2 used in their experiments.

In a similar experiment using the root of Zea (as described on p. 152) the maximum temperature rise observed when the root plus junction was moved across the beam was found to be 3.7°C for the 0.82 W/cm^2 continuous beam. For the 0.21 W/cm^2 pulsed beam, the highest temperature rise was found to be 0.9°C. The quoted values of the intensity are average values measured by pressure balance in the absence of the roots and jig, and represent the highest average intensities of the pulsed and continuous beams obtainable with the present equipment. Since the maize roots can be grown at a temperature of 30°C, a temperature rise of about 4°C seems unlikely to be significant. This was confirmed by an experiment in which the root tips of Zea were subjected to a rapid temperature change from 19°C in the culture tank to water at 30°C, 45°C and 60°C respectively, as described earlier (p. 153). The growth curves in Figure 4.28 indicate that a temperature change from 19°C to 30°C (for 15 min.) does not affect the subsequent growth of the roots. For the roots exposed to a temperature of 45°C, however, the growth curve decreases rapidly and reaches a minimum within the first day after

treatment (Fig. 4.28). There was virtually a complete recovery of all the roots in the following two days. The roots exposed to 60°C died immediately. The response of the roots to a sudden temperature change of 45°C may be compared with the curve which represents the corresponding variation in fractional growth rate following sonication at an average intensity of 0.16 W/cm^2 (pulsed beam) for 150 minutes (Fig. 4.29). Figure 4.30 shows the variation of the fractional growth in ten days (G_{10}) for the roots treated for 15 minutes at temperatures of 7, 30, 45 and 60°C respectively. This figure indicates that the threshold for the onset of damage caused by a sudden change in temperature (lasting for 15 min.) lies between 30 and 45°C. When the roots were allowed to grow in water at 7°C (instead of 19°C) they continued to do so at a reduced rate ($\approx$ 2mm/day) but the growth rate returned to normal when the temperature was raised to 19°C. The growth curve for the roots at a temperature of 19°C is shown in Fig. 3.1 .

Roots were also sonicated at the average intensities of 0.82 W/cm^2 (cont. beam) and 0.21 W/cm^2 (pulsed beam), but with the water in the sonication tank at 7°C instead of the normal 18-22°C as described earlier (p. 82). The values of the fractional growth in ten days (G_{10}) observed for the roots treated for various times at this low temperature are listed in Table 4.7 together with the corresponding values obtained when the roots were sonicated in the normal way. There is no sig-

nificant difference between the values of G_{10} obtained for the sonications at the two different temperatures. The shapes of the growth curves were also similar. In Figures 4.31, 4.32 and 4.33 the growth curves obtained when the roots were kept at 7°C for 48 hrs. and were then sonicated (at 7°C) at the average intensities of 0.21 W/cm^2 (pulsed beam) and 0.82 W/cm^2 (cont. beam) respectively, are compared with the ones obtained for the roots grown at 19°C and sonicated at 18 - 22°C. After sonication, both groups of roots were grown at 19°C. The ultrasonic intensity distributions at the position of the roots in water at 7°C were found to be similar to those plotted in water at 18 - 22°C, for the pulsed as well as the continuous beams. The areas under these profile curves (average of three) did not differ by more than 6% from those obtained at the higher temperature. The method for plotting these curves is described elsewhere.

nificant difference between the values of G_{10} obtained for the sonications at the two different temperatures. The shapes of the growth curves were also similar. In Figures 4.31, 4.32 and 4.33 the growth curves obtained when the roots were kept at 7°C for 48 hrs. and were then sonicated (at 7°C) at the average intensities of 0.21 W/cm^2 (pulsed beam) and 0.82 W/cm^2 (cont. beam) respectively, are compared with the ones obtained for the roots grown at 19°C and sonicated at 18 - 22°C. After sonication, both groups of roots were grown at 19°C. The ultrasonic intensity distributions at the position of the roots in water at 7°C were found to be similar to those plotted in water at 18 - 22°C, for the pulsed as well as the continuous beams. The areas under these profile curves (average of three) did not differ by more than 6% from those obtained at the higher temperature. The method for plotting these curves is described elsewhere.

FIGURE 4.28 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) FOLLOWING TREATMENT FOR 15 MIN. IN WATER AT VARIOUS TEMPERATURES AS INDICATED.

Each curve represents the mean of at least two experiments.

For errors, see p. 173 .

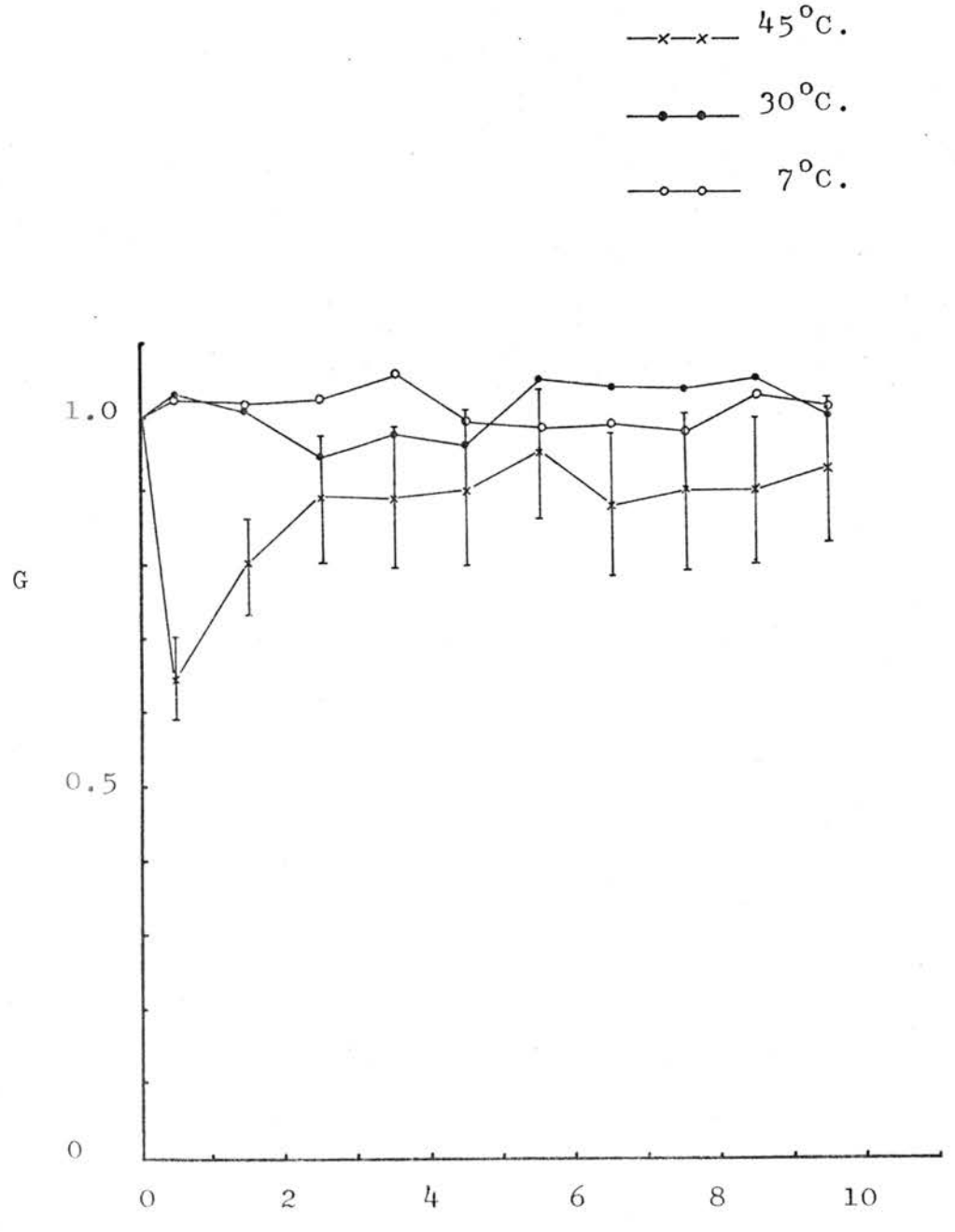


FIGURE 4.29.

COMPARISON OF THE PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER TREATMENT WITH ULTRASOUND AND IN WATER AT A TEMPERATURE OF 45°C RESPECTIVELY.

———— ULTRASONIC RADIATION AT AN AVERAGE INTENSITY OF 0.16 W/cm^2 (PULSED BEAM) FOR 150 MINUTES.

- - - - - - - - - TREATMENT IN WATER AT 45°C FOR 15 MINUTES.

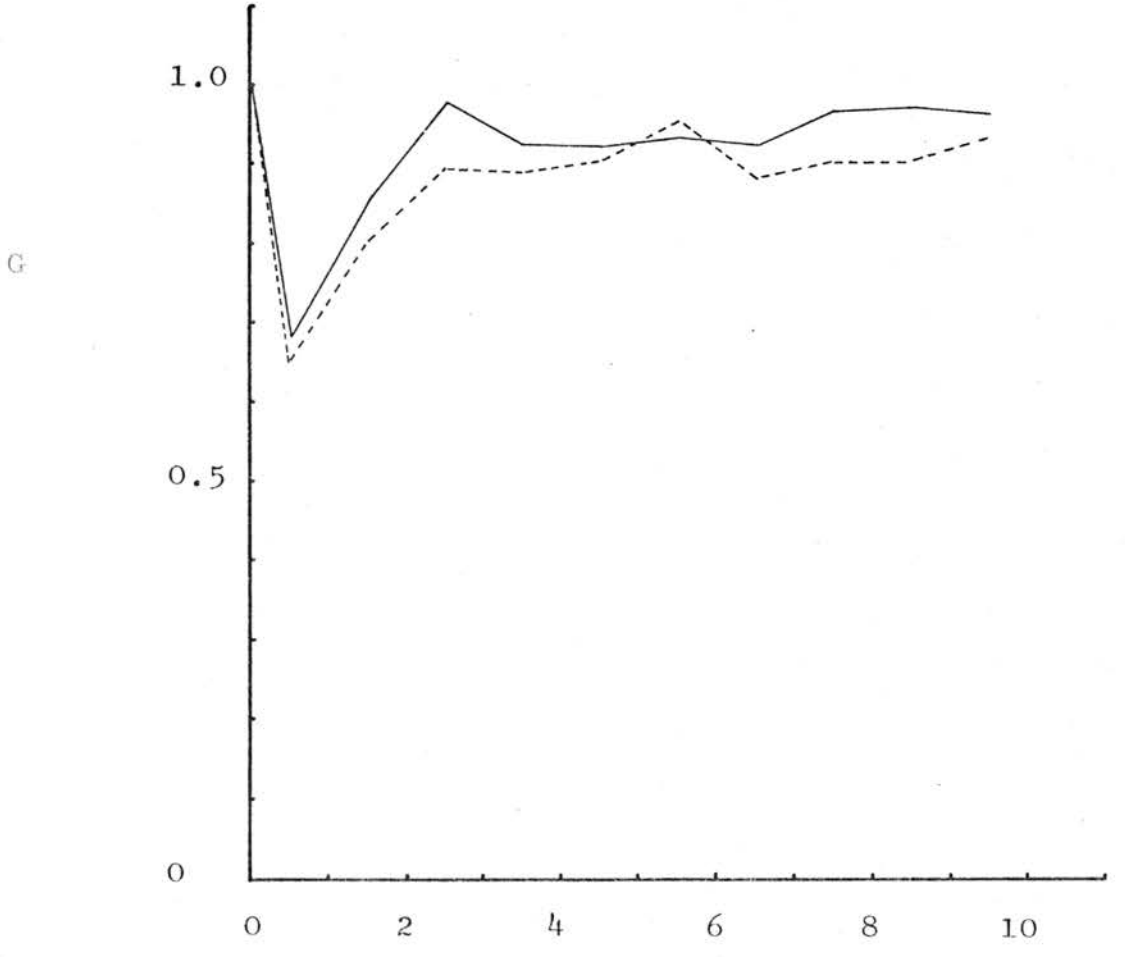


FIGURE 4.30 .

THE VARIATION OF THE FRACTIONAL GROWTH IN TEN DAYS (G_{10}) WITH THE TEMPERATURE TO WHICH THE ROOTS HAVE BEEN EXPOSED FOR 15 MINUTES.

Each value of G_{10} represents the mean of at least 2 experiments. For errors, see p. 173 .

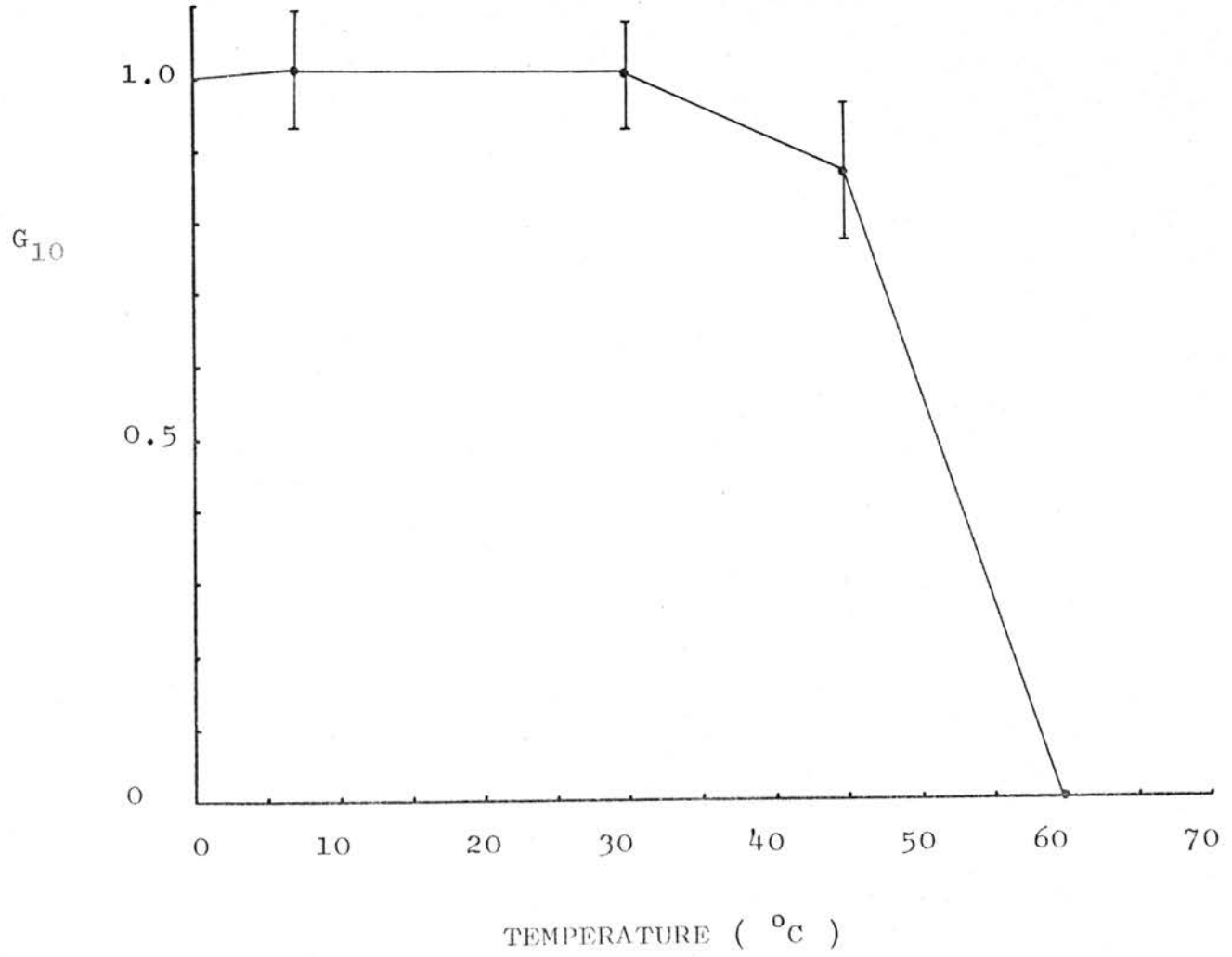


FIGURE 4.31.

EFFECT OF SONICATION OF THE ROOTS AT A LOW TEMPERATURE USING AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM)FOR 30 MINUTES.

Each curve represents the mean of at least two experiments.

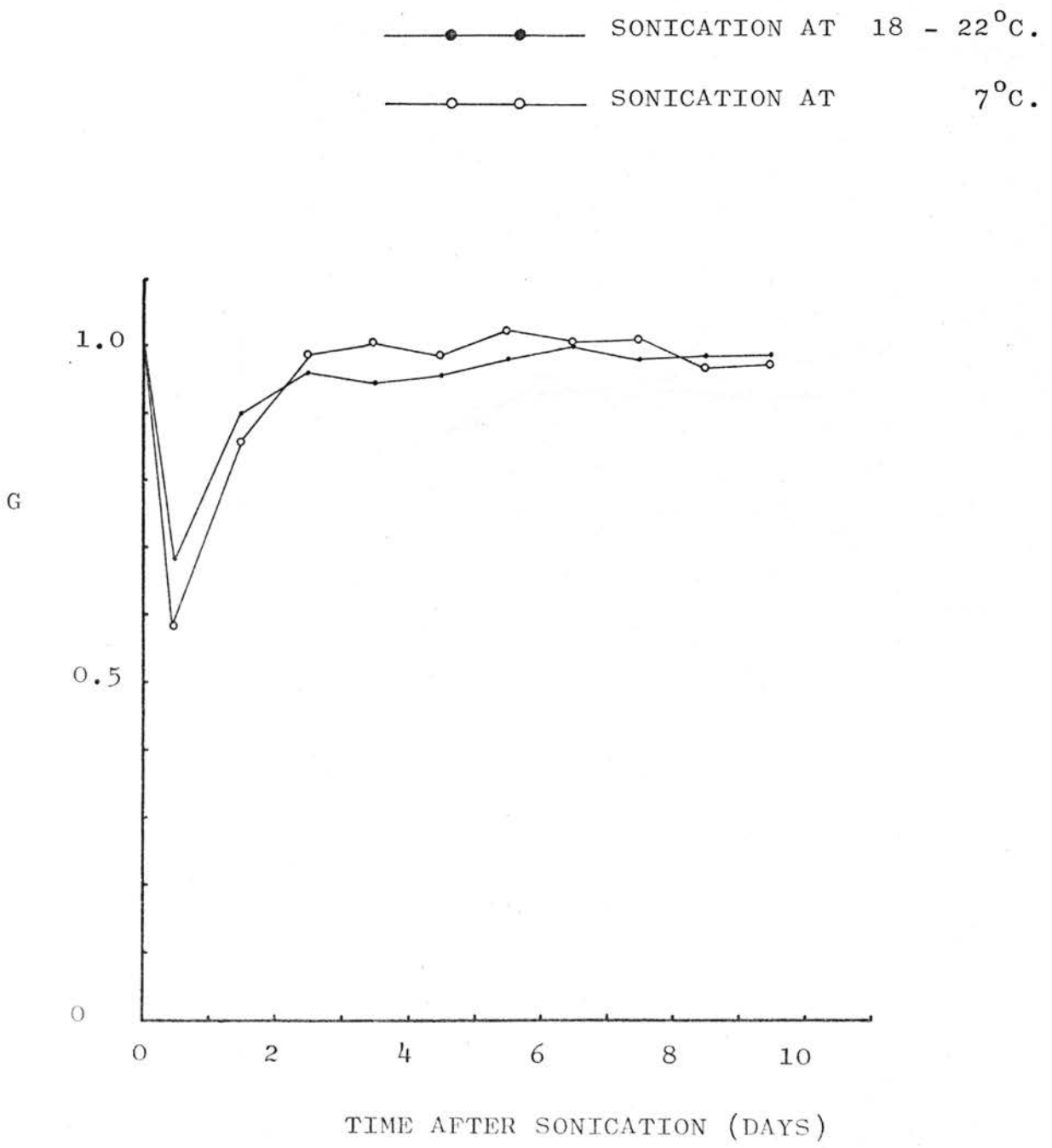


FIGURE 4.32.

AS FOR FIGURE 4.31 , BUT AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR 60 MINUTES.

Each curve represents the mean of at least two experiments.

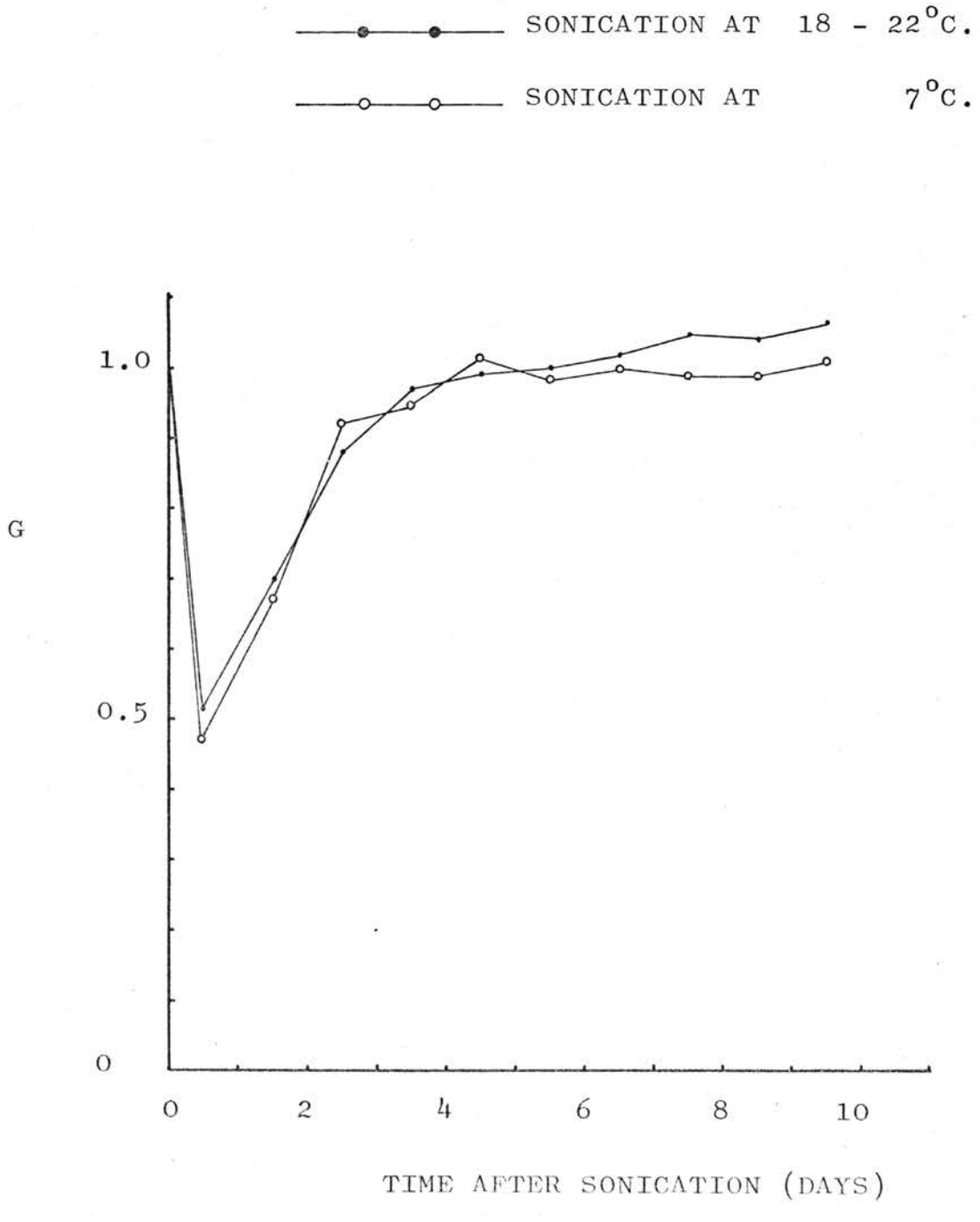


FIGURE 4.33.

AS FOR FIGURE 4.31 , BUT AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) FOR 30 MINUTES.

Each curve represents the mean of at least two experiments.

For errors see p. 173 .

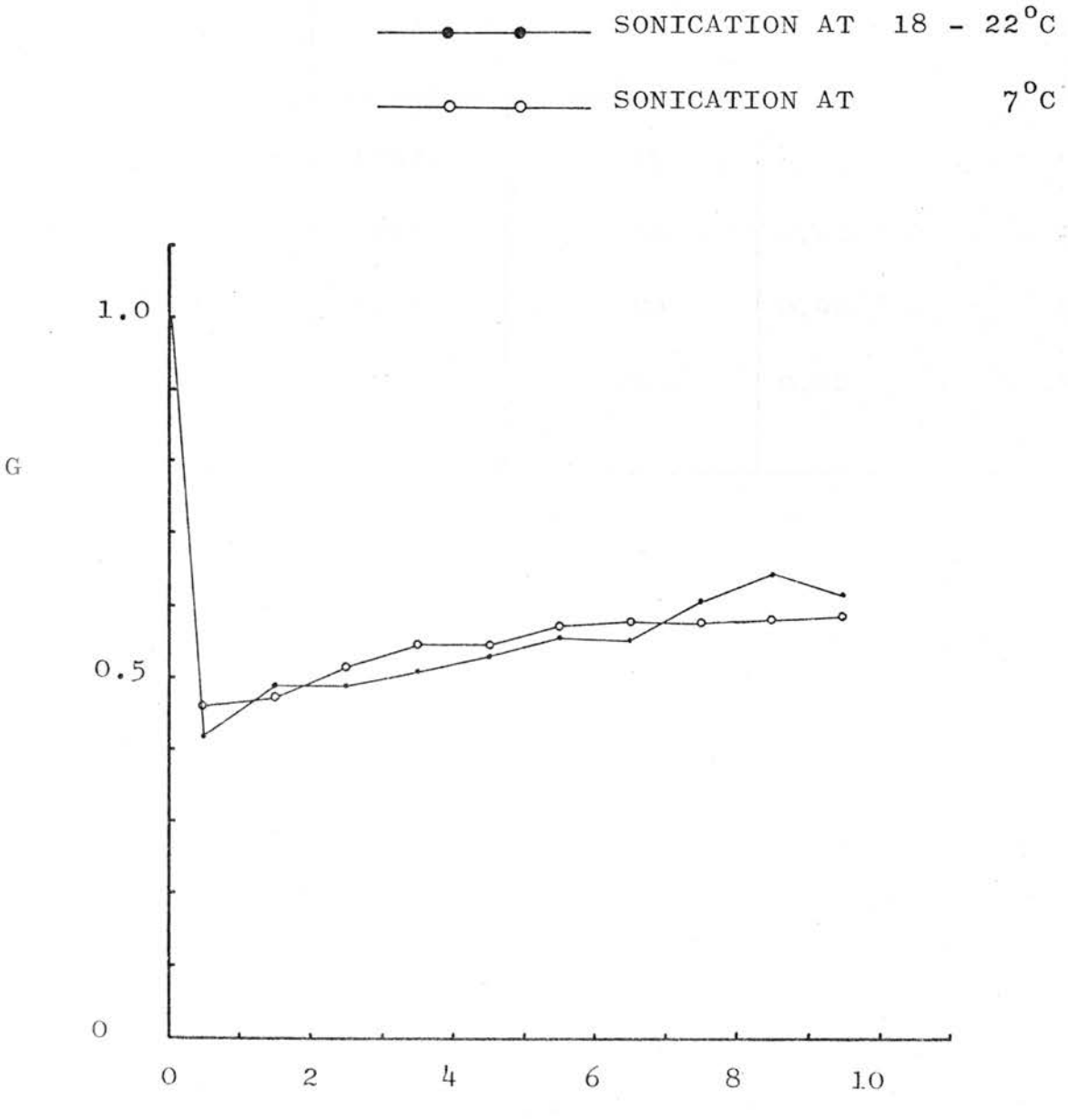


TABLE 4.7 .

EFFECT OF SONICATING THE ROOTS OF ZEA AT A LOW TEMPERATURE ($7^{o}C$).

AVERAGE ULTRASONIC INTENSITY W/cm^2	MODE	SONICATION TIME MIN.	G_{10}	
			$7^{o}C$	18 -$22^{o}C$
0.82	CONT.	15	0.70 ± 0.13	0.68 ± 0.08
0.82	CONT.	30	0.61 ± 0.11	0.56 ± 0.08
0.21	PULSED	60	0.90 ± 0.13	0.92 ± 0.06
0.21	PULSED	180	0.82 ± 0.10	0.86 ± 0.06

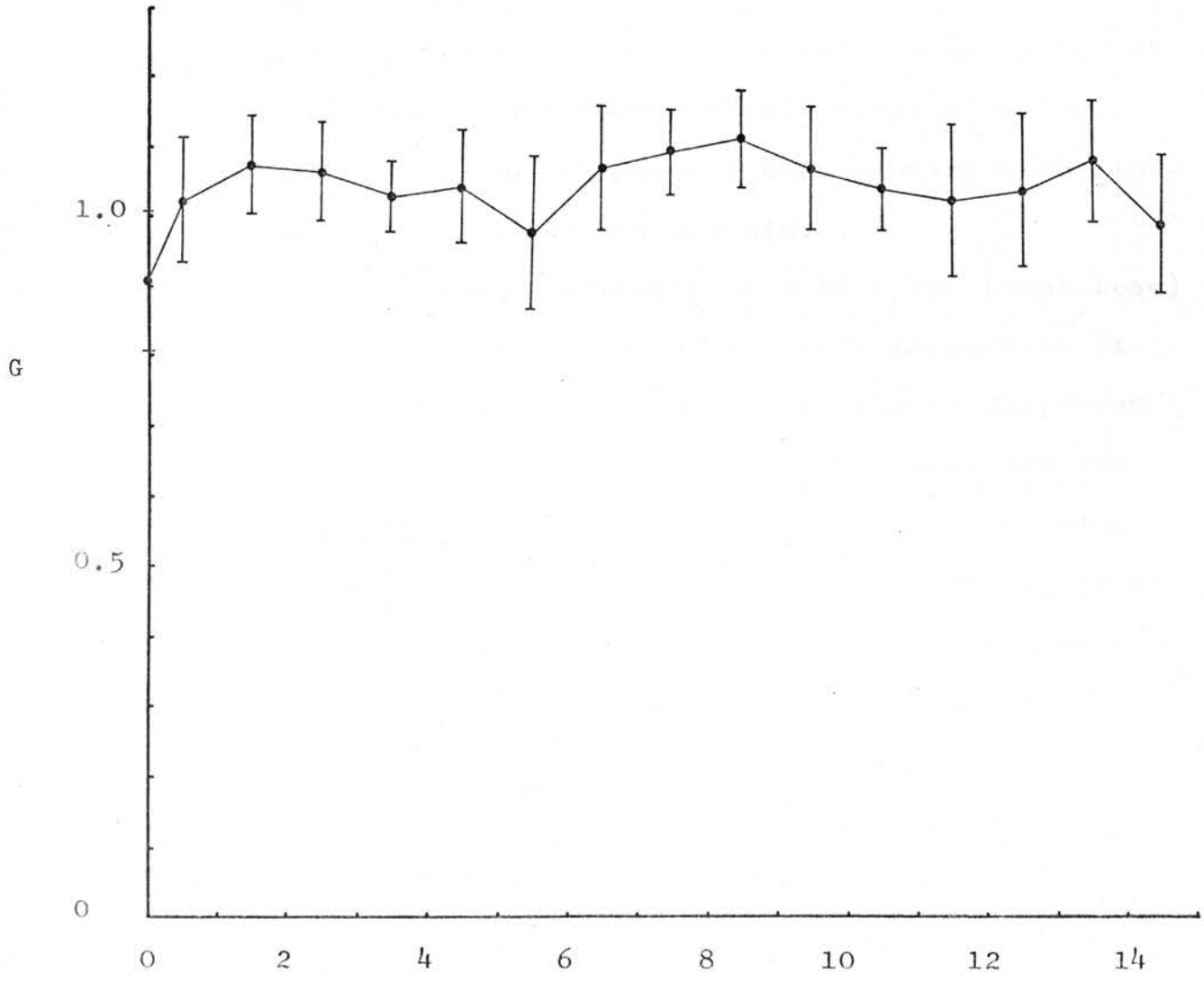
Results of the experiments in which the seeds of Zea were exposed to ultrasound.

All the seeds used in the present experiments germinated, except for one single seed in one of the control groups and there was no delayed germination of the sonicated seeds. Also, all the roots were still growing at the end of two weeks. It may therefore be concluded that the sonication time and average intensity of the beam were not sufficient to induce any "delayed killing" as observed by Smith and Kersten (1941) for ionizing radiation.

The graph of the growth rate as a fraction of controls of equal age (G) as a function of time is given in Figure 4.34 . Similar results were obtained in each of three experiments and thus the results were pooled. Each point in Figure 4.34 represents the mean obtained for the three experiments. The error is expressed as the standard error of the mean for the ratio of the means of the irradiated and cotrol groups.

FIGURE 4.34.

PATTERN OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) FOR THE ROOTS OF ZEA MAYS WHEN THE SEEDS ARE EXPOSED TO ULTRASOUND (0.82 W/cm^2 CONT. BEAM) FOR 60 MIN.



THE RESPONSE OF THE ROOTS OF ZEA TO ULTRASOUND GIVEN IN CONJUNCTION WITH X-RAYS.

Effect of exposing the roots first to ultrasound and then to X-rays or vice versa.

The growth curves obtained for the roots exposed to X-rays on their own, (Figures 4.35 and 4.39) are typical of the response to ionizing radiation as described earlier (Chapter I).

For the X-ray dose of 775 rads used in the present experiments, there was virtually complete recovery of all the roots and the growth curves level off at about 0.8. The few roots that did not recover were growing more slowly than the controls, but did not die.

For the average intensity of 0.82 W/cm^2 (cont.beam) given alone for 30 minutes an appreciable proportion of the roots died and some were growing slowly as discussed previously (p. 170). An additional X-ray dose did not change the number of roots which were dying and growing slowly as a result of the sonication. Although G_{10} is a rather doubtful entity in this case (see p. 78), it has been calculated for these groups as well. The observed values of this parameter, for the various sonication times and intensities delivered in conjunction with the X-ray dose, are listed in Table 4.8 . It seems, therefore, that it is more suitable to assess the effect of the radiation on the roots in terms of G_{min} rather than in terms of G_{10}

(G_{min} and G_{10} are defined on page 78).

The errors in these experiments are expressed as the standard error of the mean for the ratio of the means of the irradiated and control groups (10 roots in each group). In order to preserve clarity of presentation, error bars have been omitted on most of the graphs, but were always of the same order as those shown in Figure 4.39.

For the ultrasonic intensities which, on their own, had virtually no observable effect on the growth of the root, the additional X-ray dose given before or after sonication resulted in growth curves similar to that obtained when the dose of X-rays was given alone. There is, however, a more rapid decrease in growth in the first day due to the immediate reduced growth resulting from sonication (Figures 4.35 and 4.36), and which has been described previously (p. 168). The subsequent rapid recovery, characteristic for roots treated with ultrasound, is modified by the effect of the X-ray dose given and the growth curve reaches a minimum equal to that if the X-rays were given on their own. For both the pulsed and the continuous beams applied for 5 and 2 minutes respectively, the growth curves are similar on the addition of a dose of 775 rads of X-rays.

For the longer sonication times (pulsed or continuous beams), the growth curves show a greater immediate drop in growth rate after sonication. This is similar to that observed when the ultrasonic radiation is applied to the roots alone (Figures 4.37 and 4.38). The recovery of

the roots after the first day post-irradiation is again modified by the X-ray dose given before or after sonication, and no effect of the latter is observed in the growth curve from about the third day onwards.

Effect of exposing the roots to ultrasound and X-rays, both radiations being delivered simultaneously.

In these experiments the roots were placed parallel to the X-ray beam axis as described earlier and the shape of the growth curve obtained when the roots were exposed in this position to 775 rads of X-rays alone, is found to be similar to the curve obtained with the roots irradiated in the "normal" way (i.e. orthogonal to the beam axis) as shown in Figures 4.35 and 4.39.

For all the experiments in which the roots were exposed to X-rays together with ultrasonic doses which did not produce a large damage (i.e. the growth rate did not decrease to below the minimum attained by X-rays when both radiations were applied separately on two different groups of roots), the growth curves are all similar to that when the roots are treated with X-rays only as shown in Figures 4.39 and 4.40.

