A MICROSCOPIC STUDY OF THE EFFECT OF MUSCLE PULL ON DEVELOPING BONE

by

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I. INTRODUCTION

The effect of functional activity on development of organs in the mammalian embryo and foetus provides a field of research to which little critical attention has been paid. Many studies have been made dealing with the relation of functional activity to the development of the nervous system, especially in amphibians. The effects of overload and underload of various portions of the spinal cord by grafting supernumery limbs or by excising limb buds have been studied in urodeles by Detwiler (2) and in the chick by Shorey (8) and Hamburger (3). Larsell (4) (5) has studied the effects on the developing optic lobe of the tree-frog resulting from excision of one eye from the tadpole after optic function had begun, but at an early stage of growth of the brain. Pronounced reduction of development resulted, but since the optic nerve fibers entering the optic lobe were cut and degenerated, the results cannot be attributed alone, with certainty, to absence of functional stimuli from these fibers. The absence of the fibers themselves would account in some measure for the reduced volume of the optic lobe, and possibly also for reduced migration of other nervous elements into this part of the brain.

With reference to growth and orientation of nerve fibers, after the initial formation of axones and dendrites has taken place, three theories have been advanced. Cajal and Forssman have regarded orientation as due to chemical factors, and the attraction of nerve fibers by substances diffused in centers of activity. Kappers, Child and others have explained outgrowth and orientation on the basis of differences in electrical potentials between the neuroblast and its environment. His, Harrison, Weiss

and others have held that nerve fibers grow along lines of force produced by mechanical stresses in the developing embryo, supplemented perhaps by chemical and electrical activities as directive influences.

Since the nervous system, even of urodele larvae, is very complex, and since electrical factors are probably more active in the nervous system than in other parts of the developing body, it was thought desirable to study the effect of reduced functional activity on other organs. The scapula of the rat foetus provides favorable material. It probably is as much affected by mechanical stimulation as any organ in the foetus, due to contractions of the numerous muscles attached to it. These contractions begin, as Angulo (1) has shown, on about the sixteenth day of development. Up to the sixteenth day the scalpula in the rat is very small and undeveloped. It grows rapidly from this stage to birth at 21 days after insemination. The broad, thin structure of this bone makes it easily measured as compared with other bones, and the arrangement of the trabeculae is also more readily studied. Moreover, the blood supply is so arranged that it is in nowise affected by amputation of the foetal forelimb.

The method of reducing the functional activity of the scapula consisted in amputating the foreleg on one side of foetuses at various stages of development, beginning at 16 days. Amputation was performed by electric cautery. The cornu of the pregnant uterus was brought to the surface, after lateral incision through the body wall of the mother, and a small incision was made in the uterine wall and through the amnion. The amniotic fluid was allowed to escape, and the foetus was manipulated so as to bring the limb bud, usually the left, through the incision. The bud was then destroyed with the cautery, with as little injury to the

uterine wall as possible. Destruction was effected in varying degree, affording varying loads on the scapula through the remaining period of its development. The operations were performed by Doctor Larsell, and he has placed the material in my hands for microscopic study. For this I wish to make acknowledgement.

The changes in development of the entire scapula, with some microscopic observations, have been described by Doctor Larsell (6). Briefly the results may be summarized as follows:

The development of the scapula on the operated side of the foetus as compared with the opposite normal side was found to have a direct relationship to the amount of fore-limb destroyed and to the stage of gestation at which the injury was inflicted. The greater the amount of forelimb destruction with corresponding lessened muscular pull, the smaller was the scapula at term. Likewise, the earlier the amputation was performed in the development of the foetus the smaller was the scapula, whose muscles were affected, at term, as compared with that of the intact side. Dr. Larsell concluded that these results pointed to a direct effect of the action of the muscles connecting forelimb and scapula on the development of the latter. In a study of the sectioned scapula his observations, based upon a comparison of the scapula of the operated side with that of the opposite un-cauterized side which served as a control, revealed a reduced growth activity in the experimental scapula. The patterns of the bony spicules and the marrow spaces of the scapular neck were different in experimental and controlled bones. The former were relatively fewer and demonstrate a trabeculation unmodified by muscle pull, while the marrow spaces were correspondingly larger and fewer. The zone of cartilage formation was found to be narrower.

In the present study more detailed attention has been given to the internal development of the bone, the arrangement of spicules and the changes observed in the latter.

II. MATERIAL AND METHODS

The operated rat foetuses were fixed at term in Bouin's fluid and preserved in 80 per cent alcohol. They were obtained as late as possible on the twenty-first day by killing the mother with ether and removing them from the uterus. This was found necessary because the mothers destroyed those born maimed. In addition to the rat foetuses two opossum pouch young were also used. Those had been operated by Doctor Larsell by amputating the forelimb by ligature.

For the present study the scapulae and attached bones of the arm were dissected out and muscles removed. With the aid of an Edinger-Leitz projectoscope an outline of each pair of scapulae was made. The bones were then decalcified in a formol-nitric acid solution containing 5 per cent formalin with 7.5 to 15 cc. nitric acid added per 100 cc. of solution. When the bones became soft, which took from two to seven days, they were placed in 5 per cent aqueous solution of sodium sulfate for 24 hours to remove the acid and then washed in running water for the same length of time. To aid visibility in orientation and sectioning surface stain, in a dilute solution of borax-carmine for 5 to 10 minutes, was used. The bones were then dehydrated, imbedded in paraffin and sectioned serially. The slides were stained with the Heidenhain's azan technique which used azocarmine Gx as the stain and a counterstain of Orange G and aniline blue. The cells and nuclei retained the red stain while the bone and cartilage matrix took the brilliant blue. The sections then were dehydrated and mounted in dammar.

Using the projectoscope, a reconstruction of these serial sections of each scapula was made. A section approximately near the center of one scapular series was selected and an outline of the neck and the spicules extending into the marrow cavity was drawn. In turn, adjacent sections were projected upon this outline making it possible to continue the spicules or to introduce new ones into the drawing as they appeared in the serial sections. As many sections were used in this manner as could be superimposed easily without making the drawing too complex. The drawings then were shaded according to the arrangement of spicules shown in this reconstruction. Because of the comparatively simple arrangement of the spicules the neck of the scapula only was used. A reconstruction of the blade and its spicules was attempted, but the spicule pattern proved too complex to insure sufficient accuracy by the method used.

From these reconstructions the number of definite spicules projecting down into the marrow cavity in the neck of the scapula were counted. Also the width of the neck of each scapula was measured at the level where the trabeculae commenced.

The serial sections were measured. Using a microscope with an ocular micrometer the diameter of the neck of each scapula in approximately the same level as mentioned above was taken. The spicules of the same section were measured likewise, including all of those which protruded down into the neck of the scapula. Three sections of the same scapula, somewhat near the center of the series, were measured to secure greater accuracy.

III. DESCRIPTION AND RESULTS

The results of this microscopic study of the effect on the developing scapula produced by varied muscle pull are demonstrated in Plates 1 to 22. The scapulae, of which the reconstructions are shown in Plates 1 and 2

(foetus EH 9246), are from a rat foetus in which part of the forearm only was destroyed at 16 days of gestation. There is some reduction in size of the experimental left scapula (Plate 2), as compared with the unoperated right scapula. This is indicated by the measurements of the neck diameter of the scapulae taken from the microscopic slides in Table 2 showing a decrease in diameter of the experimental of 2.3 per cent. The pattern of trabeculation is somewhat more unorganised on the experimental side, with the spicules and marrow spaces becoming larger and fewer. Table 2 shows an increase in the size of spicules, 8.7 per cent, on the operated side, while Table 1 indicates a decrease of 15.1 per cent in the number of spicules.

A more marked difference between the two scapulae shown in Plates 3 and 4 is observed. These are reconstructions of a pair of scapulae from foetus EH 9114 in which most of the left forelimb, leaving only a small portion of the humerus, was destroyed at 16 days. The atrophy is decidedly more marked on the experimental side. As shown by the tables there is a decrease of 8 per cent in neck diameter in the left scapula with an increase of 14.6 per cent in size of the spicules and 27 per cent decrease in spicule number. The arrangement of the trabeculae is not orderly as compared with the normal, and the zone of cartilage formation is decidedly lessened.

Plates 5 and 6 show the scapulae of a foetus (EH 6949) from which the forearm and approximately one-half of the humerus was removed at 19 days. The normal bone pattern is somewhat disturbed but not to the extent of those operated at an earlier stage and with a greater amount of the forelimb amputated, as in the case of EH 9114. However, spicules are larger on the average showing an increase of 32 per cent as compared with the left

normal scapula. There is a decrease of 21.1 per cent in spicule number. The size of the neck shows a decrease of 6.9 per cent.

