FINAL PROGRESS REPORT

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Evaluation of the Effects of Hypergravity Exposure and Caging Restraint on Bone Mineralization in the Beagle by *In Vivo* Photon Absorptiometry

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(NASA-CR-137710) EVALUATION OF THE EFFECTS OF HYPERGRAVITY EXPOSURE AND CAGING RESTRAINT ON BONE MINERALIZATION IN THE BEAGLE BY IN VIVO PHOTON ABSORPTIOMETRY Final Progress Report (California Univ.)

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Photon absorptiometry was used to evaluate bone mineral kinetics associated with normal development and the possible perturbations to bone development resulting from hypergravity exposure over a period of six months in developing Beagles. A series of seven measurements to evaluate bone mineral kinetics were performed at specific times with the first measurement prior to treatment and subsequent measurements at 2, 5, 9, 14, 20 and 26 weeks from the onset of the experiment. Four groups of six male Beagle pups, ranging in age from 85-92 days were studied. Two groups were chronically exposed to hypergravity treatments by centrifugation of 2.0 G (18.0 RPM, 11.7 ft radius) and 2.6 G (18.0 RPM, 19.8 ft radius) for the 26 week period at Ames Research Center. A third group of six dogs served as a caged control to evaluate possible changes due to confinement in small plexiglass cages similar to those of the centrifuge. Thus this control group was subjected to limited exercise due to caging restraint. The fourth group of animals was housed in open runs to allow exercise without the spatial confinement of the smaller plexiglass cages.

Bone mineral changes were studied using a recently developed, jointly sponsored ^{125}I photon absorptiometer under NASA contract NAS2-7375 and AEC contract AT(0Y-31472), capable of reproducibly measuring bone mineral and associated bone parameters *in vivo* in the Beagle. The bone scanner is a semi-automated, rectilinear scanner with variable scan speeds and path lengths and has been described in the final report for the NASA Contract NAS2-7375 entitled "Study on Photon Absorptiometer" and in two publications (Fisher *et al.*, Biomed. Eng. 9: 196, 1974 and Schwind *et al.*, Biomed. Eng. 9: 208, 1974).

Optimal scan sites were selected after evaluating the results of preliminary scanning of two developing male Beagles over a three month time interval beginning at three months of age at the Radiobiology Laboratory. In agreement with previously published work on photon absorptiometry in humans (Proc. Symp. on

Bone Mineral Determinations, Vol. 2, Stockholm, Sweden, p. 192, 1974), it was found that the midshaft of the radius-ulna was a convenient site for scanning due to the ease of positioning. Also, in agreement with the human findings, the one-third distal site of the radius-ulna was found to be a very reproducible site since the measurement of bone mineral content at this site is not greatly affected by slight errors in positioning due to its relatively constant geometry. A site one centimeter distal from the radial tuberosity was also chosen for scanning since it is representative of the more active trabecular bone. However, this site is difficult to accurately reposition since it represents an area of bone with rapidly changing geometry.

Three sites on the radius-ulna were scanned in each dog: at one cm distal from the radial tuberosity, at one third the distance to the styloid process from the radial tuberosity, and at the midshaft. The first two scan sites were relative sites determined by measuring the linear distance from the radial tuberosity to the styloid process. This distance was defined as the "bone length". The data from the third scan site, one cm distal from the radial tuberosity, should be interpreted considering two important sources of error: (1) as the bone develops a proportionately different site is being studied because this site is determined from a fixed distance, and (2) the rapidly changing geometry introduces difficulty in reproducibly positioning each dog.

At the time of measurement the dogs were anesthetized with surital or nembutal, the right foreleg shaved, the leg positioned and the bone length, the distance from the radial tuberosity to the styolid process on the radius, was measured. Positioning was accomplished utilizing a potentiometric system. Three scan sites for measurement were determined: (1) one cm distal from the radial tuberosity, (2) the distal third of the bone length, and (3) one-half of the measured bone length. The limb was then packed in tissue equivalent material and scanned at these predetermined sites. Data were computer processed and the following parameters were calculated: (1) bone mineral content, (BMC) (2) ash weight, (3) bone width (BW), (4) ash weight:width (AWW) ratio, (5) marrow cavity width (MCW), (6) geometric regularity ratio, (7) paradensity value, (8) average cortical thickness (ACT), (9) bone width:marrow cavity width ratio, and (10) a plot of point density in grams of ash equivalent per centimeter length of bone vs. cross-sectional distance through bone for each bone scan.

During the course of the experiment two of the dogs died under anesthesia. One of these dogs was replaced by a dog in the trial maintained under the same run conditions. The other dog was a 2.6 G-treated animal for which a replacement was not possible.

Because of instrumental difficulties the scheduled 20 week post treatment (sixth) measurements were not performed on one dog exposed to 2.0 G and the five dogs exposed at 2.6 G.

The data were compiled and preliminary statistical analyses were completed. For each site at each measurement time the mean, standard deviation, variance, sum of individual values, sum of their squares, and a single factor analysis of variance was computed for the following parameters: bone width (mm), length to width ratio, ash weight (g/cm), ash weight to width ratio (g/cm^2), paradensity (g/cm^3), the marrow cavity width (mm), bone width to marrow cavity width ratio, and average cortical thickness (mm). The MCW, BW/MCW and ACT were not calculated for the combined radius ulna at the midshaft site since this was done for the radius-ulna individually at this site. Similarily statistical analysis were performed for each treatment period for bone length and body weight. Computer plots were prepared for each of the above parameters as a function of age. The following items will be delivered directly to Dr. Jiro Oyama at Ames Research

Center, Moffett Field, California:

(1) Raw absorptiometric data cards

(2) Point density plots for each animal at every bone site for each measurement

(3) Computer listing of standard statistics for each group

(4) Computer plots for each parameter vs. age

(5) Data summary sheets for each animal at every bone site for each measurement

Summary tables of the one-factor analysis of varience (ANOVA) (Tables 1-5) are presented. The ANOVA results (Table 1) indicate highly significant ($p<0.001$) differences in body weight directly after exposure and highly significant ($p<0.001$) differences in bone length beginning 9 weeks after treatment and continuing throughout the experiment. The body weight difference (Fig. 1) was due to the depressed body weight of the hypergravity treated animals after two weeks of exposure and was maintained throughout the study. Bone length differences can be observed after 3 weeks of treatment (Fig. 2) becoming significant after 9 weeks of treatment. There appears to be a dose-response in this parameter with the 2.6 G-treated animals showing a greater decrease than the 2.0 G-treated animals relative to controls. The forelimbs of the gravity treated animals appeared to be bowed, which may account for the decrease in bone length.

Summary of bone mineral results at specific bone sites:

(1) Midshaft: Ulna Only

There appeared to be no consistent trends in any parameter studied for the midshaft-ulna. The paradensity (g/cm^3) for the midshaft-ulna (Fig. 3) was significantly different ($p<0.05$) for the seventh measurement due to high values for run controls and low values for caged controls with the treated animals having values between those of the caged and run controls. Ash wt:width ratios were different ($p<0.10$) for the seventh measurement with the caged control animals again having the lowest values. For the 3rd and 4th measurements the average cortical thickness (Fig. 4) appeared lower ($p<0.10$) for the 2.6 and 2.0 G-treated dogs compared to other groups. In general no clear cut treatment effects were observed for the midshaft ulna.

(2) Midshaft: Radius Only

The ash weight:width ratio differences (Fig. 5) were significant ($p<0.10$) for the fifth measurement and highly significant ($p<0.01$) for the seventh analysis. These differences appear to be due to higher values for the caged controls and lower values for the 2.6 G-treatment group. It appears that after the first two weeks of treatment the caged controls have consistently higher paradensity (Fig. 6) and AWW ratios than the other groups. A markedly lower AWW ratio is found for the 2.6 G-treated dogs at the time of the seventh measurement, apparently due to lower ash weight values at this time. A consistent trend indicating lower ash weights (Fig. 7) for gravity treated animals appears to begin at the time of the third measurement. Bone width (Fig. 8) was consistently greater in run controls than the other groups, with significant differences at the time of the fourth ($p<0.1$) and fifth ($p<0.5$) measurements. The ACT (Fig. 9) appeared to be greatest for the run controls (excepting the second measurement) with significant differences during the second ($p<0.01$) and fifth ($p<0.05$) measurements due to high values for caged controls on the second measurement and higher values for run controls and lower values for the 2.6 G-treatment group on the fifth measurement.

(3) One-third Distal Site: Combined Radius and Ulna

The paradensity (Fig. 10) values were consistently higher for the caged control group than the others after the second measurement. These differences were significant for the second ($p<0.1$), third ($p<0.1$), fifth ($p<0.05$) and seventh ($p<0.01$) measurements with the 2.6-G group having a 26% lower value than the cage controls at the time of the seventh measurement. The AWW (Fig. 11) ratios

($p<0.05$) and very significantly ($p<0.001$) lowered AWW for the 2.6-G group at the time of the seventh measurement. Similarly ash weight (Fig. 12) was significantly ($p<0.05$) depressed for the 2.6-G group at the time of the seventh measurement and run controls were consistently greater than caged controls. At this time, it appears that the 2.6-G group also had the greatest bone width. Thus, the highly significant differences observed for AWW and paradensity during the seventh measurement were due to the wider bones and lower ash weights of the 2.6 G-treatment dogs.

The marrow cavity widths for the run control dogs were consistently higher than the caged controls although there were no significant differences. The bone width to marrow cavity width demonstrated no trends and no significant differences. The average cortical thickness was not significantly different between groups, however, a trend did seem to be apparent from the third to the seventh measurement when run control values are higher than caged control values.