The growth curve for the roots when irradiated to an X-ray dose of 775 rads in 60 mins. and at an average ultrasonic intensity of 0.21 W/cm^2 (pulsed beam) for 60 mins. (given at the same time), shows again the immediate drop in growth rate due to sonication. The subsequent

rapid recovery is modified by the effect of the X-ray dose given and the growth curve reaches a minimum equal to that if the X-rays were given on their own (Fig.4.41). For the average intensity of 0.82 W/cm^2 (cont. beam) given in conjunction with the X-ray dose, a growth curve was obtained indicating a rapid initial decrease in growth rate but the damage due to the X-ray dose is hardly apparent in this curve (Fig.4.42).

FIGURE 4.35.

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER IRRADIATION WITH ULTRASOUND AT THE AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) AND 775 RADS OF X-RAYS, THE RADIATIONS BEING DELIVERED SEPARATELY.

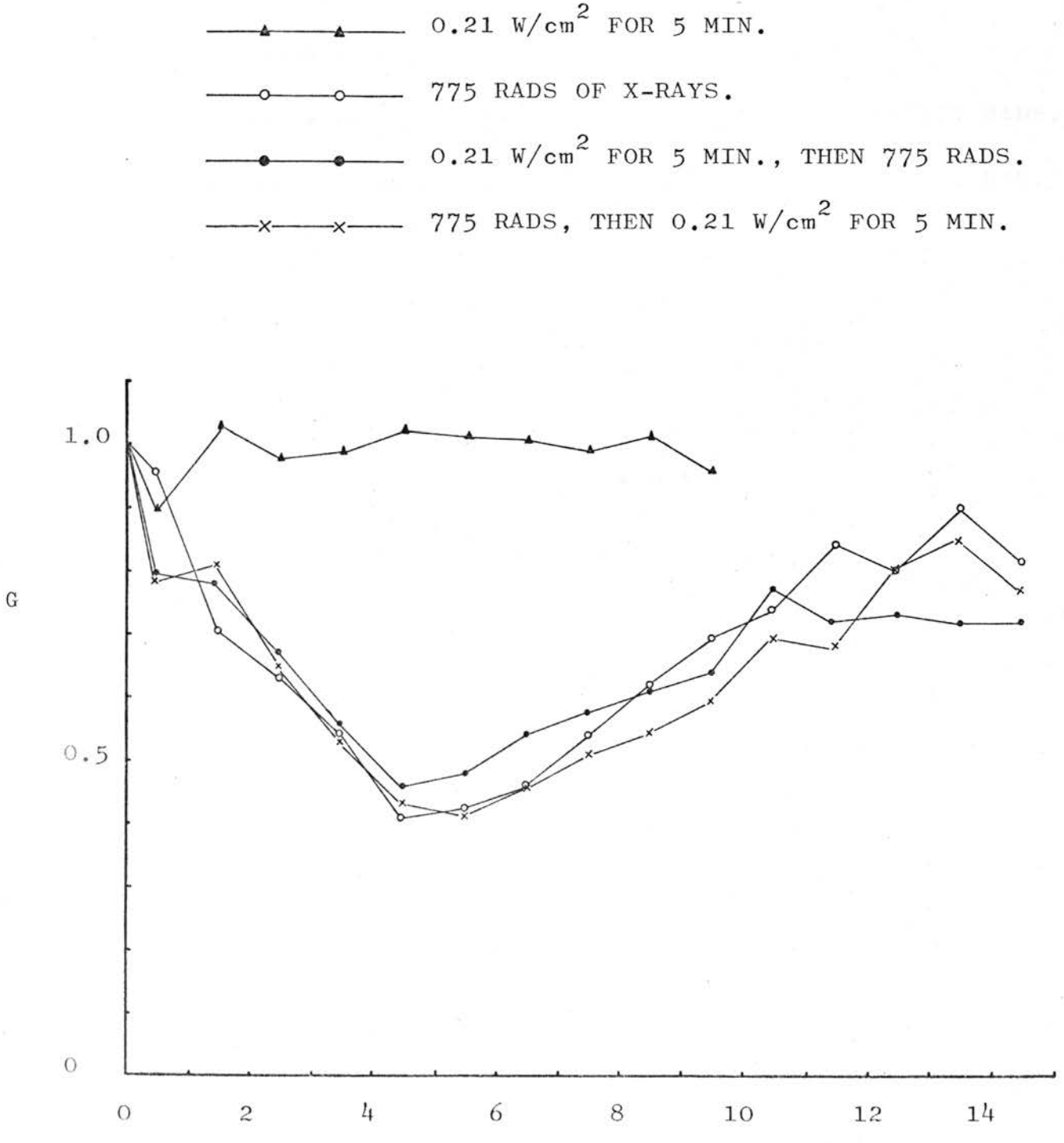


FIGURE 4.36.

AS FOR FIGURE 4.35, BUT AT AN AVERAGE INTENSITY OF 0.62 W/cm^2 (CONT. BEAM) AND 775 RADS OF X-RAYS.

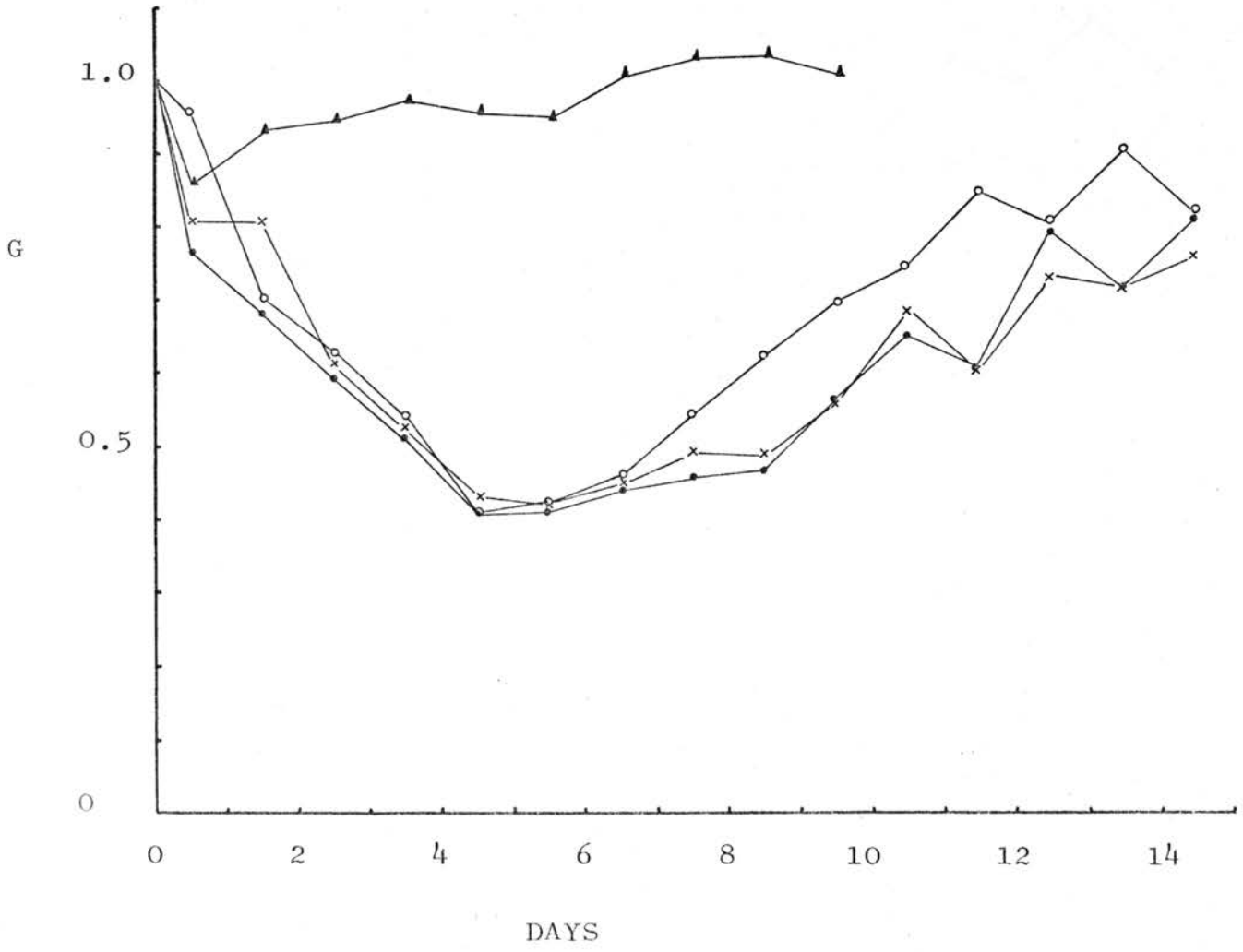


FIGURE 4.37 .

AS FOR FIGURE 4.35 , BUT AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) AND 775 RADS OF X-RAYS.

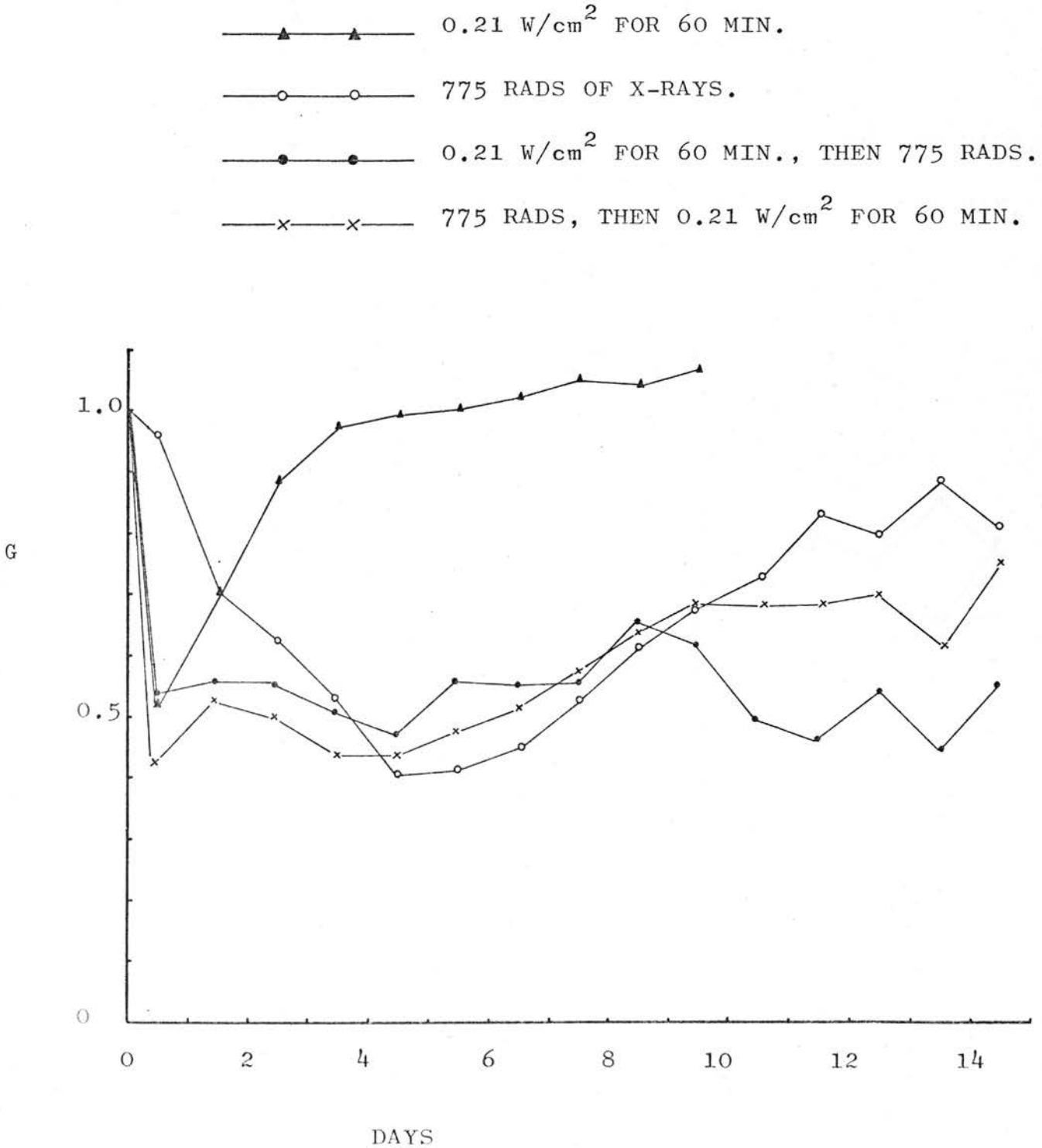


FIGURE 4.38 .

AS FOR FIGURE 4.35 , BUT AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) AND 775 RADS OF X-RAYS.

—▲—▲— 0.82 W/cm^2 FOR 30 MIN.

—○—○— 775 RADS OF X-RAYS.

—●—●— 0.82 W/cm^2 FOR 30 MIN., THEN 775 RADS.

—×—×— 775 RADS, THEN 0.82 W/cm^2 FOR 30 MIN.

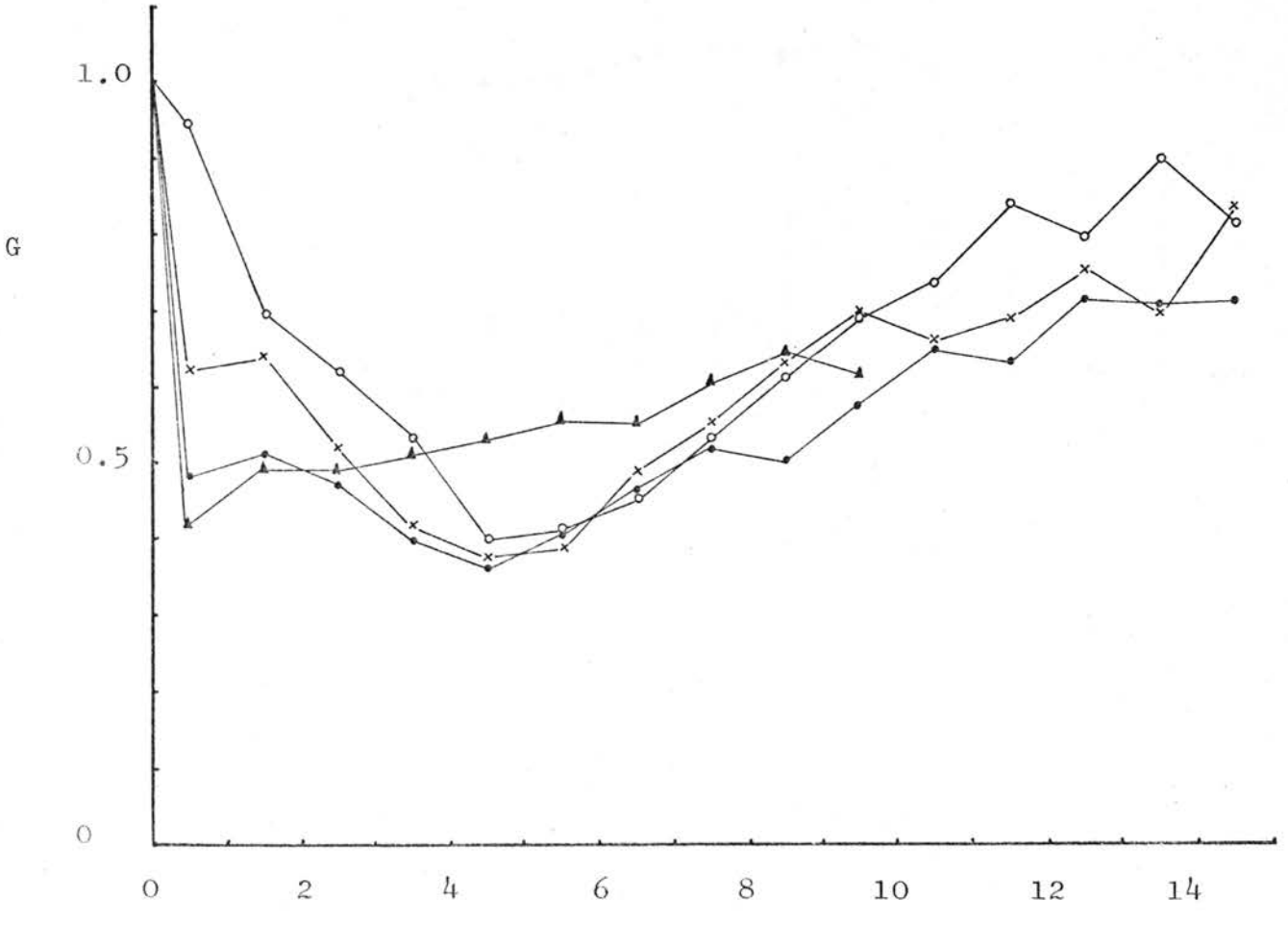


FIGURE 4.39 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER IRRADIATION WITH ULTRASOUND AT THE AVERAGE INTENSITY OF 0.05 W/cm^2 (PULSED BEAM) AND 775 RADS OF X-RAYS, THE RADIATIONS BEING DELIVERED SIMULTANEOUSLY.

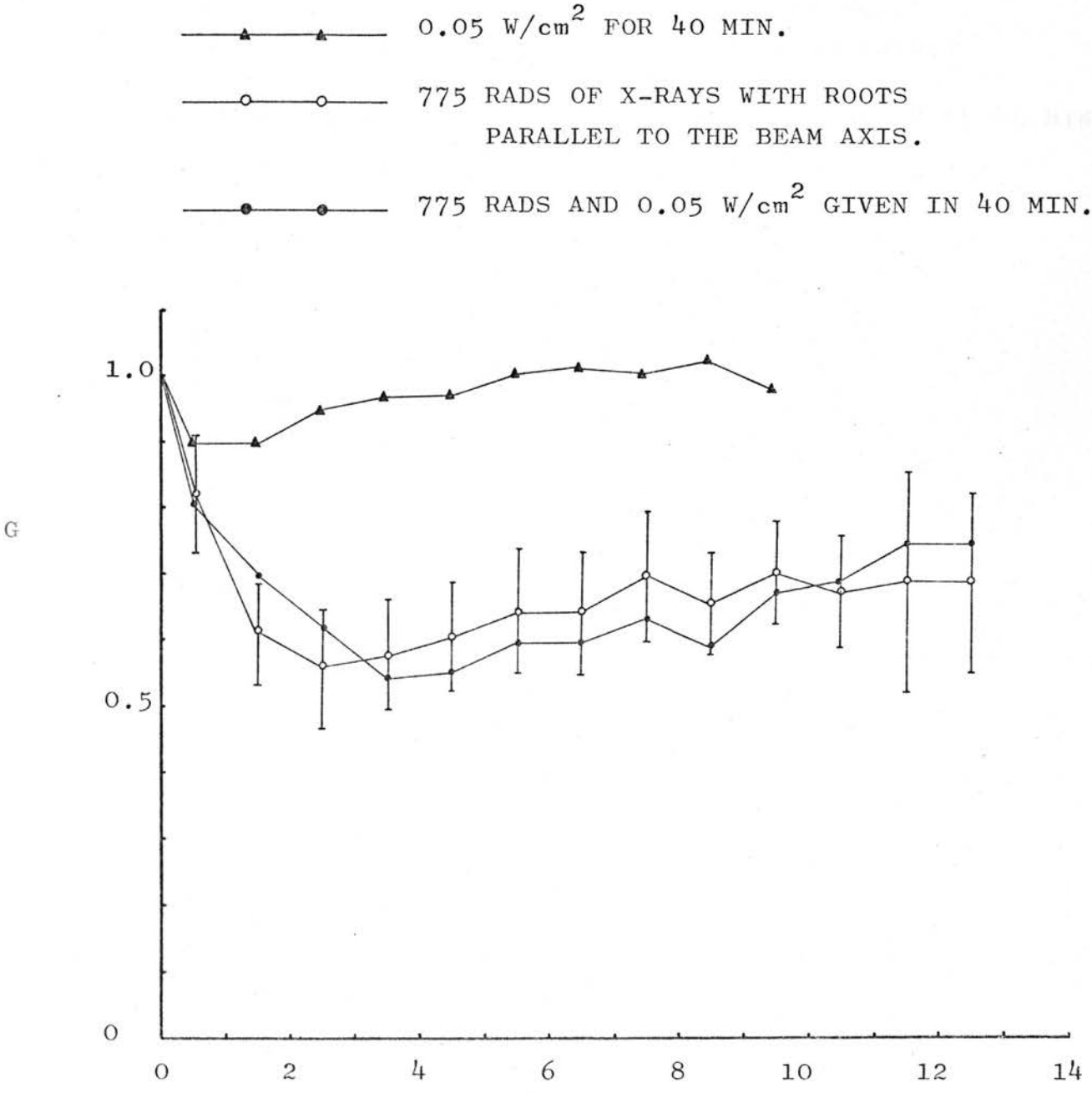


FIGURE 4.40 .

AS FOR FIGURE 4.39 , BUT AT AN AVERAGE INTENSITY OF 0.10 W/cm^2 (CONT. BEAM) AND 775 RADS OF X-RAYS.

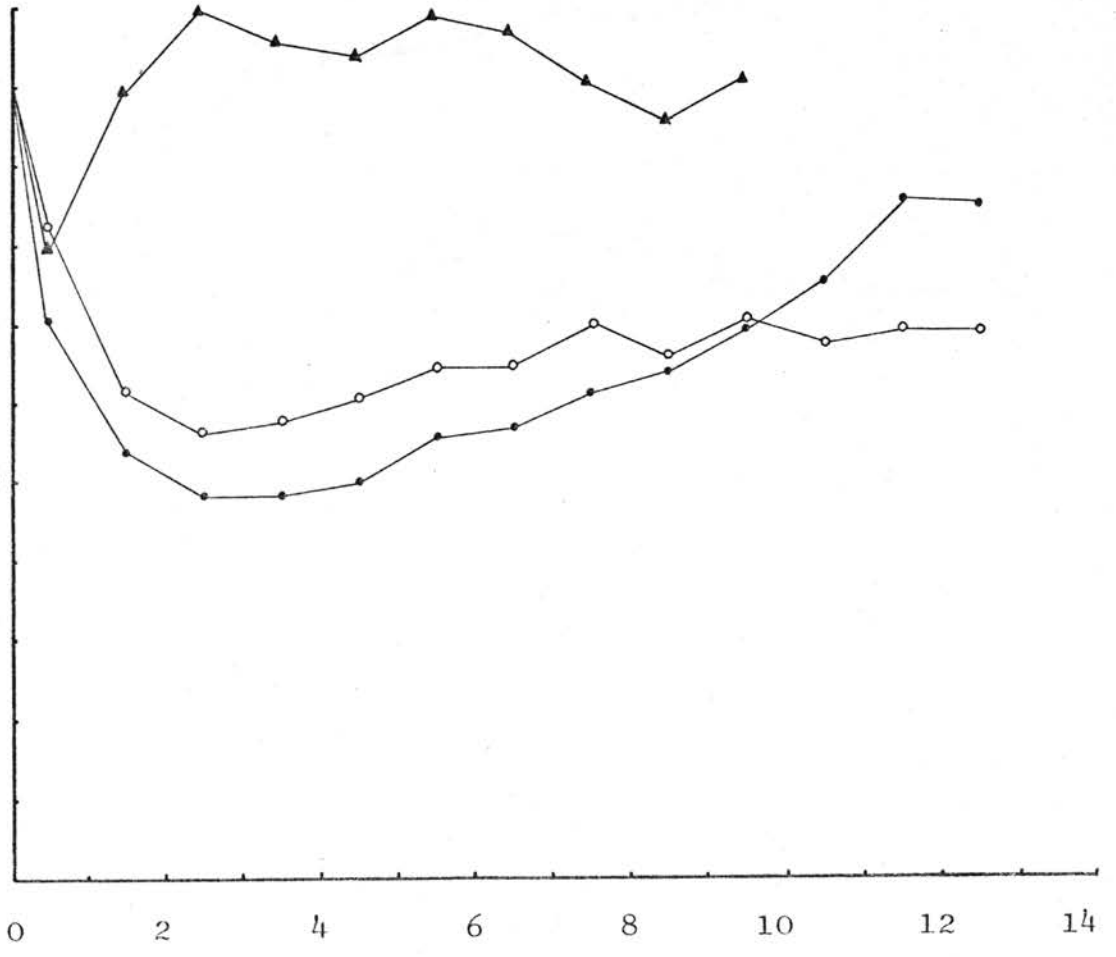


FIGURE 4.41.

AS FOR FIGURE 4.39, BUT AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) AND 775 RADS OF X-RAYS.

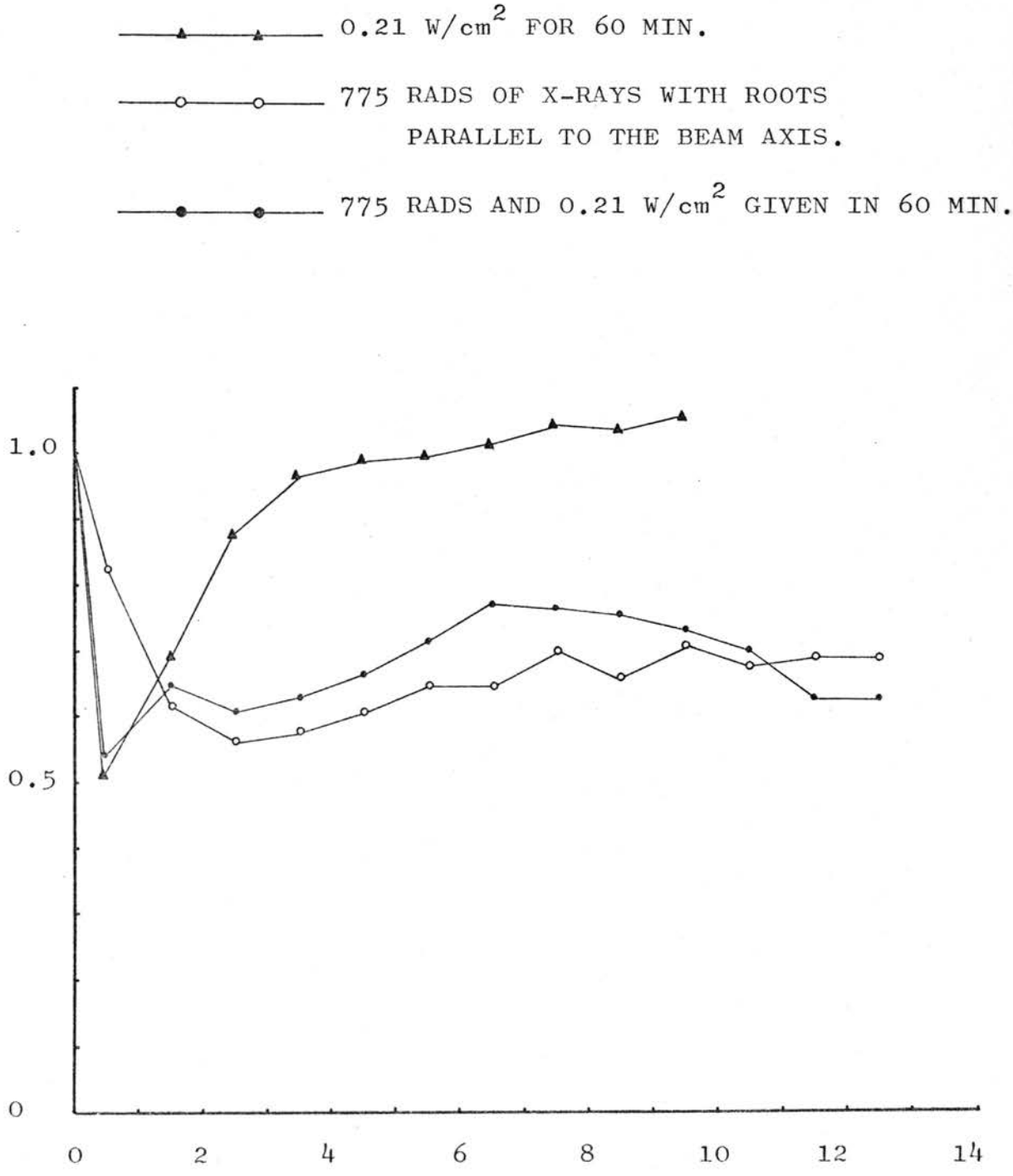


FIGURE 4.42 .

AS FOR FIGURE 4.39 , BUT AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) AND 775 RADS OF X-RAYS.

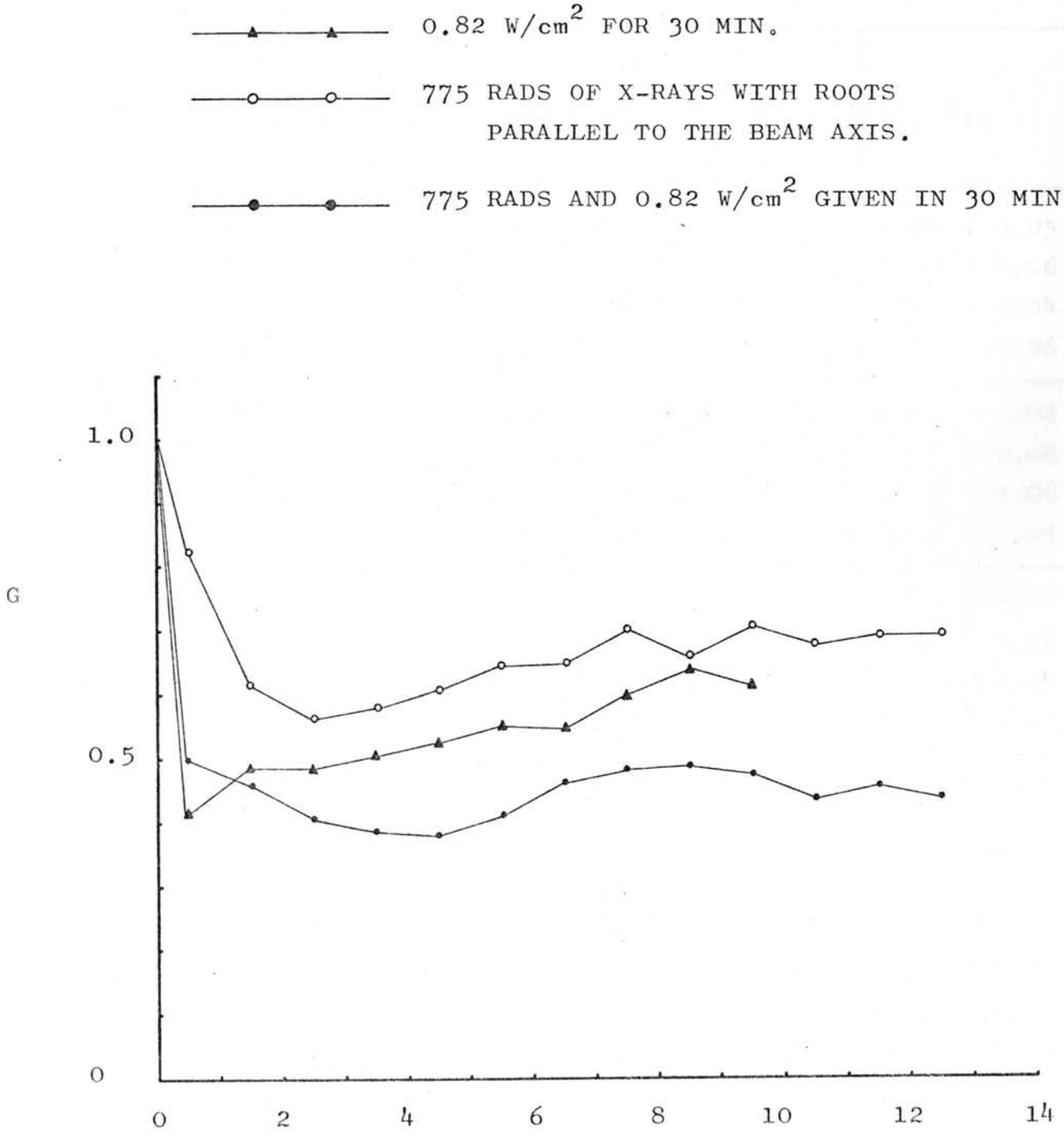


TABLE 4.8 .

AVERAGE GROWTH OF ROOTS IN TEN DAYS POST-IRRADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS (G_{10}).

AVG. ULTRA-SONIC INT. W/cm^2	MODE	SONICATION TIME MIN.	X-RAY DOSE RADS	G_{10}
0.21	PULSED	5	-	0.99 ± 0.05
-	-	-	775	0.59 ± 0.06
0.21	PULSED	5	775 AFTER SONICATION	0.59 ± 0.04
0.21	PULSED	5	775 BEFORE SONICATION	0.57 ± 0.06
0.62	CONT.	2	-	0.97 ± 0.06
-	-	-	775	0.59 ± 0.06
0.62	CONT.	2	775 AFTER SONICATION	0.48 ± 0.06
0.62	CONT.	2	775 BEFORE SONICATION	0.55 ± 0.05
0.21	PULSED	60	-	0.92 ± 0.06
-	-	-	775	0.59 ± 0.06
0.21	PULSED	60	775 AFTER SONICATION	0.57 ± 0.09
0.21	PULSED	60	775 BEFORE SONICATION	0.52 ± 0.05
0.82	CONT.	30	-	0.56 ± 0.08
-	-	-	775	0.59 ± 0.06
0.82	CONT.	30	775 AFTER SONICATION	0.47 ± 0.07
0.82	CONT.	30	775 BEFORE SONICATION	0.53 ± 0.06
0.21	PULSED	60	775 SIMULTANEOUSLY	0.68 ± 0.09
0.82	CONT.	30	" "	0.44 ± 0.11
-	-	-	775 (ROOTS PARALLEL TO BEAM AXIS.)	0.66 ± 0.08
0.05	PULSED	40	775 SIMULTANEOUSLY	0.63 ± 0.05
0.10	CONT.	40	" "	0.57 ± 0.06
0.05	PULSED	40	-	0.98 ± 0.10
0.10	CONT.	40	-	1.02 ± 0.09

THE RESPONSE OF THE ROOTS OF ZEA TO ULTRASOUND GIVEN IN CONJUNCTION WITH VINCRISTINE.

Effect of vincristine on the roots.

The two vincristine treatment times chosen, viz. one hour and two hours, resulted in growth curves as shown in Figure 4.43. The shapes of the curves for the two treatment times seem to be identical and are characterized by an initial decrease in growth followed by a gradual recovery. The minimum in growth rate as a fraction of controls (G_{min}) is reached at about 2 - 3 days after treatment in both cases. The value of G_{min} (defined on p. 78) for the roots treated for 2 hours (≈ 0.2) is, however, much less than the value of G_{min} for the roots treated for 1 hour (≈ 0.5). For the 1 hr. treatment there was virtually a complete recovery of all the roots and the growth curve levels off at about 0.95. For the two-hour treatment the recovery was not complete and the growth curve levels off at about 0.45. It was found that in both experiments in which the roots were treated with vincristine for 2 hrs., only about half of the roots recovered. For those roots that did recover, the growth rate was about equal to that of the control roots at about ten days after treatment. The growth rate of the roots that did not recover continued to decrease and growth virtually ceased after ten days. The diameter of such roots had increased by about 25% (as compared to the average diameter of the control roots at this stage) at the time when growth ceased. The

response of the roots to vincristine treatment may be compared with the curve which represents the corresponding variation in fractional growth rate following an X-radiation dose of 775 rads (Figure 4.44).

Effect of low ultrasonic exposures given in conjunction with vincristine treatments.

For the average ultrasonic intensities which, on their own, had virtually no observable effect on the growth of the roots, the additional vincristine treatment for 1 or 2 hours before or after sonication resulted in growth curves similar to the ones obtained when vincristine treatment was given alone (Figures 4.45, 4.46, 4.47 and 4.48).

For both the pulsed and continuous beams applied for 5 and 2 minutes respectively, the additional vincristine treatment for 1 hour before or after sonication resulted in growth curves with values of the minimum growth rate as a fraction of controls (G_{min}), which seem to be slightly less than that obtained when vincristine treatment was given alone (Figures 4.45 and 4.46). There is also an indication of a more rapid decrease in growth rate during the first day in the curves obtained for a vincristine treatment of 1 hr.

Effect of higher ultrasonic exposures given in conjunction with vincristine treatments.

For the longer sonication times (pulsed or continuous beams) used in conjunction with vincristine treatments of one or two hours, the growth curves show a more rapid decrease in growth in the first day due to the immediate reduced growth resulting from sonication (Figures 4.50 - 4.53).

For the vincristine treatment of 1 hour given in conjunction with the pulsed ultrasonic irradiation at an average intensity of 0.21 W/cm^2 for 60 min. the subsequent rapid recovery, characteristic for roots treated with ultrasound, is modified by the effect of the vincristine treatment given and the growth curves reach a minimum equal to that attained if the vincristine treatment was given alone (Figure 4.50). No effect of the ultrasound is observed in these growth curves from about the second day onwards.