The scapulae of foetus W 2310-2 on Plates 7 and 8 are the results of the removal of part of the left humerus on the seventeenth day of gestation. However, when the mother rat was killed and the foetus removed it was found that the right forelimb, which was to be the control, projected through the uterine wall. According to Doctor Larsell (6) this must have placed an additional muscle pull on the scapula which was found slightly larger than some of the normal scapulae of the other foetuses. The possibility of variation in size of the different individual animals at birth may give misleading conclusions as to the increased size. In accordance with the observations of Doctor Larsell (6), measurements from the serial sections of the neck diameter show a greater percentage decrease of the left experimental scapula as compared with the right, than occurs in the other pairs measured. However, this comparison of disparity does not hold true, for the spicule size in the experimental is only 18.6 per cent and that of the decrease of spicule number, 22.3 per cent. The same lack of organization of spicules, noticed in the other experimental scapulae, occurs. In each pair the direction of the spicules in the normal is at a decided angle to the sides of the neck while in the experimental the spicules run in a direction more parallel to the neck. This difference in spicule lines is shown very clearly in the reconstructions (Plates 7 and 8).

In the serial sections of foetus W2310-1 the normal left scapula was cut transversely and the experimental scapula was sectioned longitudinally, as shown in Plates 9 and 10. In this foetus part of the left forelimb had been removed on the seventeenth day leaving a portion of the humerus. The

normal side was more completely developed at term than the opposite experimental side. The normal spicules were smaller and larger in number and there was an increase in the size of the normal neck. Graphic reconstructions of lines were made at different levels of the neck and measurements were taken at the first level shown, which seemed to correspond as nearly as possible to that level measured in the longitudinal sections. Tables 1 and 2 give the results of these measurements.

The drawings on Plates 11 and 12 are from the scapulae of foetus EH 6290. Upon amputation of most of the left forelimb on the sixteenth day approximately one half of the humerus was left. These scapulae were sectioned transversely and the four reconstructions of the experimental on Plate 12 correspond approximately to the same level as that taken for the drawings of the normal on Plate 11. This angle of sectioning shows to better advantage the presence of a greater number of spicules in the normal while the enlarged marrow spaces are portrayed inthe experimental. The diameter through the long axis of the neck cross section is greater in the normal than the experimental, the latter showing an atrophy of 4.1 per cent. The measurements and the number of spicules in the transverse sections were much more difficult to take than in the longitudinal slides as the width, considered through the long axis of the cross section, merges with the depth, taken through the short axis of the neck. These values vary greatly to show a very intricate network of bone which does not readily differentiate between spicules. However, those lines which seemed to run at right angles to the flat surface of the bone and near the center of the section were considered to correspond to those spicules recorded in the longitudinal section, and were measured and counted as such.

In the study of the two pairs of scapulae from the opossum the gross

observations reveal a slight atrophy on the experimental side, thus agreeing with the results observed in the rat. Plates 13 and 18 are outlines of these gross structures showing the slight increase in size of the control in both cases. The microscopic studies expose a spicule pattern much less complex than that of the rat but with similar results. Plates 14 and 15 are reconstructions of different sections from the same control scapula of opossum #55-2 in which the forearm and part of the humerus was removed 8 days after birth. The pouch young was killed at 31 days. The spicules shown in Plate 15 especially, take a direction with a definite angle to the neck while those of the experimental in Plates 16 and 17 run approximately straight up and down with a more primitive bony pattern. From the tables it is observed that the experimental spicules are larger showing an increase of 37.6 per cent over the normal and the number decreasing 9.1 per cent less than those in the control. The diameter of the neck is likewise smaller showing a decrease of 2.5 per cent as compared with the size of the control.

Plates 19 to 22 compare the control and experimental scapulae of opossum #55-1 killed at 31 days after birth with the left forelimb having been amputated near the middle of the humerus when 8 days in the pouch. These show results similar to those found in #55-2 namely, the patterns of the experimental show a trend of the spicules to parallel the sides of the scapula; the spicules are 8.7 per cent fewer and show a 11.9 per cent increase in size over those of the control.

The measurements from these drawings of the diameter of the scapula neck given in Table 1 do not seem to be in proportion to the figures recorded in Table 2 from the microscopic sections. This is most readily accounted for

by the fact that the sections for the reconstructions were selected for their representation of the trabeculae and were approximately in the center of the scapula. However, in all cases but one these measurements show a percentage decrease in the size of the neck in the experimental. The exception, #55-2, which shows a slight increase over the normal can be explained because the sections for one reconstruction of the control were those nearer the surface of the scapula, which is much narrower than those of the center. These were selected because they illustrate very well the slanting direction of the normal spicules. In reality the figures for this scapula, taken from the microscopic measurements in Table 2, show this control larger than its experimental. Despite this discrepancy, these figures illustrate the atrophy of the experimental scapulae which is apparently due to the lessened muscular stresses.

The number of spicules counted in each case from the reconstructions (Table 1) were fewer in number in the experimental scapulae. These were counted by including all of those spicules extending from the cartilage downward which could be seen as if the bone had been cut in half longitudinally and parallel to the blade to give a more accurate figure. The number of spicules in the control scapulae range from 25 to 18 in the rat and from 23 to 19 in the opossum. The experimental range was from 20 to 13 for the rat while the opossum showed a smaller difference of 21 to 18. The largest decrease in number was found in the rat foetus in which the greatest amount of forelimb was destroyed and at the earliest stage operated, thus giving the greatest per cent in decrease, 27.8 per cent, as compared with the other rat scapulae. Also it is interesting to note that the least in number and percentage difference, 13.1 per cent, occurred in HH 9246 in which a very small portion of the forelimb was removed making a slightly lessened muscular pull on the scapula. The decrease in the opossum was approximately the

same for both experiments, 9.1 and 8.7 per cent.

Table 2 gives the measurements for the individual spicules in a single section, and the average size of these. The number of spicules measured for a section is indicated at the top of the columns. These figures in one colymn do not pertain to the measurement of a corresponding spicule in another scapula, for such a comparison of an individual spicule would require a more accurate technique than was feasible. The average size of a spicule in the normal rat was found to vary from 0.54 to 0.93 and in the opossum 1.17 to 1.19, with 1 equal to 0.0916 mm. The experimental was 0.84 to 1.01 in the rat and 1.22 to 1.60 for the opossum. The percentage increase over the control varies greatly with BH 9246 again having the lowest value of 8.7 per cent increase only. The highest percentage difference is not in BH 9114 as might be expected but in W 2510-1 which was operated at 17 days with only a small portion of the humerus removed. This percentage increase was 55.0 per cent.

For a comparison of these changes in spicule size with the average number, a graph was charted of these values for each the normal and experimental (Chart 2). These relationships in the normal and experimental are somewhat similar as shown by the likeness of the two curves. The decrease in spicule number and increase of spicule size shifts the experimental curve below and to the right of the normal. This includes values for both the rat and opossum.

The proportions of the percentage decrease in spicule number to the percentage increase of spicule size is shown in Table 1. Also Charg 6 was taken from these values. The inconsistancy of the curve, together with the wide variance of ratio values between the different specimens, points to little if any relationship between the decrease of spicule number to the

increase of spicule size in the individual experiment regardless of degree of muscle pull.

As to the comparison in size of the scapula neck, Table 2, it has already been stated that the greatest differences are in that experiment in which the least muscle pull was exerted, BH 9114, giving an 8 per cent decrease but this isnot considered of value as the normal scapula had an increased strain which was produced by the forelimb protruding through and pulling against the uterine wall. Likewise, the least amount of decrease, 2.3 per cent, was found in BH 9246 which developed with only a slightly lessened strain on the scapula. The other scapular neck sizes varied similarly according to the amount of amputation and developmental stage at which it was performed.

These values for the neck size were plotted against the average size of the spicules for both normal and experimental (Chart 1). The two curves follow a course similar to each other, with the increased size of spicule values causing a shift in the experimental curve to the right. Chart 4, which compares the percentage decrease of spicules with the percentage decrease in scapular neck gives a very uneven curve showing no apparent connections between the reduced size of the scapula and enlarged spicules of different experimental specimens.

The relationship of the number of spicules to the average size of the neck in the experimental and control are illustrated by Chart No. 3. Both factors in the experimental vary to a greater extent than that of the control and give a curve with less resemblance to the normal than those which occurred when the number and size of spiculesin the control were compared with those of the experimental. The percentages of decrease in the neck size and spicule number were plotted against each other in Chart No. 5 to

give another irregular curve. These numbers, together with the wide variations in ratio values for these same figures given in Table 1, do not show a direct proportion between the percentage decrease of neck diameter and of spicule number.

IV SUMMARY AND CONCLUSIONS

In the aforegoing discussion an attempt has been made to describe the effect of various amounts of muscle pull on developing bone. It has been shown by Doctor Larsell that the gross structure of the scapula varied in size directly in proportion to the amount of stress exerted on the bone by the muscles, the less the muscle pull the more the resulting atrophy of the bone. Also, the earlier the stage in which the normal pull is lessened the greater is the arrest in further development of the bone. Further influences of external forces are indicated by the enlargement of the spicules and the smaller number formed when these forces are decreased.