(4) <u>One cm</u>: <u>Radius and Ulna Combined</u>

Both hypergravity-treated groups had significantly decreased paradensity (Fig. 13) values at the fourth ($p<0.05$), fifth ($p<0.01$) and seventh ($p<0.1$) measurements. The run and caged control dogs had values that appeared similar. The AWW (Fig. 14) ratio was only significantly different at the third measurement time with the run control and caged control groups greater than the treated groups. Ash weight was not significantly different for any measurement. The bone width (Fig. 15) was significantly greater for the hypergravity-treated groups at the time of measurement four ($p<0.1$) and five ($p<0.01$), suggesting a widening of the epiphyses. It appears that the bone width, of the run control animals are generally greater than the caged controls. The larger bone widths of the gravity-treated groups was probably the major factor contributing to the observed decreases in AWW ratios and paradensities.

SUMMARY:

Highly significant decreases in the body weights and bone lengths of dogs exposed to 2.0-G and 2.6-G were observed. The body weight differences appeared directly after initial exposure while the bone length changes appeared to begin 5 weeks after the initiation of the gravity exposures. The bone length differences were probably a result of a bowing of the forelimbs.

No pattern of effects was observed in any bone parameter of the midshaft-ulna. At the midshaft-radius the caged control group appeared to have consistently higher paradensity and ash weight:width ratios, while run controls had higher ash weights than caged controls. At the midshaft of the radius the run control group demonstrated a consistently wider bone than the others. Also at this site, a markedly lower (23%) ash weight:width ratio is observed for the 2.6 G-treated group at the time of the last measurement.

Similar to the midshaft of the radius, the paradensity values at the third-distal radius-ulna were consistently higher for the cage control group relative to all other groups. Also markedly lower paradensity, ash weight:width, and ash weight values were observed at the time of the last measurement for the 2.6 G-treated group. Significant decreases in paradensity and ash weight at the one-cm site were observed for both gravity-treatment groups.

Further statistical analysis and mathematical modelling is necessary to fully evaluate these data. These preliminary analyses suggest a general trend toward increased bone density in the caged control group relative to all others and a decrease in bone mineral in the two gravity-treated groups. The higher AWW ratios and paradensity values in the caged controls compared to run controls appear to be due to narrower bone widths in this group.

Table 1. SUMMARY OF ANALYSIS OF VARIANCES FOR BODY WEIGHT AND BONE LENGTH AS MEASURED FROM THE RADIAL TUBEROSITY TO THE STYLOID PROCESS

Treatment Time (wks)	0	2	5	9	14	20[a]	26
Body weight	-	****	****	****	****		****
Bone Length	-	-	-	****	****		***

* $p<.1$

** $p<.05$

*** $p<.01$

**** $p<.001$

[a] Due to instrumental difficulties the measurement of bone parameters 20 weeks post exposure were not performed on one dog exposed at 2.0 G and five dogs exposed at 2.6-G. Thus no analysis of variance is reported for this time period.

Table 2. SUMMARY OF ANALYSIS OF VARIANCES OF ABSORPTIOMETRICALLY DETERMINED BONE PARAMETERS FOR COMBINED ONE-THIRD DISTAL RADIUS-ULNA SITE

Treatment Time (wks)	0	2	5	9	14	20[a]	26
Bone width (BW)	-	-	-	-	-		*
Ash weight	-	-	-	-	-		**
Ash wt/width ratio (AWW)	-	-	-	-	**		****
Marrow cavity width (MW)	-	-	-	-	-		-
BW/MCW ratio	-	-	-	-	-		-
Average cortical thickness	-	-	-	-	-		-
Paradensity	-	*	*	-	**		***

* $p<.1$
** $p<.05$
*** $p<.01$
**** $p<.001$

[a] Due to instrumental difficulties the measurement of bone parameters 20 weeks post exposure were not performed on one dog exposed at 2.0-G and five dogs exposed at 2.6-G. Thus no analysis of variance is reported for this time period.

Table 3. SUMMARY OF ANALYSIS OF VARIANCES OF ABSORPTIOMETRICALLY DETERMINED BONE PARAMETERS FOR COMBINED ONE CENTIMETER DISTAL RADIUS-ULNA SITE

Treatment Time (wks)	0	2	5	9	14	20[a]	26
Bone width	-	-	-	*	***		-
Ash weight	-	-	-	-	*		-
Ash wt/width ratio (AWW)	-	-	**	-	*		-
Paradensity	-	-	-	**	***		*

* $p<.1$

** $p<.05$

*** $p<.01$

**** $p<.001$

[a] Due to instrumental difficulties the measurement of bone parameters 20 weeks post exposure were not performed on one dog exposed at 2.0-G and five dogs exposed at 2.6-G. Thus no analysis of variance is reported for this time period.

Table 4. SUMMARY OF ANALYSIS OF VARIANCES OF ABSORPTIOMETRICALLY DETERMINED BONE PARAMETERS FOR THE RADIUS AT THE MIDSHAFT SITE

Treatment Time (wks)	0	2	5	9	14	20[a]	26
Bone width (BW)	-	-	-	*	**		-
Ash weight	-	-	-	-	-		-
Ash wt/width ratio (AWW)	-	-	-	-	*		***
Marrow cavity width (MCW)	-	-	-	-	-		-
BW/MCW ratio	-	**	-	-	-		-
Average cortical thickness	-	***	-	-	**		-
Paradensity	-	-	-	-	**		**

* p<.1

** p<.05

*** p<.01

**** p<.001

[a] Due to instrumental difficulties the measurement of bone parameters 20 weeks post exposure were not performed on one dog exposed at 2.0-G and five dogs exposed at 2.6-G. Thus no analysis of variance is reported for this time period.

Table 5. SUMMARY OF ANALYSIS OF VARIANCES OF ABSORPTIOMETRICALLY DETERMINED BONE PARAMETERS FOR THE ULNA AT THE MIDSHAFT SITE

Treatment Time (wks)	0	2	5	9	14	20[a]	26
Bone width (BW)	-	-	-	-	-		-
Ash weight	-	-	-	-	-		-
Ash wt/width ratio (AWW)	-	-	-	-	-		*
Marrow cavity width (MCW)	-	-	-	-	-		-
BW/MCW ratio	-	-	*	-	-		-
Average cortical thickness	-	-	*	*	-		-
Paradensity	-	-	-	-	-		**

* $p<.1$

** $p<.05$

*** $p<.01$

**** $p<.001$

[a] Due to instrumental difficulties the measurement of bone parameters 20 weeks post exposure were not performed on one dog exposed at 2.0-G and five dogs exposed at 2.6-G. Thus no analysis of variance is reported for this time period.

APPROVALS

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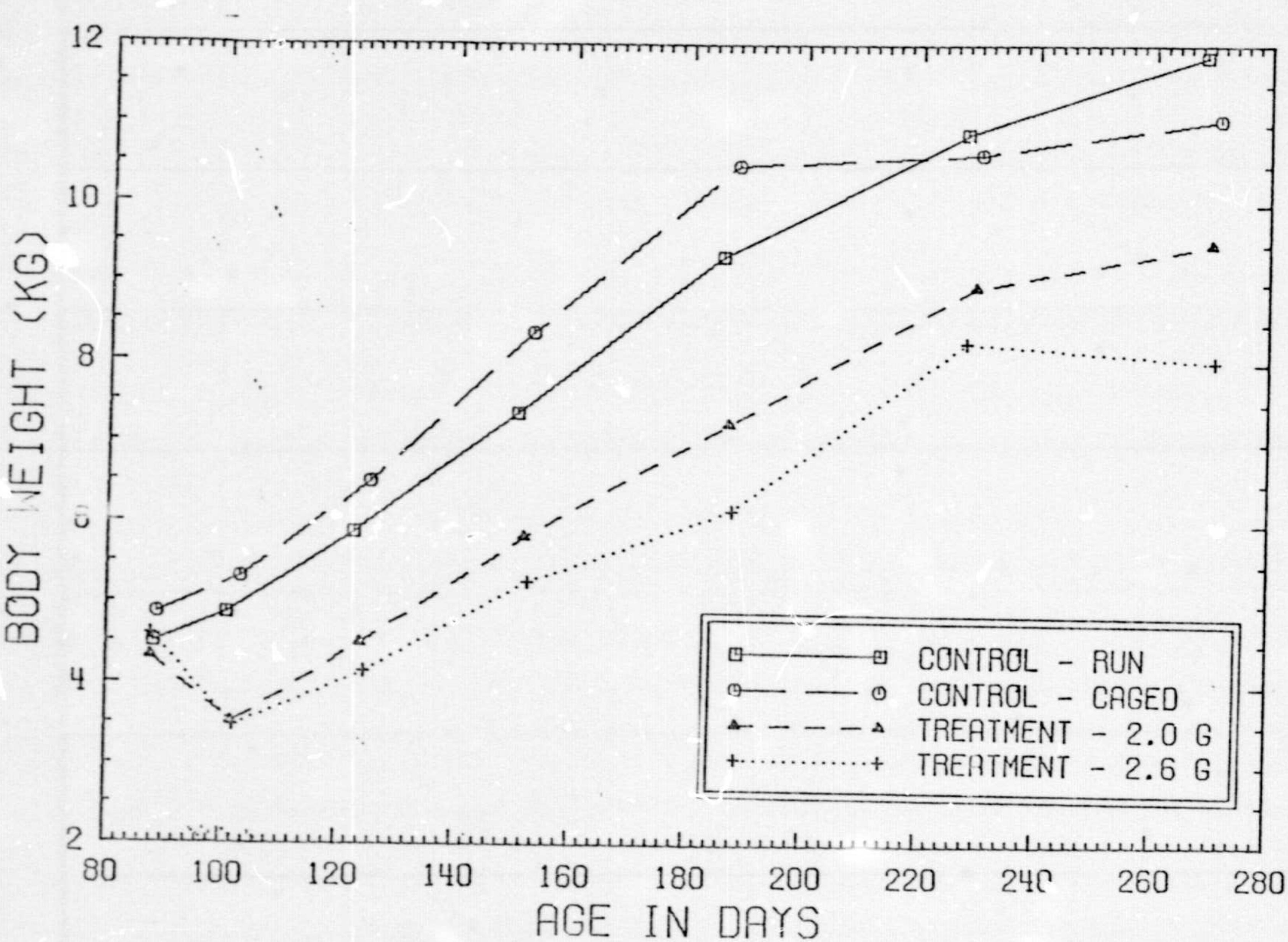

Fig. 1. Plots of Average Body Weight vs Age for the Four Experimental Groups.