The values of G_{min} of the growth curves obtained for a vincristine treatment of 1 hour and the continuous ultrasonic beam at an average intensity of 0.82 W/cm^2 for 30 min., seem to be slightly less than that of the curve obtained when vincristine treatment was given alone.

For the 2-hour vincristine treatments given in conjunction with the pulsed (at an average intensity of 0.21 W/cm^2 for 60 min.) or continuous (at an average intensity of 0.82 W/cm^2 for 30 min.) ultrasonic beams, the growth curves are all similar to those when the roots are

treated with vincristine only, except for a more rapid decrease in growth in the first day resulting from sonication (Figures 4.52 and 4.53).

The various sonication times and the average intensities of the pulsed and continuous beams delivered in conjunction with the vincristine treatments, are listed in Tables 4.9 and 4.10 . Each value of G_{10} in these tables represents the mean obtained for at least two experiments. Although G_{10} is a rather doubtful entity if an appreciable proportion of the roots in a group die and/or grow only slowly as described earlier, the values of G_{10} have been calculated for these cases as well. It seems, therefore, that it may be more suitable to assess the effects of ultrasound and vincristine on the roots in terms of G_{min} rather than in terms of G_{10}. The vincristine treatment did not change the number of roots that were growing slowly or were dying because of treatment with ultrasound when the ultrasound was the dominating modality. The ultrasonic dose on the other hand, did not change the number of roots that were dying because of the vincristine treatment when the vincristine was the dominating modality.

The errors in Figure 4.43 are standard errors of the means for the ratios of the means of the treated and control groups. The error bars on all other graphs have been omitted in order to preserve clarity of presentation, but were always of the same order as those depicted. Each experiment was repeated at least once

and all graphs drawn represent the mean obtained for at least two experiments. The two graphs in Figure 4.54 were both obtained for roots of Zea treated with vincristine for 1 hr. as described earlier. The curves indicate the consistency in the results obtained when experiments were repeated. The consistency in all the other experiments was of the same order as that depicted.

In Figures 4.55 and 4.56 some of the growth curves obtained when X-rays were used in conjunction with ultrasound are compared with those obtained when vincristine was used in combination with ultrasound.

FIGURE 4.43.

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER TREATMENT WITH VINCRISTINE (0.004% SOLUTION) FOR 1 AND 2 HOURS RESPECTIVELY.

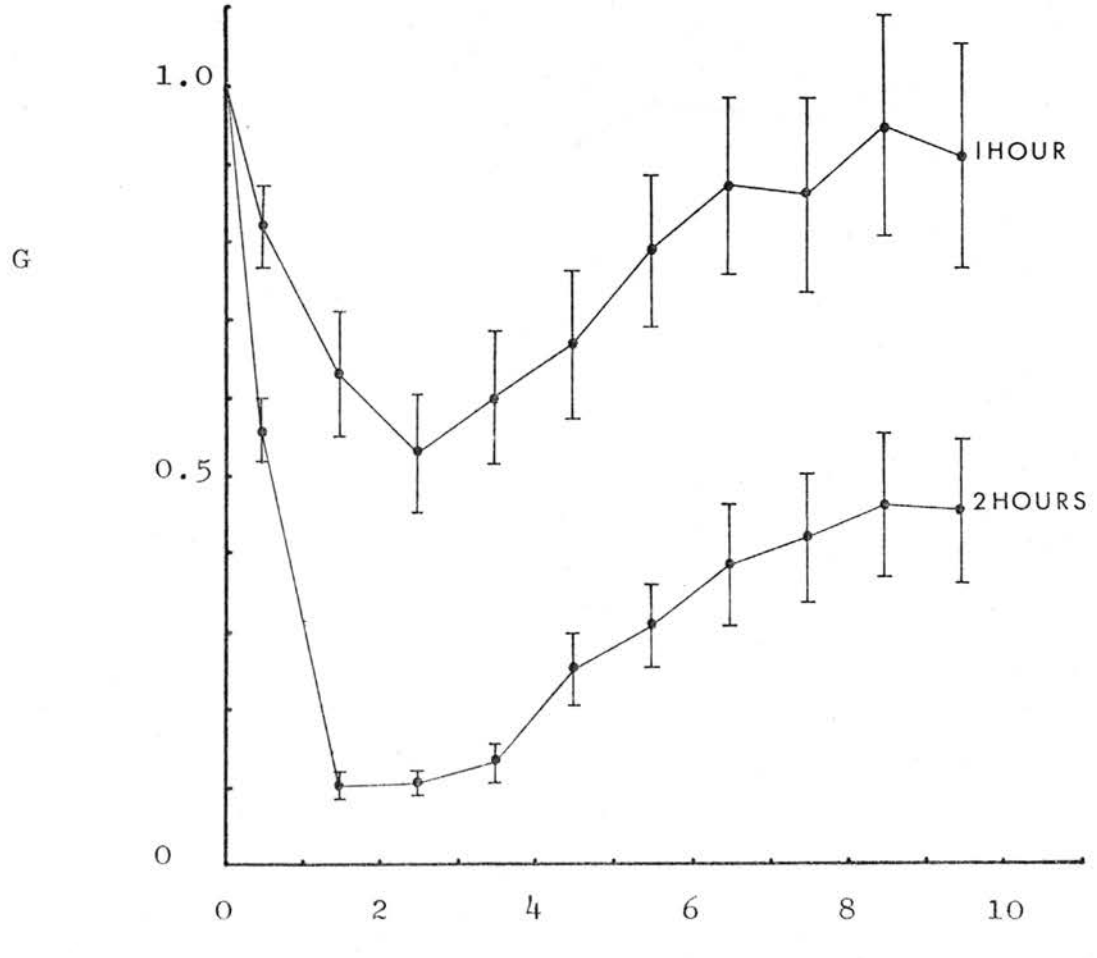


FIGURE 4.44 .

COMPARISON OF THE PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) WHEN THE ROOTS OF ZEA ARE TREATED WITH ULTRASOUND, X-RAYS AND VINCRISTINE RESPECTIVELY.

EXPOSURE TO ULTRASOUND
(0.21 W/cm^2 PULSED FOR 150 MIN.).

EXPOSURE TO X-RAYS
(775 RADS).

VINCRISTINE TREATMENT
(0.004% SOLUTION FOR 2 HRS.).

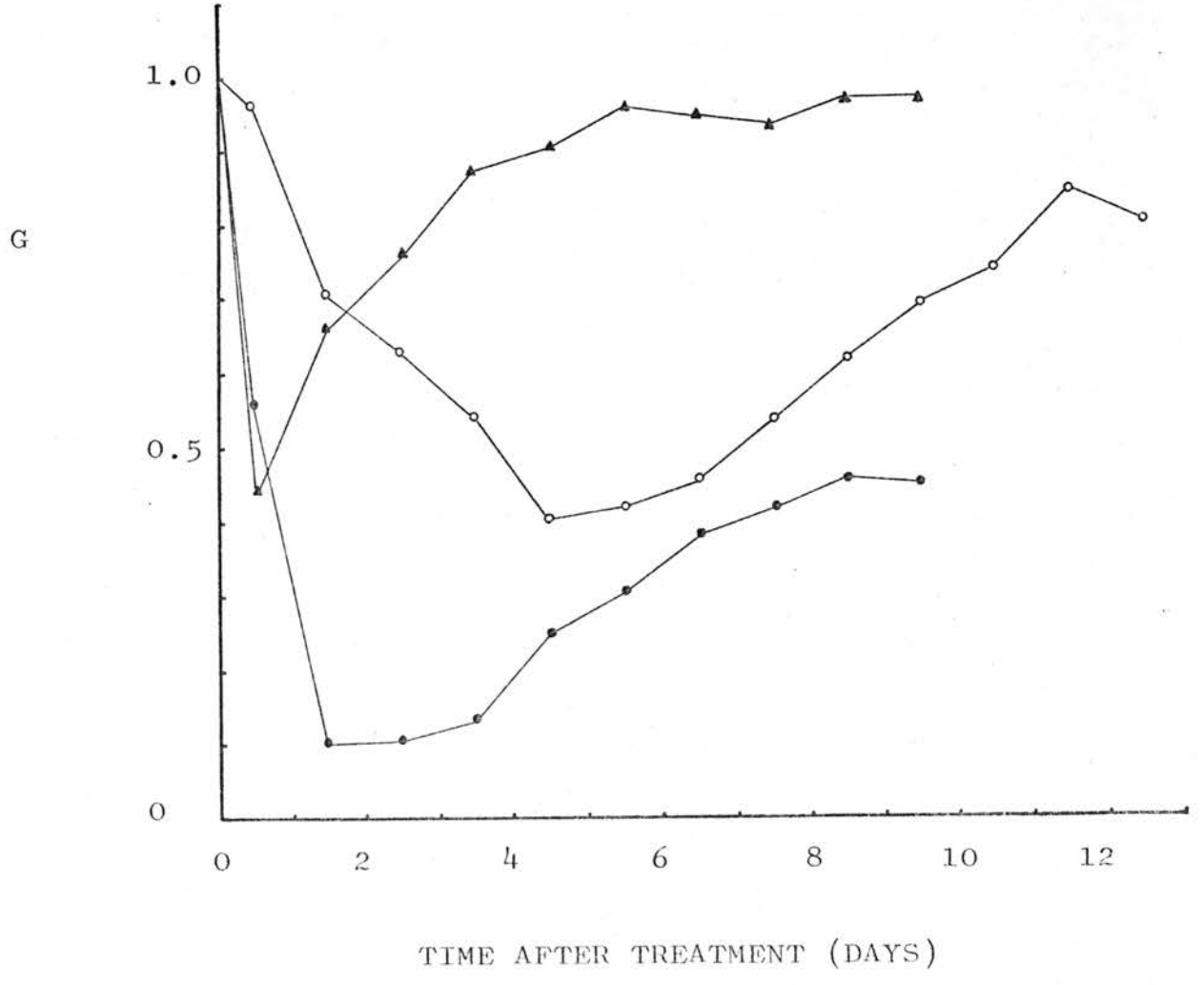


FIGURE 4.45 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER TREATMENT WITH VINCRISTINE FOR 1 HOUR AND SONICATION AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 FOR 5 MIN. (PULSED BEAM).

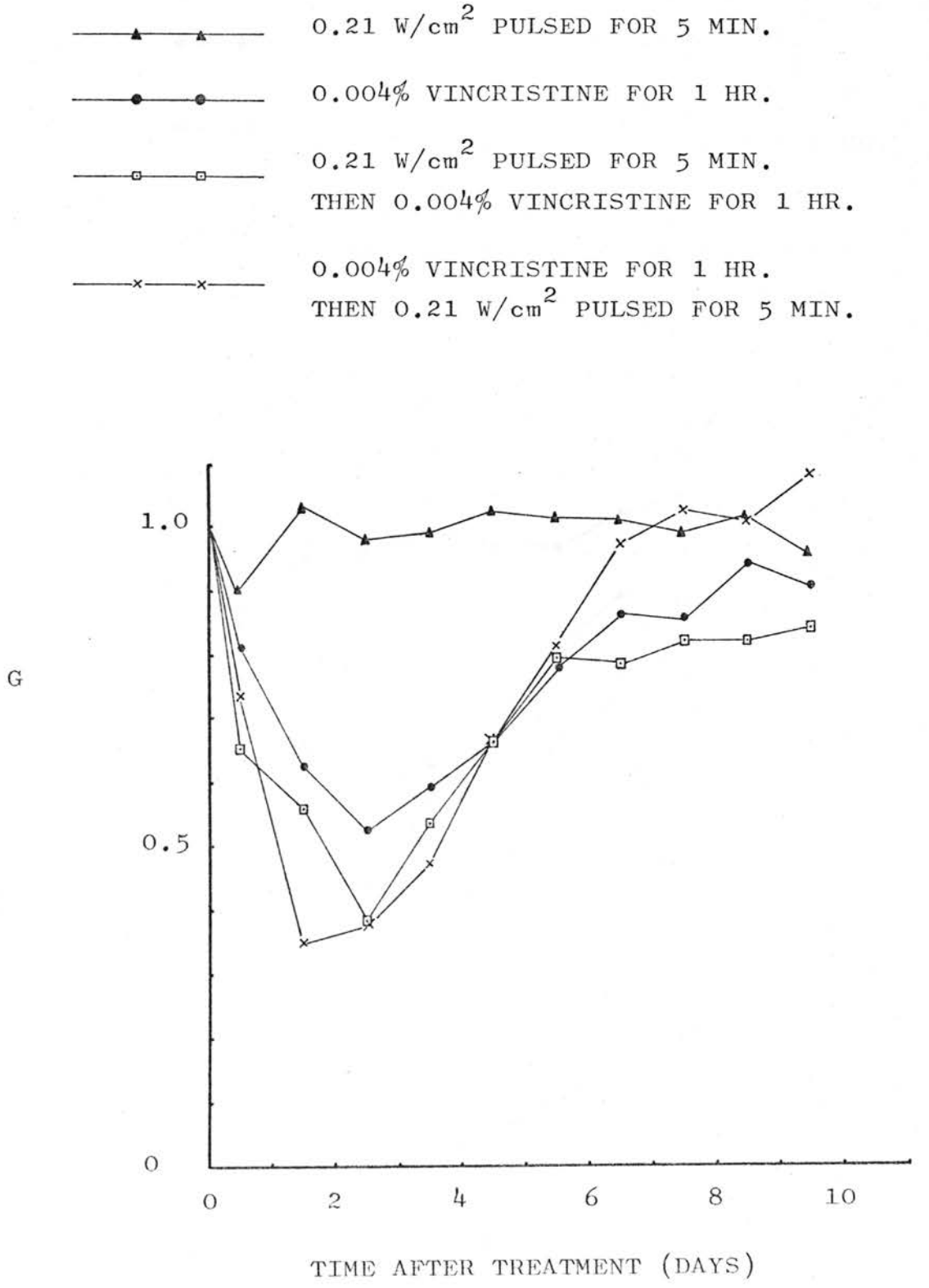


FIGURE 4.46.

AS FOR FIGURE 4.45, BUT AT AN AVERAGE ULTRASONIC INTENSITY OF 0.62 W/cm^2 (CONT. BEAM) FOR 2 MIN.

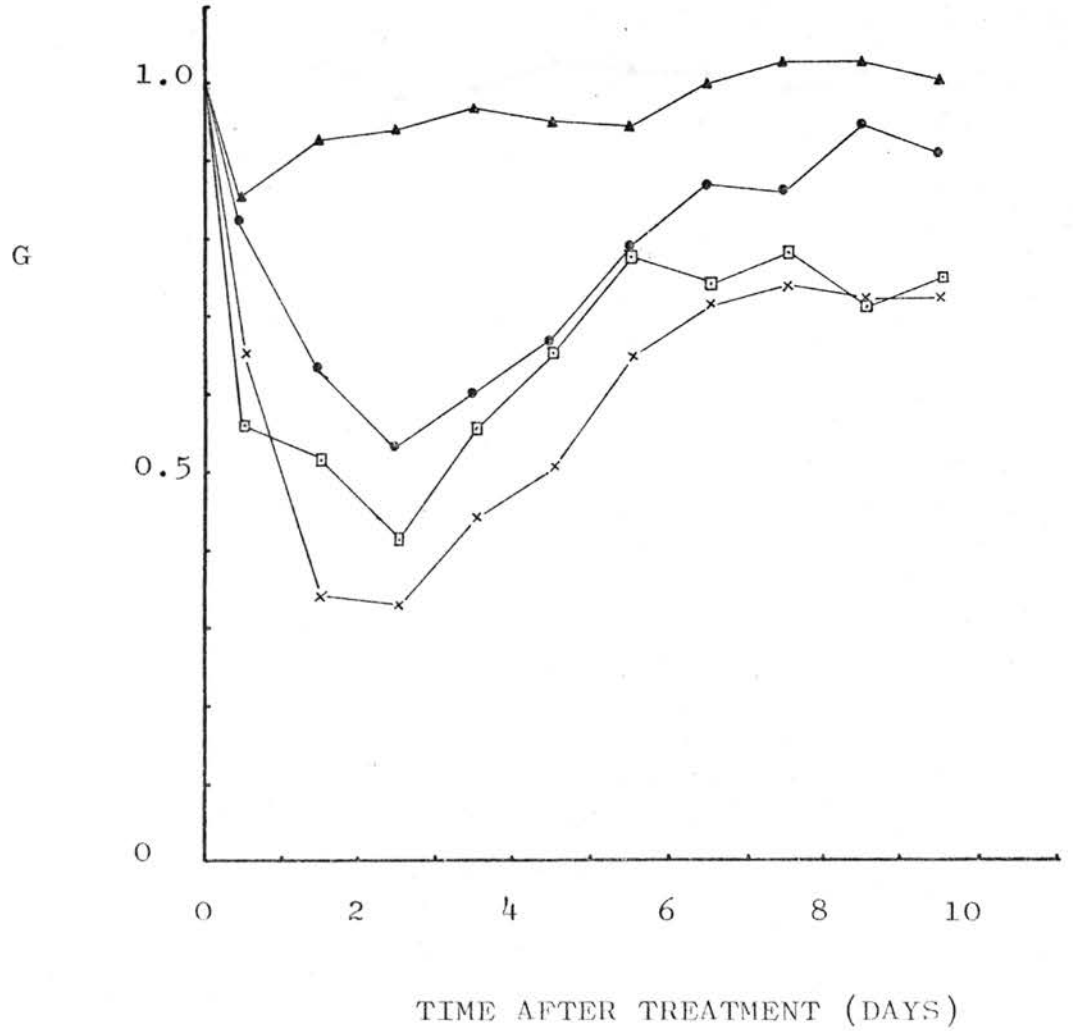


FIGURE 4.47.

AS FOR FIGURE 4.45, BUT WITH A 2-HOUR VINCRISTINE TREATMENT.

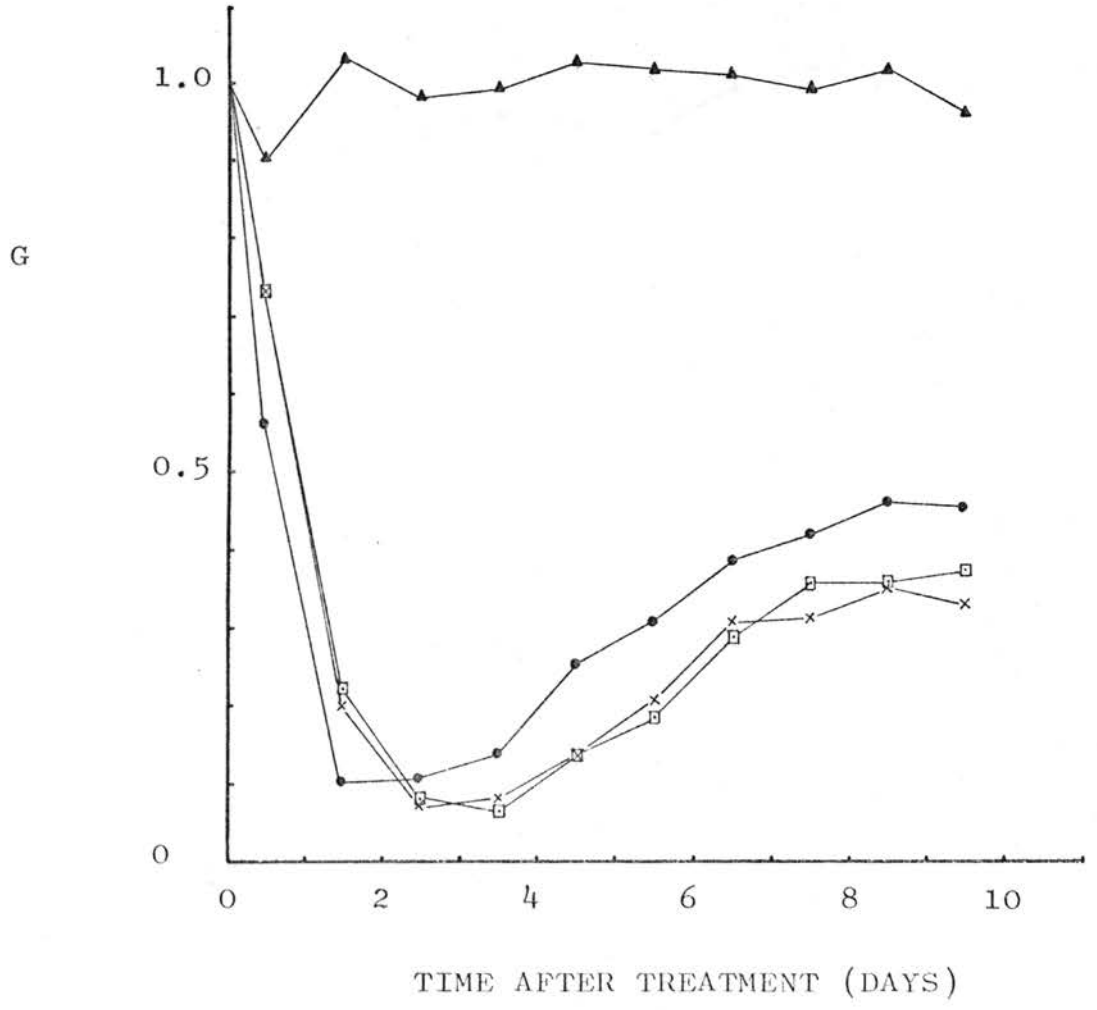


FIGURE 4.48 .

AS FOR FIGURE 4.46 , BUT WITH A 2-HOUR VINCRISTINE TREATMENT.

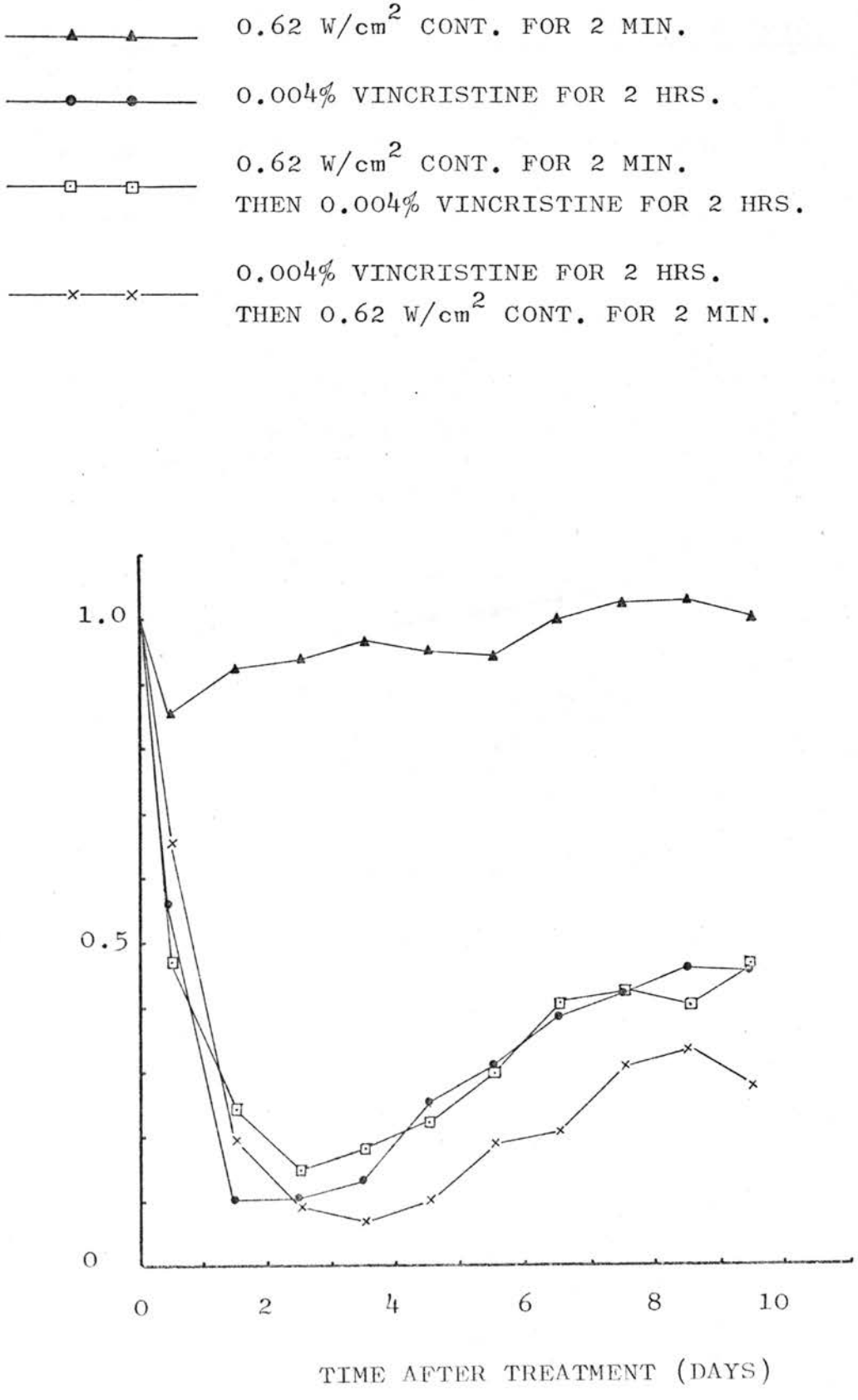


FIGURE 4.50 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER TREATMENT WITH VINCRISTINE FOR 1 HOUR AND SONICATION AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR 60 MIN.

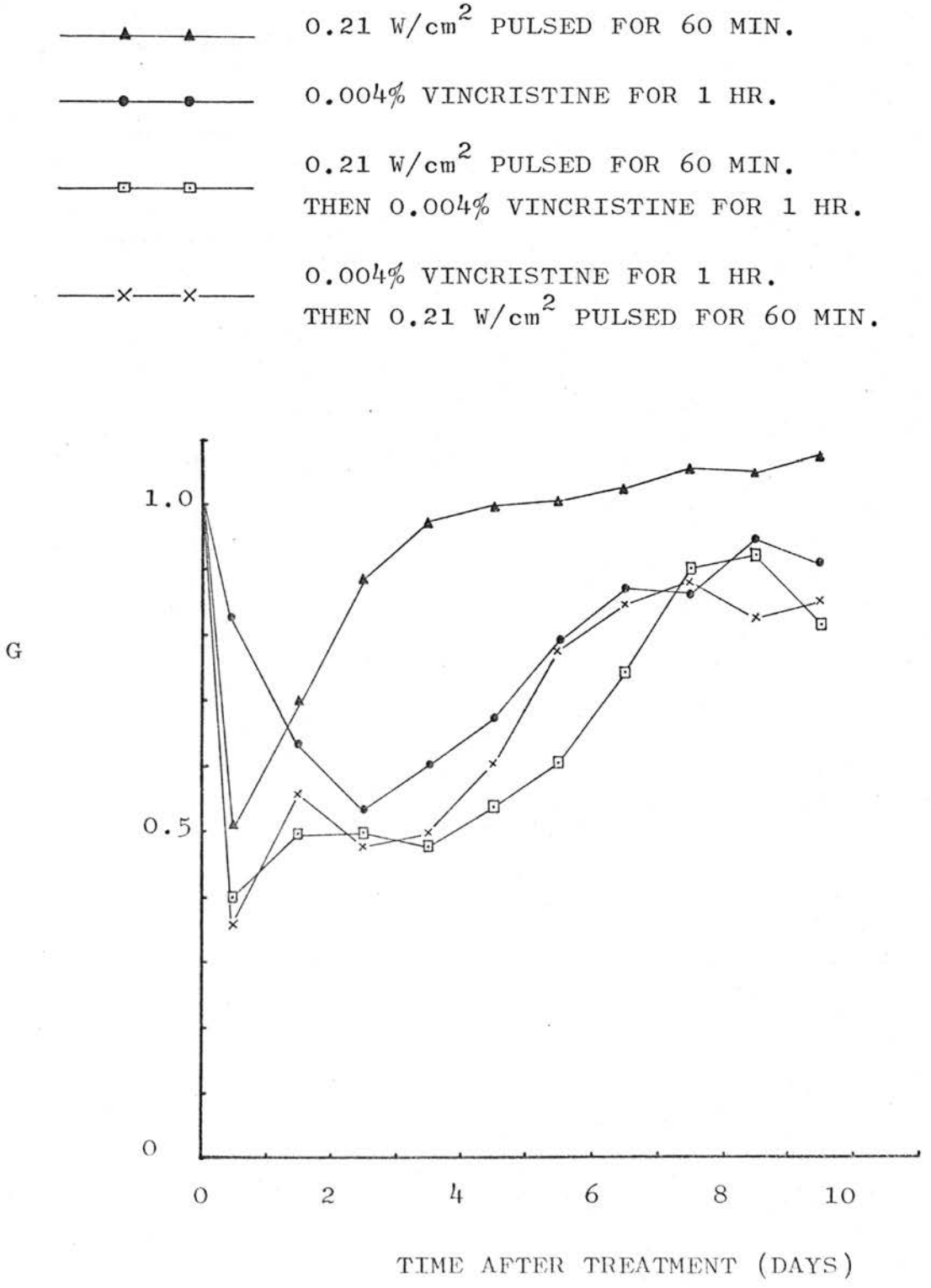


FIGURE 4.51.

AS FOR FIGURE 4.50, BUT AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 (CONT. BEAM) FOR 30 MIN.

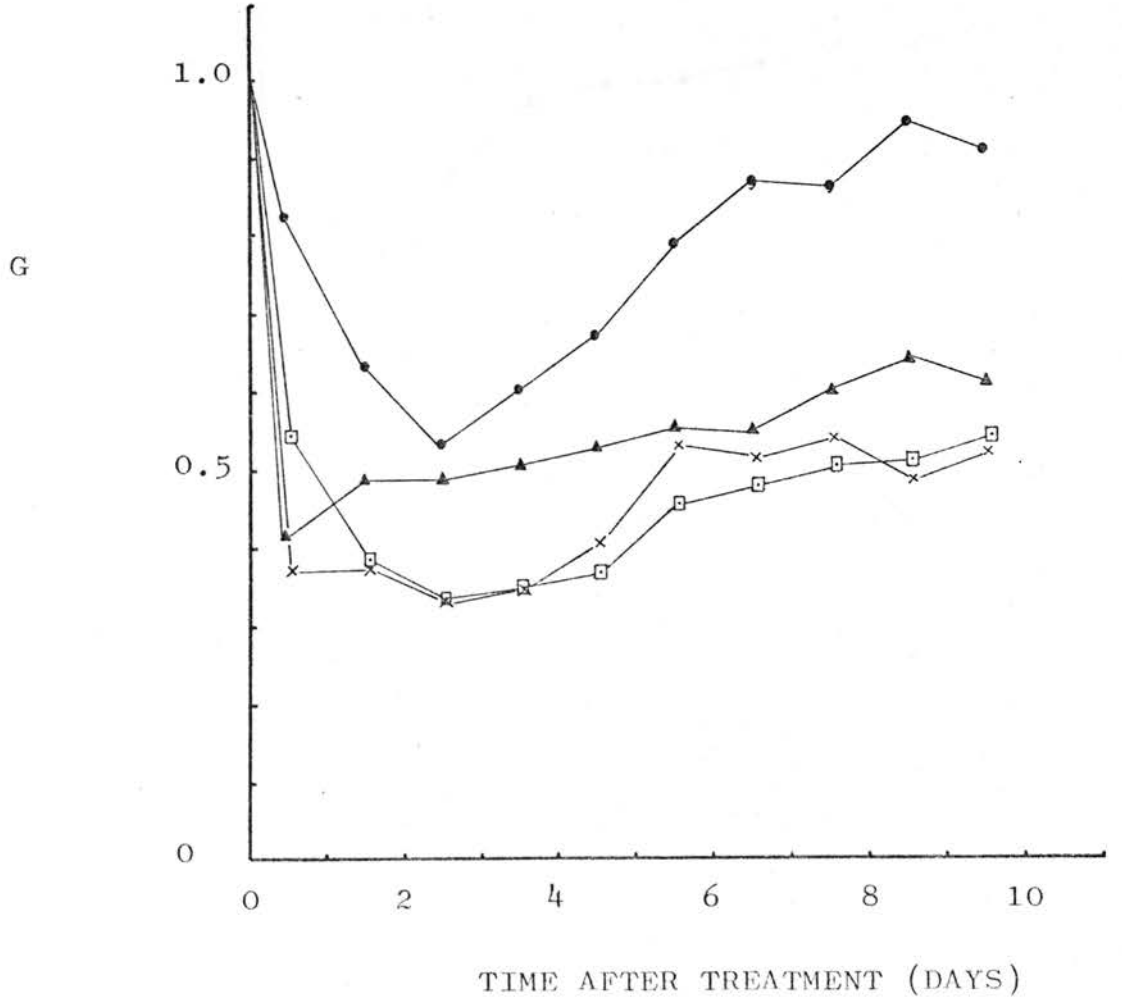


FIGURE 4.52 .

AS FOR FIGURE 4.50 , BUT WITH A 2-HOUR VINCRISTINE TREATMENT.

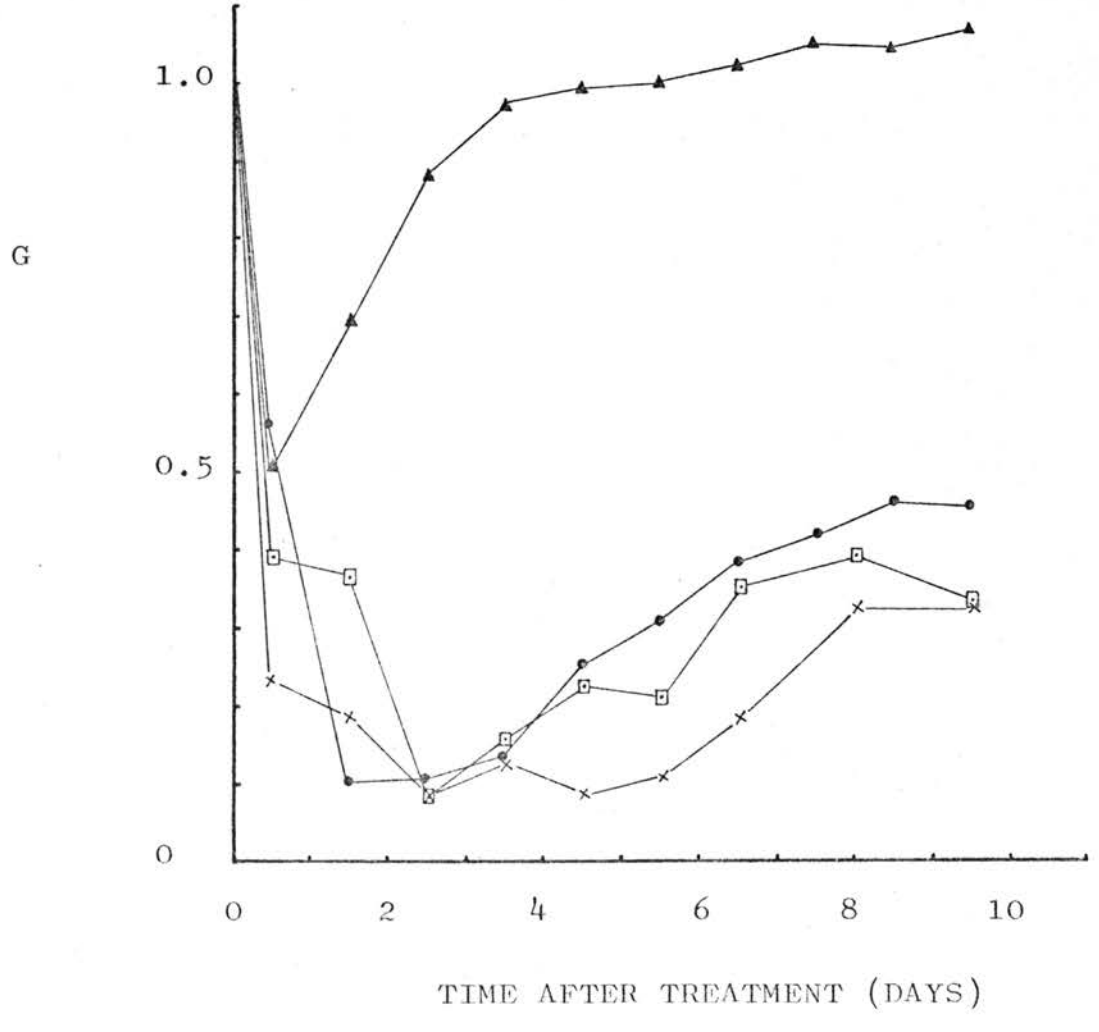


FIGURE 4.53 .