The lack of organization in the bone pattern of the experimental scapula probably has its explanation in being the primitive arrangement of spicules unmodified by muscular pull. The gross size decrease of the operated scapula does not seem to show any relationship to the decrease in spicule number and increase in spicule size, while no apparent ratio is between the enlargement of the spicules and their number decrease in all of the scapulae. However, this latter comparison in the experimental and its corresponding control indicates a possible relationship between the two scapulae of the same foetus.

The opossum experiments gave the same results as those observed in the rat. The microscopic structure of the opossum, however, was found to be more primitive, the spicules being much larger and fewer with very simple normal pattern as compared to the more complex one of the rat.

In the experimental scapulae the haphazard formation of bone shows a slight tendency of the spicules to take a parallel position in relation to the scapula surface, while those of the normal arrange themselves in a definite angle pattern along the probable lines of force produced by the muscle pull.

An explanation of the results described is probably related to the lines and planes of stress produced in the developing bone by the activity of the attached muscles. These lines of stress are regarded as zones of increased metabolic activity or zones of irritation. That irritation causes one of two things, either a necrosis or a proliferation of cells is well established. It is thus probable that in this case these so called zones of irritation, or increased metabolism, cause a proliferation of cells, the osteoblasts.

From the many theories of calcification that have been put forth, Robison (7) offers one that seems very plausible. His theory holds that a very active enzyme, phosphatase, in preparatory areas of ossification hydrolyses the phosphoric esters of the blood to bring about a local increase in the phosphate ion concentration. This in turn exceeds the solubility of the calcium phosphate or its compound, which is considered as the bone salt, to cause its precipitation in this region. Transeen and McLean (9) agree with Robison in that the osteoblasts and hypertrophic cartilage cells have assumed the function of synthesis and excretion of phosphatase. If this theory proves true, it seems probable that through this proliferation of osteoblasts a possible explanation for the formation of bone along such lines of force can be offered. In some similar manner this increased metabolism would enlarge the number of osteoclasts and increase their sculpturing action to result in the highly organized spicule pattern of the normal bone. With no increased metabolism it is probable that the formation of osteoclasts from

osteoblasts or wandering body cells is not thoroughly stimulated. Thus, without this check on the work of the osteoblasts the large irregular spicules of the experimental result.

It is apparent from the data here collected that there is a definite and decided arrest in the development of bone when deprived of stimulation normally afforded it by the pull of the developing muscles. Such arrest is demonstrated by decreased size grossly and variance in spicule size, pattern and number, microscopically. From this it is shown that muscle pull determines the direction and pattern of the forming spicules.

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VII DESCRIPTION OF TABLES, CHARTS AND PLATES

EXP. NO.	EXPERIMENT	DECREASE DIAMETER NECK, C.M.	PERCENT DECREASE IN EXP.	NO. OF SPICULES	%DECREASE OF SPICULES IN EXP.	RATIO $\frac{\text{\%DECREASE SIZE NECK}}{\text{\%DECREASE NO. SPICULES}} \times 100$	RATIO $\frac{\text{\%INCREASE SIZE SPICULES}}{\text{\%DECREASE NO. SPICULES}} \times 100$
1.	RAT B.H. 9246-RT. LEFT EXP.	10.2 10.1	1.0	23 20	13.1	10.8	66.4
2.	B.H. 9114-RT. LEFT EXP.	8.8 7.1	19.6	18 13	27.8	2.9	52.7
3.	B.H. 6949-LT. RIGHT EXP.	9.8 8.4	14.3	19 15	21.1	32.2	151.6
4.	W2310-2RT. LEFT EXP.	7.9 7.4	6.4	18 14	22.3	39.0	83.4
5.	W2310-1 LT. EXP. RT.	8.25 7.7	6.7	20 16	20.0	25.0	275.0
6.	B.H. 6290 RT. LEFT EXP.	8.8 7.9	10.5	22 18	18.2	22.5	265.4
7.	OPOSSUM NO. 55-2 LT. RT. EXP.	15.4 15.3 16.0 16.1	* 3.8	19 22 20 20	9.1	26.8	413.1
8.	NO. 55-1 RT. LEFT EXP.	14.8 15.7 13.0 13.3	15.3	21 22 18 21	8.7	81.7	136.7

* = INCREASE RATHER THAN DECREASE.

‡ = USED FIGURES FROM TABLE 2.

TABLE 1.

MEASUREMENTS FROM THE PLATES OF DIAMETERS OF SCAPULAR NECKS; NUMBER OF SPICULES; AVERAGES AND PERCENTAGES OF EXPERIMENTALS WITH CONTROLS.