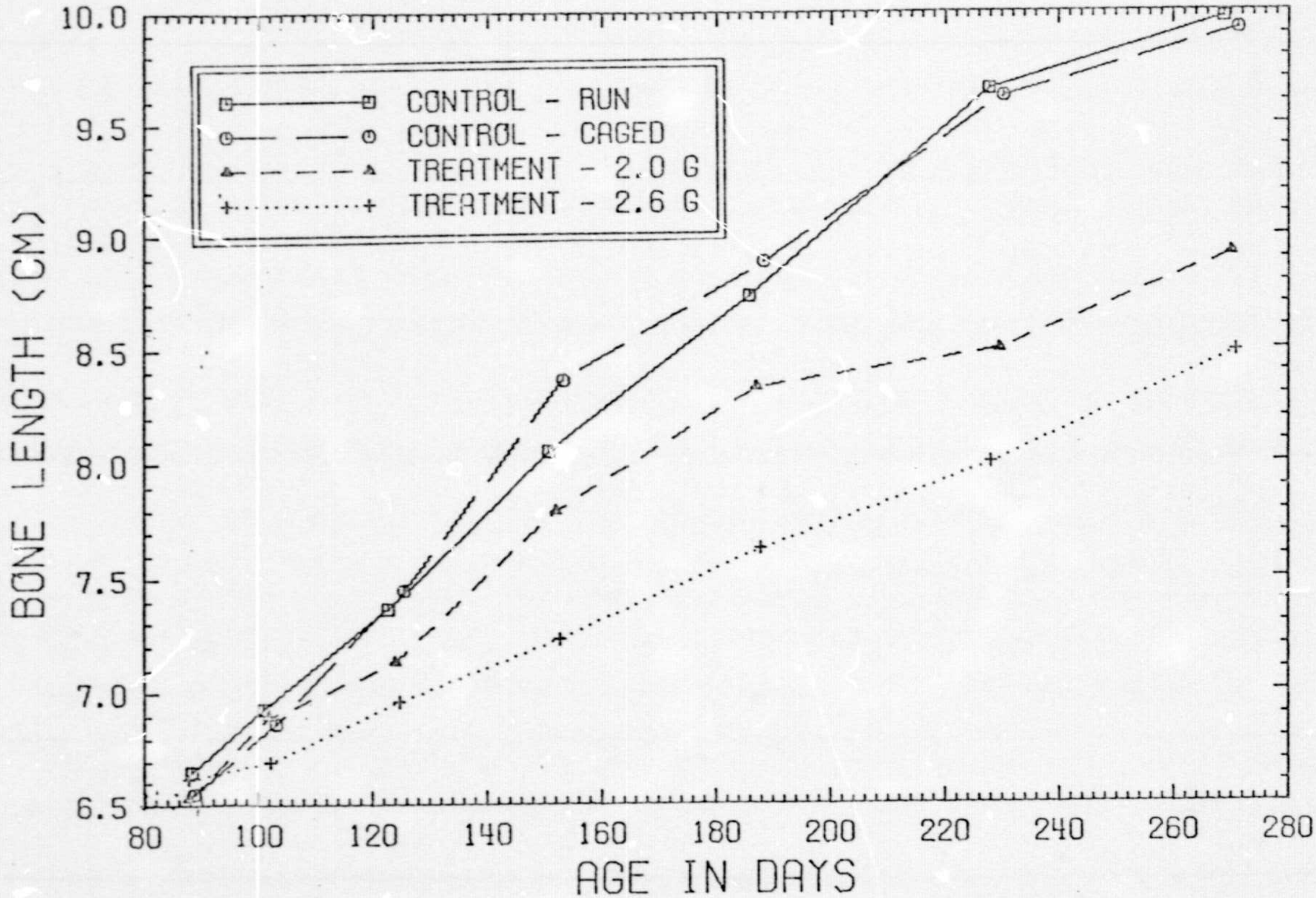

Fig. 2. Plots of Average Bone Length vs Age for the Four Experimental Groups.

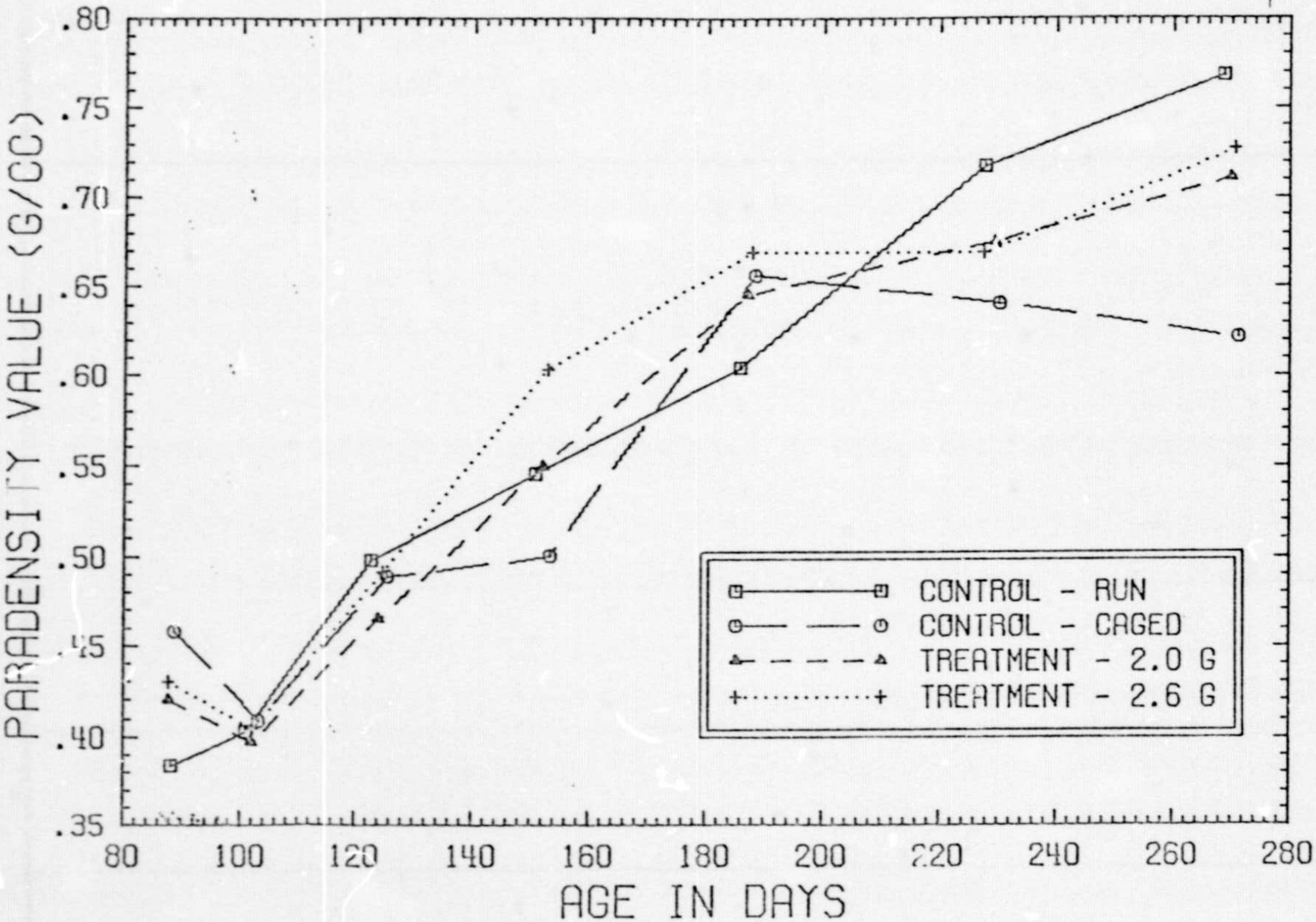

Fig. 3. Plots of Average Paradensity Values at the Midshaft Ulna Site vs Age for the Four Experimental Groups.