AS FOR FIGURE 4.51 , BUT WITH A 2-HOUR VINCRISTINE TREATMENT.

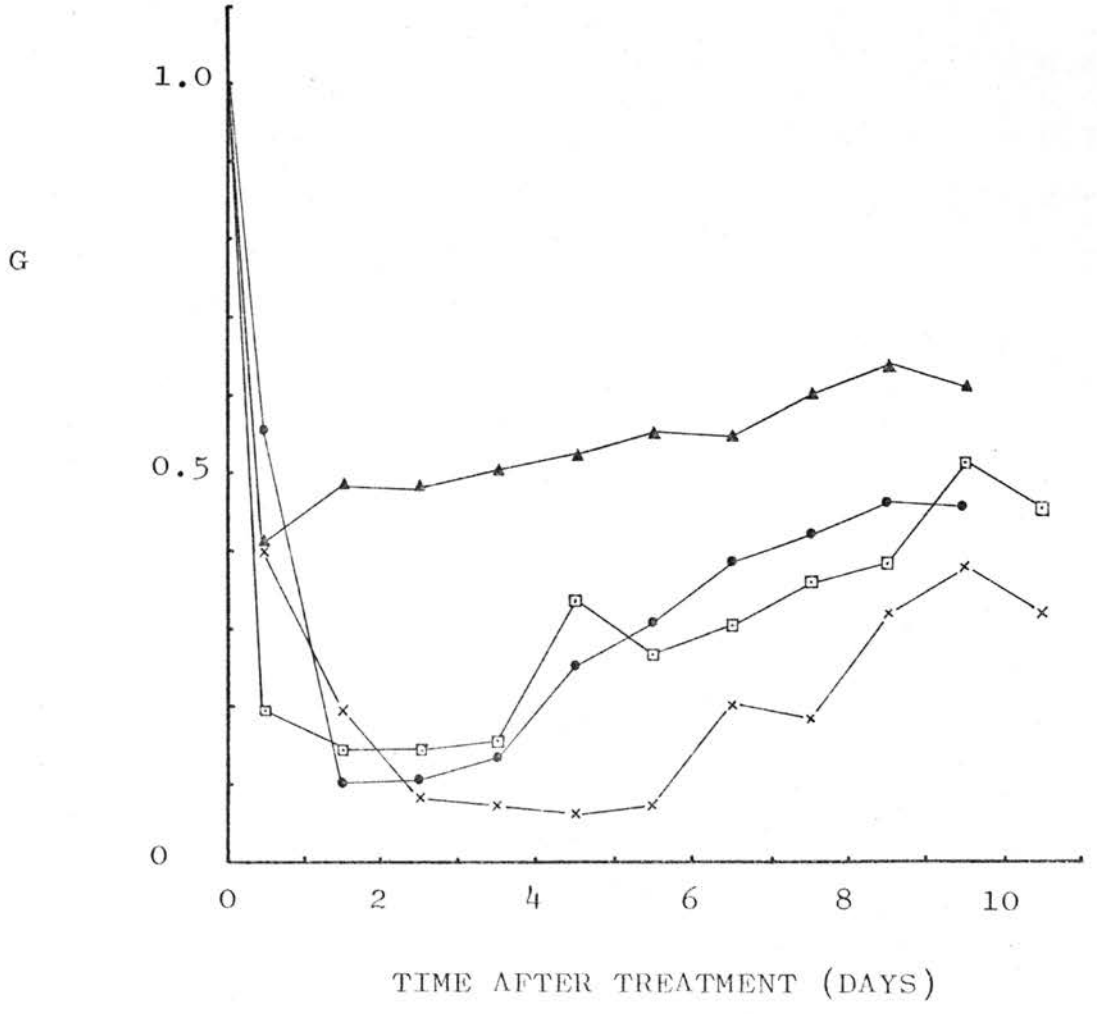


FIGURE 4.54.

VARIATION OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) AFTER TREATMENT WITH VINCRISTINE FOR 1 HR. TO INDICATE THE VARIATION IN THE RESULT OBTAINED WHEN REPEATING EXPERIMENTS.

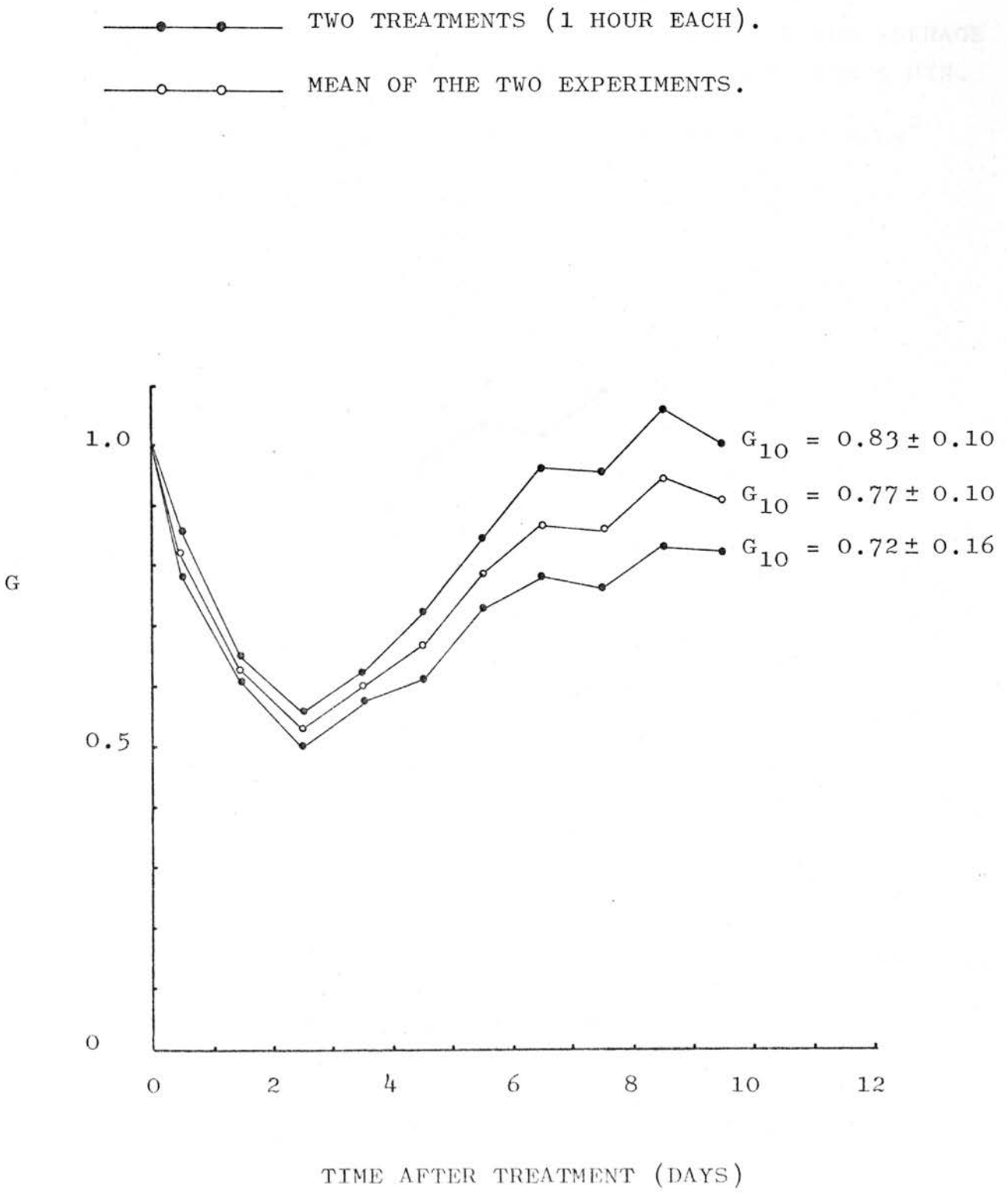


FIGURE 4.55.

COMPARISON OF THE GROWTH CURVES OBTAINED IN THE EXPERIMENTS IN WHICH A VERY LOW EXPOSURE OF ULTRASOUND WAS USED IN CONJUNCTION WITH X-RAYS AND VINCRISTINE RESPECTIVELY.

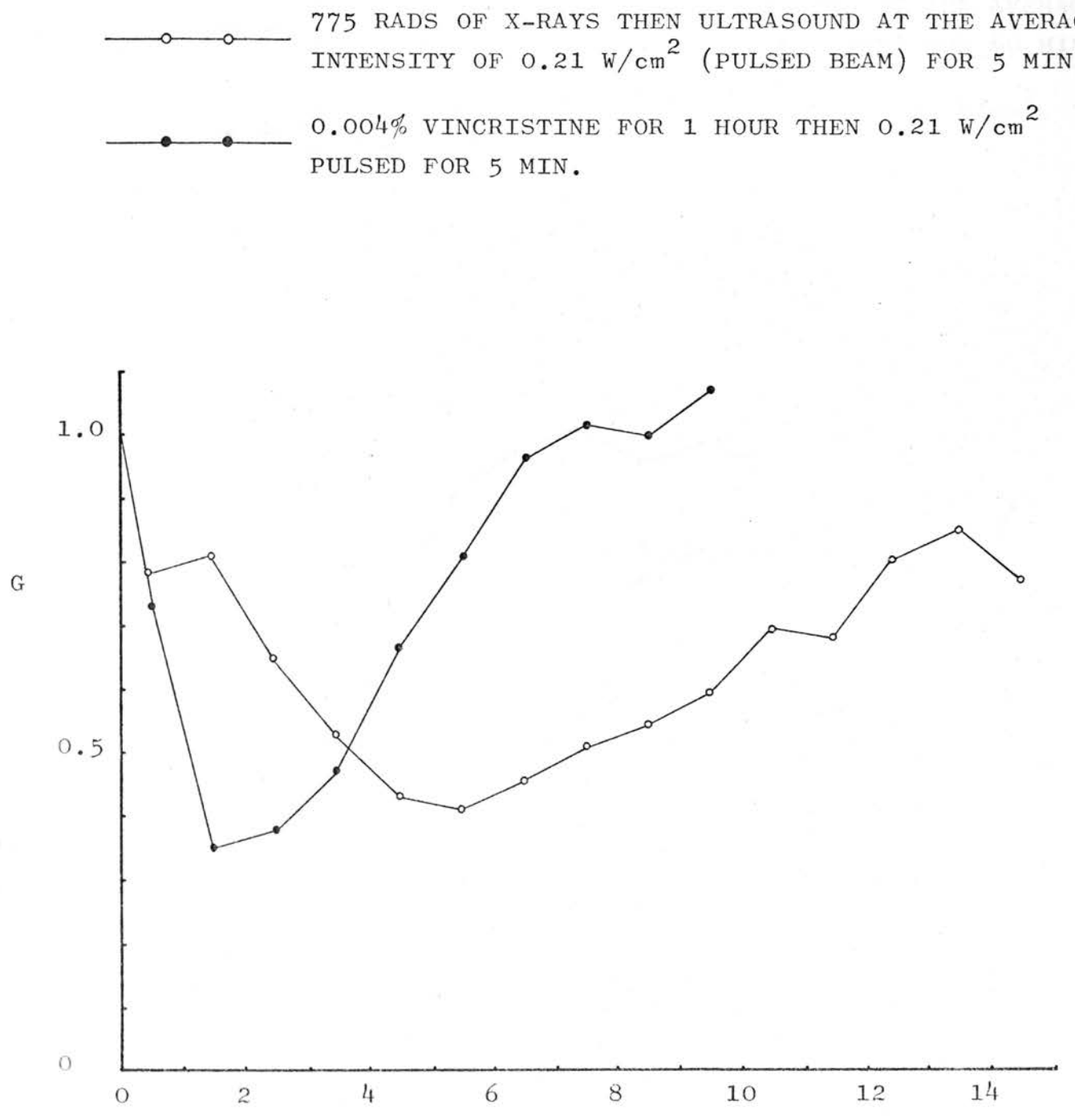


FIGURE 4.56.

COMPARISON OF THE GROWTH CURVES OBTAINED IN THE EXPERIMENTS IN WHICH ULTRASOUND WAS USED IN CONJUNCTION WITH X-RAYS AND VINCRISTINE RESPECTIVELY.

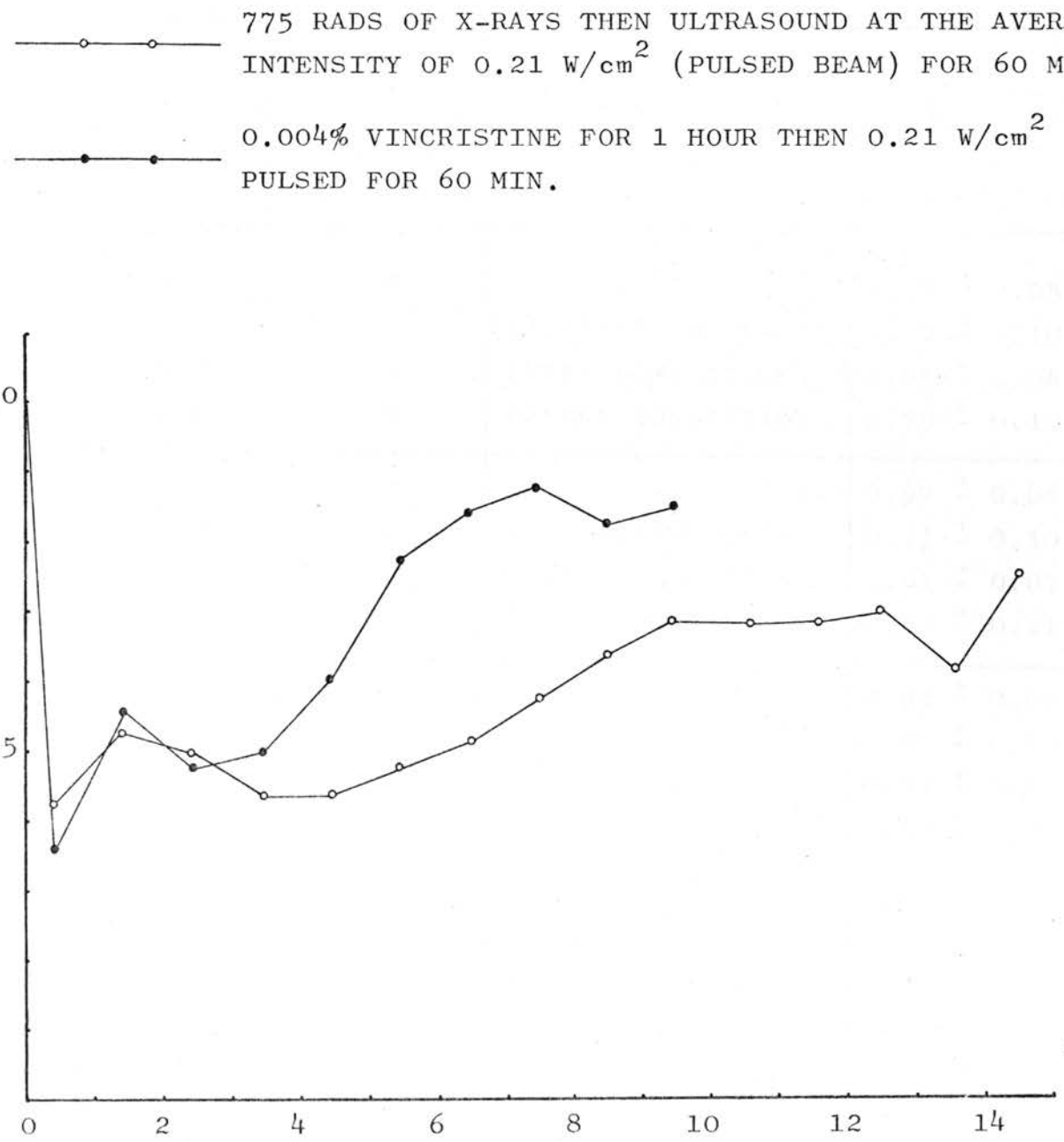


TABLE 4.9 .

AVERAGE GROWTH OF ROOTS IN TEN DAYS AFTER SONICATION AND/OR VINCRISTINE TREATMENT FOR 1 HR., AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS(G_{10}).

AVG. ULTRASONIC INT. (W/cm^2)	MODE	SONICATION TIME (MIN.)	VINCRISTINE TREATMENT (0.004% SOLUTION)	G_{10}
0.21	PULSED	5	-	0.99 ± 0.05
-	-	-	VINCRISTINE ONLY	0.77 ± 0.10
0.21	PULSED	5	AFTER SONICATION	0.65 ± 0.08
0.21	PULSED	5	BEFORE SONICATION	0.70 ± 0.10
0.62	CONT.	2	-	0.97 ± 0.06
-	-	-	VINCRISTINE ONLY	0.77 ± 0.10
0.62	CONT.	2	AFTER SONICATION	0.61 ± 0.07
0.62	CONT.	2	BEFORE SONICATION	0.54 ± 0.11
0.21	PULSED	60	-	0.92 ± 0.06
-	-	-	VINCRISTINE ONLY	0.77 ± 0.10
0.21	PULSED	60	AFTER SONICATION	0.65 ± 0.10
0.21	PULSED	60	BEFORE SONICATION	0.68 ± 0.08
0.82	CONT.	30	-	0.56 ± 0.08
-	-	-	VINCRISTINE ONLY	0.77 ± 0.10
0.82	CONT.	30	AFTER SONICATION	0.45 ± 0.09
0.82	CONT.	30	BEFORE SONICATION	0.44 ± 0.09

The values of G_{10} represent the mean of at least two experiments.

TABLE 4.10 .

AVERAGE GROWTH OF ROOTS IN TEN DAYS AFTER SONICATION AND/OR VINCRISTINE TREATMENT FOR 2 HRS., AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS (G_{10}).

AVG. ULTRASONIC INT. W/cm^2	MODE	SONICATION TIME MIN.	VINCRISTINE TREATMENT (0.004% SOLUTION)	G_{10}
0.21	PULSED	5	-	0.99 ± 0.05
-	-	-	VINCRISTINE ONLY	0.31 ± 0.04
0.21	PULSED	5	AFTER SONICATION	0.28 ± 0.04
0.21	PULSED	5	BEFORE SONICATION	0.27 ± 0.05
0.62	CONT.	2	-	0.97 ± 0.06
-	-	-	VINCRISTINE ONLY	0.31 ± 0.04
0.62	CONT.	2	AFTER SONICATION	0.33 ± 0.04
0.62	CONT.	2	BEFORE SONICATION	0.24 ± 0.05
0.21	PULSED	60	-	0.92 ± 0.06
-	-	-	VINCRISTINE ONLY	0.31 ± 0.04
0.21	PULSED	60	AFTER SONICATION	0.26 ± 0.04
0.21	PULSED	60	BEFORE SONICATION	0.17 ± 0.06
0.82	CONT.	30	-	0.56 ± 0.08
-	-	-	VINCRISTINE ONLY	0.31 ± 0.04
0.82	CONT.	30	AFTER SONICATION	0.29 ± 0.07
0.82	CONT.	30	BEFORE SONICATION	0.20 ± 0.06

The values of G_{10} represent the mean of at least two experiments.

RESULTS OF THE AGE-RESPONSE EXPERIMENTS.

Toxicity of hydroxyurea.

Treating seedlings in a 1.25 mM solution of hydroxyurea (HU) resulted in some root damage, as is evident from Figure 4.57 and Table 4.11. The drug reduced growth by about 5% which is somewhat less than the 9% reduction in growth obtained by Hall, Brown and Cavanagh (1968) when treating the roots of Vicia for 24 hrs. in a 1.25 mM solution of HU.

The growth curve in Figure 4.57 indicates that the growth rate reaches a minimum during the first day after treatment. The subsequent recovery is rapid and the growth curve levels off by about 2-3 days post-treatment. There was virtually a complete recovery of all the roots and the growth curve levels off at about unity.

Degree of synchrony.

Figure 4.61 shows the mitotic indices measured at different times after the removal of the seedlings from HU. A peak in the M.I. seems to occur at about 16 hours after treatment. The M.I. of about 20% at this time, however, is very much less than the value of about 40% obtained by Hall, Brown and Cavanagh (1968). Clowes (1965a), treating the roots of Zea with 5-Amino-Uracil, also obtained a M.I. of about 40%.

Effect of an acute dose of ultrasound at various times after removal from HU.

The average ultrasonic intensities of 0.82 W/cm^2 (cont. beam) and 0.21 W/cm^2 (pulsed beam) were chosen since Clarke and Hill (1969) have found a reduced percentage (as compared to controls) of cells in mitosis in survivors of a sonicated asynchronous population of mouse leukaemia cells (using a 1 MHz continuous beam at an average intensity of 5 W/cm^2 for ten seconds). They also found that a pulsed beam (at an average intensity of 5 W/cm^2 for 5 hours, 1 ms pulses and a mark/space ratio of 1:10) did not affect the progress of cells through the cell cycle, i.e. the labelling index (using tritiated thymidine) at various times after sonication was equal to that of the controls.

Figure 4.57 and 4.58 show the growth curves that were obtained when the roots were sonicated at 0.21 W/cm^2 (pulsed beam) for 15 minutes at 2 and 17 hours after treatment with HU respectively. These growth curves are similar to the one obtained for the roots treated with HU alone, i.e. they reach approximately the same minimum growth rate as a fraction of controls (for definition of G_{min}, see page 78) in the first day after treatment and the subsequent recovery is also similar. The ultrasonic damage is not apparent in these growth curves. The effect of sonication at an average intensity of 0.21 W/cm^2 (pulsed beam) for 15 minutes, given alone, is also expressed by a minimum in the growth rate in the first day after sonication

which is, however, greater (see p. 78) than that for the roots treated with HU alone (Figure 4.57).

Figures 4.59 and 4.60 show the growth curves for an ultrasonic exposure at an average intensity of 0.82 W/cm^2 (cont. beam) for 30 minutes, 1 and 16 hours after the treatment with HU respectively. These growth curves are very similar to the ones obtained when the roots are treated with ultrasound alone and the damage due to HU is not apparent in these growth curves (Figure 4.59 and 4.60). The ultrasonic exposure at an average intensity of 0.82 W/cm^2 (cont. beam) for 30 minutes, given alone, causes much greater damage than treatment with HU alone as shown in Figures 4.59 and 4.60 .

FIGURE 4.57 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G), FOLLOWING SONICATION AT AN AVERAGE INTENSITY OF 0.21 W/cm^2 (PULSED BEAM) FOR 15 MINUTES 2 HOURS AFTER SYNCHRONIZATION PRE-TREATMENT WITH HYDROXYUREA.

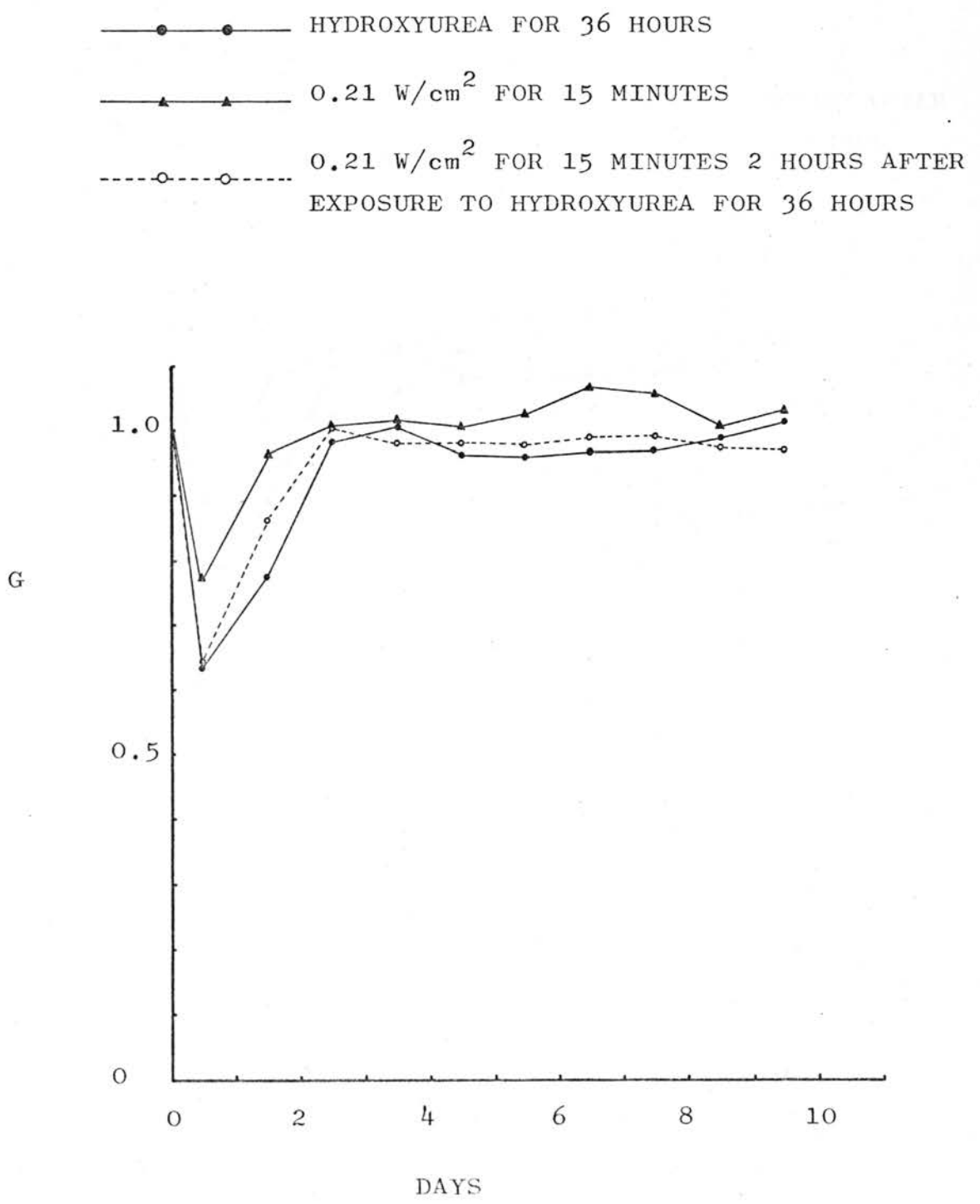


FIGURE 4.58.

AS FOR FIGURE 4.57, BUT WITH SONICATION 17 HOURS AFTER SYNCHRONIZATION PRE-TREATMENT.

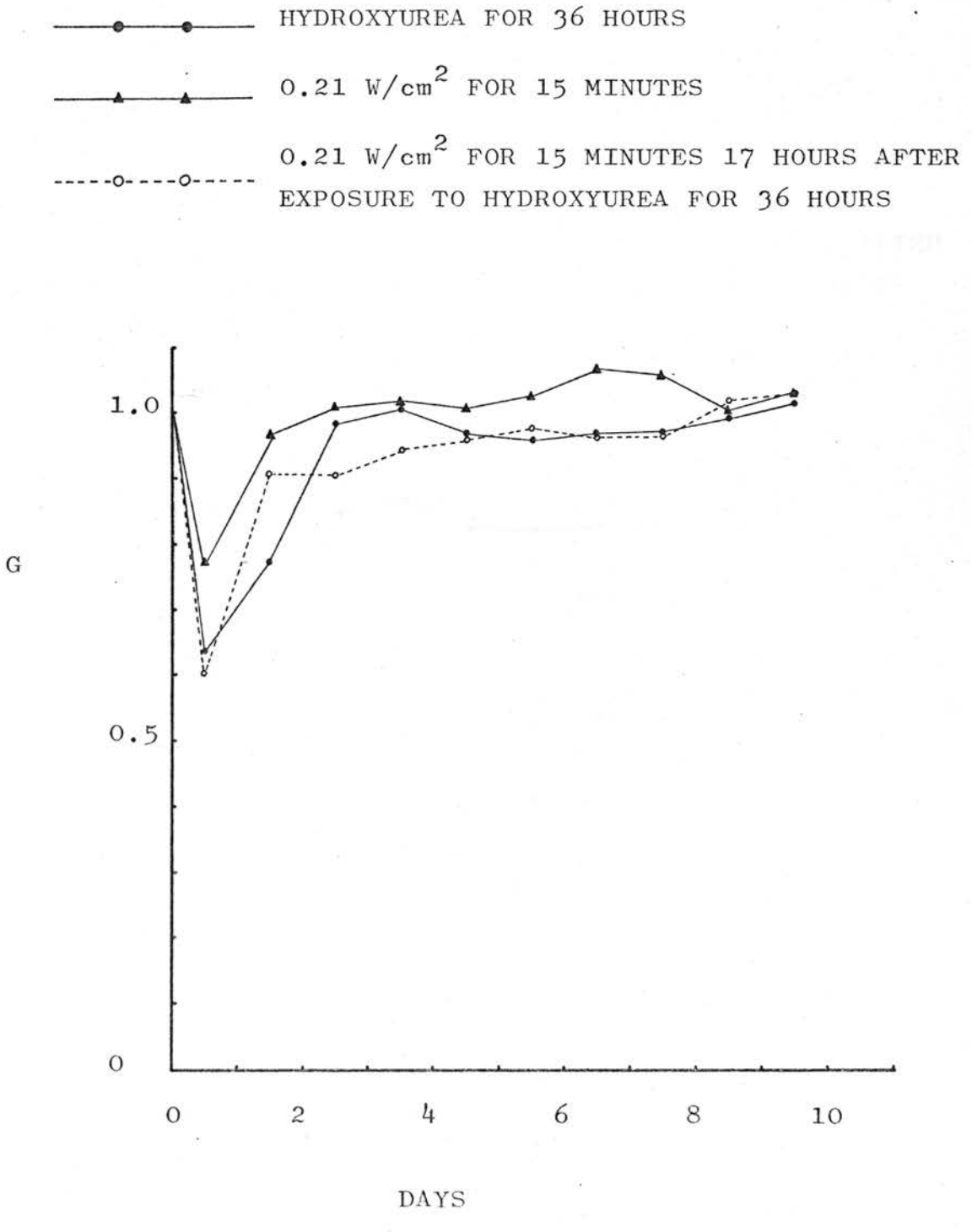


FIGURE 4.59 .

PATTERNS OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G), FOLLOWING SONICATION AT AN AVERAGE INTENSITY OF 0.82 W/cm^2 FOR 30 MINUTES (CONT. BEAM) 1 HOUR AFTER SYNCHRONIZATION PRE-TREATMENT WITH HYDROXYUREA.

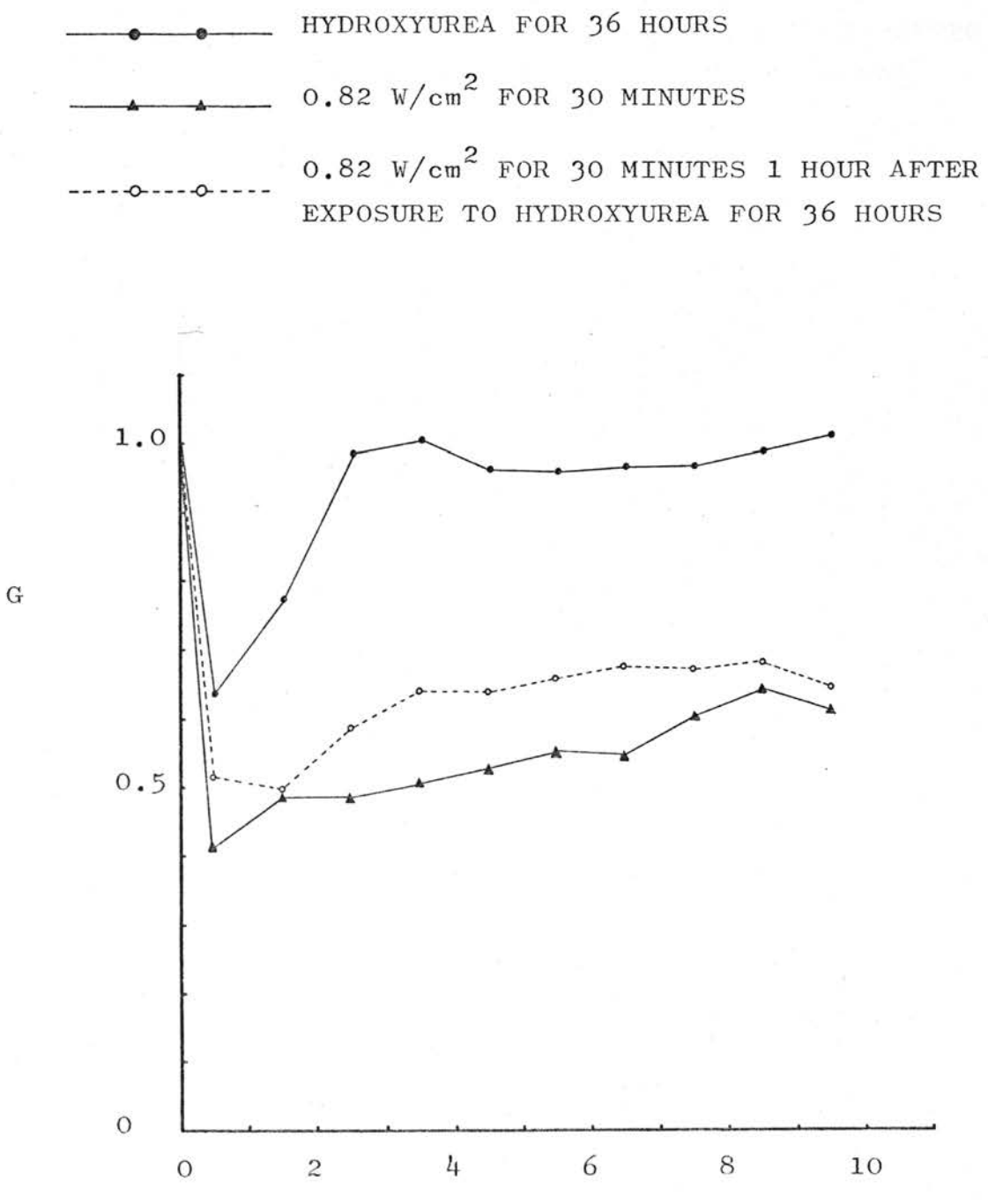


FIGURE 4.60 .

AS FOR FIGURE 4.59 , BUT WITH SONICATION 16 HOURS AFTER SYNCHRONIZATION PRE-TREATMENT.

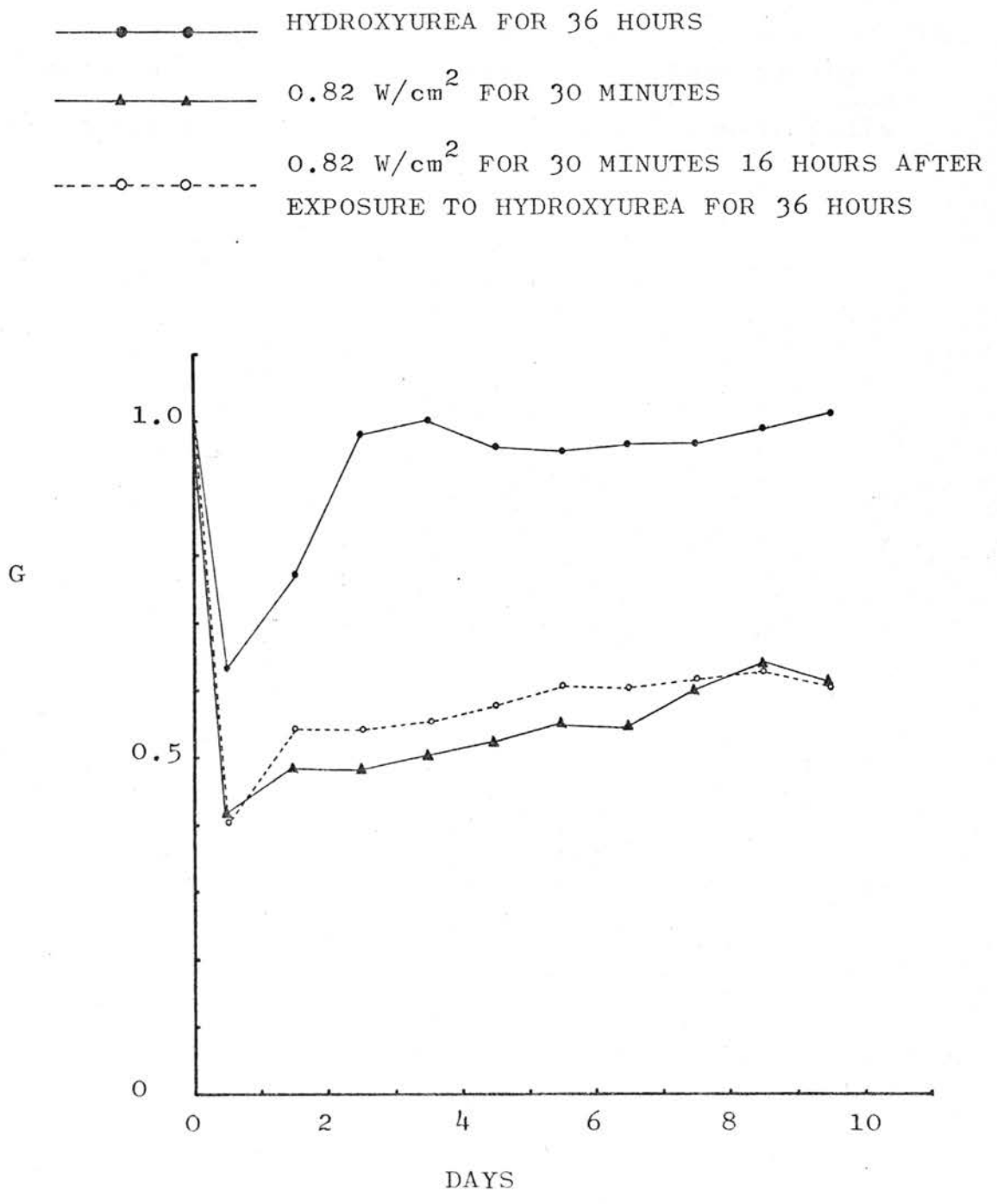


FIGURE 4.61 .