EXP. NO.	EXPERIMENT	SLIDE ROW SECTION	WIDTH AT NECK IN M.M.	AVERAGE WIDTH	PERCENT OF DECREASE IN EXP.	SIZE OF SPICULE I = .0916 M.M. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	AVERAGE SIZE	AVERAGE SIZE OF THE 3	PERCENT OF INCREASE IN EXP.
	RAT																																	
1.	B.H.9246	1-3-9	1.34	1.33	2.3	1.2	1.8	1	1	.8	.8	.8	1.	1.	1.	.9	1.	.8	.8	.8	1.	1.	1.2									.99	.93	8.7
	RIGHT	1-3-14	1.32			1.0	1.5	.7	.7	1.0	1.0	.8	.8	.7	.8	.8	.8	.9	.9	.8	1.0	1.0	1.2									.91		
	CONTROL	1-3-2	1.33			2.0	1.0	1.0	.6	1.0	.8	.7	.7	.7	.8	1.0	.9	.6	.8	1.2	.5	.6	1.0									.88		
	LEFT	1-3-14	1.30	1.30		1.5	.9	1.0	1.0	.8	1.0	.5	.5	1.3	1.0	1.0	.8	1.1	.5	.9	1.5	.9										.95	1.01	
		1-3-8	1.29			2.0	.8	1.0	1.0	1.2	1.5	.6	1.5	1.2	1.1	.8	.8	1.8	.8	.9	1.0	1.9										1.11		
	EXP.	2-1-8	1.30			1.5	.8	.9	.9	1.0	1.0	1.5	.8	1.1	1.2	1.1	1.3	1.1	1.2	.9	1.0	1.0										1.07		
2.	B.H. 9114	1-3-12	1.05	1.06	8.0	1.8	.5	.6	.6	.8	.8	1.2	.6	1.0	.6	.7	.6	.5	.6	1.0												.77	.82	14.6
	RIGHT	2-1-5	1.07			1.6	.5	.7	.6	.7	1.7	1.1	.7	.5	.5	.7	.5	.5	1.0	1.3												.77		
	CONTROL	1-3-9	1.05			2.0	.9	.5	1.1	.8	1.1	1.2	.9	.8	.5	.8	1.0	.7	.5	1.1	.8	1.0										.92		
	LEFT	1-3-10	.97	.98		1.6	1.0	1.0	.8	.8	.7	1.2	.6	.8	.5	1.0	.5	.5	1.3													.87	.94	
		1-2-9	1.00			2.0	.7	.9	.9	1.2	.9	.6	.8	1.0	.7	1.2	.9	1.5														1.02		
	EXP	1-3-1	.98			1.8	.9	.9	1.0	.5	.7	.9	1.0	.8	.7	.7	.8	1.5														.93		
3.	B.H.6949	2-2-1	1.18	1.16	6.9	1.3	.7	.8	.5	.6	.5	.5	.8	.8	.6	1.2	.9	.8	.6	.5	.6	.7	1.1									.75	.75	32.0
	LEFT	2-2-8	1.15			1.0	.6	1.0	.5	.8	.8	1.0	.5	.6	.4	.7	.8	.5	.7	.6	.4	.6	1.0									.70		
	CONTROL	2-2-4	1.15			1.8	.5	1.0	.8	1.0	.8	1.0	.8	.5	.4	.8	.7	.8	.6	.6	.7	.7	1.0									.80		
	RIGHT	2-2-6	1.08			1.5	.8	.8	.8	.6	1.0	.8	1.0	1.0	1.0	.7	1.0	.5	1.0													.90	.99	
		2-2-8	1.10			1.3	.7	1.5	1.6	1.3	1.5	1.2	1.3	.6	.9	.6	1.0	1.0														1.10		
	EXP	2-3-2	1.07			1.2	1.2	.9	.9	1.3	.5	1.3	.8	.8	.9	.9	1.0	.9	1.0													.97		
4.	W 2310-2	1-1-10	1.00	1.04	8.7	1.5	.5	.7	.4	.5	.5	.8	1.0	.7	.5	.7	.5	.5	.8	1.0	1.4											.78	.75	18.6
	RIGHT	1-2-3	1.07			1.2	.5	.7	.5	.8	.7	.8	.5	.4	.5	.5	.7	.4	.5	.6	1.4											.67		
	CONTROL	1-1-13	1.05			1.5	.6	.5	1.0	.5	.5	.8	.7	.8	1.0	.8	1.2	.5	.7	.8	.7												.80		
	LEFT	1-2-3	.95	.95		1.3	.7	.9	1.0	.8	1.0	1.0	.8	1.0	.7	.6	1.0	.8	.7	1.2													.90	.89	
		1-2-1	.95			1.5	1.0	.9	.9	.6	1.0	1.0	1.0	.7	.8	.5	.5	.7	1.0														.88		
	EXP.	1-2-4	.94			1.1	1.2	.6	.7	.8	.9	1.0	1.0	.7	.6	1.0	1.0	.7	1.4														.90		
5.	W 2310-1 LEFT	5-1-18	1.23	1.21	5.0	.8	.8	.5	.5	1.0	.7	.5	.4	.5	.5	2	.2	.5	.5	.6	1.0	.6	.3	.2	.4	.6	.6	.2	1.0			.54	.54	55.0
	CONTROL	5-1-13	1.22			.8	1.0	.5	.6	.9	1.0	.8	.5	.9	.5	.4	.5	.5	.5	.6	.2	.6	.2	.2	.6	.2	.4	.5	.5			.55		
	TRANS.	5-5-2	1.17			1.2	.5	.3	.5	.4	.7	.2	1.2	.3	.3	.5	.3	.4	.3	.4	.8	.4	.4	.7	.5	.7	.5	1.0				.54		
	RIGHT	1-3-15	1.10	1.15		1.0	1.0	.7	.5	1.0	1.0	1.0	.7	1.0	1.5	.7	.8	.6	.7	.9	1.0	1.0	1.0									.89	.84	
	EXP	1-3-12	1.15			1.1	1.0	.8	.8	.5	1.2	1.2	.8	.8	.8	1.1	1.0	1.2	.6	.8	.6	.5	1.0									.85		
	LONG.	2-1-1	1.20			1.2	1.0	.6	.5	.5	.8	.8	.8	.8	.5	.6	1.0	.8	1.0	1.0	1.0	.5	1.0									.80		
6.	B.H 6290	3-2-18	1.25	1.24	4.1	1.0	1.0	.5	.5	.6	.5	.5	.5	.5	.5	1.0	.7	.7	.7	.4	.4	.3	.5	.5	.7	.4	.7	.4	.5	1.0	1.0	.58	.61	52.4
	RIGHT	3-3-6	1.25			1.2	1.1	.9	.7	.8	.7	.4	.2	.4	.4	.5	.5	.4	.3	.4	.5	.5	.4	.2	.6	.2	.6	.7	.7	1.1	.7	.58		
	TRANS	3-3-2	1.22			1.5	.7	.3	.5	.3	.5	.5	.7	.5	.5	.7	1.0	.5	.5	1.0	.8	.5	.6	.6	.5	.8	1.0	.5	1.0	1.0		.66		
	LEFT	6-3-1	1.18	1.19		1.5	1.0	1.0	.7	1.0	.4	1.2	1.2	1.0	.9	.8	.6	1.0	1.8	1.0	1.0	1.0										1.00	.93	
	EXP	6-2-15	1.20			1.0	.7	.7	.8	.7	.8	1.1	1.0	1.2	1.1	.8	.7	1.0	1.2	1.0	1.0	.7	.8	.8	.5	1.0						.88		
		6-3-4	1.20			1.5	1.2	1.5	.7	.9	1.1	.6	1.0	.8	.9	.9	1.4	1.0	1.1	.5	.4	.7	.5	.8	1.0	.5						.90		
7.	OPOSSUM	6-2-1	1.65	1.66	2.5	1.2	2.2	1.0	.5	1.2	1.8	1.8	1.8	1.0	1.0	1.2	.6	.9	.7	1.5	2.0											1.27	1.17	37.6
	NO 55-2	6-1-6	1.68			2.3	.8	.7	1.0	1.0	1.0	.5	1.0	.7	1.7	2.0	2.0	1.0	1.0	1.0	1.5											1.20		
	LT CONTROL	6-1-4	1.67			1.9	1.0	.9	1.1	.5	.6	1.0	1.5	.5	1.2	.5	.6	.6	.8	1.0	2.0											1.04		
	RIGHT	6-2-5	1.67	1.63		2.5	1.3	1.5	1.0	.3	.8	1.5	1.5	1.2	1.0	1.0	.8	1.7	1.8	2.5												1.40	1.60	
	EXP	6-1-9	1.60			2.0	1.0	1.5	1.5	2.0	1.8	1.1	1.0	1.5	1.3	2.0	2.5	1.5	1.8	2.0												1.63		
		6-1-4	1.62			1.0	3.0	7	1.5	1.3	2.0	1.3	2.0	1.8	2.0	2.5	2.7	1.3	.5	1.5	3.5											1.78		
8.	NO 55-1	5-1-6	1.55	1.57	7.1	2.0	7	.9	1.0	1.0	.8	1.0	2.1	1.0	7	.8	1.0	1.5	1.0	2.0												1.18	1.09	11.9
	RIGHT	5-2-2	1.58			2.0	1.0	1.0	1.0	.5	.8	1.0	.8	1.0	.5	1.0	.8	.9	1.0	.8	2.7											1.05		
	CONTROL	5-1-3	1.58			1.5	1.8	.5	1.0	1.0	1.0	.7	.8	.9	1.0	1.0	.7	.7	2.0													1.04		
	LEFT	5-2-3	1.50	1.46		2.2	.7	.8	1.0	1.0	1.0	.8	.8	.5	1.7	1.7	1.0	1.5	1.9													1.17	1.22	
		5-2-5	1.45			2.8	1.5	1.2	.5	1.0	1.2	.9	.9	1.9	1.0	.9	.9	1.5	.8	.9	2.3											1.28		
	EXP	5-2-1	1.45			1.5	.7	.9	.9	1.0	1.2	.6	2.2	1.5	2.5	1.9	1.0	1.0	2.0	.8	1.0	1.9											1.25		

TABLE 2

MICROSCOPIC MEASUREMENTS OF DIAMETER AND SIZE OF SCAPULAR NECKS AND SPICULES FROM SERIAL SECTIONS, WITH AVERAGES AND RELATIONSHIPS OF EXPERIMENTALS TO CONTROLS

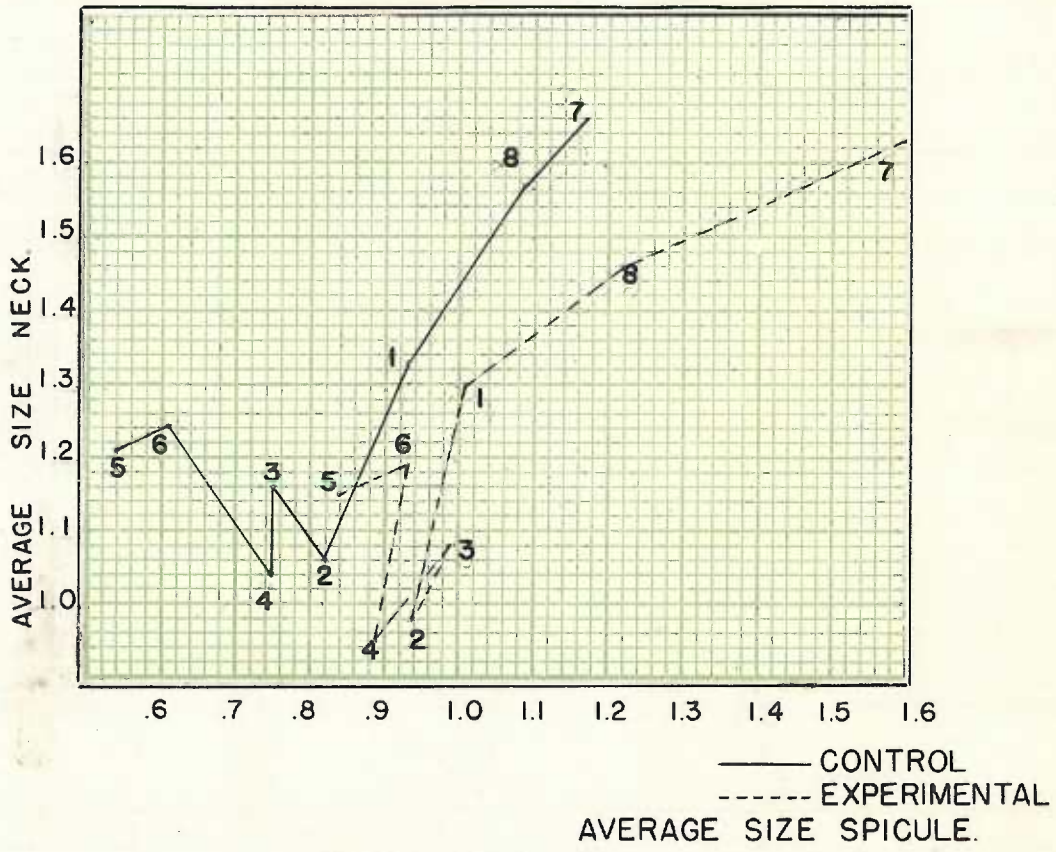


CHART NO. I.