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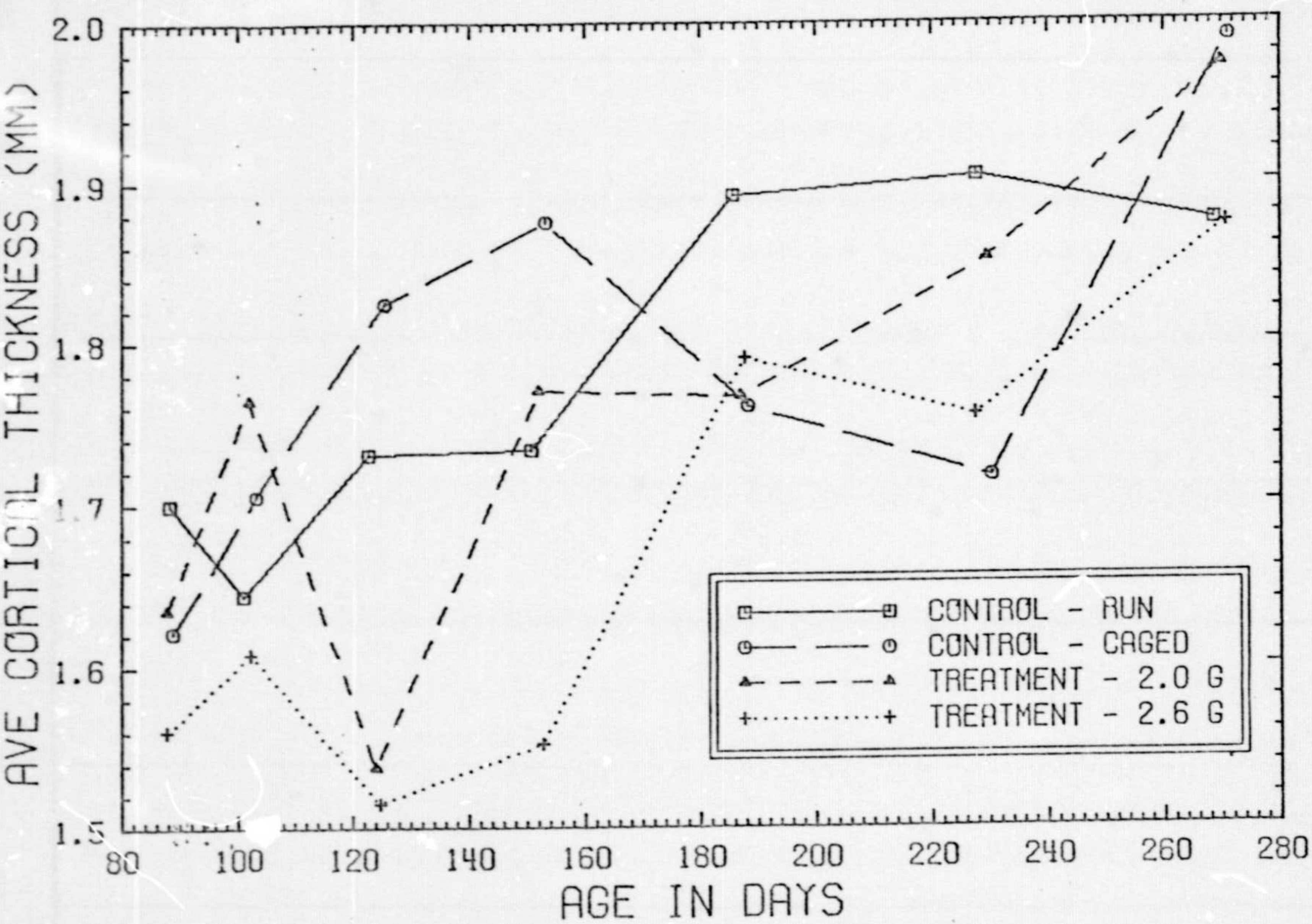

Fig. 4. Plots of Average Cortical Thickness at the Midshaft Ulna Site vs Age for the Four Experimental Groups.

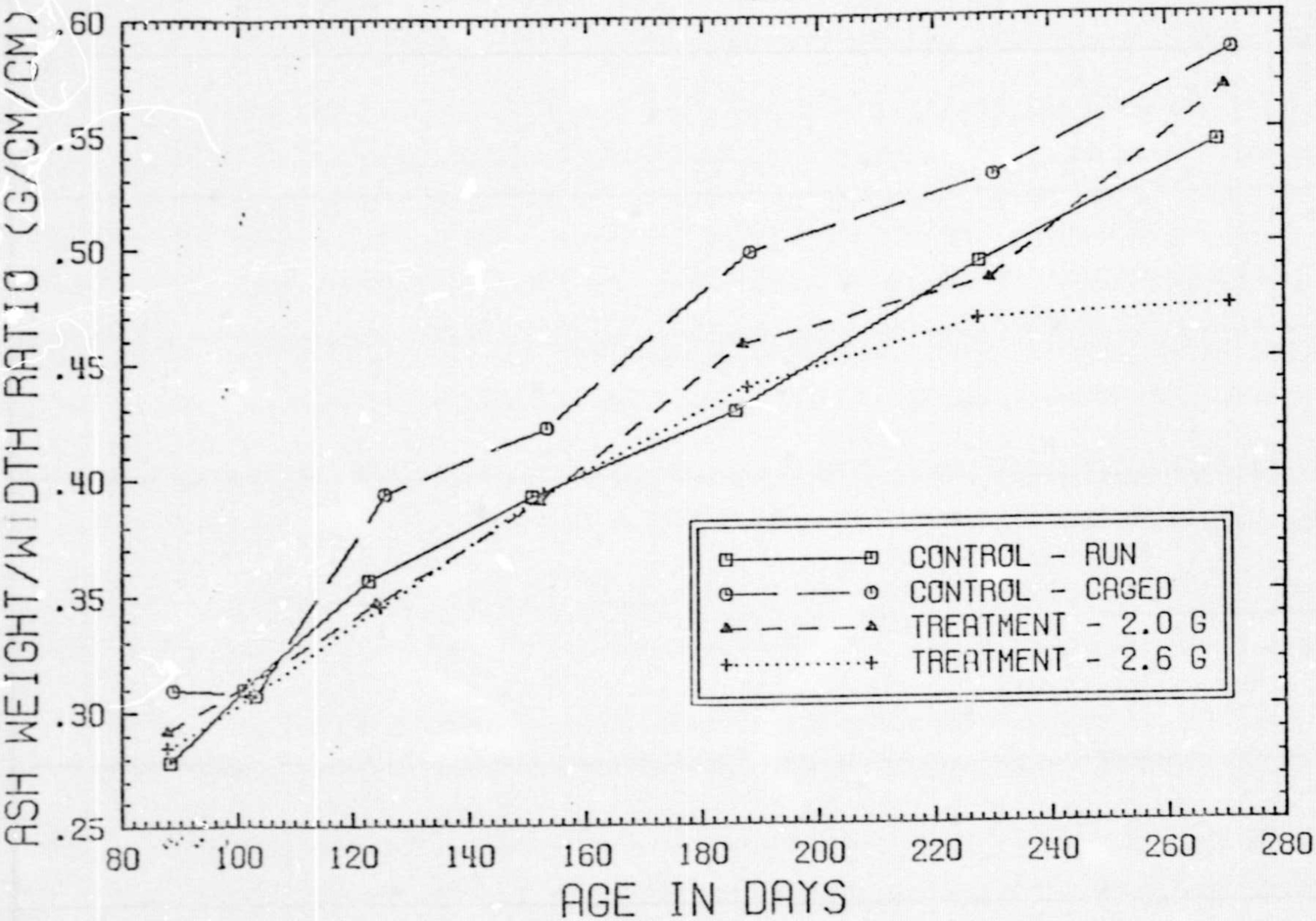

Fig. 5. Plots of Average Ash Weight to Width Ratios at the Midshaft Radius Site vs Age for the Four Experimental Groups.