VARIATION OF MITOTIC INDEX WITH TIME AFTER REMOVAL FROM A 36-HOUR EXPOSURE TO HYDROXYUREA.

The error in the number of cells in metaphase is the standard error of the mean for 4000 meristematic cells scored.

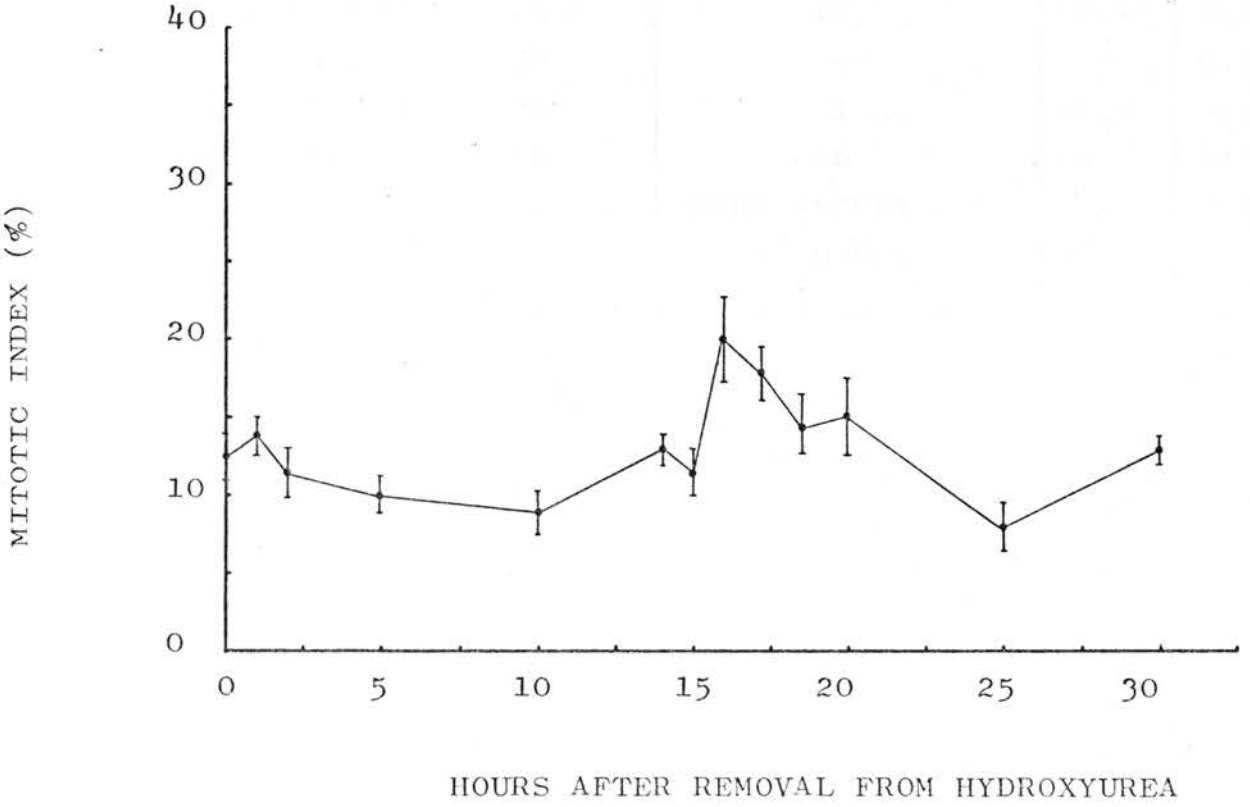


TABLE 4.11.

COMPARISON OF THE VALUES OF G_{10} OBTAINED WHEN THE ROOTS ARE SONICATED AT VARIOUS TIMES AFTER TREATMENT IN A 1.25 mM SOLUTION OF HYDROXYUREA FOR 36 HOURS, WITH THE VALUES OBTAINED WITH NO HYDROXYUREA TREATMENT.

AVG. ULTRASONIC INTENSITY (W/cm^2)	MODE	SONICATION TIME (MIN.)	TIME AFTER REMOVAL FROM HYDROXYUREA (HOURS)	M.I.	G_{10}
0.21	PULSED	15	-	-	1.00 ± 0.06
0.21	PULSED	15	2	11.5	0.94 ± 0.08
0.21	PULSED	15	17	18.1	0.93 ± 0.09
0.82	CONT.	30	-	-	0.56 ± 0.08
0.82	CONT.	30	1	14.0	0.62 ± 0.10
0.82	CONT.	30	16	19.8	0.57 ± 0.10
-	-	-	HYDROXYUREA FOR 36 HOURS	-	0.95 ± 0.05

Each value of G_{10} represents the mean of at least two experiments.
The errors in the Mitotic Index are as indicated in Figure 4.61.

CHAPTER V.

DISCUSSION.

DISCUSSION.

The results given in Table 4.3 and Figures 4.10 and 4.11 indicate that exposure to continuous and pulsed beams of ultrasound at an average intensity ranging from 0.16 to 0.82 W/cm^2 produces some inhibition of root growth in Zea mays seedlings. For sonication times of up to 180 minutes the fractional growth in ten days (G_{10}) decreases with increasing duration of sonication, but tends to a minimum value for any given intensity (Figures 4.10 and 4.11). For a given total exposure (intensity x duration) the sonication effect is greater the higher the intensity. A similar response is observed (Figures 4.12 and 4.13) for the fractional growth in the first day after sonication (G_1). These observations are similar to those obtained by Bleaney and Oliver (1972a) in their studies on the effects of a continuous beam of ultrasound on the roots of Vicia faba.

A response of the kind described above has also been observed when certain animals were exposed to ultrasound. Figure 5.1 shows the "% Survival" of Cyclops exposed to different intensities of ultrasound for various times. The number of Cyclops surviving a dose of ultrasound also decreases with increasing duration of sonication, but tends to zero for any given intensity.

For Zea, there is an indication (Figures 4.12 and 4.13) that at the lower average ultrasonic intensities, sonication damage only becomes apparent if the roots are

treated long enough. For sonicated tadpoles (Figure 1.1) a more pronounced threshold for the onset of sonication damage has been observed.

By using suitable mathematical models of the meristem to interpret the growth curve of X-irradiated roots relative to controls it has been possible to derive a dose-response curve with respect to reproductive integrity for the cells of the meristem of Vicia (Hall, Lajtha and Oliver, 1962).

The dose-response curve for Vicia exposed to X-rays is shown in Figure 5.2 , the log-survival linear-dose graph being linear after an initial "shoulder" at low doses. The curve in this instance has the same general form as those obtained with a range of mammalian cell types (Morkovin and Feldman, 1960). Such a survival curve is associated with a multi-target process (Oliver and Shepstone, 1964), the initial ineffective shoulder being taken to correspond to the build up of latent sub-lethal damage.

In order to explain the effect of ionizing radiation on organisms the target theory has been formulated (Crowther, 1924; Lea, 1955), which states that the production of ionization (a "hit") in or very near to some particular molecule or structure ("target") is responsible for the measured effect. Certain organisms contain more than one target and in order to inactivate the entire unit, each of the targets must receive a hit. This can be expressed mathematically by the equation:

$$\frac{N}{N_0} = 1 - (1 - e^{-\lambda D})^m$$

where m represents the number of targets in this model, N_0 is the number of biological entities present initially and N is the number of entities surviving a dose D. The curve described by the above equation is sigmoid and the model has been used to explain the dose-response of Vicia (Figure 5.2) exposed to X-rays (Oliver, 1964).

An attempt has been made to use the method by Hall, Lajtha and Oliver (1962) to find the initial surviving fraction of cells in the meristem after treatment of the roots with ultrasound. The method has been found to be of limited value for the roots of Zea exposed to ultrasound since many of the growth curves level off at values of the growth rate as a fraction of controls (G) less than unity. Since the sonication damage is expressed immediately (Bleaney and Oliver, 1972a) the values of the average growth in the first day post-sonication as a fraction of the corresponding average growth for control roots (G_1) may provide a good approximation of the initial surviving fraction of meristematic cells.

The log-G_1 linear-exposure graph obtained for the roots of Zea exposed to ultrasound (at an average intensity of 0.21 W/cm^2, pulsed beam) is non-linear as shown in Figure 5.3 . Similar results have been obtained for "semi-log" plots of the results for all the other average intensities used. Thus the ultrasonic exposure (intensity multiplied by duration) is not related to damage produced in the same way as dose (rads) and % survival for ionizing radiation.

In Figure 4.15 the average growth in the first day following ultrasonic exposure expressed as a fraction of the corresponding growth for controls, is related to the average ultrasonic intensity of the pulsed and continuous beams used (for 60 minutes). The damage for the pulsed beams seems to be much greater than that for the continuous beams at the same average power. The pulses may be long enough for the higher power in the pulse to be the relevant parameter rather than the average power (Bleaney and Oliver, 1972b). These results are similar to those obtained by Bleaney and Oliver (1972b), who have also demonstrated that the damage for very short pulses (20 μs with 0.25 duty cycle) is not greater than that for the continuous beam of ultrasound at the same average power (Figure 1.3). The reduced effectiveness of very short pulses is expected to be associated with the time required for the build-up of aperiodic (radiation pressure) forces (Bleaney and Oliver, 1972b).

Figure 5.4 shows the variation of log-G_1 with the average ultrasonic intensity of pulsed as well as continuous beams of ultrasound, for various sonication times ranging from 15 minutes to 150 minutes. The curves seem to have an initial "shoulder" followed by a linear portion. Similar curves are also obtained for a "semi-log" plot of the results that Bleaney and Oliver (1972b) have published in their article on the effect of pulsed ultrasound on Vicia (Figure 5.5). The shoulders of the curves in Figures 5.4 and 5.5 seem to be more pronounced for the lower

average intensities. These curves may be compared with the X-ray dose-response curve depicted in Figure 5.2 for Vicia. In view of the similarity of these curves it may be possible to interpret the damage produced by a certain intensity of ultrasound in terms of a number of targets that have to receive a hit in order to inactivate the entire unit (cell). The hit in the case of ultrasound may be a force of a certain magnitude required to produce an effect on a target, which may be a molecule such as DNA, the nucleus or even the entire cell.

According to Taylor and Dyson (1972) the primary sites of action may lie at a subcellular level. Lysosomal damage has been observed in sonicated liver (Taylor and Pond, 1972) and DNA degradation has been described in vitro (Hawley, Macleod and Dunn, 1963), but DNA is very unstable in solution so that the effects cannot be compared with the situation in vivo (Taylor and Dyson, 1972).

The shoulders of the curves in Figures 5.4 and 5.5 may correspond to the build up of forces until they are large enough to cause an observable sonication damage. There is also an indication that the reduction in growth due to sonication does not continue to increase with increasing intensity but starts to level off gradually, which may be ascribed to the disruptive forces reaching a certain magnitude beyond which any increase in the size of the force only causes a small increase in sonication damage.

Experimental growth rate curves.

Except for the very low average intensities (Figures 4.8 and 4.15) and some of the short sonication times at higher intensities (Figures 4.3 and 4.6), the growth curves show an immediate reduced growth rate after sonication for the pulsed as well as the continuous ultrasonic beams (Figures 4.1 - 4.7), the growth rate during the first day being lower for higher intensity and longer sonication times (Figures 4.12 and 4.13). This may indicate interference with the process of elongation of the already differentiated cells in the meristem (Bleaney and Oliver, 1972a).

For the lower average intensities (up to 0.38 W/cm^2) and the short sonication times at the higher intensities, the growth rate recovers rapidly immediately after the initial decrease and reaches the control value by some 6 to 8 days after sonication (Figures 4.1 - 4.7).

In view of the similarity of the growth pattern to that following X-irradiation (Figures 4.44 and 1.4), it may again be possible to interpret the inhibition in root growth in terms of damage to the dividing cells of the meristem (Bleaney and Oliver, 1972a). However, as the minimum in the growth rate curve is not delayed, it appears that cells sterilized by ultrasound are not able to undergo any divisions, the radiation damage being expressed immediately (Bleaney and Oliver, 1972a).

Delayed death of the kind caused by ionizing radiation (see also Chapter I) does not seem to be present when

the roots of Zea (and Vicia) are exposed to ultrasound at a frequency of 1 MHz and an average intensity of up to 0.82 W/cm^2. The damage to cells caused by X-rays is partly due to the formation of short-lived chemical free-radical species (e.g. Read, 1959) and although such free radicals have also been detected in ultrasonic beams which are capable of producing transient cavitation (Elpiner, 1964), cell breakage seems to be independent of free radical formation (Hughes and Rogers, 1960). At the frequency and average ultrasonic intensities of the continuous beams used in the present experiments transient cavitation is unlikely to occur since the threshold for the onset of transient cavitation in air-equilibrated water has been found to be at about 0.3 to 10^2 W/cm^2 for a 1 MHz beam (Figure 1.14). Hill (1972) has also found evidence that this type of cavitation cannot be readily stimulated in mammalian tissues when using a 1 MHz ultrasonic beam. For a pulsed beam, the cavitational activity is less than for a continuous beam at the same average intensity (Hill,1968) and cavitation also decreases rapidly as the pulse length is reduced towards 1 ms (Hill, Clarke, Crowe and Hammick, 1969; Hill, 1972) and thus for the pulsed beams used in the present experiments cavitation can be considered as being absent.

In Figure 5.6 the growth curve for roots of Zea sonicated at an average intensity of 0.62 W/cm^2 (continuous beam) for 15 minutes is compared with the curve obtained by Bleaney and Oliver (1972a) for the roots of Vicia exposed at an intensity of 3.84 W/cm^2 for the same time.

The shapes of the two curves are similar, but the roots of Zea seem to be much more sensitive to ultrasonic radiation than the roots of Vicia. The greater susceptibility to sonication damage of the roots of Zea as compared to the roots of Vicia can possibly be explained in terms of the geometry of the root tip. Although the diameter of the root tip of Zea is about equal to that of Vicia (Figure 5.7), the meristem of Zea consists of about 1.25×10^5 cells whereas the meristem of Vicia comprises 2.5×10^5 cells (Clowes, 1967). Compared to Zea, a larger number of cells have to be destroyed in the meristem of Vicia to cause the same fractional reduction in its size.

Clowes (1963) has shown that after X-irradiation of the roots of Zea mays the average rate of mitosis falls in the normally meristematic cells and increases in the quiescent centre; proliferation in the quiescent centre then gives rise to a new meristem which continues the growth of the root. The quiescent centre of Zea consists of about 600 cells surrounded by 1.25×10^5 cells whereas the quiescent centre of Vicia comprises 10^3 cells surrounded by 2.5×10^5 cells (Clowes, 1967). The quiescent centre of Vicia would thus be more protected against ultrasonic damage as compared to Zea. If the behaviour of the quiescent centre after sonication is similar to that after X-radiation, the greater shielding may also account for the more rapid repopulation of the meristem of Vicia.

Maize roots seem to be much more affected by ultrasound than the roots of Vicia but they are, on the other

hand, much less sensitive to X-rays than bean roots (Shepstone, 1964; Fenner, 1970; Hering, 1971). Sparrow (1964) has shown that plant species with big nuclei are very sensitive to X-rays; species with small nuclei are relatively resistant. The mean DNA content per cell in Zea is about 1.8×10^{-5} μg, with a calculated nuclear volume of 230 μ^3. The equivalent values for Vicia are 4×10^{-5} μg DNA per cell and 500 μ^3 respectively. Although similar experiments have not been carried out using ultrasound, the sonication results seem to indicate that DNA content and nuclear volume are not related to damage in the same way as for ionizing radiation, i.e. the DNA does not seem to be affected by ultrasound in the same way as for X-rays. This may be an indication that the DNA is not one of the possible "targets" that must be "hit" during sonication in order to inactivate the cell.

It seems that the cells of the meristem play a major role in the expression of the sonication damage to the roots. This is also evident if the elongating and meristematic regions of the root tip are exposed to ultrasound separately, the damage being larger when the meristematic cells are sonicated than when the elongating cells are treated as shown in Figures 4.23 and 4.24. There is, however, no sharp boundary between the meristematic and elongating regions (Clowes, 1961a), and there is also always some penetration of ultrasound into the orifice of the metal cylinder used in these experiments (Figure 3.23). Thus a sonication of either one of these regions will always involve some cells

of the adjacent region as well.

The surgical experiments in which various lengths of the root tips were cut off, have shown that when the meristematic region is completely removed (i.e. when 3mm of the root tip is cut off) the growth as a fraction of controls decreases to 0.25 within the first 3 hours and growth ceases altogether within the first day (Figure 4.25). Thus the elongating process does not seem to contribute significantly to the length of the root once the meristematic region has been removed. Although this does not indicate that the ultrasound has no effect on the elongating cells (Oliver, personal communication), it does seem that damage to the meristematic region is primarily responsible for the immediate reduced growth after sonication.

For the longer sonication times at the average intensities of 0.62 W/cm^2 and 0.82 W/cm^2 (continuous beams) the recovery of the roots is much slower than that for the lower average intensities and shorter sonication times, and the growth curves level off at values of G less than unity by some 9 to 10 days after sonication (Figures 4.1 and 4.2). An appreciable proportion of the roots sonicated at these intensities grow slowly or die (Table 4.1) and have a slightly smaller diameter (Table 4.2) than the control roots. This may indicate a reduction in the equilibrium size of the meristem which may result in a slower recovery and a reduced steady growth rate of the sonicated roots.

A constant reduced growth rate can also be achieved

if the size of the meristem is reduced by some other means, e.g. by cutting off the terminal 0.4 or 0.5 mm portions of the root tip (Figure 4.25). When these lengths are cut off the root tips, the incision is made at or very near to the meristem (Clowes, 1959).

For very high exposures (0.82 W/cm^2 for times $\geq$ 60 minutes) the meristem seems to loose its ability to recover altogether and the roots continue to grow at a constant reduced rate. The values at which the growth rates level off, or to which they are reduced, decrease with increasing exposure time for a given average intensity or with increasing average intensity for a given exposure time, as shown in Figure 4.9 . This response is similar to that observed for the roots that recover completely after sonication and for which G_1 and G_{10} are the parameters used to assess sonication damage, indicating possibly that the number of cells which are reproductively intact after sonication determines the extent to which the meristem will recover. The threshold for this damage to the ability of the meristem to recover seems to occur when the roots are exposed at the average intensities of 0.62 W/cm^2 and 0.82 W/cm^2 (continuous beams) for 15 and 10 minutes respectively.

It has been observed that when certain tissues are exposed to a large enough dose of X-rays the subsequent recovery is not complete, e.g. in the rat eye (Tansley, Spear and Gluecksmann, 1937), in roots of Zea mays (Fenner, 1970; Hering, 1971). This is thought to be due to the appearance of degenerating cells in the irradiated tissue (Lea, 1955, p. 309). The presence of degenerate cells may

also account for the lower steady growth rate observed for the exposures at the high ultrasonic intensities.

Theoretical growth rate curves.

Figures 5.8 and 5.9 show the theoretical growth rate curves computed for various values of P (the proportion of meristematic cells lost due to sonication in the first one tenth of the first cell cycle), and the results using Models A and B are compared. Here it is assumed that cell death occurs during and immediately after sonication (interphase death) and that the cell cycle time (T) remains constant after exposure to ultrasound. It has also been assumed that dying cells are removed from the population and do not contribute to root growth.

The theoretical growth curves have been computed on the basis of these assumptions since it is believed that cells sterilized by ultrasound are not able to undergo any divisions before dying (Bleaney and Oliver, 1972a).

The cell cycle time has been considered as remaining constant after sonication since there is at present no evidence of a change (lengthening or shortening) of the cell cycle time of the meristematic cells in the root tip of Zea or Vicia when the latter are exposed to ultrasound. Clarke and Hill (1969) in their studies on the biological action of ultrasound in relation to the cell cycle did not find any detectable changes in the relative duration of the phases of the cell cycle of mouse leukaemia cells exposed in vitro to ultrasound.

Delayed effects of the kind that are known to follow ionizing radiation, i.e. that cells are able to undergo one or more divisions after being "hit" by the radiation (Puck and Marcus, 1956), have not been observed in sonicated mammalian cells (Clarke and Hill, 1970).

Figures 5.8 and 5.9 represent the theoretical curves of the growth rate as a fraction of controls with:

(a) the depleted meristem being repopulated completely and the growth curves level off at unity ($G_{max} = 1$) as shown in Figure 5.8 , and

(b) the depleted meristem being unable to recover completely and the growth rate reduced by a constant fraction (k), resulting in growth curves which level off at values of G less than unity ($G_{max} < 1$).

From the above comparisons it can be seen that there is only a small difference in the growth curves obtained using Models A and B respectively. The initial reduction in the population of cells is a little larger for Model A than for Model B, resulting in a lower G-value at the end of the first one tenth of the first cell cycle in the case of Model A. The subsequent recovery, however, is more rapid for Model A than for Model B and at the time when the curves start to level off, the growth rate for Model A equals or exceeds that of Model B. Model B, however, seems to be more plausible since in this model an attempt is made to provide the right type of feedback control for the repopulation of the meristem after ultrasonic sterilization of some of its cells, on the basis of a possible biological

response to the population change (Oliver and Shepstone, 1965).

Comparison of experimental and theoretical growth curves.

In Figures 5.10-5.15 the curves computed on the basis of both models are compared with some selected experimental results. Here the growth rate as a fraction of controls (G) is plotted against the time after treatment in cell cycles. Each value of P in Figures 5.10-5.15 is applicable to both curves (for Model A and B) which are compared with a particular experimental curve.

For the unirradiated meristem the cell cycle time of these cells in the case of Zea cultivated at 19°C has been taken as 31.1 hours. This value has been determined experimentally using a metaphase accumulation technique as described previously, and it is in close agreement with a published determination (Table 1.3) in which also a metaphase accumulation technique has been used. Also, as a general approximation, it is assumed that the cycle time derived for cells of the stele just above the meristem is representative of the meristem as a whole because they constitute the major proportion of the latter.

Although the fit to the experimental results is by no means absolute, the computed curves (on the basis of the assumption that the cells sterilized by ultrasound are removed immediately from the population and do not contribute to root growth, and that the ultrasonic dose given does not change the cell cycle time) have the correct shape with

respect to the observed results. The hypothesis that the size of the meristem at equilibrium can be reduced as a result of sonication and that the recovery of the sonicated meristem to this reduced equilibrium size is also proportionally slowed down, provides theoretical growth curves of the correct general form. This response is reminiscent of that of a second-order system with a high damping ratio to a step-function input signal. Thus there appears to be reasonable agreement between experimental results and the application of a simple model system.

From a comparison of the experimental and theoretical growth curves it seems that the maximum proportion of cells (P) that can be removed before a reduction in the equilibrium size of the meristem (or in the already reduced equilibrium size of the meristem) occurs, is of the order of 0.50. The lowest P-values are obtained for the very short sonication times where there is an immediate complete recovery of the sonicated roots (Figures 5.10 - 5.15), and also for the long sonication times at the highest average intensity of 0.82 W/cm^2 (continuous beam) where there is a large reduction in the steady growth rate reached after sonication ($G_{max} \approx 0.2$). There are most probably too few cells left for any recovery when the roots are sonicated for a long time (≥ 60 minutes) at the highest average intensity. The highest P-values are obtained for the average intensity (0.62 W/cm^2, continuous beam) at which a reduction in the steady growth rate just becomes apparent.

Growth curve for the roots of sonicated seeds.

From Figure 4.34 it can be seen that the growth rate of the roots of the sonicated seeds of Zea does not show the significant initial decrease which has been observed for sonicated roots as mentioned previously. Any embryonic damage caused by the ultrasound may not be apparent at the time of the first measurement of the root length since this measurement and the sonication are separated in time by about 6 days, which may be long enough for complete recovery to have taken place since the latter has been found to commence immediately after sonication of growing roots. On the other hand, the sonication time and the intensity of the ultrasonic beam may be insufficient to affect the embryo. The intensity of the ultrasonic beam used in these experiments is the highest that the Impulsaphon is capable of producing and from the results obtained it seems that a very much longer sonication time would be necessary to cause an observable effect at this intensity. The average ultrasonic intensity of 0.82 W/cm^2 used in this experiment is very much lower than that of 35 W/cm^2 used by Haskell and Selman (1950) on maize seeds. The only effect observed by these authors is a decrease in germination rate which has been attributed to a rise in temperature during sonication.

It seems that the growth rate of the roots of the sonicated seeds is slightly higher than that of the control roots although the error bars shown in Figure 4.34 indicate that this increased growth rate is most probably

insignificant. This indication of a higher growth rate of the roots of sonicated seeds, however, has been observed in each of the three experiments.

Temperature effects.

A temperature rise inside the root of 4°C, which is the maximum increase measured during sonication at the intensities used in the present experiments, is unlikely to have any effect on the subsequent growth of the roots since damage becomes apparent only when the temperature is increased from 19°C to 45°C as reflected in the growth curve in Figure 4.28 . The temperature of 45°C is also quoted by Meyer and Anderson (1949) as being the maximum growth temperature for the entire maize plant. These authors have also mentioned a minimum growth temperature of about 10°C, an optimum temperature of about 30-35°C, and 60°C at which death occurs. The growth of the roots of Zea virtually ceases at 7°C (≈ 2mm/day) and a treatment of short duration at 7°C and 30°C has no adverse effect on the subsequent growth of roots which have been cultured at 19°C (Figures 4.28 and 4.30). Death occurs when the roots are treated at 60°C.

There is no difference in the growth curves obtained when the roots are grown at 19°C and sonicated at 7°C and 19°C respectively (Table 4.7), also indicating that the temperature change of 4°C during sonication is most probably insignificant. Bleaney and Oliver (1972a) have obtained a similar result, i.e. that the fractional

growth in eight days for the roots of Vicia sonicated at 7°C is not significantly different from that for 19°C.

At 7°C the cell cycle time will be much longer than that for the roots cultured at 19°C since growth virtually ceases at 7°C. This is also evident from the results of Evans and Savage (1959) on the cycle time of Vicia roots at different temperatures, shown in Figure 5.16 . For a decrease in temperature from 19°C to 7°C the percentage increase in the cycle time of Zea will probably be equal to that for Vicia since the internal structure of the root tip of Zea closely resembles that for Vicia as mentioned in Chapter I . Thus a possible effect on the sonication damage due to the progress of the cells through the cell cycle during the long exposures at 18 - 22°C should not be present when the roots are sonicated at 7°C. Since the growth curves for the roots sonicated at 7°C are similar to those for the roots sonicated at the higher temperature (Figures 4.31 , 4.32 and 4.33), progress of the cells through the cell cycle does not seem to be significant for the sonication times used.

For the roots of Pisum exposed to ionizing radiation it is believed (Van't Hof and Sparrow, 1963) that the temperature in itself is unimportant in determining the amount of damage suffered because, when plotted against dose per cycle, all their results fall on the same curve. Thus the effect of temperature on X-ray damage is merely that of varying the duration of the cell cycle and

hence the total dose received by the average cell (Van't Hof and Sparrow, 1963). Following a study on the population kinetics of the root meristem of Vicia faba exposed to continuous γ - radiation, Hall, Oliver, Shepstone and Bedford (1966) support the hypothesis that it is the dose per cell cycle that determines the degree of damage produced in a population of dividing cells exposed continuously to ionizing radiation. In the experiments by the above authors the roots were exposed to ionizing radiation for times equalling a few cell cycles. The sonication times used in the present experiments are short (maximum sonication time used equals 180 minutes) compared to the cell cycle time of 31.1 hours which has been determined using a metaphase accumulation technique as described previously. A possible dependence of the sonication damage on the dose per cell cycle is unlikely to be observed for these short sonication times.

It is perhaps of interest to note that the growth curve for the roots exposed to a temperature of $45^{o}C$ reaches a minimum during the first day after treatment and is similar to those obtained for the majority of the ultrasonic exposures used (Figure 4.29). This may indicate that there is an analogy between the damage caused by both modalities. The damage to plant cells caused by a high temperature has been ascribed to be largely, if not entirely, due to the coagulation of the protoplasm (Lepeschkin, 1935; Meyer and Anderson, 1949).

A reduction of viscosity in plant cell protoplasm

using a low ultrasonic intensity of 0.04 W/cm^2 (1 MHz continuous beam) has been observed by Johnsson and Lindvall (1969). The expression of possible damage accompanying a change in viscosity may be similar to that due to coagulation.

Enzymes in the cells are, however, also destroyed by excessive heating (Meyer and Anderson, 1949). Not all enzymes are similarly affected by ultrasound (Hogeboom and Schneider, 1952). Some show a marked increase in activity, e.g. amylase, while for others, e.g. catalase, there is a decrease in activity (Rubar and Dolgopolov, 1953; Obolensky, 1957a). The analogous results obtained may, therefore, be due to the effect of heat and ultrasound on certain enzymes.

Effect of dissolved oxygen.

For the various concentrations of dissolved oxygen brought about by passing air, helium, oxygen and nitrogen through the water in the sonication tank, there is a marked reduction in the damage to the roots when compared to the sonication effect in air-equilibrated water (Figure 4.26 and Table 4.6).

The damage at the various oxygen concentrations ranging from 2.0 ml/l to 22.2 ml/l is similar, except for the lowest oxygen concentration of 1.4 ml/l at which the damage is somewhat larger (Figure 4.26, Table 4.6). The growth curve obtained when air is passed through the water is similar to that for all the other gases although

the change in oxygen concentration from 6.7 ml/l in air-equilibrated water to 6.9 ml/l in aerated water is very small. These results suggest that the nature of the ambient gas does not influence the sonication damage. Rouyer and Grabar (1947) have also found that the percentage of B. paradysenteriae of a given titre killed in 30 minutes by irradiation with ultrasound is similar in the presence of different gases (air, oxygen, nitrogen, argon and hydrogen).

In contrast to this response, the presence of molecular oxygen at the time of X-irradiation acts as a sensitizing agent, and the biological effects of the radiation are greater in the presence of oxygen than in its absence. This sensitizing effect of oxygen is illustrated in Figure 5.17 , which depicts the survival curves obtained by Dewey (1960) when human liver cells in tissue culture were irradiated in the presence and absence of gaseous oxygen. In this case the slopes of the two curves differ by a factor of 2.2, and cells in oxygen are 2.2 times as sensitive to radiation as cells in pure nitrogen.

The effect of ultrasound on biological material has been found to be dependent on the nature of the ambient gas only under conditions of transient cavitation (Elpiner and Sokolskaya, 1962). Since transient cavitation is unlikely to occur in the present situation (discussed previously) any effect due to the presence of free radicals can be ruled out.

Although the oxygen content of the aerated water is

about equal to that of the air-equilibrated water, the average ultrasonic intensity at the position of the roots is reduced from about 0.82 W/cm^2 in air-equilibrated to about 0.68 W/cm^2 in the aerated water. Thus the reduction in average intensity seems to be due to the presence of gas bubbles formed by the diffuser stone and not so much on the amount of dissolved gas. The reduced effectiveness of the ultrasound to produce sonication damage to the roots may possibly be ascribed to a "filtering effect" of the sound by the gas bubbles. Such an effect has, however, not been detected with the thermistor probe.

Dose-fractionation experiments.

The split dose experiments (Table 4.4 and Figure 4.17) indicate that any possible damage in surviving cells produced as a result of exposure to ultrasound at an average intensity of 0.82 W/cm^2 (continuous beam), is not repaired within a period of 24 hours. In fact, the values of the fractional growth in ten days are lower for time intervals between doses larger than or equal to $2\frac{1}{2}$ hours and there seems to be an indication of an increase in damage with the time interval between the doses. These results seem to be again a confirmation of the "all or nothing" effect (see also page 1) of ultrasound, the cells either being damaged beyond repair or not damaged at all.

Bleaney and Oliver (1972a) have found that the sonication effects on the roots of Vicia faba are similar for a single 30 minute exposure and two exposures of 15 minutes

each at the same intensity level but separated in time by 4 and 24 hours, i.e. no repair of damage has been observed between doses.

For X-rays, however, it has been found that the effect of a single dose is larger than that of a dose of the same magnitude but given in two equal fractions separated by various time intervals as shown in Figure 5.18 for Vicia, indicating that there is recovery of sublethal X-radiation damage between doses (Elkind and Sutton, 1959; 1960).

Age-response of partially synchronized meristematic cells.

The maximum mitotic index (MI) of 20% (Figure 4.61) obtained when the roots of Zea are partially synchronized with hydroxyurea (HU) is much less than that of 40% obtained by Hall, Brown and Cavanagh (1968). The concentration of HU used for Zea is equal to that used by Hall et al for Vicia and although the roots of Zea have been treated for 36 hours as compared to 24 hours for Vicia, the drug has reduced the growth of Zea by only 5% compared to the 9% for Vicia. This may indicate that the roots of Zea respond differently to HU-treatment than those of Vicia. Clowes (1965a) obtained a maximum MI of 86% in the root tip of Zea but he used a different drug, viz. 5-amino-uracil.

The growth curves (Figures 4.57 - 4.60) and the values of G_{10} (Table 4.11) obtained for roots sonicated immediately after treatment with HU are similar to those for the ultrasonic irradiations at the time when a peak

in the MI occurs. This may be an indication that cells in mitosis and cells in the other phases of the cell cycle are affected similarly by ultrasound. On the other hand, a possible age-response of the meristematic cells to ultrasound may not be detected if only 20% of the population of cells are in mitosis during sonication. The MI of 20% differs only by about 10% from the MI in a control root (Table 3.5). Also, hydroxyurea and ultrasound seem to act independently of each other, the damage apparent in the growth curve either being due to HU or ultrasound depending on the modality which dominates at the time.

Clarke and Hill have found (as described previously) a reduced percentage of surviving cells in mitosis after sonication of an asynchronous population of mouse leukaemia cells as compared to control populations. The organized population of cells in the root meristem may, however, respond differently to ultrasound compared with cells in tissue culture.

The effect of simultaneous X-irradiation on the sonication damage.

The shape of the growth curve obtained when the roots of Zea are exposed to X-rays (Figure 4.44) is similar to that for Vicia (Figure 1.4), and is typical of the response to ionizing radiation (Read, 1959). When the roots of Zea are perpendicular to the X-ray beam axis during X-irradiation, G_{min} (≈ 0.4) is reached at about

5 days post-irradiation whereas when the roots are parallel to the beam axis (necessary when ultrasound and X-rays were delivered simultaneously), G_{min} (≈ 0.6) is reached already after about 3 days. This difference can possibly be ascribed to a gradually decreasing dose rate along the length of the roots when they are parallel to the beam axis.

When both types of radiation (i.e. ultrasound and X-rays) are applied to the roots, the X-rays either being given before, after or simultaneously with the continuous or pulsed beams of ultrasound, the effects of both modalities are apparent in the growth curves (Figures 4.35 - 4.42). During the first one or two days after radiation the effect of the X-ray dose is not expressed fully and consequently the ultrasonic damage is always apparent in the growth curve at that time. The subsequent immediate recovery, characteristic for the roots treated with ultrasound as described previously, is modified by the X-ray dose given and the growth curve reaches a minimum at about 3-5 days after irradiation. Thus the radiations seem to act independently of each other, suggesting that the mechanism of damage due to X-rays is different from that due to ultrasound, possibly as an immediate type of death in the case of the latter, as opposed to a delayed (? reproductive) type of death in the case of the former (Bleaney and Oliver, 1972a).