AVERAGE SIZE NECK OF SCAPULA IN RELATION TO SIZE OF SPICULE FOR CONTROL AND EXPERIMENTAL (TABLE 2).

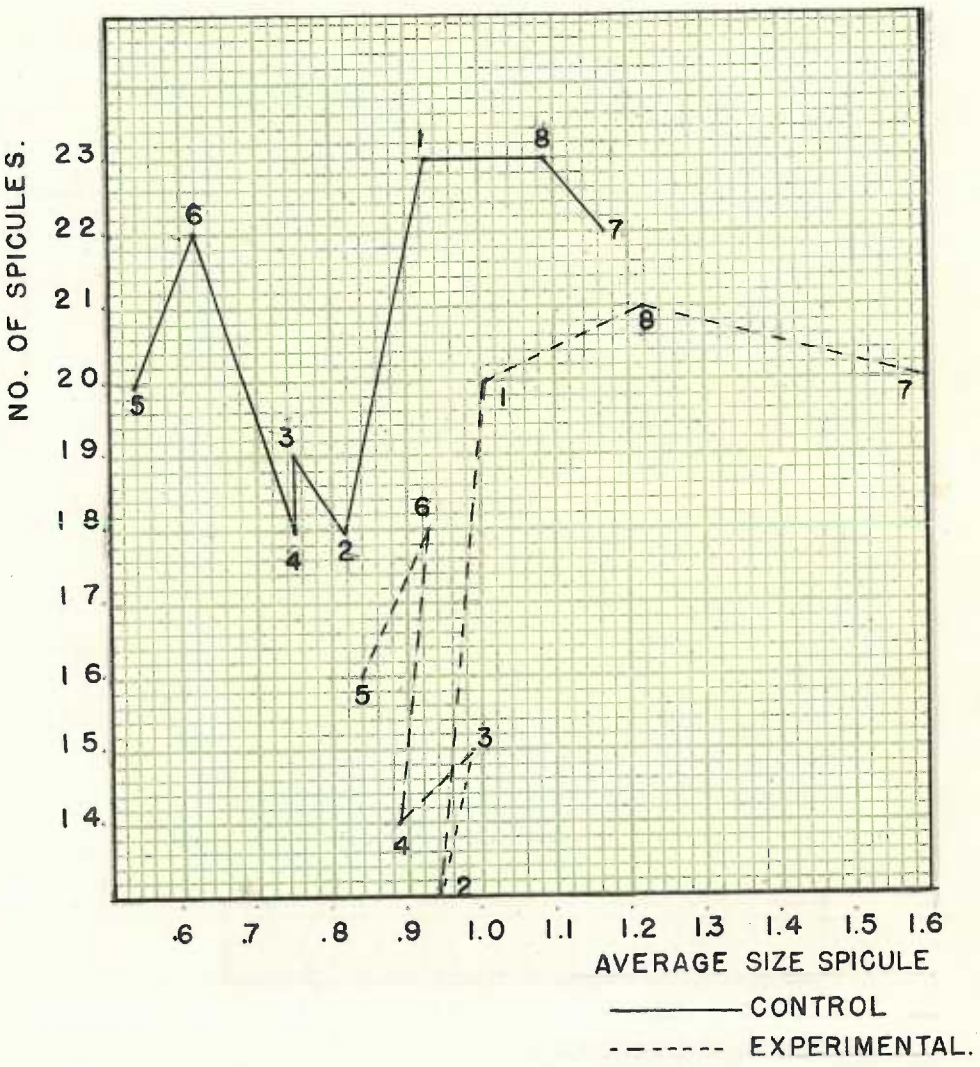


CHART NO. 2.

AVERAGE NUMBER OF SPICULES IN RELATION TO AVERAGE SIZE OF SPICULE FOR CONTROL AND EXPERIMENTAL (TABLES 1 AND 2).

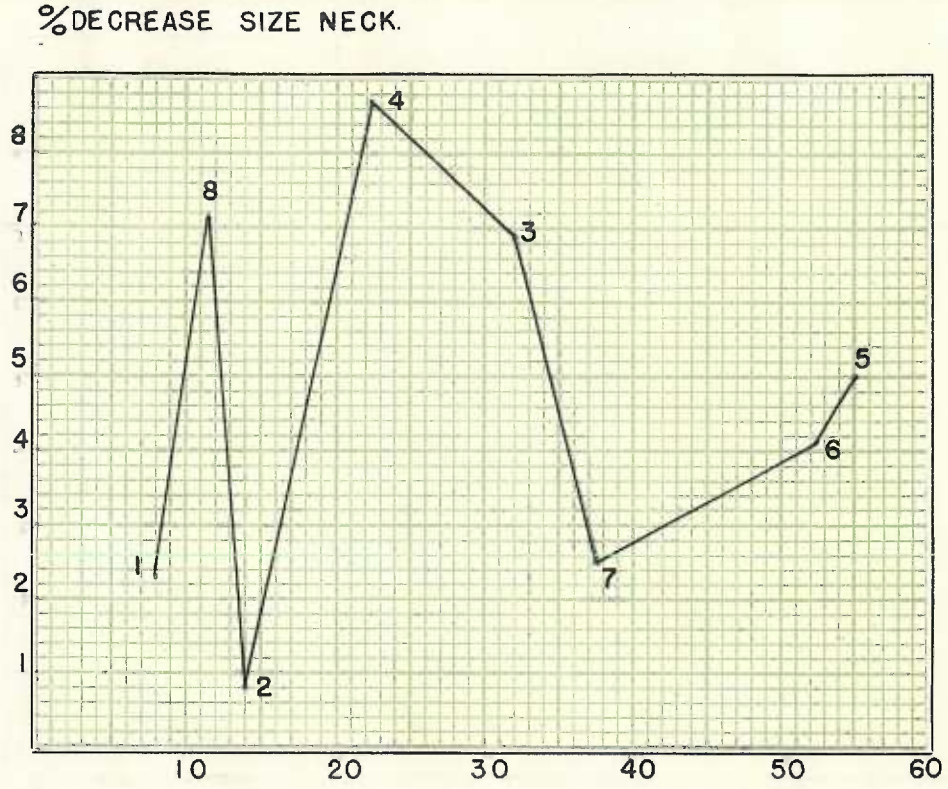


CHART NO. 4.

PERCENTAGE DECREASE IN SIZE OF SCAPULAR NECK OF EXPERIMENTAL IN RELATION TO PERCENTAGE INCREASE IN SPICULE SIZE IN EXPERIMENAL (TABLE 2).