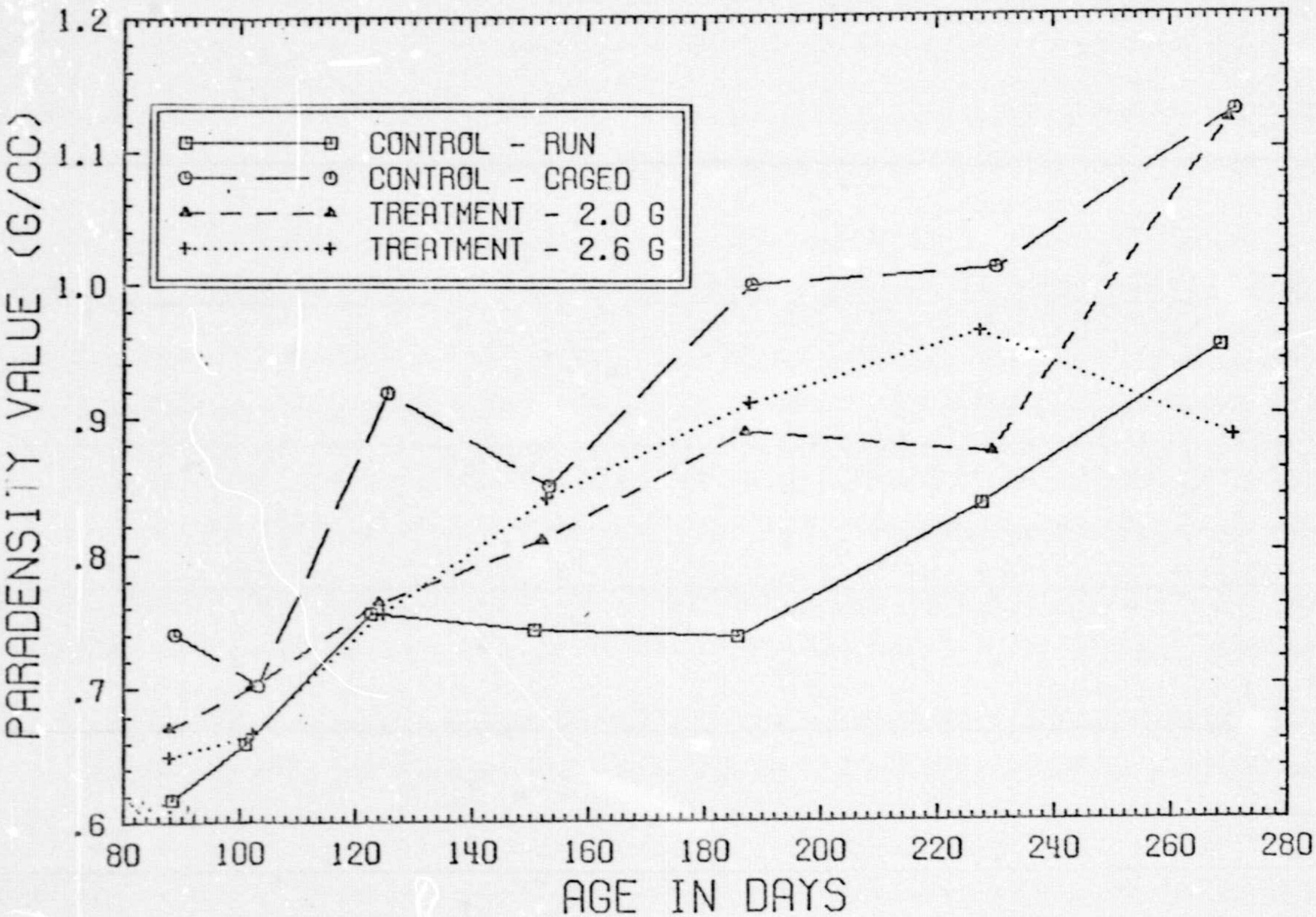

Fig. 6. Plots of Average Paradensity Values at the Midshaft Radius Site vs Age for the Four Experimental Groups.

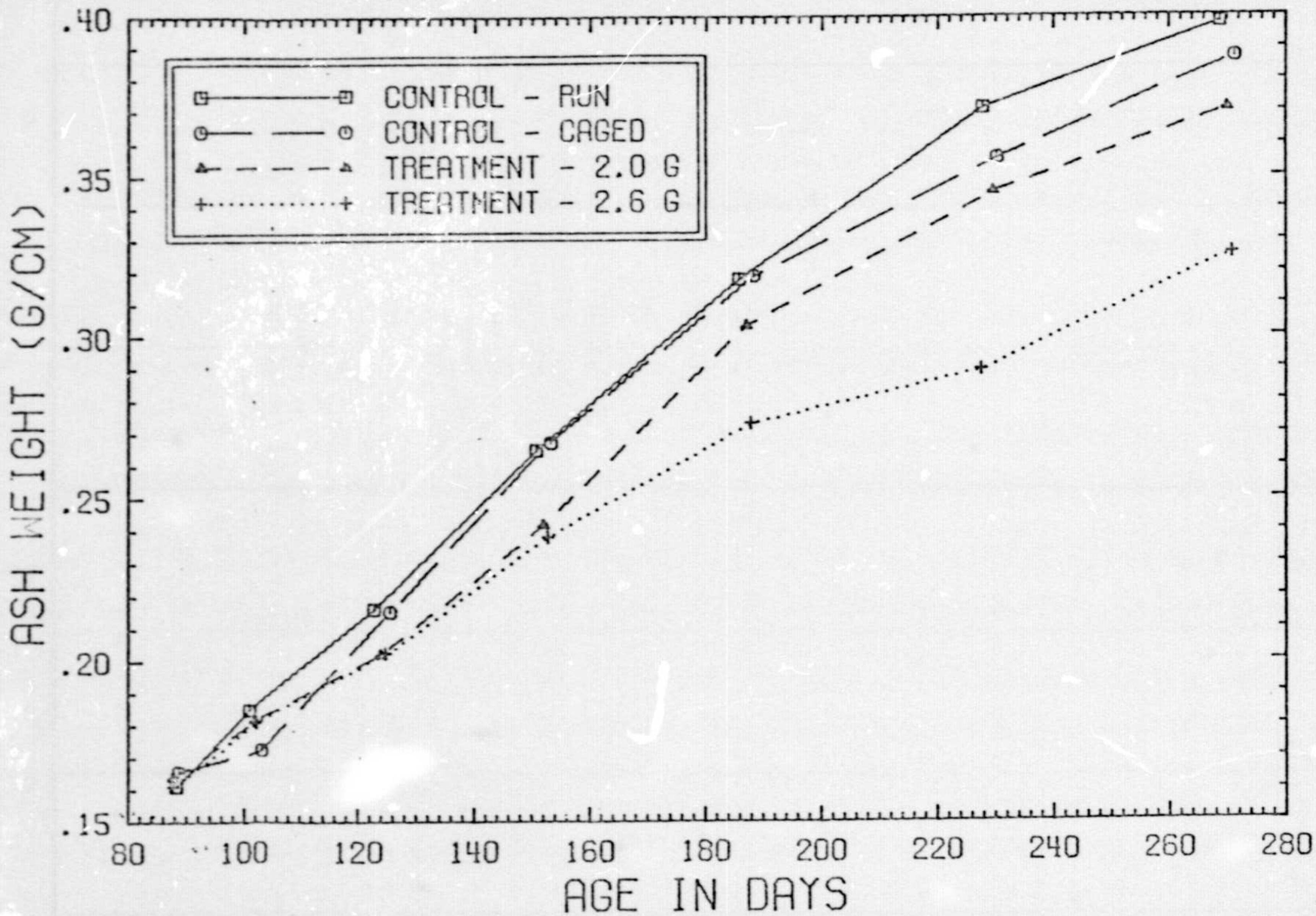

Fig. 7. Plots of Average Ash Weight at the Midshaft Radius Site vs Age for the Four Experimental Groups.

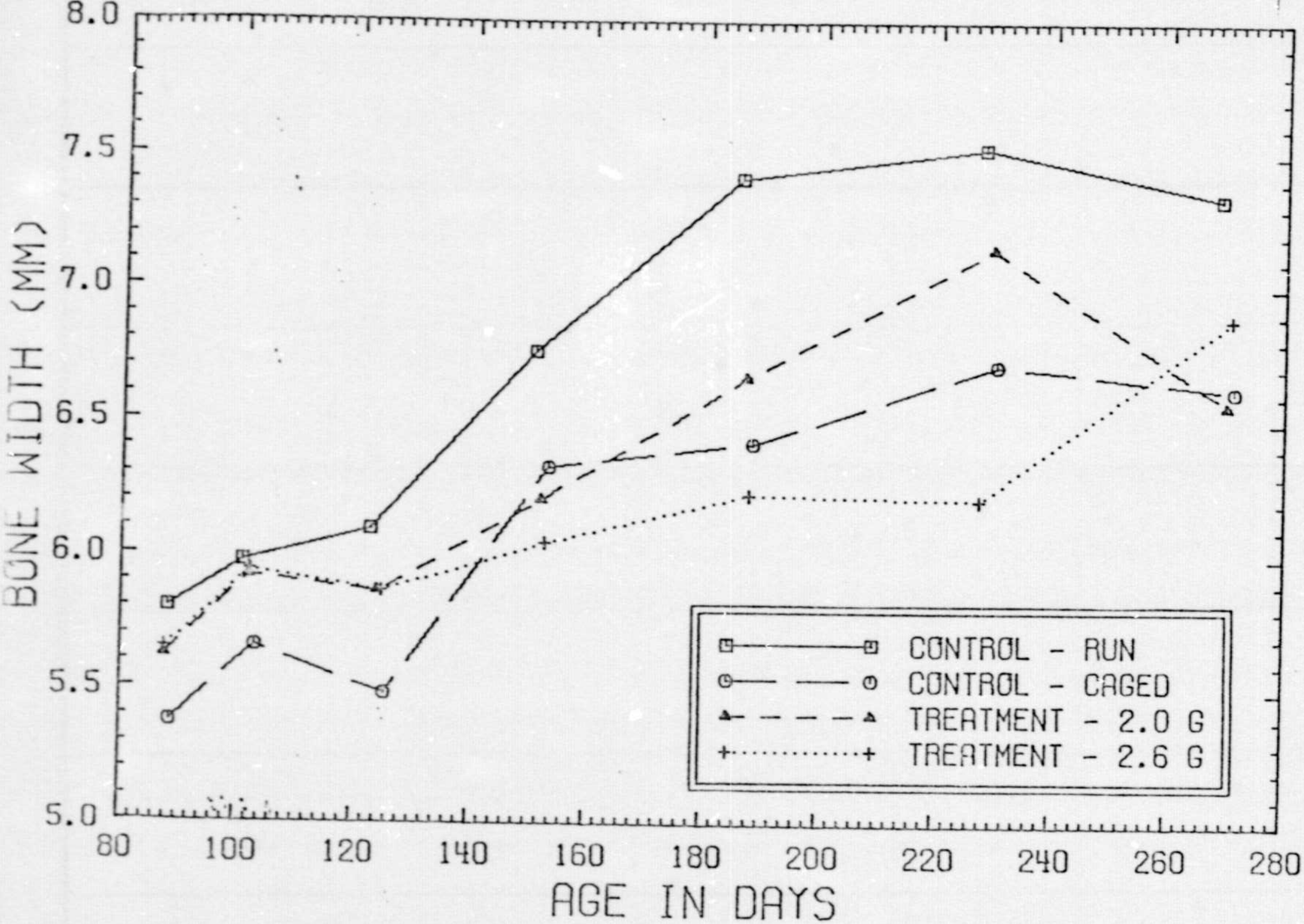

Fig. 8. Plots of Average Bone Width at the Midshaft Radius Site vs Age for the Four Experimental Groups.