An indication of an additive effect of the ultrasound and X-rays is only apparent in the growth of the roots during the first day after irradiation, except perhaps for the

simultaneous irradiation with 775 rads and 0.82 W/cm^2 (continuous beam) for 30 minutes (Figure 4.42). Most of the values of the average growth in the first day as a fraction of controls (G_1), after a combined irradiation with ultrasound and X-rays are less than the values of G_1 for the roots exposed to ultrasound alone (Figures 4.35 - 4.42). These differences are, however, within the inter-experimental error referred to on pages 173 and 235, and are probably not significant.

An increase in the X-ray effectiveness by a factor of 1.7 under the action of ultrasound as reported by Woeber (1959, 1965) is not apparent in the growth curves nor in the values of G_{10} (Table 4.8) obtained. The sensitivity of the experiments is such that a possible enhancement in radio-sensitivity of the order of that obtained by the various authors listed in Table 1.4 , should have been detected. These results are in keeping with those obtained by Clarke, Hill and Adams (1970) who attribute Woeber's results to a possible increase in the temperature of the irradiated tissue.

Effect of vincristine on sonication damage.

For X-irradiated roots the expression of the radiation damage is delayed (as described previously) and the minimum growth rate is reached at about 5 days post-irradiation (Figure 4.44).

For the vincristine-treated roots there is also a gradual decrease in growth rate, reaching a minimum at

about $2\frac{1}{2}$ days, and then increases gradually and levels off by some ten days post-irradiation (Figures 4.43 and 4.44). This similarity in the expression of the damage produced by vincristine and that due to X-rays indicates that the cells damaged by vincristine may also be able to undergo one or more divisions before dying. In fact Read (1959) mentioned that the meristematic cells of Vicia are able to undergo some divisions before dying when damaged by certain radiomimetic drugs, e.g. 8-ethoxycaffeine and nitrogen mustard. The shape of the growth curves obtained by Read for these drugs (Read, 1959; Figure 1.13) are similar to the ones obtained in the present experiments with vincristine (Figure 4.43).

The minimum growth rate is reached sooner in the case of roots of Zea treated with vincristine ($2\frac{1}{2}$ days) as compared to that of the X-rayed roots (5 days). This result is similar to that obtained for the roots of Vicia treated with 8-ethoxycaffeine (EOC) and X-rays (Read and Kihlman, 1956; Read, 1959).

For ultrasonic exposures sufficiently high to affect the growth rate of the roots, the latter falls immediately after sonication, recovers in the next few days and levels off by about 10 days post-sonication (Figure 4.44). As discussed previously it appears that cells sterilized by ultrasound are not able to undergo any divisions and as a result of this the sonication damage is expressed immediately, without any delay in the minimum of the growth curve.

When roots are treated with vincristine in con-

junction with ultrasound, the curves seem to indicate that the damage due to ultrasound is somewhat masked by the damage produced by vincristine. During the first day after treatment, however, the effect of the vincristine treatment is not expressed fully and consequently the ultrasonic damage (i.e. a more rapid decrease in growth rate) is always apparent in the growth curves at that time. The immediate drop in growth rate seems to be greater for the combined treatments with ultrasound and vincristine, than when ultrasound is given alone. For the experiments in which low exposures of ultrasound are used in combination with a vincristine treatment of 1 hour (Figures 4.45 and 4.46), the minimum growth rate as a fraction of controls reached after 2-3 days is less than that obtained when the vincristine is used alone. Most of these differences are, however, within the inter-experimental error referred to on page 243 and are most probably insignificant.

In Figures 4.50 and 4.51 the rapid recovery after the initial drop in growth rate in the first day is modified by the effect of the vincristine treatment and the growth curves reach a minimum 2-3 days after treatment.

The ultrasound and vincristine seem to act independently of each other, suggesting that the mechanism of damage due to vincristine is different from that due to ultrasound, possibly as an immediate type of death in the case of the latter, as opposed to a delayed (? reproductive) type of death in the case of the former if vincristine can be assumed to affect cells in the same way as

other radiomimetic drugs (i.e. the effect being similar to that due to X-rays) as described earlier.

There seems to be a marked similarity between these results and those obtained in the experiments using ultrasound and X-rays (Figure 4.56), which can possibly be explained in terms of the similarity in the expression of the damage due to X-rays and that produced by radiomimetic agents (Read, 1959; Bruce, 1967; Elkind, Sutton-Gilbert, Moses and Kamper, 1967; Berry, 1968; Berry, 1969; Elkind and Sakamoto, 1969). The decrease in growth rate of X-rayed roots is, however, slower than that for vincristine-treated roots and thus in the experiments in which low exposures of ultrasound are given in conjunction with X-rays the recovery from ultrasonic damage is apparent in the growth curves, whereas in similar experiments using vincristine the recovery from ultrasonic damage is mostly masked by the more rapid (as compared to X-rays) expression of damage by vincristine (Figure 4.55).

In contrast to the above response of the roots to vincristine, the growth curve for the roots treated with hydroxyurea shows an immediate decrease in growth rate after treatment and a rapid subsequent recovery similar to the growth curve for the roots treated at the lower ultrasonic intensities (Figures 4.57 and 4.58). Vincristine causes an arrest of mitosis at the metaphase stage (Palmer, Livengood, Warren, Simpson and Johnson, 1960) whereas it has been shown that hydroxyurea kills Chinese hamster cells that are synthesizing DNA but does not affect the progression of cells through the non-DNA-synthesizing portion of

the cycle (Sinclair, 1965 and 1967). Such a difference in the mechanism of action of these drugs may be responsible for the observed effect. Nevertheless, hydroxyurea and ultrasound also seem to act independently of each other, the damage apparent in the growth curve either being due to hydroxyurea or to ultrasound depending on the modality which dominates at the time (Figures 4.57 - 4.60).

FIGURE 5.1 .

LETHAL EFFECTS ON CYCLOPS OF ULTRASOUND OF DIFFERENT INTENSITIES.

(FROM: POHLMAN, 1950)

—o—o— 1.8 W/cm^2

—•—•— 2.6 W/cm^2

—×—×— 3.1 W/cm^2

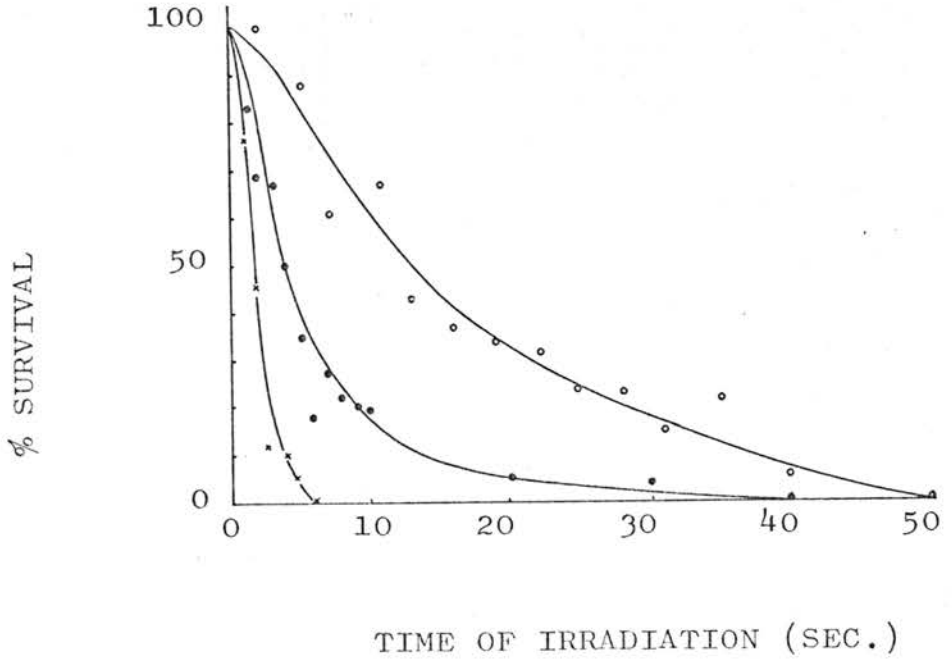


FIGURE 5.2 .

INITIAL SURVIVING FRACTION OF CELLS IN THE MERISTEM OF VICIA PLOTTED AGAINST X-RAY DOSE.

(FROM: HALL, LAJTHA AND OLIVER, 1962)

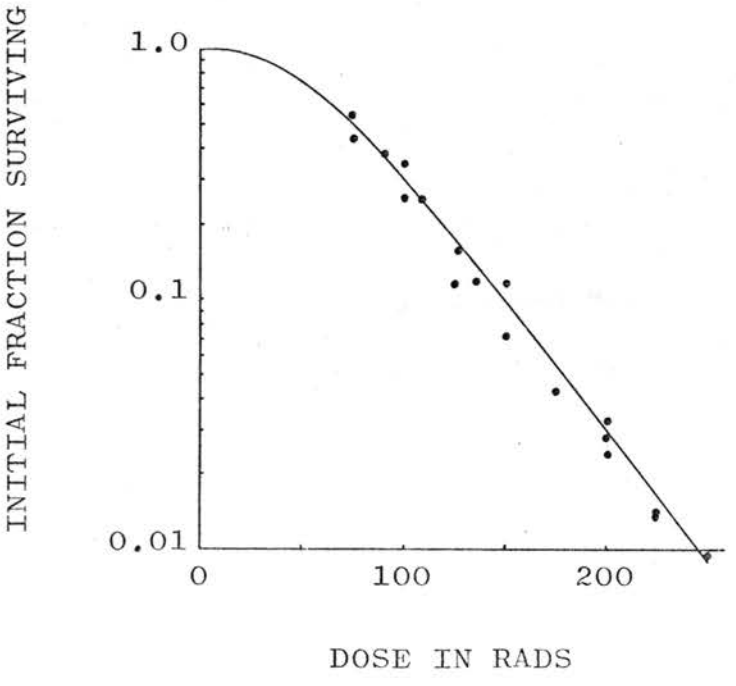


FIGURE 5.3 .

VARIATION OF G_1, THE AVERAGE GROWTH IN THE FIRST DAY POST-SONICATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, AS A FUNCTION OF TOTAL EXPOSURE (W/cm^2 MULTIPLIED BY DURATION OF EXPOSURE IN HOURS) FOR AN AVERAGE ULTRASONIC INTENSITY OF 0.21 W/cm^2.

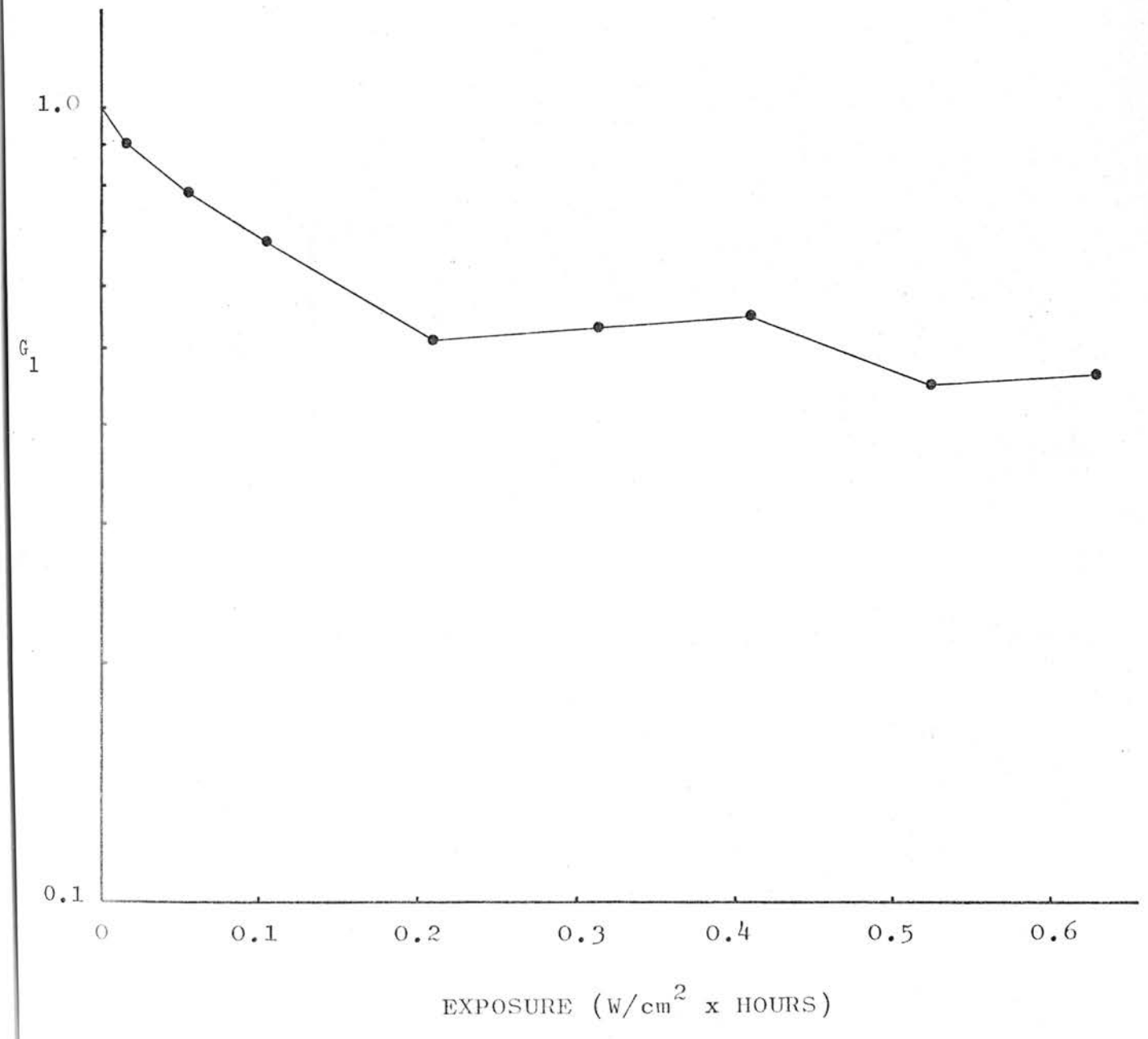


FIGURE 5.4 .

VARIATION OF G_1, THE AVERAGE GROWTH OF ZEA MAYS ROOTS IN THE FIRST DAY POST-SONICATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, AS A FUNCTION OF THE AVERAGE ULTRASONIC INTENSITY OF THE PULSED (DASHED LINES) AND CONTINUOUS (FULL LINES) BEAMS FOR VARIOUS SONICATION TIMES AS INDICATED (PULSED BEAM WITH 1ms PULSES AND 0.2 DUTY CYCLE).

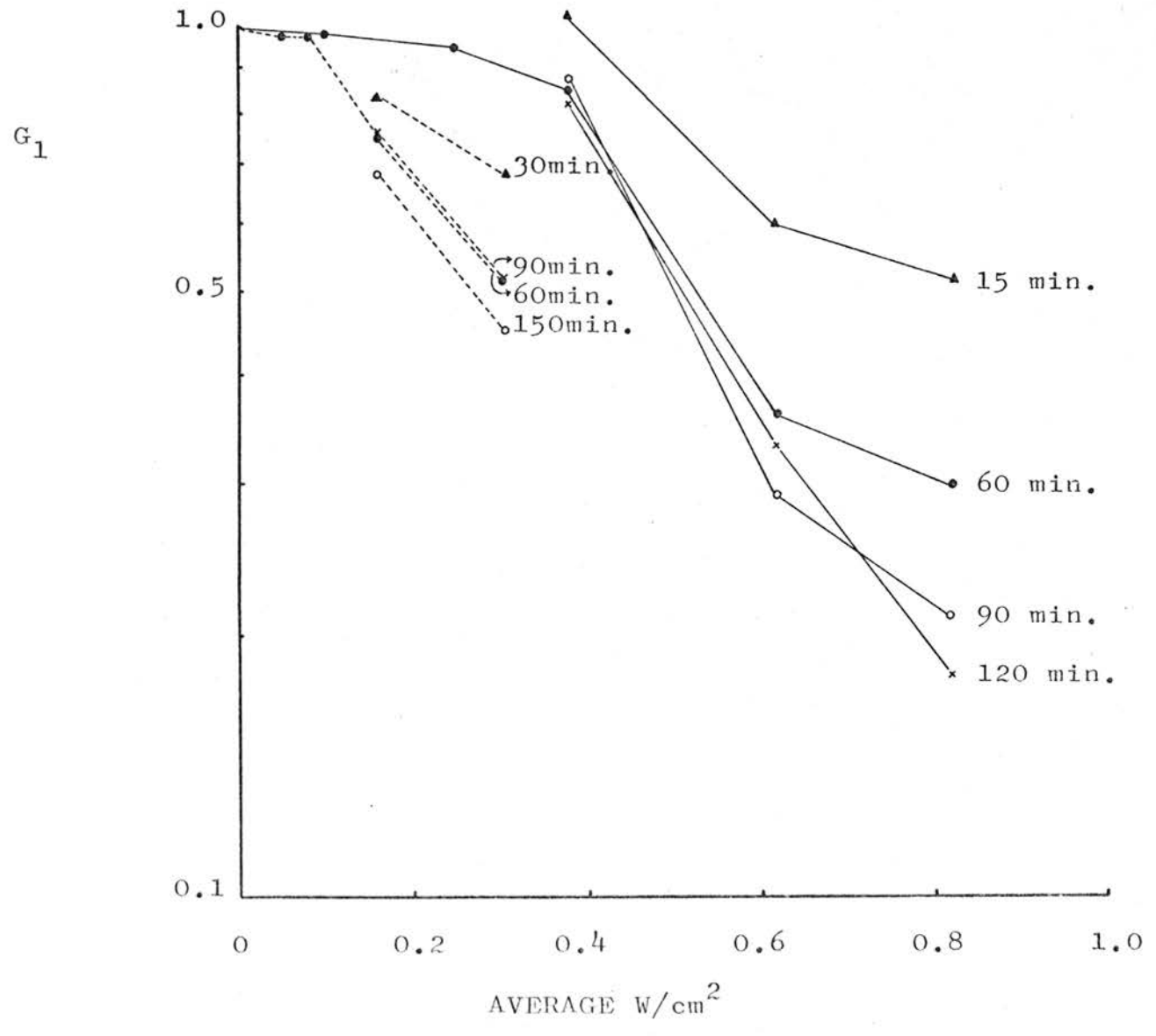


FIGURE 5.5 .

AVERAGE GROWTH (G_8) OF VICIA FABA ROOTS IN EIGHT DAYS FOLLOWING EXPOSURE TO 1.5 MHz ULTRASONIC RADIATION AS A FRACTION OF THE CORRESPONDING AVERAGE GROWTH FOR CONTROL ROOTS, USING CONTINUOUS RADIATION (——•——•——), 1 ms PULSES WITH 0.25 DUTY CYCLE (------o-----o-----). ABSCISSA REFERS TO AVERAGE POWER IN ALL CASES.

(FROM: BLEANEY AND OLIVER, 1972b)

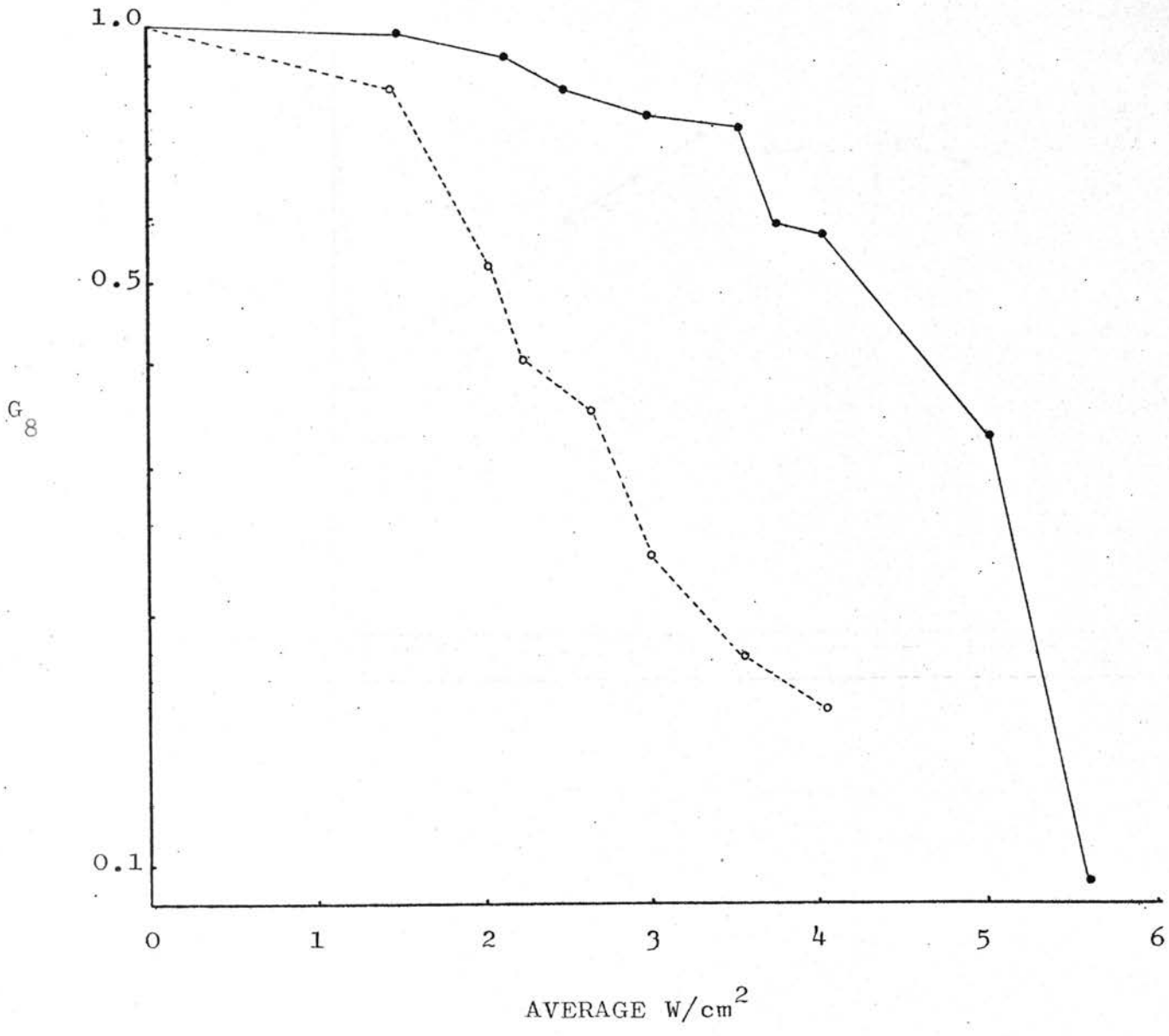


FIGURE 5.6 .

VARIATION OF G WITH TIME AFTER EXPOSURE.

1.0 MHz ULTRASONIC RADIATION AT THE AVERAGE INTENSITY OF 0.62 W/cm^2 FOR 15 MIN., CONTINUOUS BEAM, FOR ZEA MAYS.

1.5 MHz ULTRASONIC RADIATION AT 3 W/cm^2 FOR 15 MIN., CONTINUOUS BEAM, FOR VICIA FABA. (BLEANEY & OLIVER, 1972a).

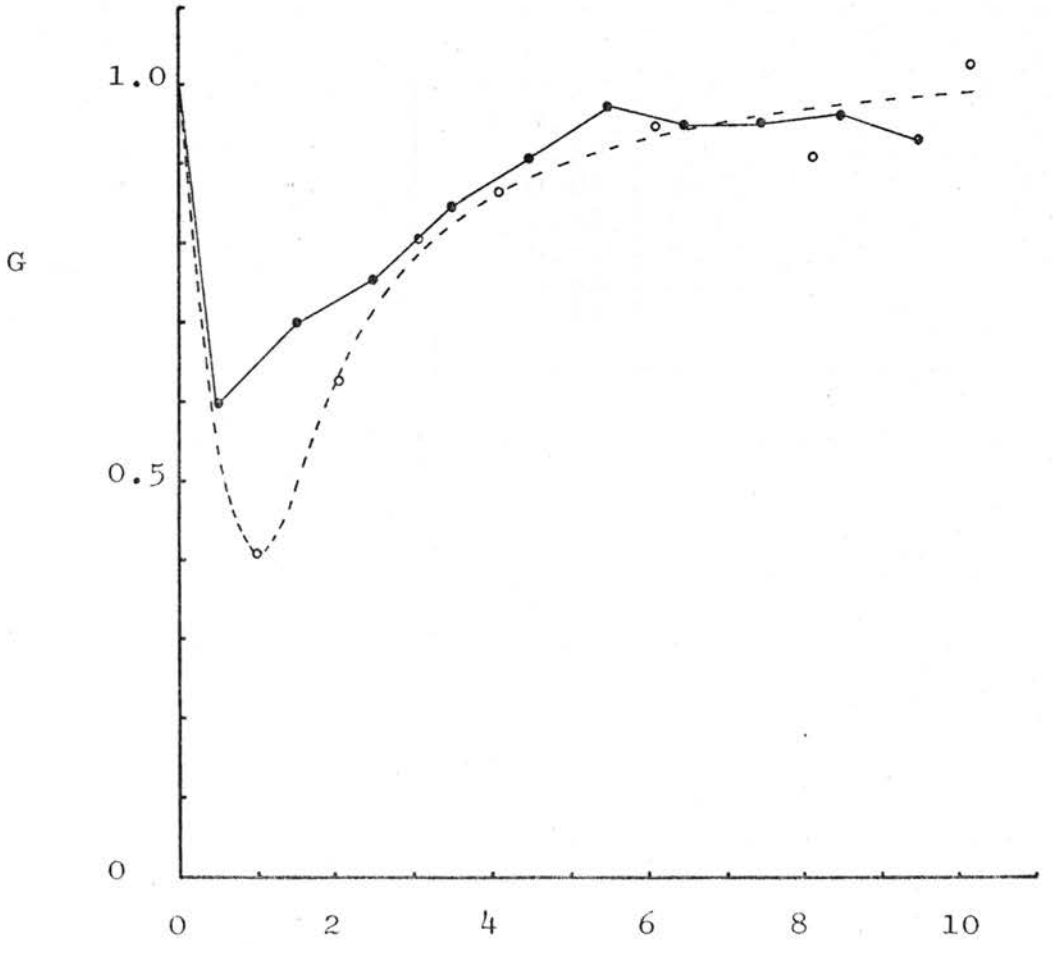
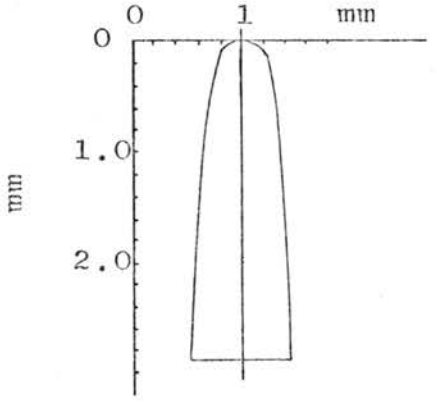


FIGURE 5.7 .

CHANGE IN THE DIAMETER (MEAN OF 5 ROOTS) OF THE ROOT OF ZEA WITH DISTANCE FROM THE ROOT TIP (3 DAYS AFTER TRANSFER INTO CULTURE TANK).

(The errors are standard errors of the means for 10 roots).

DISTANCE FROM TIP (MM)	MEAN DIAMETER (MM)
0.1	0.27 ± 0.03
0.2	0.36 ± 0.05
0.3	0.45 ± 0.06
0.4	0.52 ± 0.08
0.6	0.61 ± 0.07
0.8	0.65 ± 0.06
1.0	0.69 ± 0.05
1.2	0.70 ± 0.04
1.4	0.75 ± 0.02
1.6	0.79 ± 0.03
1.8	0.81 ± 0.02
2.0	0.82 ± 0.02
2.5	0.82 ± 0.02
3.0	0.82 ± 0.02
3.5	0.82 ± 0.02



OUTLINE OF THE ROOT OF VICIA (MEAN OF 4 ROOTS)

AS MEASURED BY GRAY AND SCHOLES, 1951.

FIGURE 5.8 .

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

---------- MODEL A

———————— MODEL B

ASSUMPTIONS: CELL DEATH DUE TO SONICATION IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; CONSTANT CELL CYCLE TIME; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH;COMPLETE RECOVERY AFTER SONICATION, MAXIMUM G-VALUE = 1.0.

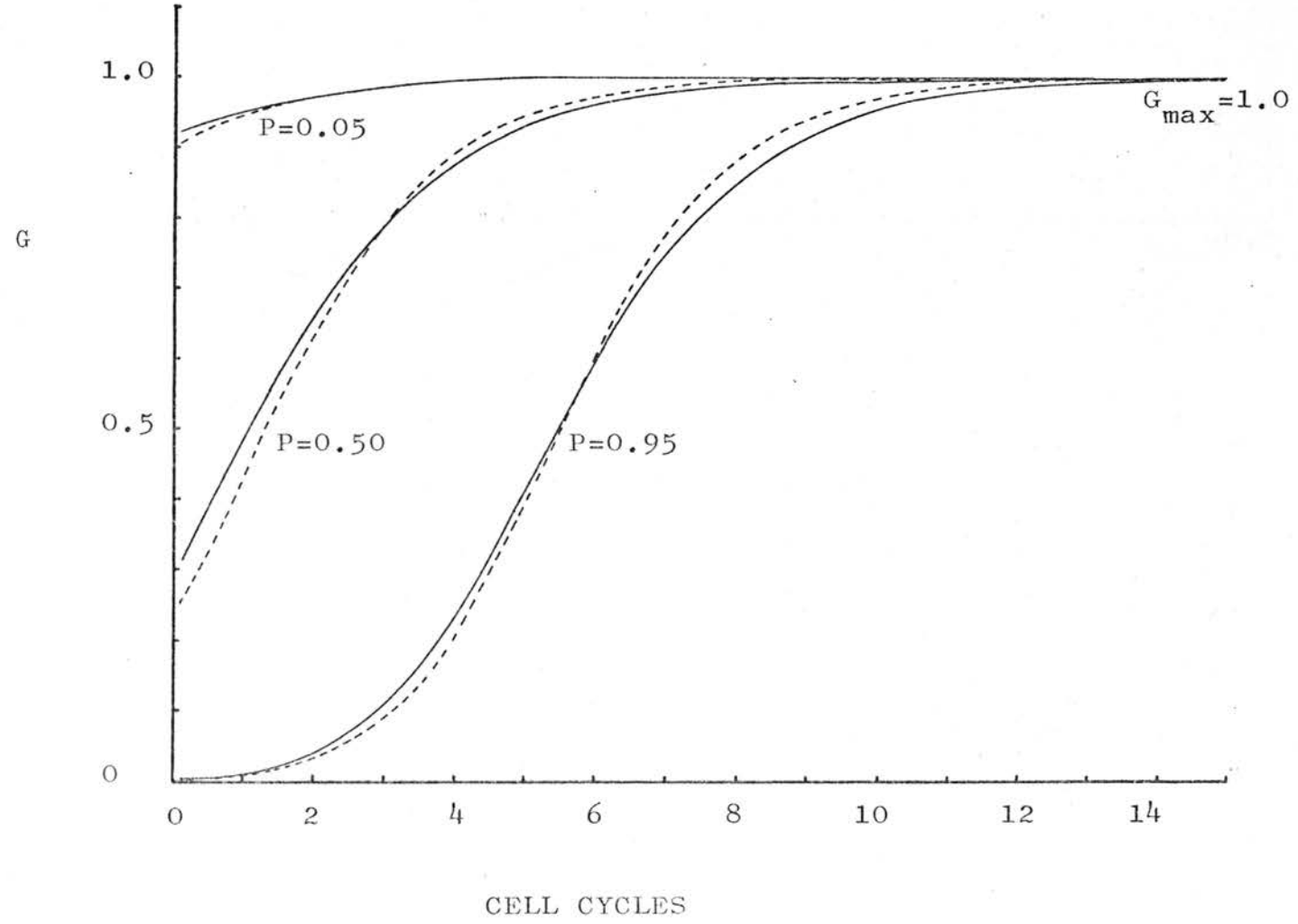


FIGURE 5.9 .

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

---------- MODEL A

———————— MODEL B

ASSUMPTIONS: CELL DEATH DUE TO SONICATION IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; CONSTANT CELL CYCLE TIME; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH; INCOMPLETE RECOVERY AFTER SONICATION, MAXIMUM G-VALUE = 0.5.

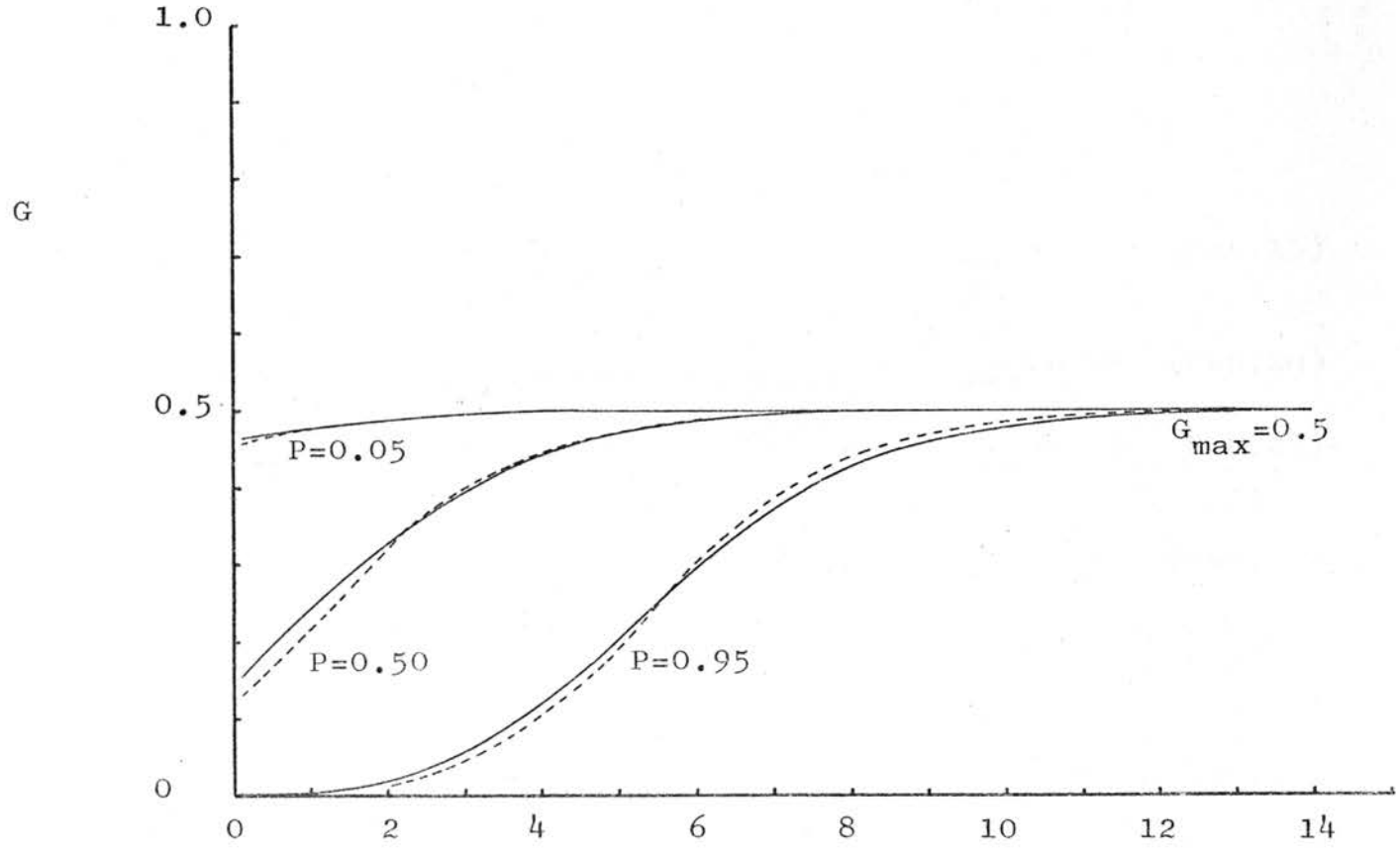


FIGURE 5.10.

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———•———•——— EXPERIMENTAL RESULTS (AVERAGE INTENSITY = 0.82 W/cm^2, CONTINUOUS BEAM, SONICATION TIMES AS INDICATED).