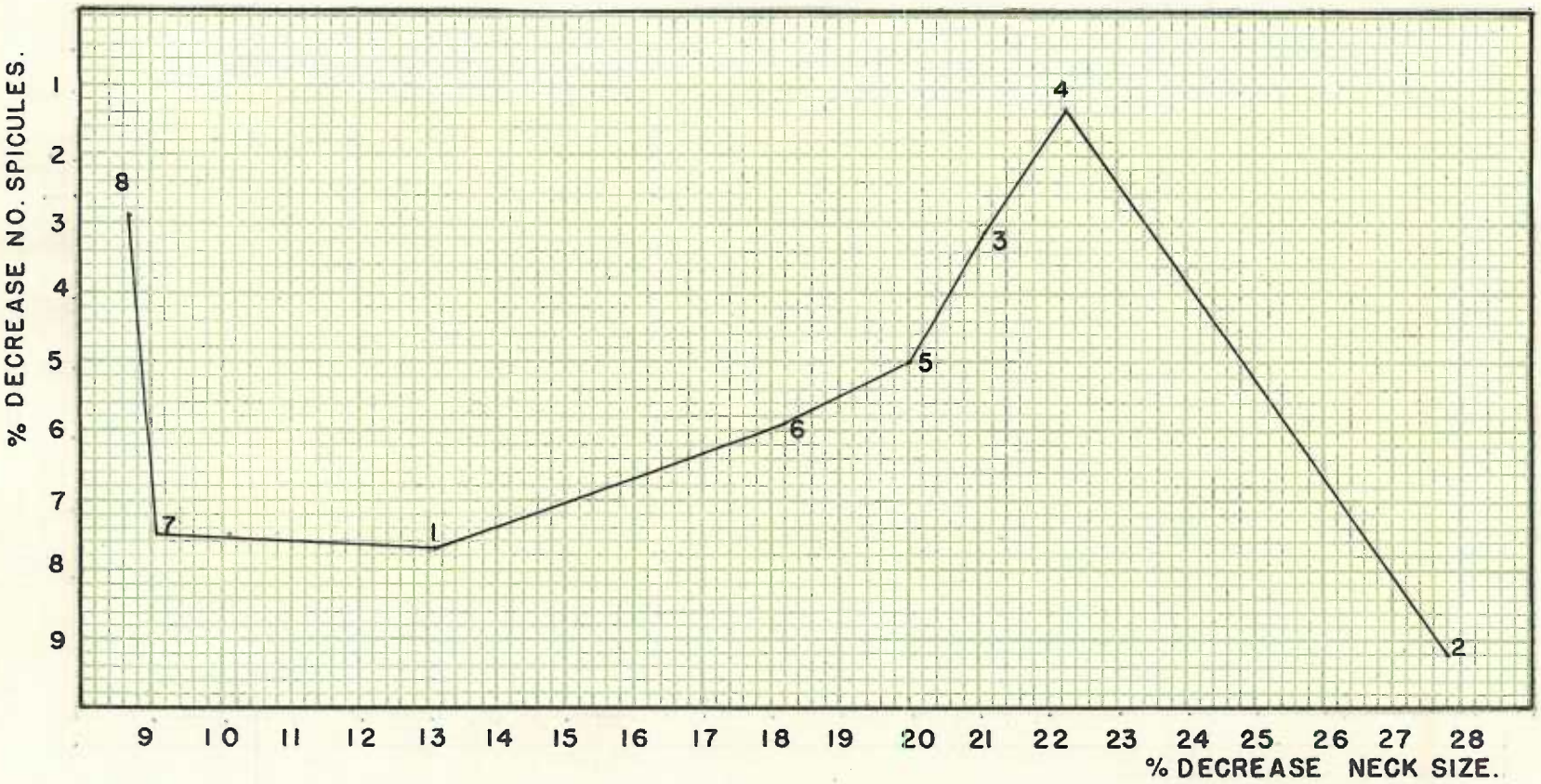


CHART NO. 5.

PERCENTAGE DECREASE IN DIAMETER OF SCAPULAR NECK OF EXPERIMENTAL IN RELATION TO PERCENTAGE DECREASE OF SPICULE NUMBER IN EXPERIMENTAL (TABLES 2 AND 1).

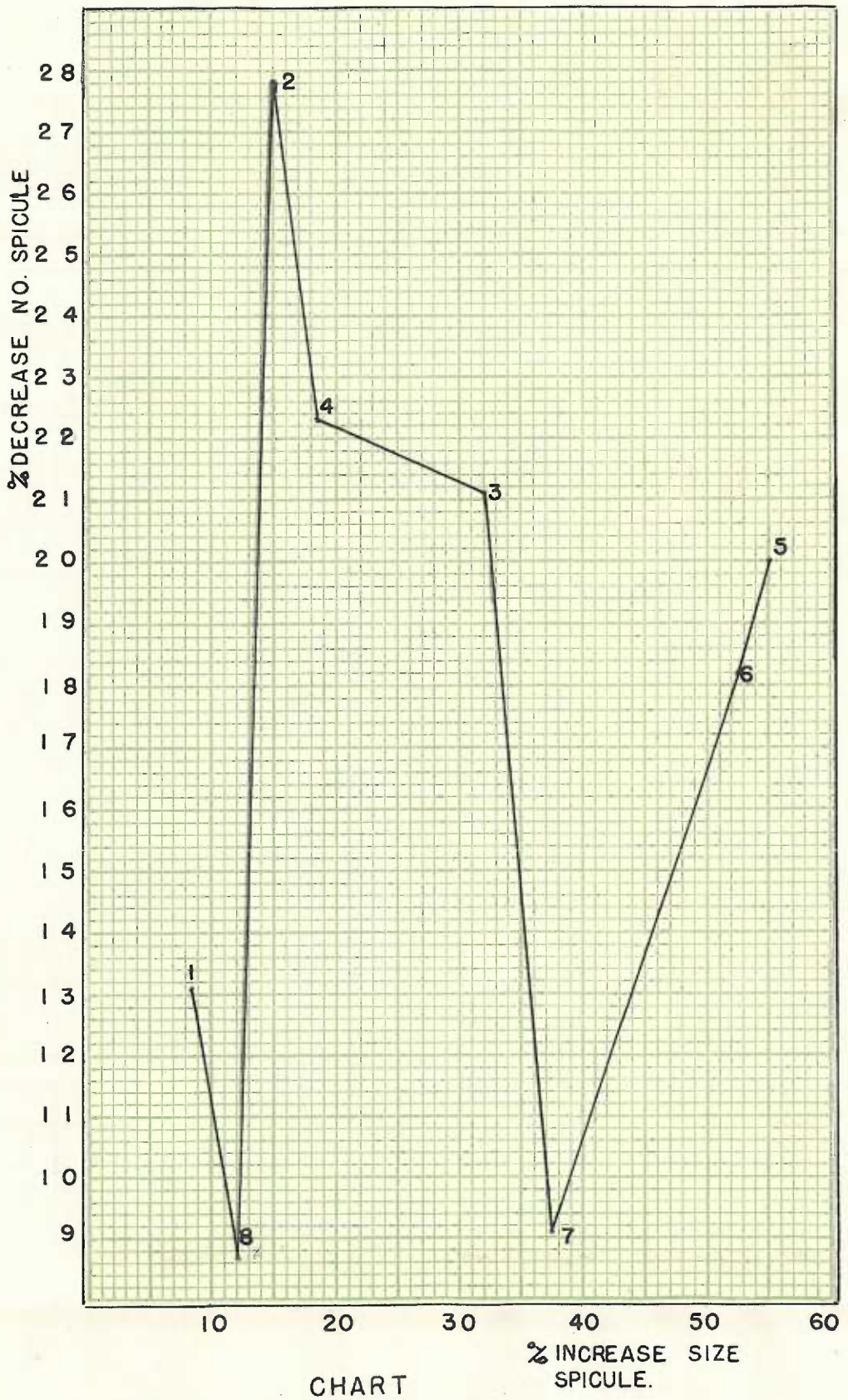
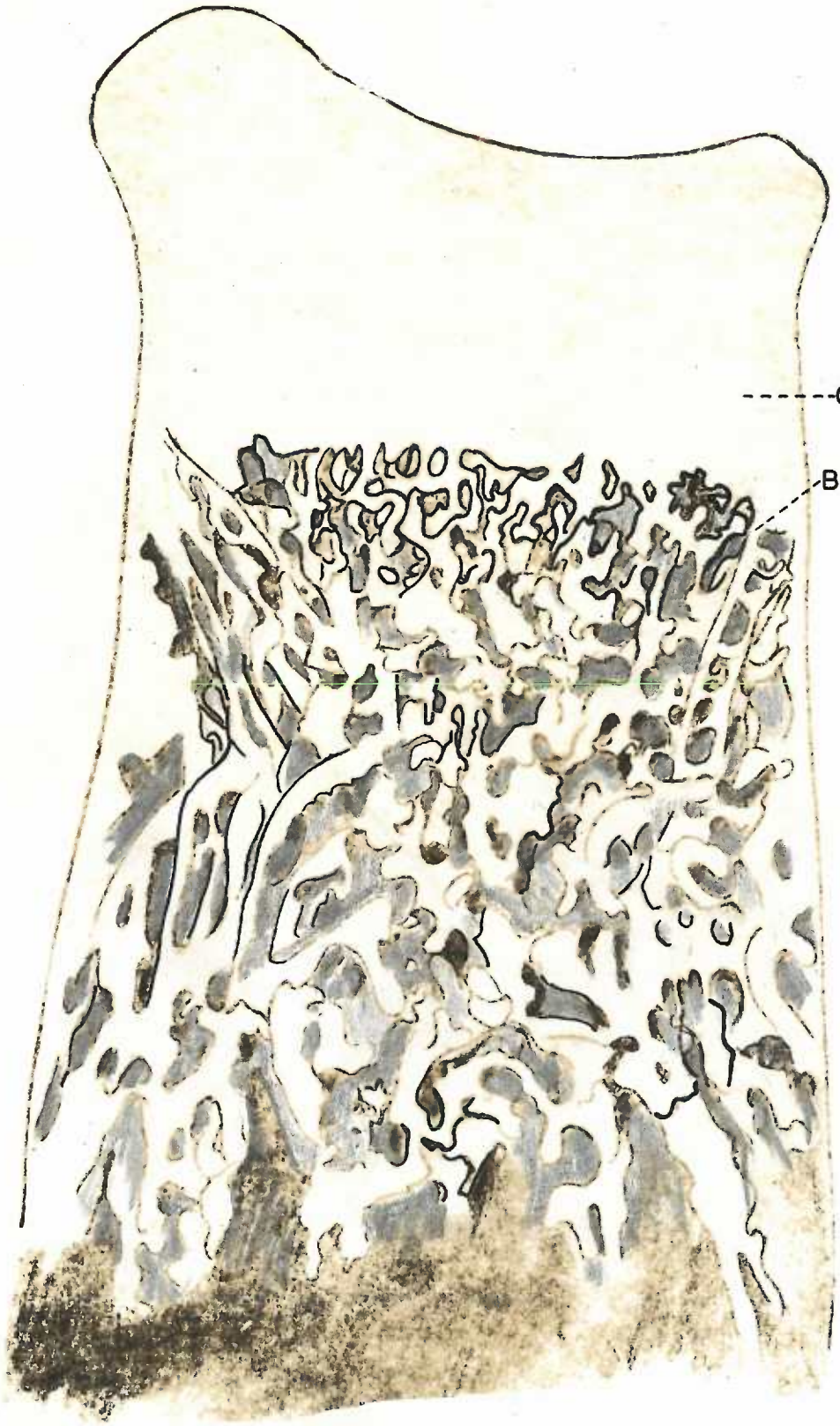


CHART NO. 6.