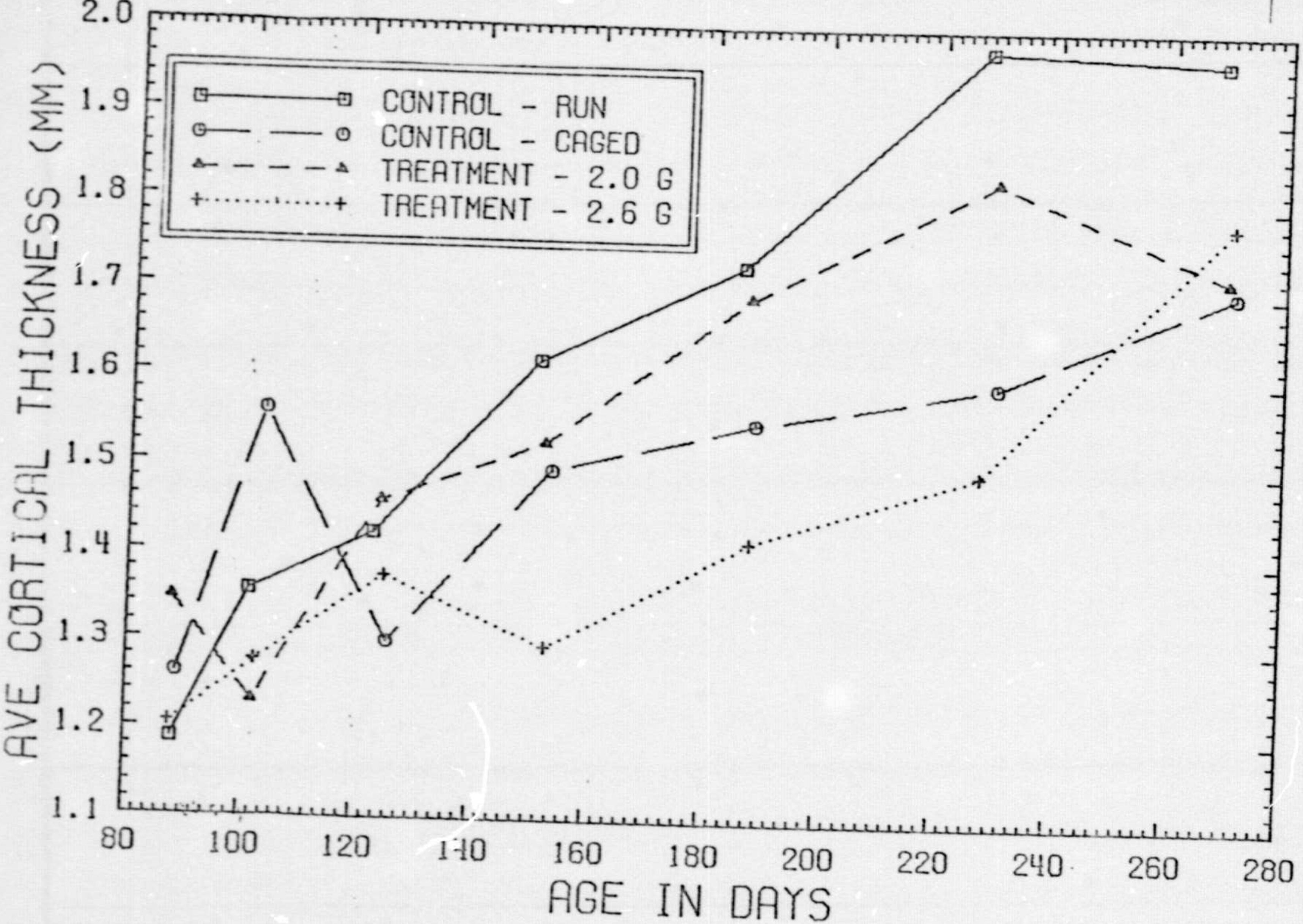

Fig. 9. Plots of Average Cortical Thickness at the Midshaft Radius Site vs Age for the Four Experimental Groups.

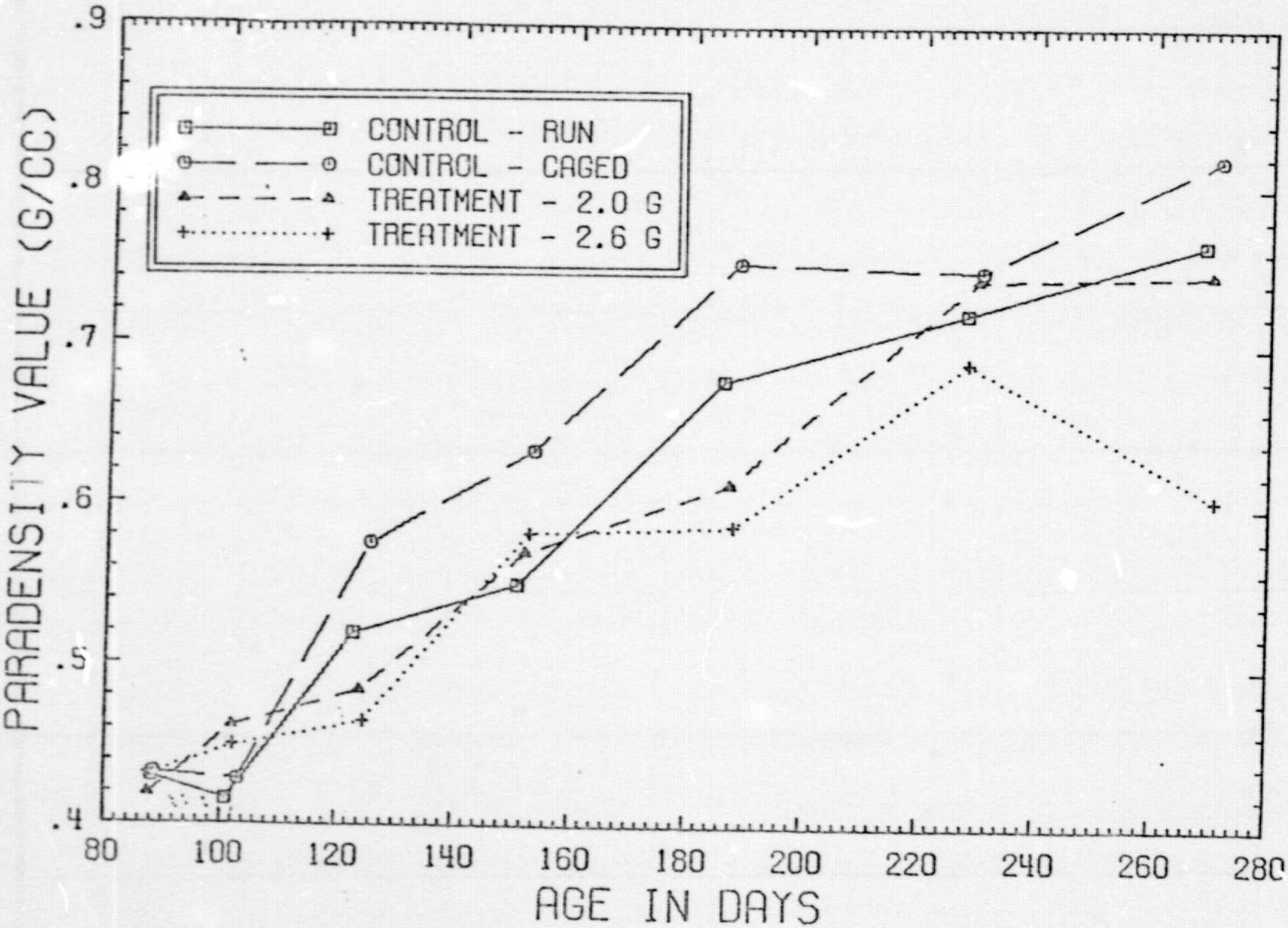

Fig. 10. Plots of Average Paradensity Values at the One-Third Distal Site on the Radius-Ulna vs Age for the Four Experimental Groups.