---------- CALCULATED, KINETIC SYSTEM OF MODEL A.

..... CALCULATED, KINETIC SYSTEM OF MODEL B.

ASSUMPTIONS: CONSTANT CELL CYCLE TIME; CELL DEATH IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

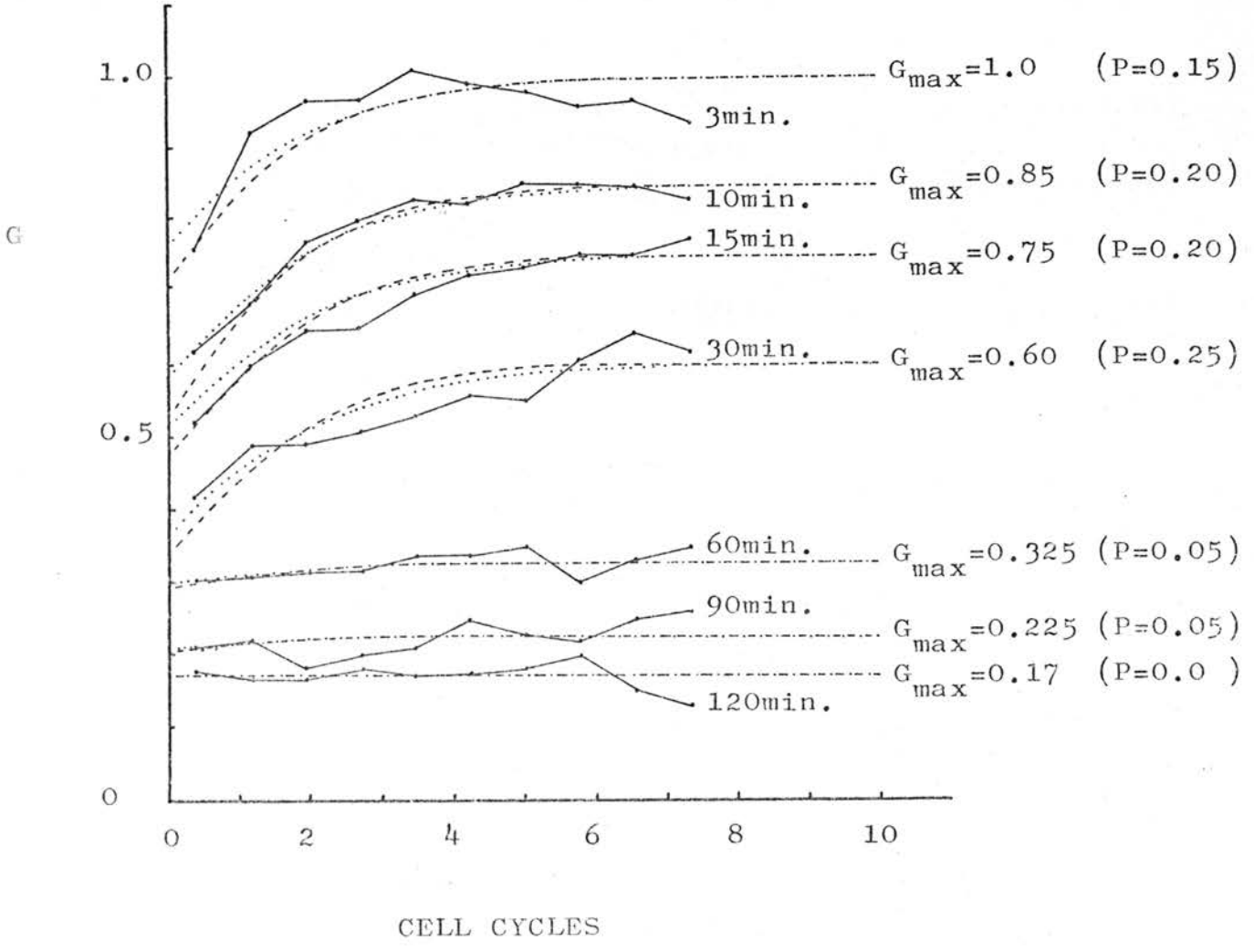


FIGURE 5.11 .

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———•———•——— EXPERIMENTAL RESULTS (AVERAGE INTENSITY = 0.62 W/cm^2, CONTINUOUS BEAM, SONICATION TIMES AS INDICATED).

---------- CALCULATED, KINETIC SYSTEM OF MODEL A.

..... CALCULATED, KINETIC SYSTEM OF MODEL B.

ASSUMPTIONS: CONSTANT CELL CYCLE TIME; CELL DEATH IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

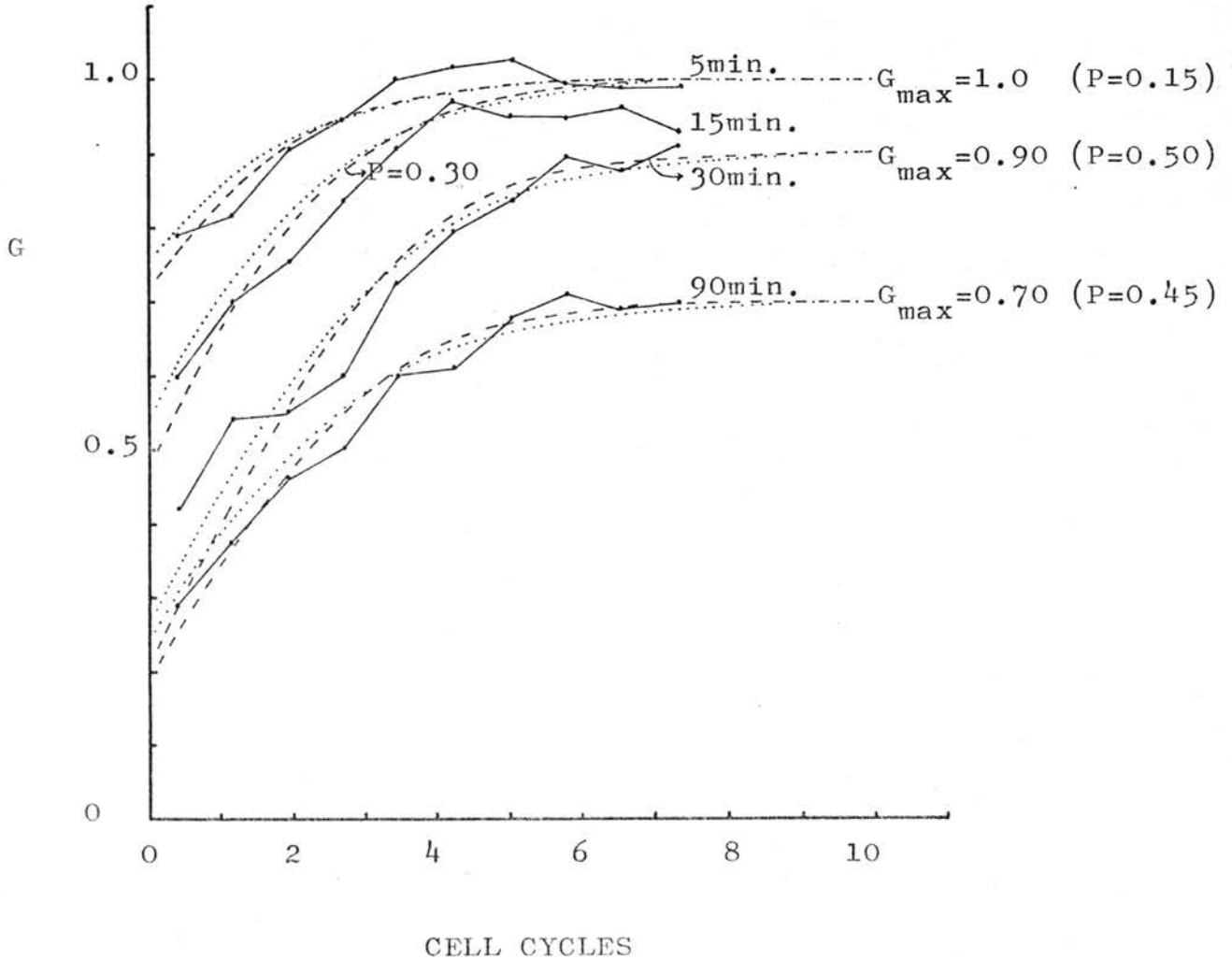


FIGURE 5.12 .

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———•———•——— EXPERIMENTAL RESULTS (AVERAGE INTENSITY = 0.21 W/cm^2, PULSED BEAM, SONICATION TIME = 5 MINUTES).

---------- CALCULATED, KINETIC SYSTEM OF MODEL A.

..... CALCULATED, KINETIC SYSTEM OF MODEL B.

ASSUMPTIONS: CONSTANT CELL CYCLE TIME; CELL DEATH IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

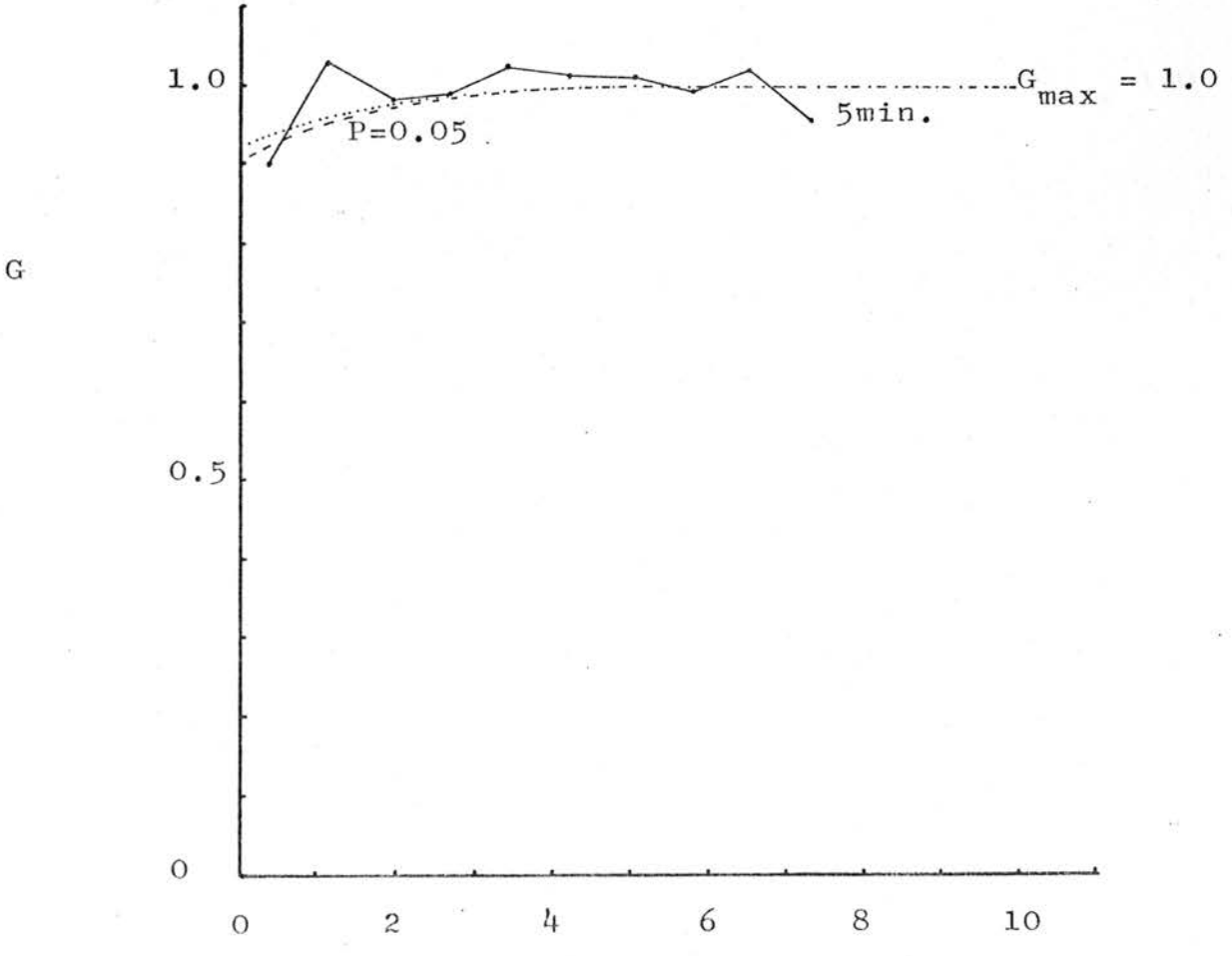


FIGURE 5.13 .

AS FOR FIGURE 5.12 , BUT FOR A SONICATION TIME OF 30 MINUTES.

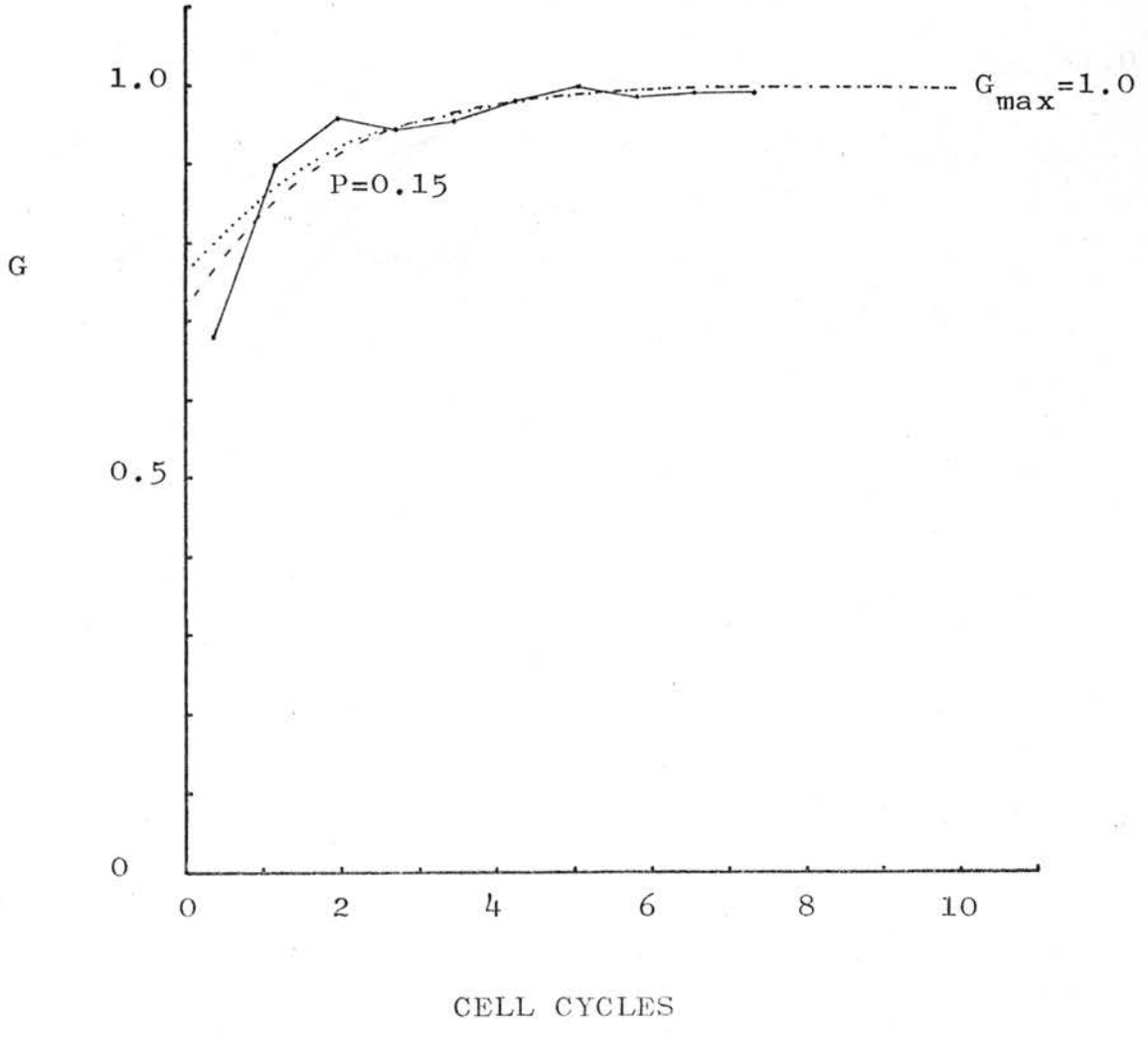


FIGURE 5.14 .

AS FOR FIGURE 5.12 , BUT FOR A SONICATION TIME OF 150 MINUTES.

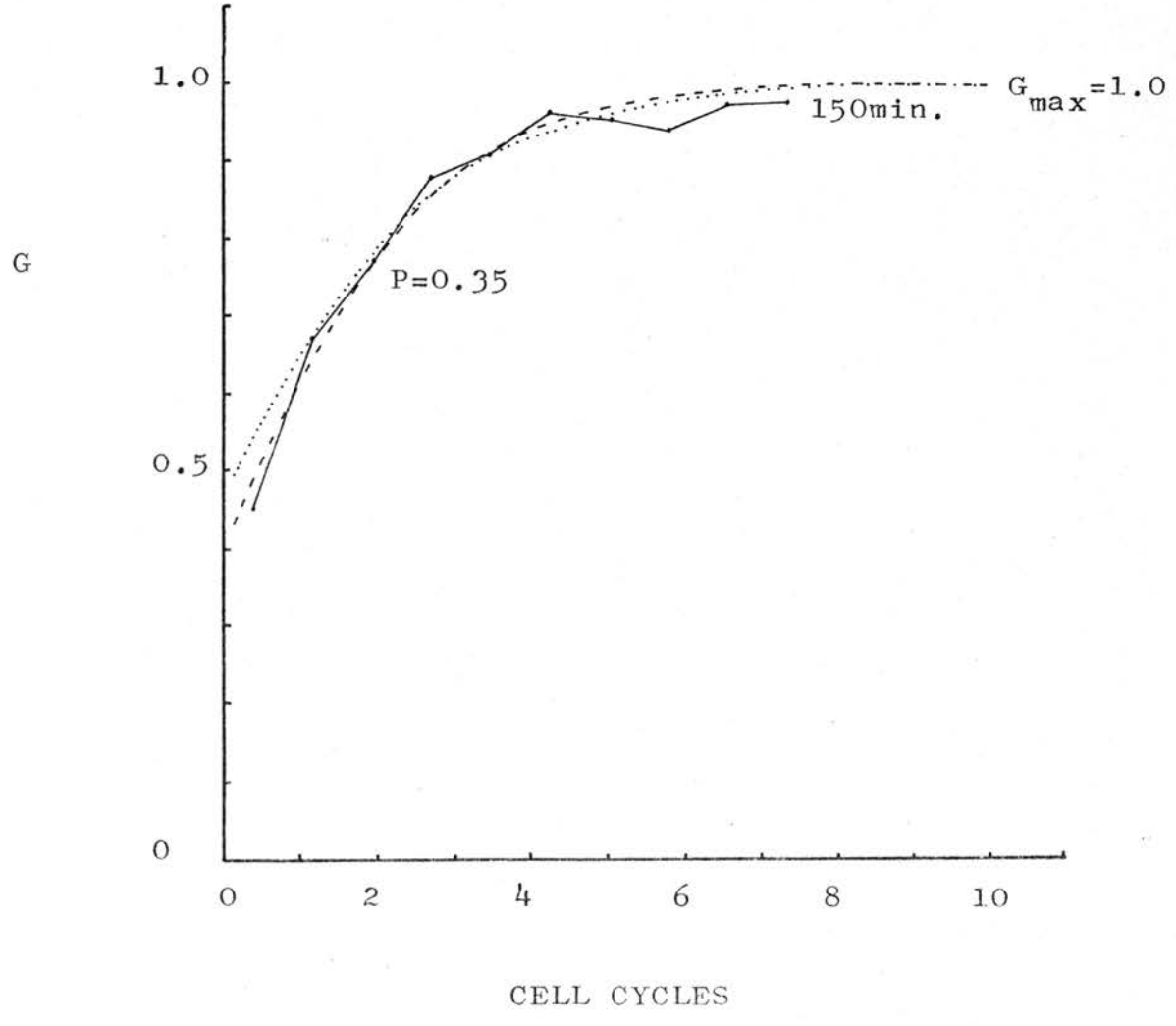


FIGURE 5.15.

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———•———•——— EXPERIMENTAL RESULTS (AVERAGE INTENSITY = 0.16 W/cm^2, PULSED BEAM, SONICATION TIME = 30 MINUTES).

---------- CALCULATED, KINETIC SYSTEM OF MODEL A.

..... CALCULATED, KINETIC SYSTEM OF MODEL B.

ASSUMPTIONS: CONSTANT CELL CYCLE TIME; CELL DEATH IN THE FIRST ONE TENTH OF THE FIRST CELL CYCLE; DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

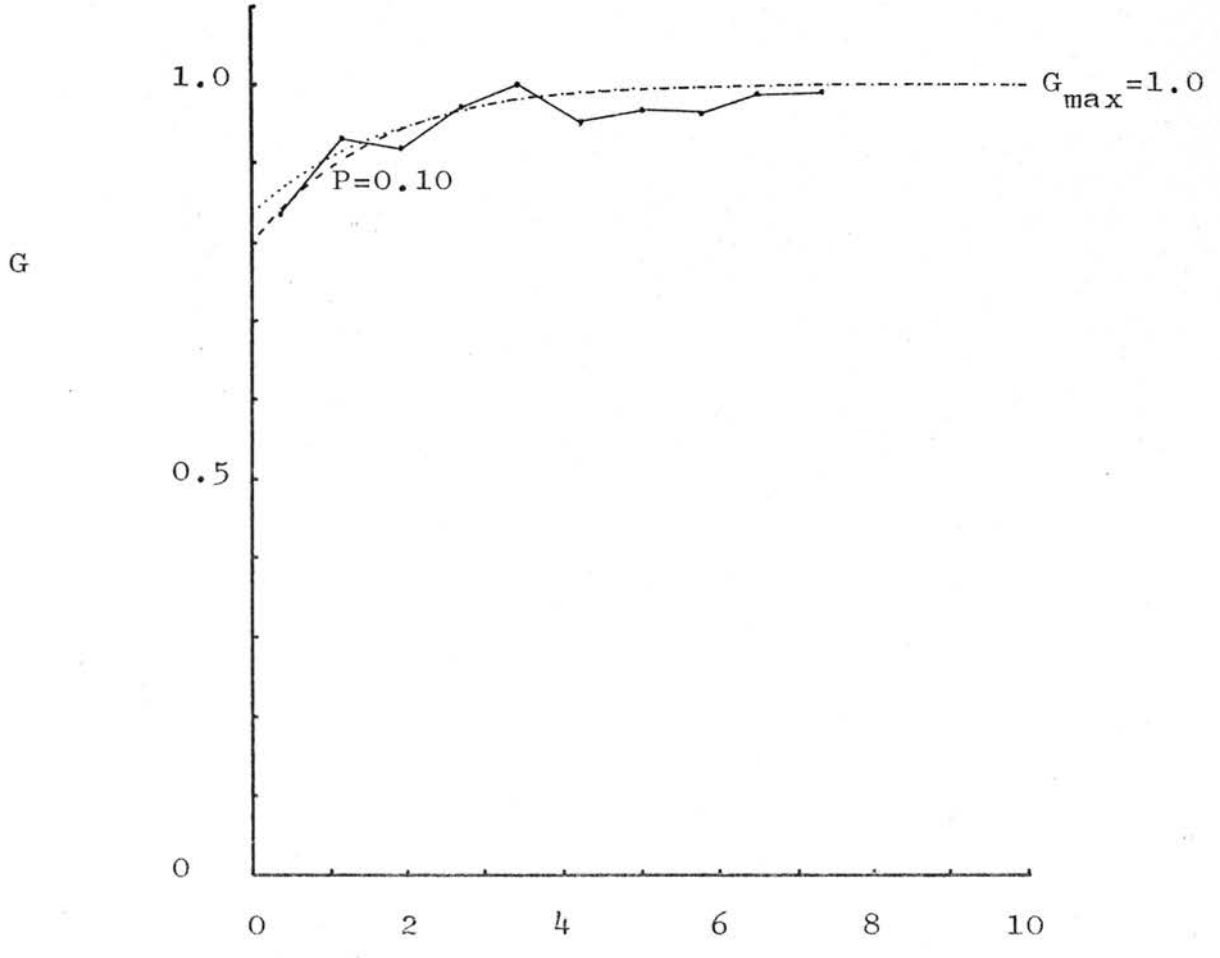


FIGURE 5.16.

MITOTIC CYCLE TIME AS A FUNCTION OF TEMPERATURE.

(DATA FROM EVANS AND SAVAGE, 1959.)

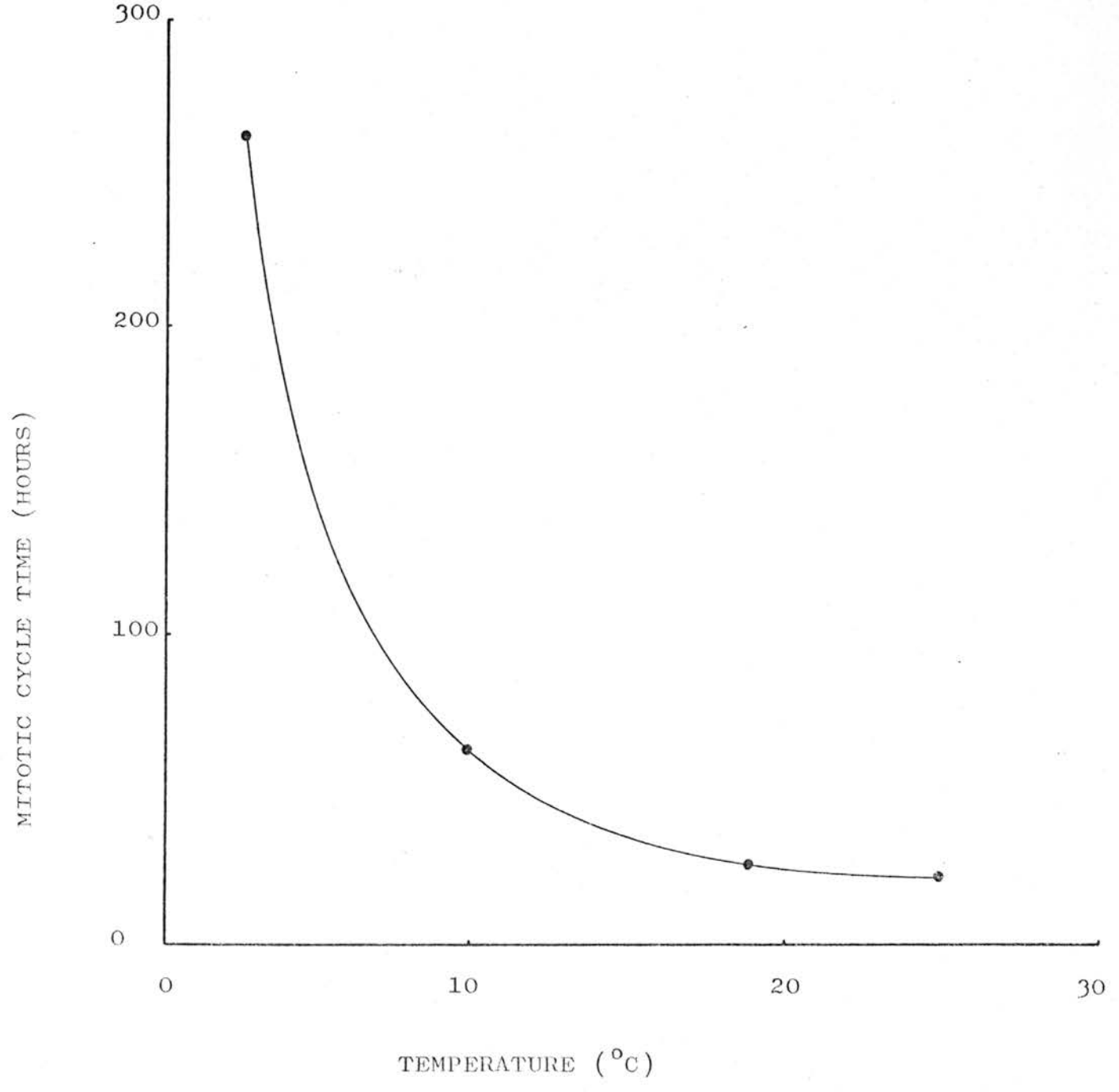


FIGURE 5.17.

SURVIVAL CURVES FOR HUMAN EMBRYO LIVER CELLS IN TISSUE CULTURE, IRRADIATED WITH IONIZING RADIATION IN THE PRESENCE AND ABSENCE OF OXYGEN.

(FROM: DEWEY, 1960)

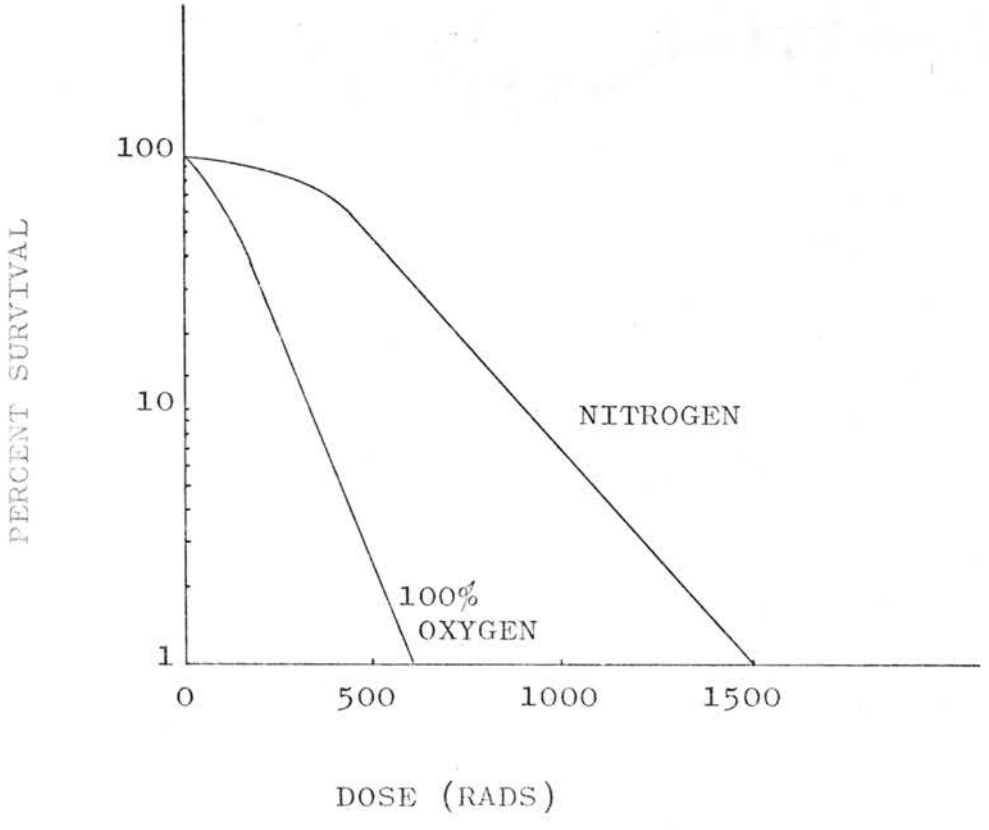
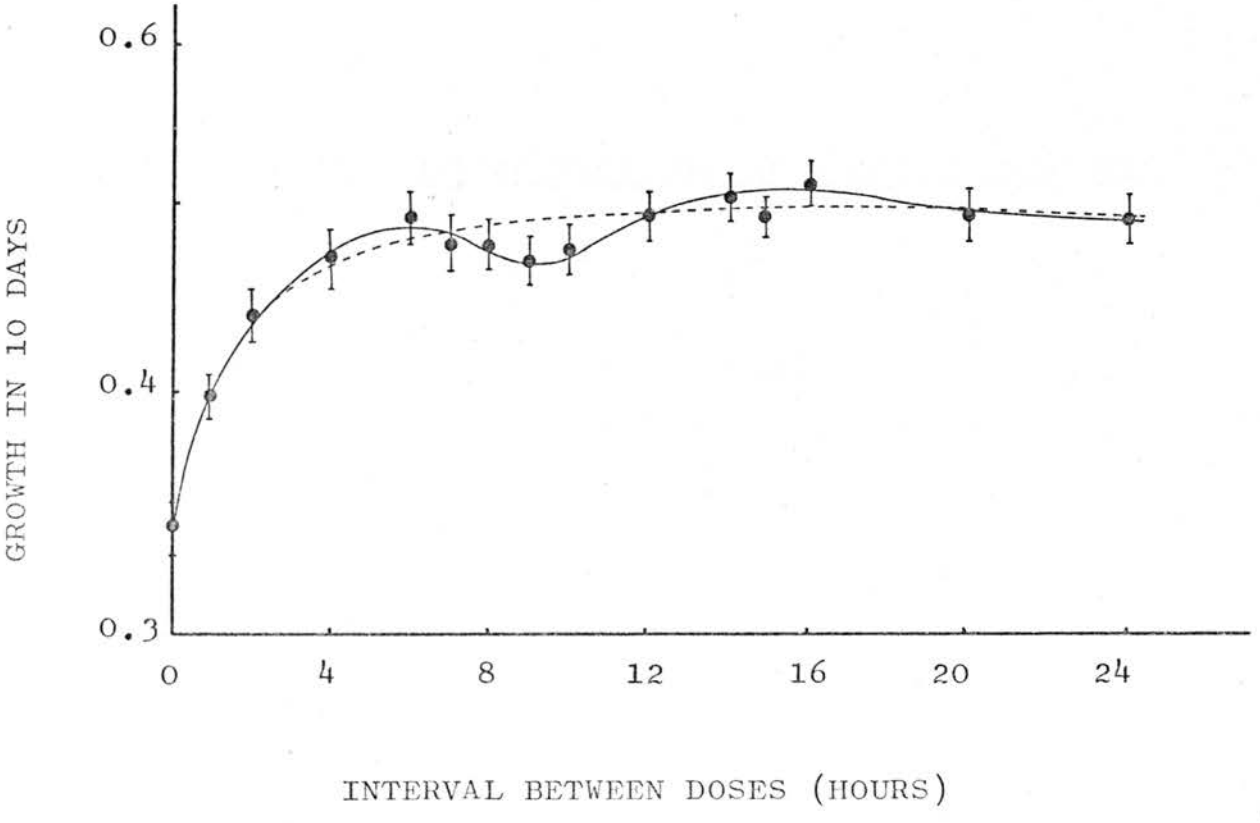


FIGURE 5.18 .

THE "GROWTH IN TEN DAYS" FOR ROOTS OF VICIA FABA EXPOSED TO TWO DOSES OF 100 RADS SEPARATED BY VARIOUS TIME INTERVALS.

(FROM: SHEPSTONE, 1964)



CHAPTER VI.

POSSIBLE CLINICAL IMPLICATIONS OF THE RESULTS OBTAINED.

POSSIBLE CLINICAL IMPLICATIONS OF THE RESULTS OBTAINED.

The first therapeutic results of treatment with ultrasound were not always those anticipated. Inability to measure and control the intensity of the ultrasonic beam accurately and inadequate knowledge of the interaction between the beam and the various tissues of the body, were both perhaps reasons for the variation in the results reported. This led Nelson, Herrick and Krusen to recommend in 1950 that ultrasound should not be used for the treatment of any human disease. It was, however, also clear that the therapeutic possibilities of ultrasonics merited more careful scientific evaluation than had been accorded to them and that there was a need for fundamental research into ultrasonics.

The present experiments have provided some information on the relationship between damage and dosage parameters such as intensity, treatment time and pulsing of the radiation. Some tentative explanations have also been put forward as to the possible biological action of the ultrasonic beams used. The relation of such action to the position of the sonicated cells in the cell cycle, the amount of dissolved oxygen present during sonication, the temperature of the irradiated tissue and the fractionation of doses has been investigated. The dosage parameters used in these experiments are similar to those employed in ultrasound therapy (Summer and Patrick, 1964) and the results obtained may ultimately contribute to an improvement of such treatment.

There have been many reports (see Chapter I) of a synergistic action between ultrasound and X-rays, and it has been thought that the use of ultrasound in conjunction with X-ray therapy could increase the effectiveness of the latter in destroying tumours. Experiments with Zea have shown that the two agents are possibly acting independently of each other. A similar result has also been obtained when vincristine, a stathmokinetic agent used in tumour therapy, was used in conjunction with ultrasound. In the roots of Zea, however, one has a homeostatic cell system and for such a system the combined action of drugs and X-rays with ultrasound may not yield the same results as when a non-homeostatic system of cells is investigated (as is found in certain tumours). Further experiments are, however, contemplated which may provide more information about the response of a non-homeostatic cell system to ultrasonic treatment.