PERCENTAGE DECREASE OF SPICULE NUMBER IN EXPERIMENTAL IN RELATION TO INCREASE IN SPICULE SIZE OF EXPERIMENTAL (TABLES 1 AND 2).

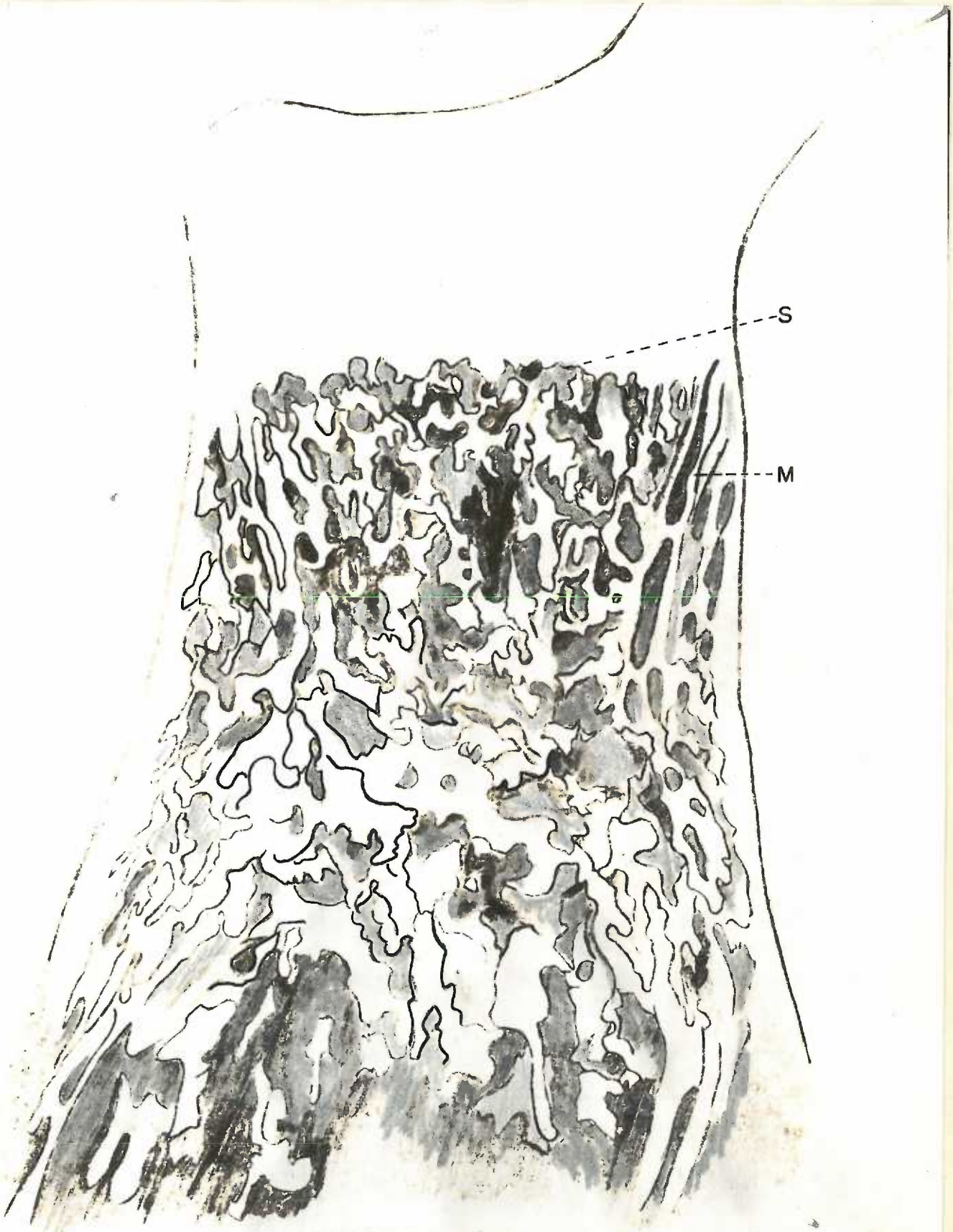
Plate 1--Microscopic reconstruction of control scapula from rat foetus BH 9246, with part of left forearm destroyed leaving humerus and head of ulna with adjacent structures at 16 days of gestation; using 24 mm. objective and 10 per ocular lenses; x 50. C,--cartilage; BS,--bony spicules.

Plate 2--Left experimental of same foetus. Same magnification. S,--few spicules; M,--large marrow spaces.



B.H. 9246
SLIDE 1 - 2
R.C.
400μ

PLATE I.



B.H. 9246
SLIDE 1-2
L.EXP.
500μ

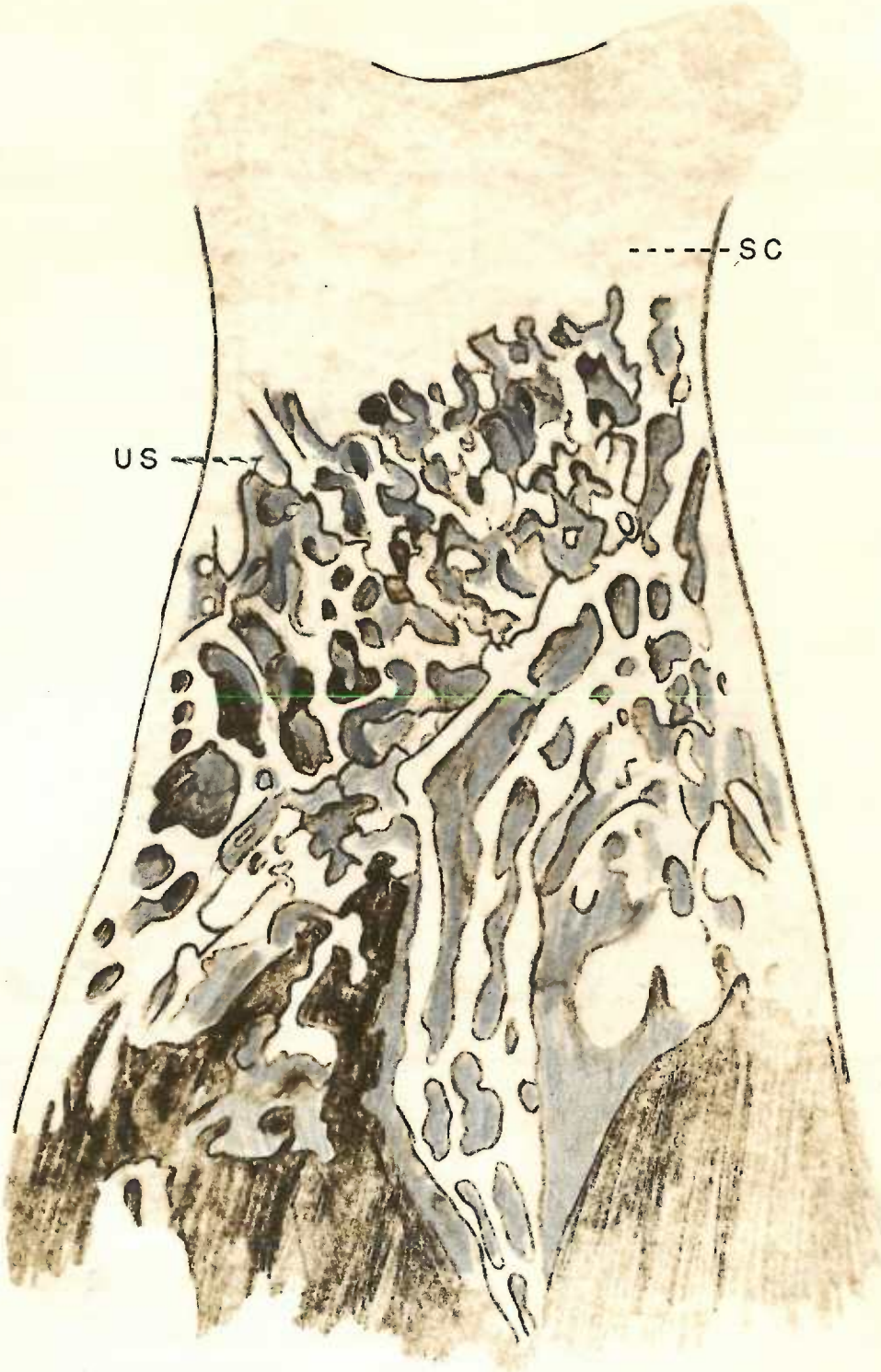
PLATE 2.