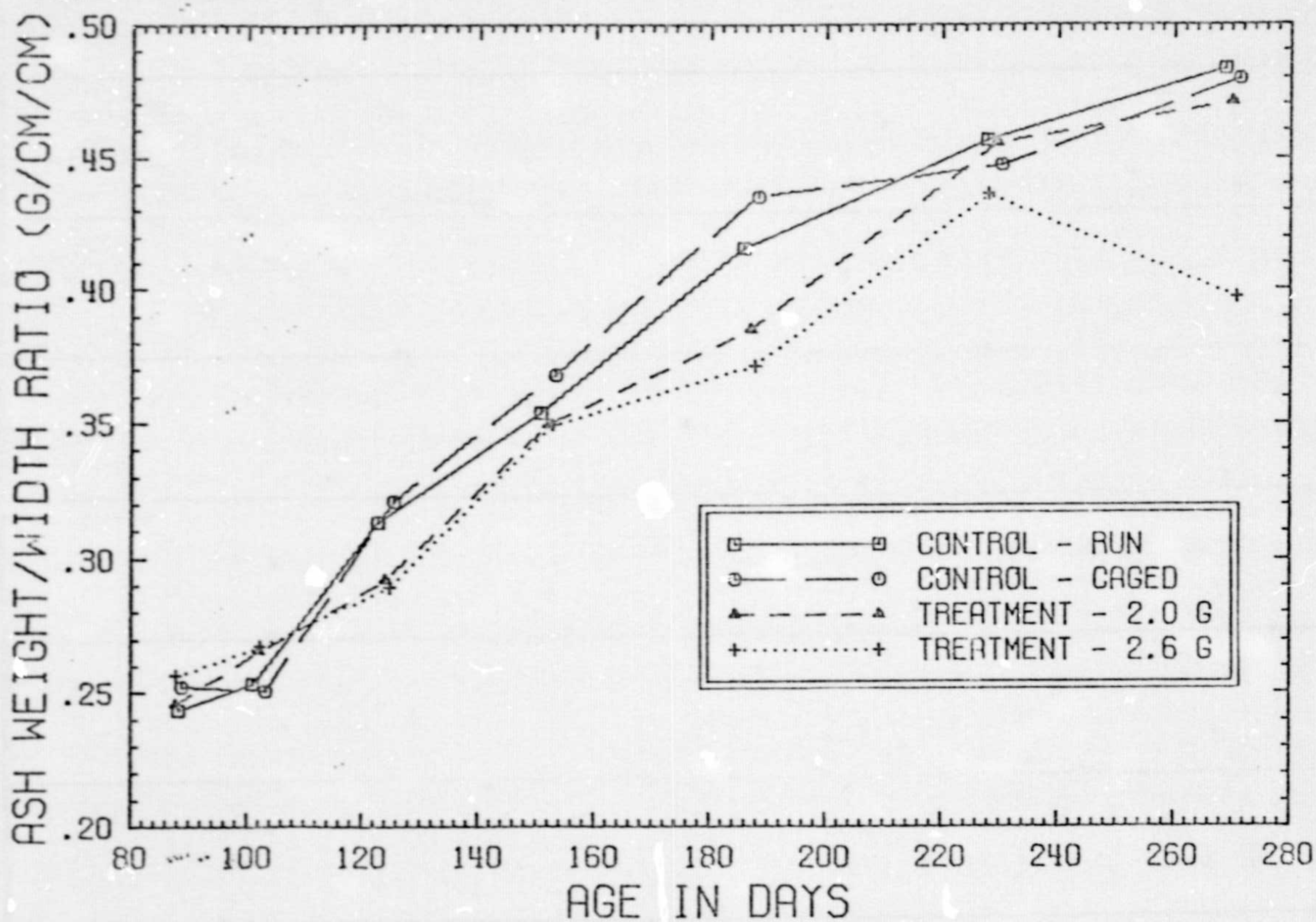

Fig. 11. Plots of Average Ash Weight to Width Ratios at the One-Third Distal Site on the Radius-Ulna vs Age for the Four Experimental Groups.

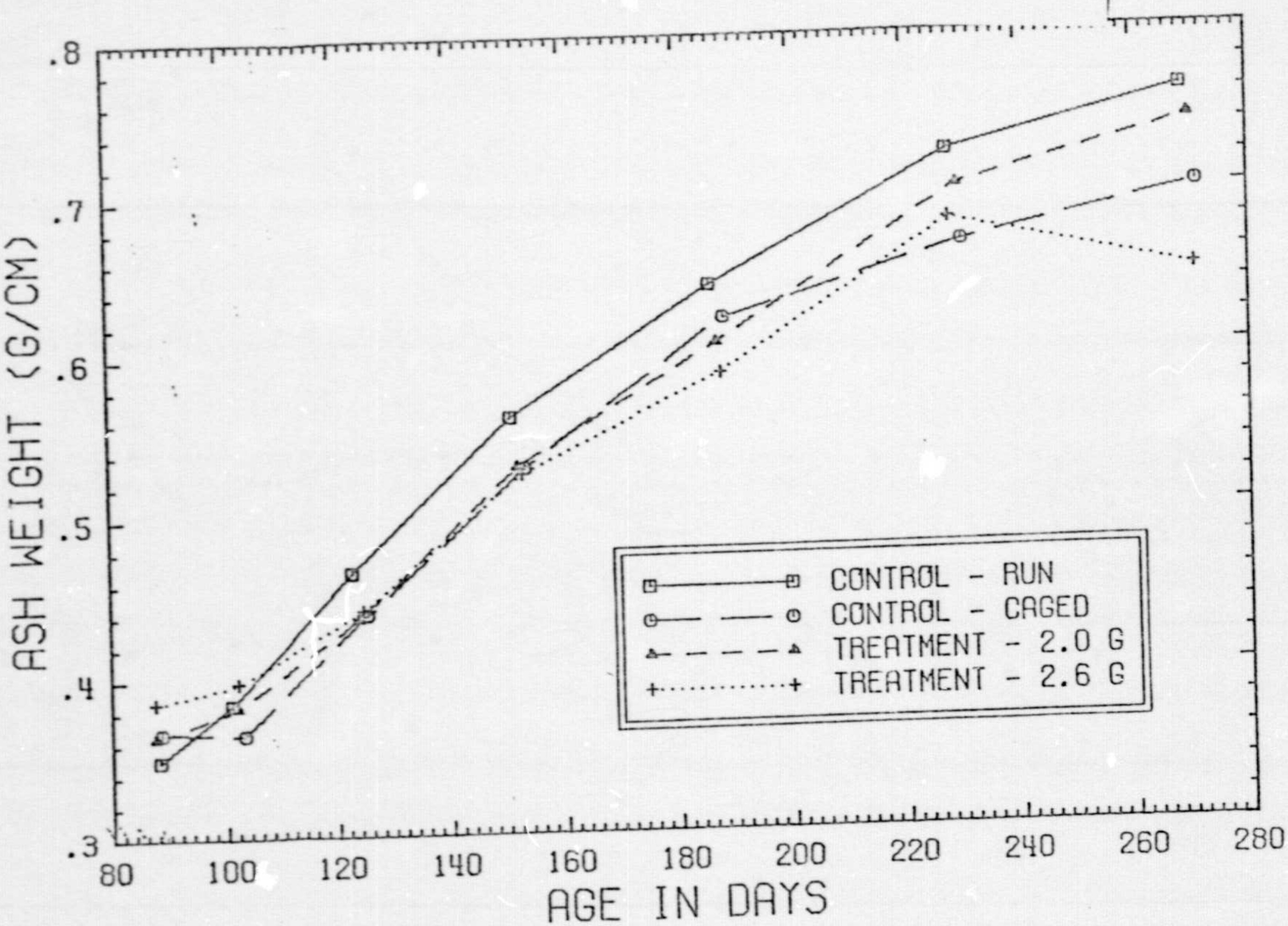

Fig. 12. Plots of Average Ash Weight at the One-Third Distal Site on the Radius-Ulna vs Age for the Four Experimental Groups.