For pulse-echo diagnostic techniques, the maximum instantaneous pulse intensity is typically about 5 W/cm^2, but may be as high as 96 W/cm^2 and the average intensity is smaller by a factor of about 10^4 (Wells and Ross,1970; Hill, 1972a). Such values exceed the intensity thresholds for damage found in experimental models under different conditions of treatment (Taylor and Dyson, 1972) and some misgivings over possible dangers, immediate and delayed, have arisen (Andrew, 1964; Connolly and Pond, 1967; Hill, 1968; Macintosh and Davey, 1970 and 1972). Since the average rate of delivery of energy is low, thermal damage is precluded and at these short pulses cavitation is

probably absent (Hill, Clark, Crowe and Hammick, 1969). Also, the reduced effectiveness of very short pulses to produce damage in Vicia roots supports the use of pulses of only a few microseconds duration in diagnostic techniques at very low average power level (Bleaney and Oliver, 1972b). Other effects of ultrasound may, however, be active and form damage mechanisms (Taylor and Dyson, 1972), but recent results of animal experimentation, using diagnostic ultrasonic devices, have failed to disclose any deleterious effects (Woodward, Pond and Warwick, 1970; Taylor and Dyson, 1972). Ziskin (1973) has reported that in over 120,000 patient examinations, there was not one ill-effect. Sound intensities, where measured, ranged from 1 - 62 W/cm^2. Duration of exposure ranged from 1 - 240 minutes.

One line of investigation, which has proved of value in ionizing radiation, has been the search for chromosomal aberrations subsequent to insonation. Macintosh and Davey (1970) have reported that after exposure of human peripheral blood cultures to levels of ultrasound similar to those used during foetal heart detection a "considerable increase in the number of chromosome aberrations over control values was found." The intensities of ultrasound used for foetal heart detection are far below those at which any significant biological damage might be expected to occur. Hence, their report has caused a great deal of concern amongst those using ultrasound machines and has stimulated others to publish their early negative data and repeat experiments, all to date with negative results (Coakley,

Hughes, Slade and Laurence, 1971; Boyd, Abdulla, Donald, Fleming, Hall and Ferguson-Smith, 1971; Bobrow, Bleaney, Blackwell and Unrau, 1971; Buckton and Baker, 1972; Abdulla, Talbert, Lucas and Mullarkey, 1972; Coakley, Slade,Braeman and Moore, 1972; Hill, Joshi and Revell, 1972; Watts, Hall and Fleming, 1972).

Since a decrease in the growth rate of the roots of Zea has been detected when exposed to an ultrasonic beam at an average intensity as low as 0.1 W/cm^2 (continuous beam) for 40 minutes, it was thought possible that a diagnostic ultrasonic beam could produce some observable effect on the roots of Zea. The roots were thus exposed to the beam of the Nuclear Enterprises "Diasonograph" (NE 4101) which is used locally in obstetric diagnosis. The sonication procedure followed was similar to the one described previously (Chapter III), but the sonication jig had to be modified to accommodate the probes of the Diasonograph. Two transducers, viz. the 1.5 MHz (NE 4161) and 2.5 MHz (NE 4163) focussed probes, were used to sonicate the roots for 15 minutes at the maximum output of the Diasonograph and a pulse rate of 300 pulses/sec. The beam parameters above are used in routine patient examinations except for the highest output of the generator which is not normally used. No change was observed in the growth of the sonicated roots. Although this result does not exclude possible sonication damage, it provides additional evidence (see also Watts, Hall and Fleming, 1972) that the Diasonograph can be safely used.

ADDENDUM.

EXPOSURE OF THE ROOTS OF ZEA TO ULTRAVIOLET (UV) RADIATION.

EXPOSURE OF THE ROOTS OF ZEA TO ULTRAVIOLET (UV) RADIATION.

Having exposed the roots of Zea to X-rays, ultrasound, drugs and even heat, it was thought that it would be of interest to see how the roots would respond when treated with UV-radiation.

The roots of Zea were thus exposed to an intense beam of UV-radiation as follows:
The roots were positioned on a perspex plate at a distance of 18 cm (at the focus) from the source. At the focus the diameter of the beam was only about 0.5 cm and the seedlings were arranged around the beam with only the terminal 2-3 mm being exposed to the UV-radiation. The root tips were irradiated in a few drops of water to prevent them from drying during treatment which lasted for 5 minutes. There was a marked increase in temperature from 22°C to 29°C during irradiation. This temperature rise was measured with a thermistor thermometer immersed in a few drops of water at the focus of the source in the absence of the roots. Five roots were exposed at a time for 5 minutes. A total of 15 roots were irradiated and 15 control roots were treated in exactly the same manner but with the source turned off. The variation of the daily growth rate as a fraction of controls (G) with time after treatment is shown in Figure 1.

Immediately after exposure many of the roots had changed colour from the normal whitish to dark brown. The roots curled up slightly after treatment, but straightened

out with subsequent growth. These observations are similar to those of Tomberg (1950) who treated the roots of Vicia faba with UV-radiation. The growth rate of the treated roots reached a minimum within the first two days after treatment and then recovered gradually back to control value in the next few days (Figure 1).

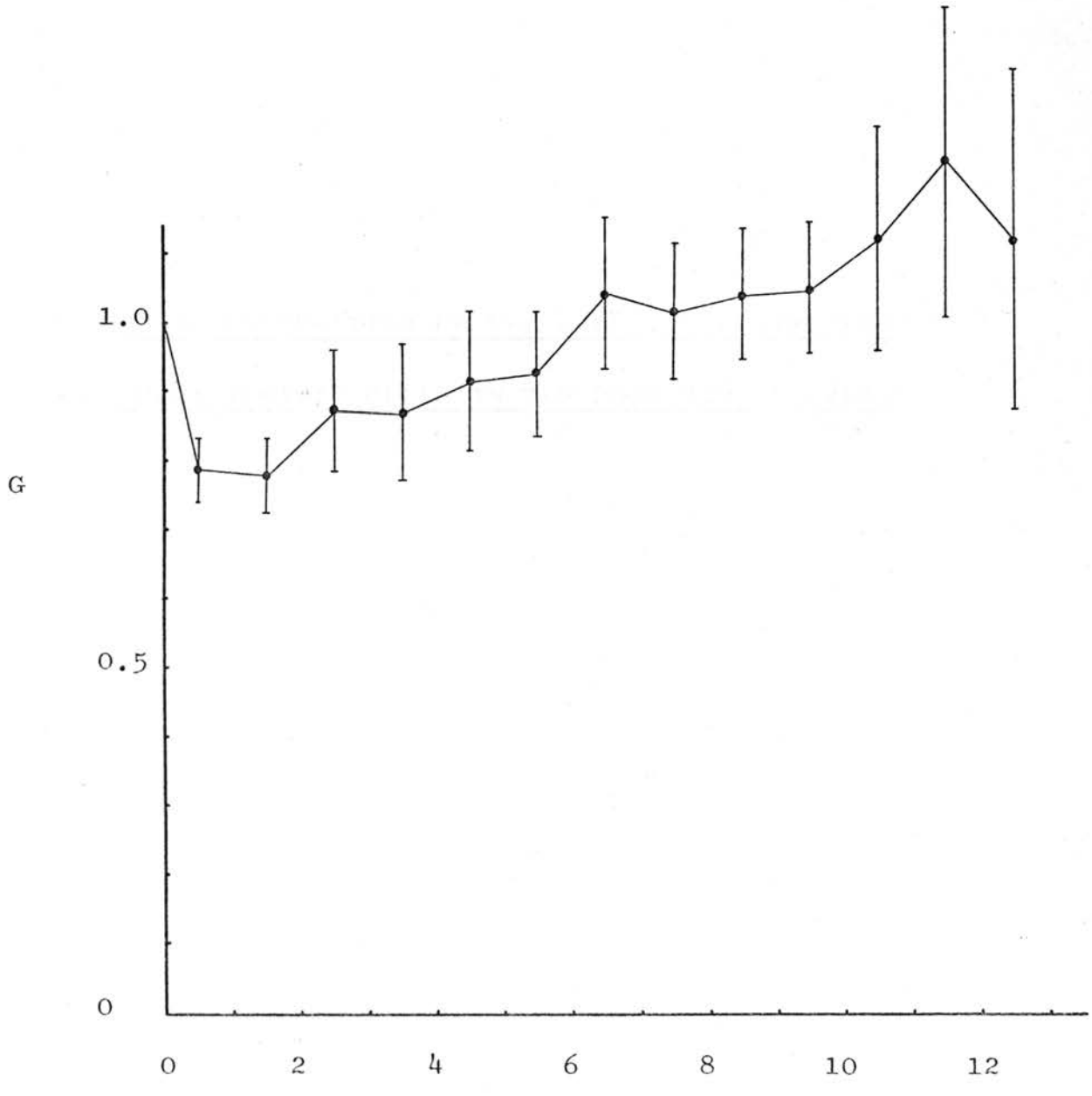
Although there was a steep temperature rise during the treatment of the roots, the increase in temperature is probably not responsible for the observed effect since a temperature rise from 19°C to 30°C has been found to have no adverse effect on the subsequent growth of the roots (Chapter IV).

The root of Zea may not be suitable for studies involving UV-radiation since a large proportion of the radiation is probably absorbed before reaching the cells inside the root.

FIGURE 1.

PATTERN OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) FOLLOWING EXPOSURE TO ULTRAVIOLET RADIATION FOR 5 MINUTES.

(The errors are standard errors of the means for 15 roots.)



APPENDIX A.

METHOD OF CALCULATION OF THE CELL CYCLE TIME FOR THE MERISTEMATIC CELLS IN THE ROOT TIP OF ZEA.

APPENDIX A

METHOD OF CALCULATION OF THE CELL CYCLE TIME FOR THE MERISTEMATIC CELLS IN THE ROOT TIP OF ZEA.

The kinetics of the cell population in a portion of the root tip:

From the observed fact that the M.I. in a root-tip portion of fixed size is constant (p. 158), it follows that this limited cell population is growing exponentially at the instant of observation. The M.I. is known to depend on the length of the root tip examined. Thus it follows that the instantaneous rate of multiplication of the growing population of cells does not remain constant. Nevertheless, it is approximately constant over short time intervals which are small compared with the generation time. Therefore, if observations on the rate of cell multiplication over short time intervals are made, e.g. by means of colchicine, it is possible to make deductions about the kinetics of the cell population, over these intervals, by using a mathematical formulation corresponding to a simple exponential increase. It is important to note that within the particular portion of the root tip there are variations in M.I. (Gray and Scholes, 1951; Clowes, 1961 and 1965), and so the exponential growth constant merely represents an effective value. When a cell of this particular population divides into two daughter cells, let L of these cells not divide further so that (2 - L) continue as meristematic cells.

The division of one parent cell thus brings about a net increase of L differentiating cells and (1 -L) meristematic cells. Thus the proportion of differentiating cells in the total population is L. The number of differentiating cells in the terminal 0.6 mm portion of the root tip of Zea was found to be about 200 for all the slides scored (p.156). Thus in these populations, $L \approx 0$.

If m is the total number of meristematic cells in the population, and since a constant proportion of these cells divide per unit time, the rate of increase of m is proportional to m or $dm/dt = \lambda m$, therefore

$$m = m_o \; e^{\lambda t} \quad \text{---------- (A1)}$$

where λ is some constant and m_o is the number of meristematic cells at time t = o. If the time interval between successive divisions, i.e. the complete mitotic cycle time, is T, and if cell division continued according to equation (A1), then m_o meristematic cells at time t = o would by time t = T have multiplied to $(2-L)m_o$. Therefore, from equation (A1),

$$e^{\lambda T} = (2 - L), \text{ so that}$$

$$\lambda = \frac{\log_e (2 - L)}{T} \quad \text{---------- (A2)}$$

Since a dividing cell contributes an additional (1 -L) meristematic cells to the population, the number of cells, R, undergoing cell division per unit time is equal to the rate of increase of the population of

meristematic cells divided by (1 -L) or

$$R = \frac{1}{(1-L)} \quad \frac{dm}{dt} = \frac{\lambda}{(1-L)} \; m_o \; e^{\lambda t} \text{ -- (A3)}$$

It may be deduced from equation (A3) that for an increasing population, the number of cells in various stages of the mitotic cycle at a given instant is not constant. The population is continually increasing by division of cells, and the number of cells coming into division in successive intervals is also increasing, thereby maintaining the M.I. at its observed constant value. At a given instant, t, the number of cells in the population which will reach the terminal stages of telophase after a further time interval lying between s and (s + ds) is the rate R from equation (A3) at a time equal to t + s, multiplied by the infinitesimal interval ds, i.e.

$$\frac{\lambda}{(1-L)} \; m_o \; e^{\lambda(s+t)} \; ds \text{ ------ (A4)}$$

There are thus relatively more cells in the earlier stages of interphase than in the later interphase stages. This difference between the number of cells in early or late interphase is in proportion to the weighting factor $e^{\lambda s}$ which from equation (A2) can be seen to vary from (2 -L) at one end of the mitotic cycle (early interphase), to 1 at the other (late telophase). Thus, from equations (A1) and (A4) we may express the M.I. of the meristematic cells as follows:

$$M.I. = \frac{1}{m_o e^{\lambda t}} \int_{s=o}^{s=\tau} \frac{\lambda}{(1-L)} m_o e^{\lambda s} e^{\lambda t} ds$$

$$= \frac{e^{\lambda\tau} - 1}{(1-L)}, \qquad (A5)$$

where τ is the mitotic time, i.e. the time taken for a cell to develop from the beginning of prophase to the end of telophase.

Let us assume that the action of colchicine may be represented as the creation of a barrier in metaphase beyond which cells cannot progress. Let the time between the application of colchicine and the production of an effective barrier be equal to c, and let the barrier be at a point from which cells would normally take a time s_1 to reach the end of telophase. Similarly, let s_2 mark the point of entry into metaphase. If the colchicine is applied at time t = o the cell population will continue to increase normally in the interval from t = o to t = c. In the interval t = c to t = $(c + s_1)$, the cells in anaphase and telophase will progress normally into interphase and the cells which should have taken their place will be held up at metaphase. In the period before t = $(c + s_1)$ the cell population will continue to multiply and there will be no change in the M.I. After t = $(c + s_1)$ there will be no further cell reproduction and the total meristematic cell population remains constant at $m_o e^{\lambda(c + s_1)}$, but cells will continue to enter prophase

and the M.I. will accordingly rise.

The rate of entry of cells into metaphase at time t is equal to the rate at which these same cells would have emerged from telophase after an interval s_2, i.e. at time $t + s_2$ if they were not held up at metaphase. The rate of entry into metaphase, dM/dt, is therefore (from equation A3)

$$\frac{dM}{dt} = \frac{\lambda}{(1 - L)} \; m_o \; e^{\lambda (t + s_2)}$$

where M(t) is the number of cells in metaphase at time t. From this we see that the number of cells accumulated at metaphase subsequent to the time $(c + s_1)$ is

$$M(t) - M(c + s_1) = \frac{m_o e^{\lambda (c+s_1)} e^{\lambda s_2}}{(1 - L)} \left[e^{\lambda (t-c-s_1)} - 1 \right]$$

or, as a fraction of the total constant number of meristematic cells $m_o \, e^{\lambda (c + s_1)}$,

$$\frac{M(t) - M(c + s_1)}{m_o \, e^{\lambda (c + s_1)}} = \frac{e^{\lambda s_2}}{(1 - L)} \left[e^{\lambda (t - c - s_1)} - 1 \right] \text{--(A6)}$$

The accumulation of metaphases after the time $(c + s_1)$ is thus an exponentially increasing function of time, although the departure from linearity is small for short times. Writing x for $(t - c - s_1)$, a very good approximation is

$$\frac{e^{\lambda s_2}}{(1 - L)} \left(\lambda x + \tfrac{1}{2} \lambda^2 x^2 \right) \quad \text{----------} \quad \text{(A7)}$$

If the observations on the accumulation are equally spaced over a total time interval $2\bar{x}$ then the regression line fitted by least squares to the data will have a slope

$$\frac{e^{\lambda s_2}}{(1-L)} \quad \lambda(1+\lambda\bar{x}).$$

It has already been shown (p.159) that the period $(c + s_1)$ is probably not more than 1 hr. and that the corrected mean rate of entry into metaphase for times of application of colchicine between 1 and 6 hrs. (i.e. $2\bar{x} = 5$ hrs.) is 0.025 ± 0.0020. It is now possible to obtain the value for λ by equating this mean rate to the mean slope derived from equation (A7), i.e.

$$\frac{e^{\lambda s_2}}{(1-L)} \quad \lambda(1 + 2\tfrac{1}{2}\lambda) = 0.025 \pm 0.0020 \text{ ---- (A8)}$$

The constant s_2 which marks the boundry between prophase and metaphase in a colchicined root is the same as in an untreated root, as may be shown from an examination of the total number of cells in metaphase and ana-telophase in the first hour. In an untreated root there are 51.3 cells in these stages per population of 1000 meristematic cells (Table 3.5 , p.164), and corresponding to equation A5 we have

$$\frac{e^{\lambda s_2} - 1}{(1-L)} = 51.3 \times 10^{-3}$$

But $1 - L = 1 - 0 = 1$, therefore, $\frac{e^{\lambda s_2}}{(1 - 0)} = 1.0513$,

and by substituting this quantity in equation (A8) for λ, λ is found to be 0.0223 $hr.^{-1}$ From equation (A2) we then have

$$T = \frac{\log_e 2}{\lambda} = \frac{0.693}{0.0223} = 31.1 \pm 1.9 \text{ hrs.}$$

The error is the standard error in T, calculated according to the method given by Topping (1971, p. 82).

APPENDIX B.

MATHEMATICAL DERIVATION OF MODEL A.

MATHEMATICAL DERIVATION OF MODEL B.

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MATHEMATICAL DERIVATION OF MODEL A .

Suppose I_s is the number of cells in the model meristem under steady growth rate conditions. If these are assumed to be all in uniform cell cycle, the number of cells dividing per unit time is μI_s where

$$\mu = \frac{\ln 2}{\text{intermitotic period}} \qquad (B.1)$$

In order to maintain equilibrium an equal number of cells must differentiate per unit time.

Suppose that after a dose of radiation, the number of integer cells in the meristem is reduced to I, and that fatally damaged cells are removed immediately.

The number of integer cells dividing per unit time is then μI. Further, suppose that the number of cells which differentiate per unit time is no longer equal to the number which divides, but is reduced in the ratio $\frac{I}{I_s}$, i.e. the number is given by $\frac{I}{I_s} \times \mu I$ or $\mu \frac{I^2}{I_s}$

As a result, I will increase as the integer cells divide. The characteristic of the meristem which governs the rate of differentiation is thus postulated to be its fractional size, the ratio of the actual meristem population to the equilibrium value. The rate of change with time of the total number of cells, I, in the meristematic department, is then the difference between the in-

crease in the number of cells resulting from division, and the loss due to differentiation.

i.e. $$\frac{dI}{dt} = \mu I - \mu \frac{I^2}{I_s} \qquad (B.2)$$

It is evident from this model that the rate of differentiation is small when I is small and increases with I.

When I equals I_s, the rate of change of the number of cells on the meristem becomes zero, i.e. steady growth rate conditions prevail. The expression in equation (B.2) can be integrated by standard methods and results in the following expression:

$$I = \frac{I_s}{1 + \left(\frac{I_s}{I_o} - 1\right) e^{-\mu t}} \qquad (B.3)$$

where I_o is the value of I just after irradiation.

The rate of differentiation at any time is given by: -

$$\frac{dD}{dt} = \mu \left(\frac{I^2}{I_s}\right) \qquad (B.4)$$

Substituting the expression for I deduced in equation (B.3) we have: -

$$\frac{dD}{dt} = \frac{\mu I_s}{\left[1 + \left(\frac{I_s}{I_o} - 1\right) e^{-\mu t}\right]^2} \qquad (B.5)$$

The area under this curve over a time interval of one day represents the total number of cells differentiating during that period. The corresponding quantity for a control root is the area under the curve: -

$$\frac{dD}{dt} = \mu I_s \qquad (B.6)$$

It is therefore possible to evaluate the total amount of differentiation and, therefore, the growth of irradiated roots, as a fraction of controls for each successive day after the initial depopulation. The growth rate is small during the early days after irradiation or depopulation, while the meristem is being repopulated, but then increases to a steady value as equilibrium is restored.

Mathematical Derivation of Model B.

It is assumed that all meristematic cells are preparing for division, but that the proportion of cells maintaining their reproductive integrity is proportional to the concentration of a specific substance - in other words, the fraction of the population dividing per unit time is proportional to this concentration. It is also assumed that the maintenance of reproductive integrity implies a 'consumption' of this substance - so that the fall in concentration of this substance in a given region or layer of cells is proportional to the number of cells present which retain their reproductive integrity. Cells having lost their reproductive integrity differentiate; for the purposes of simplification of the mathematical calculations it is assumed here that such cells are unable to divide even once.

Let N be the number of cells expected to divide per unit time in a population with 100 per cent reproductive integrity (no cells differentiating) corresponding

to the concentration, C_o, of the postulated specific substance. As the substance diffuses through such a region, its concentration would fall from C_o to C_N. From the above assumptions:

$$C_N = C_o\, e^{-KN} \qquad \text{(where K is a constant)} \qquad \text{(B.7)}$$

The proportion of cells with reproductive integrity at a concentration level of C_N is $\frac{C_N}{C_o}$, and from equation (B.7)

$$\frac{C_N}{C_o} = e^{-KN} \qquad \text{(B.8)}$$

In any infinitesimal part of the region containing dN cells, (the number expected to divide per unit time if 100 per cent reproductive integrity is maintained), e^{-KN} dN cells will in fact divide. In the whole region, therefore, instead of the possible N cells, the total number of cells dividing per unit time will be

$$\int_0^N e^{-KN}\, dN = \frac{1}{K}\,(1-e^{-KN}) \qquad \text{(B.9)}$$

This corresponds to a proportion $\frac{1}{KN}\,(1-e^{-KN})$.

For steady state to be achieved this proportion must be 0.5 : whence $KN = 1.595$.

Considering now the total population reduced to proportion F (following, for example, radiation damage) one is concerned with FN instead of N in the above formulae. Therefore the proportion of the possible FN cells to divide per unit time will be

$$X = \frac{1}{KFN}\,(1-e^{-KFN}) \qquad \text{(B.10)}$$

and the proportion differentiating (1-X).

The number differentiating, D , will be FN (1-X), that is: -

$$D = \frac{1}{K} \left(KFN + e^{-KFN} - 1\right) \qquad (B.11)$$

This is a measure of growth rate (the corresponding value for steady state equilibrium being 0.5N). Consequently, G , the growth rate as a fraction of that for a steady state population is given by: -

$$G = \frac{KFN + e^{-KFN} - 1}{0.5N}$$

$$\text{i.e.} \quad G = \frac{1.595F + e^{-1.595F} - 1}{0.7975} \qquad (B.12)$$

An approximate solution, taking the first three terms only of the exponential term will be

$$G = 1.595\ F^2 \qquad (B.13)$$

APPENDIX C.

FLOW DIAGRAM AND COMPUTER PROGRAMS FOR THE CALCULATION OF THEORETICAL GROWTH CURVES USING THE KINETIC SYSTEMS OF MODEL A AND MODEL B.

APPENDIX C.

FLOW DIAGRAM AND COMPUTER PROGRAMS FOR THE CALCULATION OF THEORETICAL GROWTH CURVES USING THE KINETIC SYSTEMS OF MODEL A AND MODEL B.

The computer programs written for Model A and Model B (with the assumption that cell death due to sonication occurs in the first one tenth of the first cell cycle) are given in Figures C1 and C3 respectively. The values of G for the various times after the sonication dose are printed by the computer as shown in Figures C2 and C4 for Model A and Model B respectively.

The flow diagram for the computation is given in Figure C5. This diagram is applicable to both models under consideration. The growth curves are calculated for values of G_{max} ranging from 0.05 to 1.0 in increments of 0.05, as follows. For a set value of k (=1-P), the number of cells in each compartment are calculated at the time t=0, using the initial conditions for the particular model under consideration. The number of cells in each compartment at the end of each successive time interval equalling one tenth of the cycle time is evaluated using the appropiate formulae as given in Chapter I . The corresponding values of G are printed (for times up to 20T) as shown in Figures C2 and C4. This process is repeated for each increment of 0.05 in the value of k, from 0.05 to 1.0.

FIGURE C1.

COMPUTER PROGRAM FOR MODEL A.

```
@RUN        CSC1,A0605-A005,,25,500
@FOR,IS

 1          DIMENSION A(100),F(100),GNCD(100)
 2          C=0.0
 3          DO 6 K15=1,20,1
 4          C=C+0.05
 5          DO 1 K=1,20,1
 6          P=K
 7          P=P/20.0
 8          Q=0.933
 9      100 FORMAT(1H1,2X,'FRACTIONAL GROWTH RATE (G) - MODEL A')
10          WRITE(5,100)
11          WRITE(5,101)P
12      101 FORMAT(1H ,2X,'K=',1X,F6.3,//)
13          WRITE(5,105)C
14      105 FORMAT(1H ,2X,'MAXIMUM G-VALUE=',1X,F6.3,/)
15          WRITE(5,102)
16      102 FORMAT(1H ,///,1X,'TIME(IN CELL CYCLES)',5X,'G')
17          A(1)=P
18          A(1)=A(1)*0.933*20.0
19          DO 2 K1=2,10,1
20          L=K1-1
21        2 A(K1)=A(L)*0.933
22          F(1)=1.0
23          DO 8 K8=1,20,1
24          M=11
25          N=20
26          DO 3 K2=1,10,1
27          IF(M-11)50,51,50
28       51 DO 4 K3=M,N,1
29          L2=K3-11
30          L1=K3-1
31          X=1.0-((1.0-Q)*F(K2))
32          IF(K3-M)10,11,10
33       11 A(K3)=2.0*A(L1)*X
34          SUMA=A(K3)
35          GO TO 4
36       10 A(K3)=X*A(L2)
37          SUMA=SUMA+A(K3)
38        4 CONTINUE
39          M=M-10
40          N=N-10
41          GO TO 53
42       50 DO 5 K3=M,N,1
43          L2=K3+9
44          L1=K3+19
45          X=1.0-((1.0-Q)*F(K2))
```

FIGURE C1 CONTINUED.

```
46          IF(K3-M)54,55,54
47       55 A(K3)=2.0*A(L1)*X
48          SUMA=A(K3)
49          GO TO 5
50       54 A(K3)=X*A(L2)
51          SUMA=SUMA+A(K3)
52        5 CONTINUE
53          M=M+10
54          N=N+10
55       53 F(K2)=SUMA/139.38
56          GNCD(K2)=F(K2)*F(K2)
57          GNCD(K2)=GNCD(K2)*C
58          IF(K8-3)70,70,71
59       70 K20=K8-1
60          E=K2
61          E=E/10.0
62          H=K20
63          E=E+H
64          WRITE(5,103)E
65      103 FORMAT(1H ,8X,F3.1)
66          WRITE(5,104)GNCD(K2)
67      104 FORMAT(1H+,25X,F5.3)
68       71 K10=K2+1
69        3 F(K10)=F(K2)
70          K2=10
71          IF(K8-3)80,80,81
72       81 WRITE(5,110)K8
73      110 FORMAT(1H ,8X,I3)
74          WRITE(5,111)GNCD(K2)
75      111 FORMAT(1H+,25X,F5.3)
76       80 F(1)=F(K10)
77        8 CONTINUE
78        1 CONTINUE
79        6 CONTINUE
80          CALL EXIT
81          END

 @XQT
 @FIN
```

FIGURE C 2.

FRACTIONAL GROWTH RATE (G) - MODEL A.

K= .500

MAXIMUM G-VALUE = .500

TIME (IN CELL CYCLES)	G
.1	.125
.2	.134
.3	.143
.4	.153
.5	.163
.6	.173
.7	.184
.8	.194
.9	.205
1.0	.216
1.1	.226
1.2	.237
1.3	.248
1.4	.258
1.5	.269
1.6	.279
1.7	.289
1.8	.299
1.9	.309
2.0	.319
2.1	.328
2.2	.337
2.3	.346
2.4	.354
2.5	.362
2.6	.370
2.7	.377
2.8	.385
2.9	.391
3.0	.398
4	.447
5	.473
6	.487
7	.493
8	.497
9	.498
10	.499
11	.499
12	.499
13	.499
14	.499
15	.500
16	.500
17	.500
18	.500
19	.500
20	.500

FIGURE C 3.

COMPUTER PROGRAM FOR MODEL B.

```
@RUN       CSC1,A0605-A005,,25,500
@FOR,IS

 1          DIMENSION A(100),F(100),GNCD(100)
 2          C=0.0
 3          DO 6 K15=1,20,1
 4          C=C+0.05
 5          DO 1 K=1,20,1
 6          P=K
 7          P=P/20.0
 8      100 FORMAT(1H1,2X,'FRACTIONAL GROWTH RATE (G) - MODEL B')
 9          WRITE(5,100)
10          WRITE(5,101)P
11      101 FORMAT(1H ,2X,'K=',1X,F6.3,//)
12          WRITE(5,105)C
13      105 FORMAT(1H ,2X,'MAXIMUM G-VALUE=',1X,F6.3,/)
14          WRITE(5,102)
15      102 FORMAT(1H ,///,1X,'TIME(IN CELL CYCLES)',5X,'G')
16          DO 2 K1=1,10,1
17        2 A(K1)=P*10.0
18          F(1)=1.0
19          DO 8 K8=1,20,1
20          M=11
21          N=20
22          DO 3 K2=1,10,1
23          IF(M-11)50,51,50
24       51 DO 4 K3=M,N,1
25          L2=K3-11
26          L1=K3-1
27          X=(1.0-EXP(-1.595*F(K2)))/(1.595*F(K2))
28          IF(K3-M)10,11,10
29       11 A(K3)=2.0*A(L1)*X
30          SUMA=A(K3)
31          GO TO 4
32       10 A(K3)=A(L2)
33          SUMA=SUMA+A(K3)
34        4 CONTINUE
35          M=M-10
36          N=N-10
37          GO TO 53
38       50 DO 5 K3=M,N,1
39          L2=K3+9
40          L1=K3+19
41          X=(1.0-EXP(-1.595*F(K2)))/(1.595*F(K2))
42          IF(K3-M)54,55,54
43       55 A(K3)=2.0*A(L1)*X
44          SUMA=A(K3)
45          GO TO 5
46       54 A(K3)=A(L2)
```

FIGURE C 3 CONTINUED.

```
47          SUMA=SUMA+A(K3)
48        5 CONTINUE
49          M=M+10
50          N=N+10
51       53 F(K2)=SUMA/100.0
52          GNCD(K2)=((1.595*F(K2))+(EXP(-1.595*F(K2)))-1.0)/0.7975
53          GNCD(K2)=GNCD(K2)*C
54          IF(K8-3)70,70,71
55       70 K20=K8-1
56          E=K2
57          E=E/10.0
58          H=K20
59          E=E+H
60          WRITE(5,103)E
61      103 FORMAT(1H ,8X,F3.1)
62          WRITE(5,104)GNCD(K2)
63      104 FORMAT(1H+,25X,F5.3)
64       71 K10=K2+1
65        3 F(K10)=F(K2)
66          K2=10
67          IF(K8-3)80,80,81
68       81 WRITE(5,110)K8
69      110 FORMAT(1H ,8X,I3)
70          WRITE(5,111)GNCD(K2)
71      111 FORMAT(1H+,25X,F5.3)
72       80 F(1)=F(K10)
73        8 CONTINUE
74        1 CONTINUE
75        6 CONTINUE
76          CALL EXIT
77          END

 @XQT
 @FIN
```

FIGURE C 4.

FRACTIONAL GROWTH RATE (G) - MODEL B.

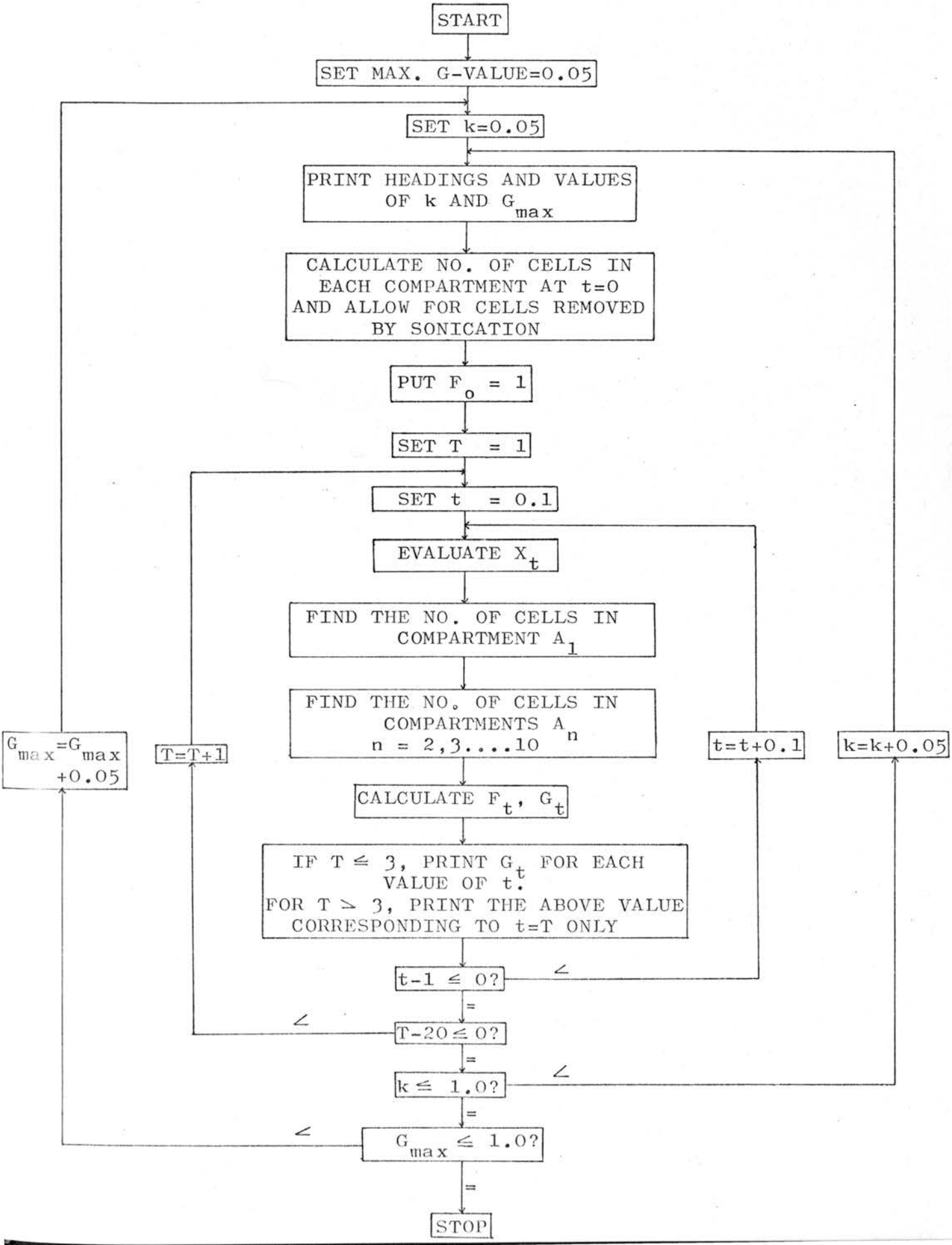
K= .050

MAXIMUM G-VALUE = 1.000

TIME (IN CELL CYCLES)	G
.1	.004
.2	.005
.3	.005
.4	.006
.5	.007
.6	.008
.7	.009
.8	.010
.9	.011
1.0	.012
1.1	.014
1.2	.016
1.3	.019
1.4	.021
1.5	.024
1.6	.027
1.7	.030
1.8	.033
1.9	.036
2.0	.039
2.1	.043
2.2	.049
2.3	.056
2.4	.063
2.5	.070
2.6	.077
2.7	.084
2.8	.092
2.9	.099
3.0	.106
4	.233
5	.411
6	.594
7	.744
8	.848
9	.913
10	.951
11	.973
12	.985
13	.991
14	.995
15	.997
16	.998
17	.998
18	.999
19	.999
20	.999

FIGURE C 5 .

FLOW DIAGRAM FOR THE CALCULATION OF THEORETICAL GROWTH CURVES USING THE KINETIC SYSTEMS OF MODEL A AND MODEL B.



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