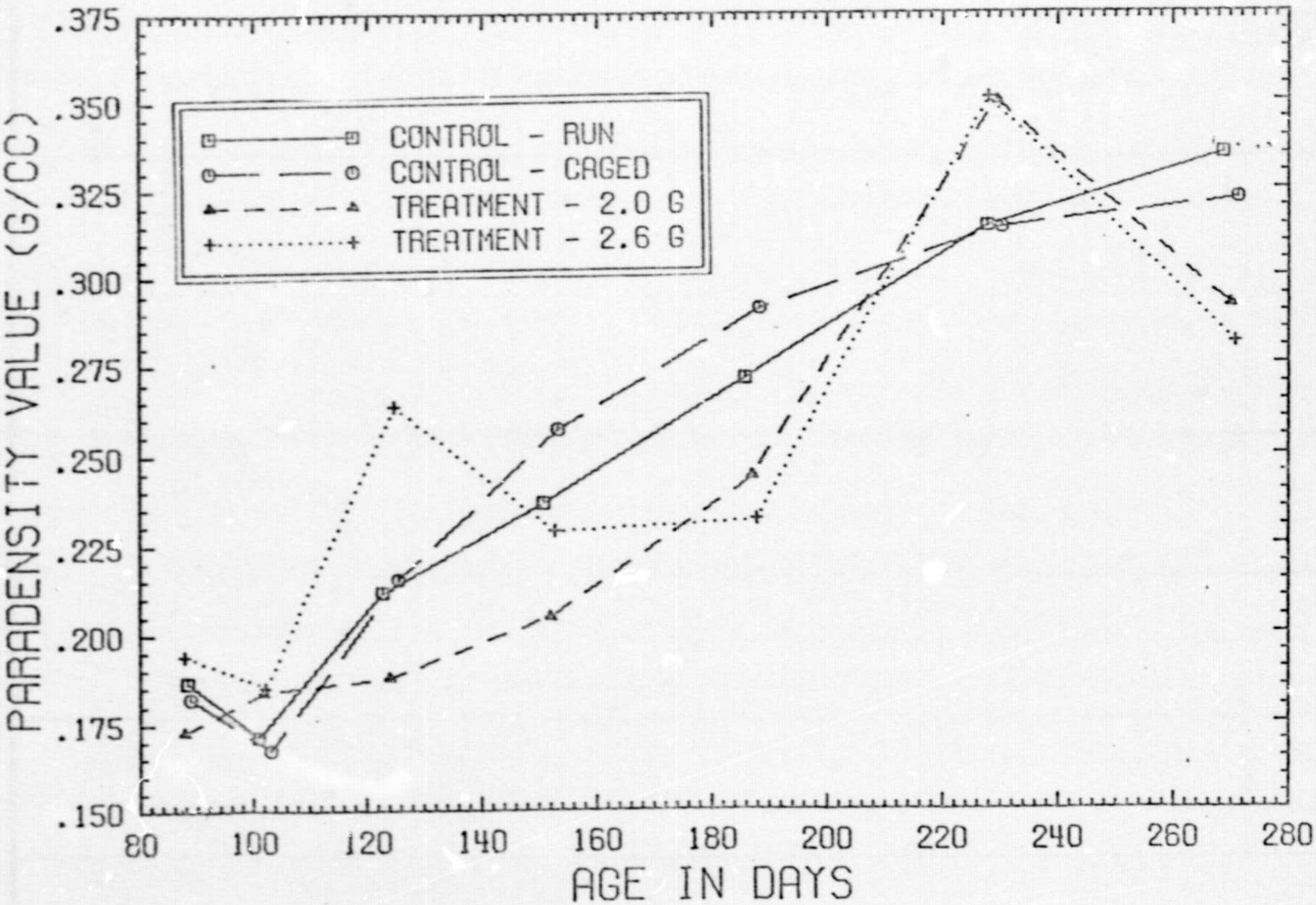

Fig. 13. Plots of Average Paradensity Values at the One-Centimeter Site on the Radius-Ulna vs Age for the Four Experimental Groups.

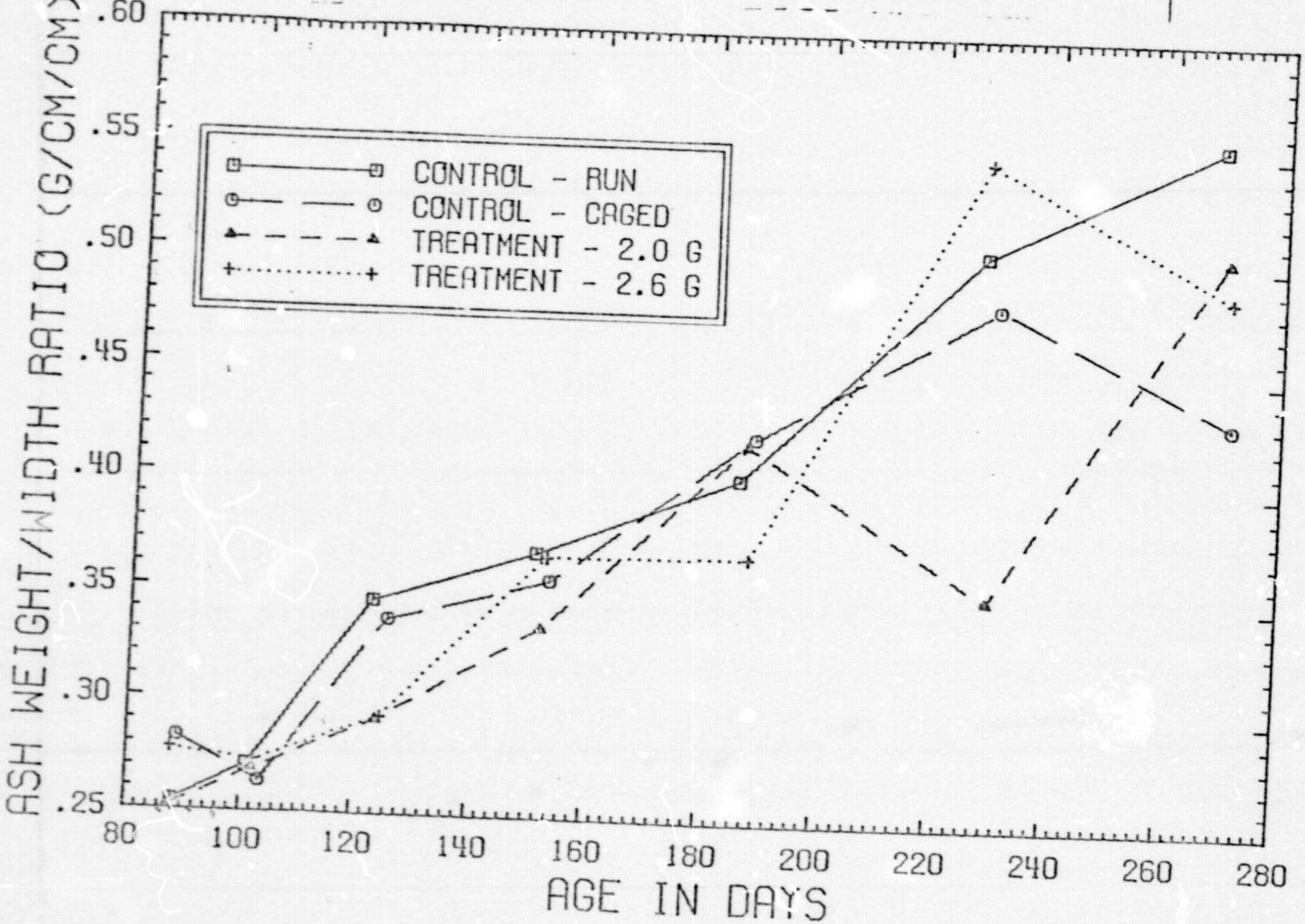

Fig. 14. Plots of Average Ash Weight to Width Ratios at the One-Centimeter Site on the Radius-Ulna vs Age for the Four Experimental Groups.

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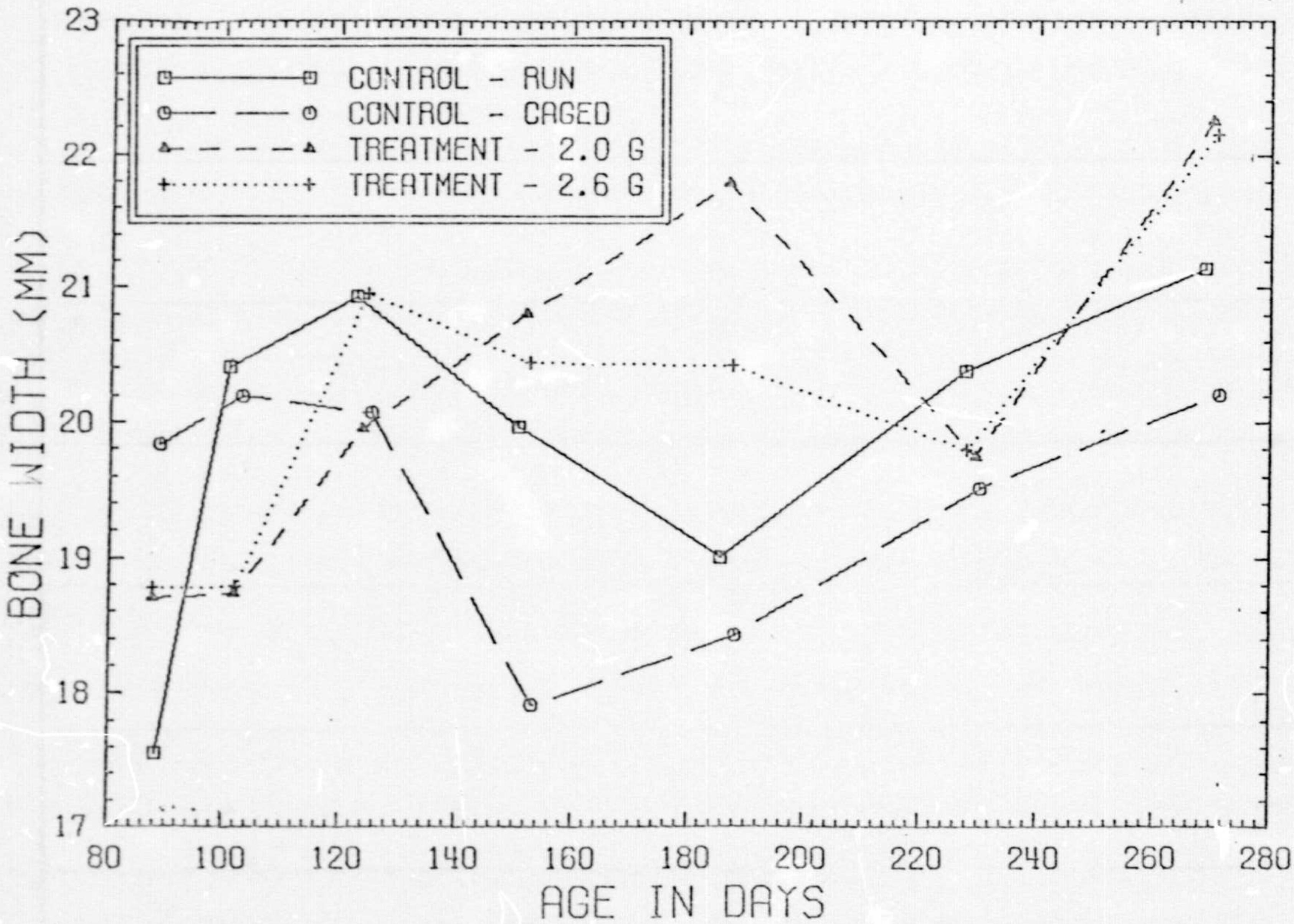

Fig. 15. Plots of Average Bone Width at the One-Centimeter Site on the Radius-Ulna vs Age for the Four Experimental Groups.