Plate 3--Microscopic reconstruction of right control scapula from rat foetus BH 9114, operated on sixteenth day of gestation to remove most of left forelimb leaving small portion of humerus; using 24 mm. objective and 10 per ocular lenses; x 50. OS, organized spicules.

Plate 4--Left experimental scapula of same foetus. Same magnification. US, unorganized spicules; SC, small cartilage zone.

B.H. 9114.
SLIDE 1 - 2
R.C.
240μ

PLATE 3



B.H. 9114.
SLIDE 1
L. EXP.
300 μ

PLATE 4.

Plate 5--Microscopic reconstruction of left normal scapula from rat foetus BH 6949 of which the forearm and approximately one half of the humerus was removed at 19 days; using 24 mm. objective and 10 per ocular lenses; x 50.

Plate 6--Right experimental scapula of same foetus with same magnification. SN, smaller neck.

B.H. 6949
SLIDE 2
LEFT NORMAL
160μ

PLATE 5

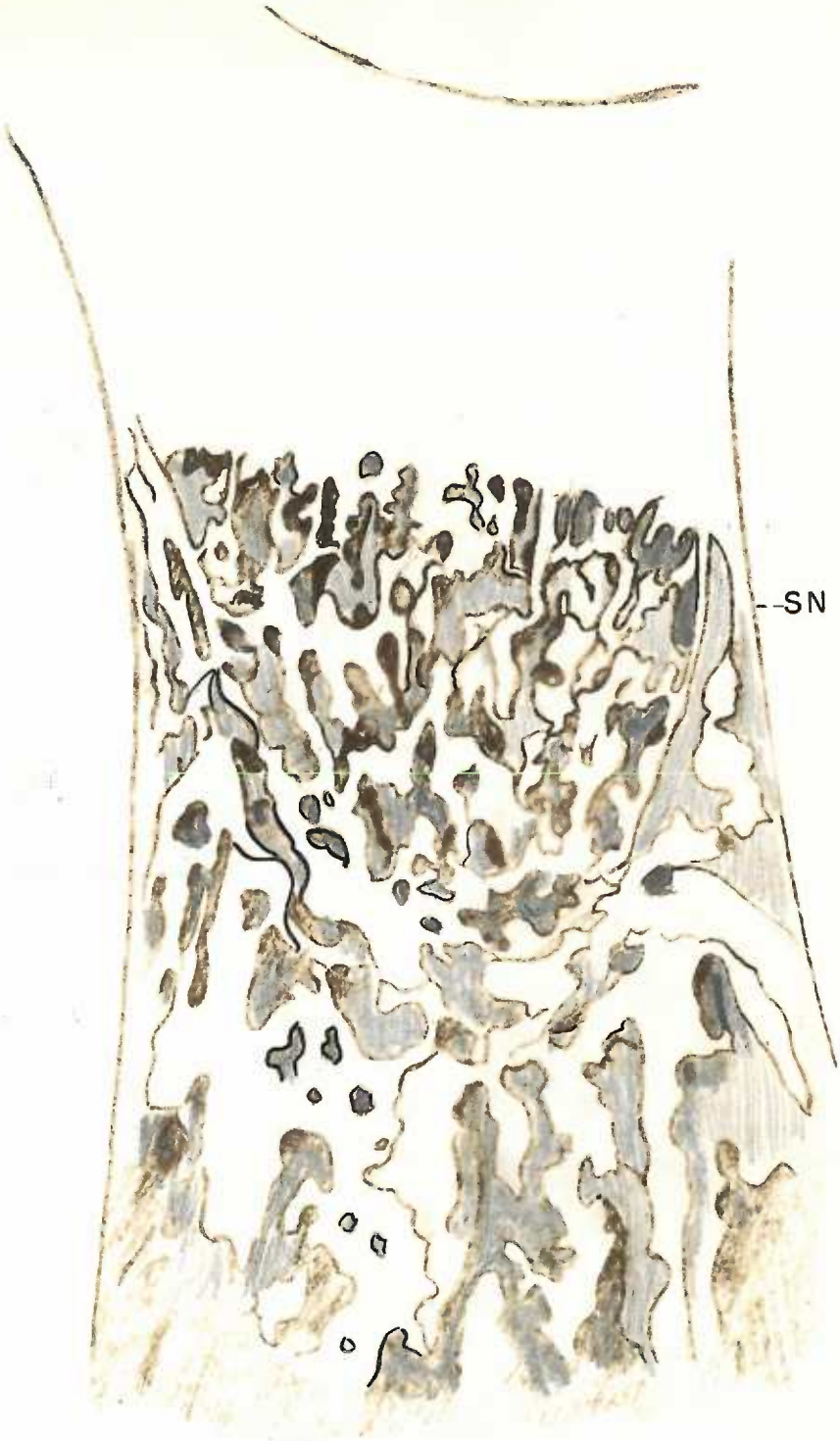


PLATE 6.

Plate 7--Microscopic reconstruction of right normal scapula from rat foetus W 2310-2 with part of the left humerus amputated on the seventeenth day of development; using 24 mm. objective and 10 per ocular lenses; x 50. DCS, direction of normal spicules.

Plate 8--Left experimental scapula of same foetus and magnification. DES, parallel direction of experimental spicules.

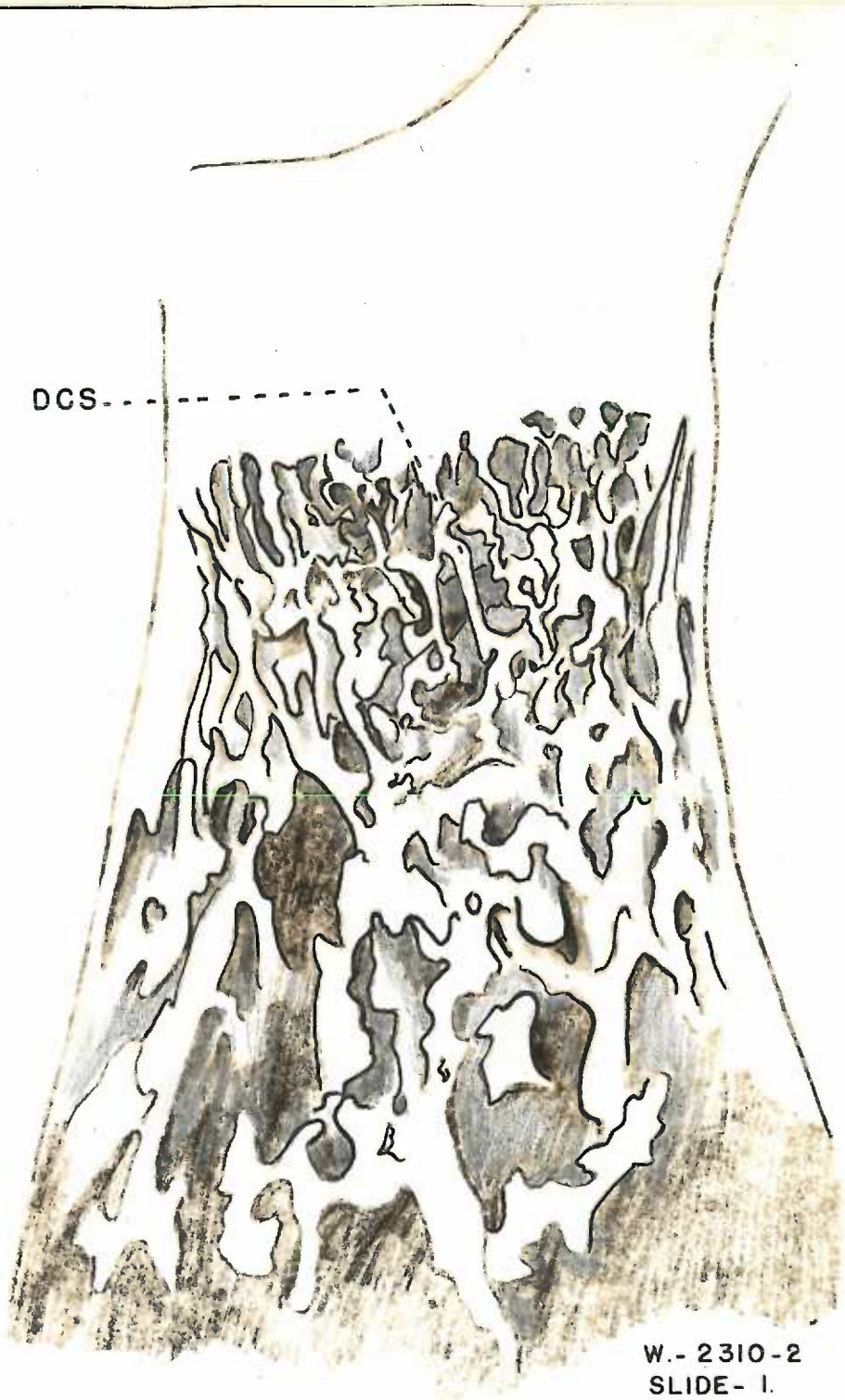


PLATE 7.

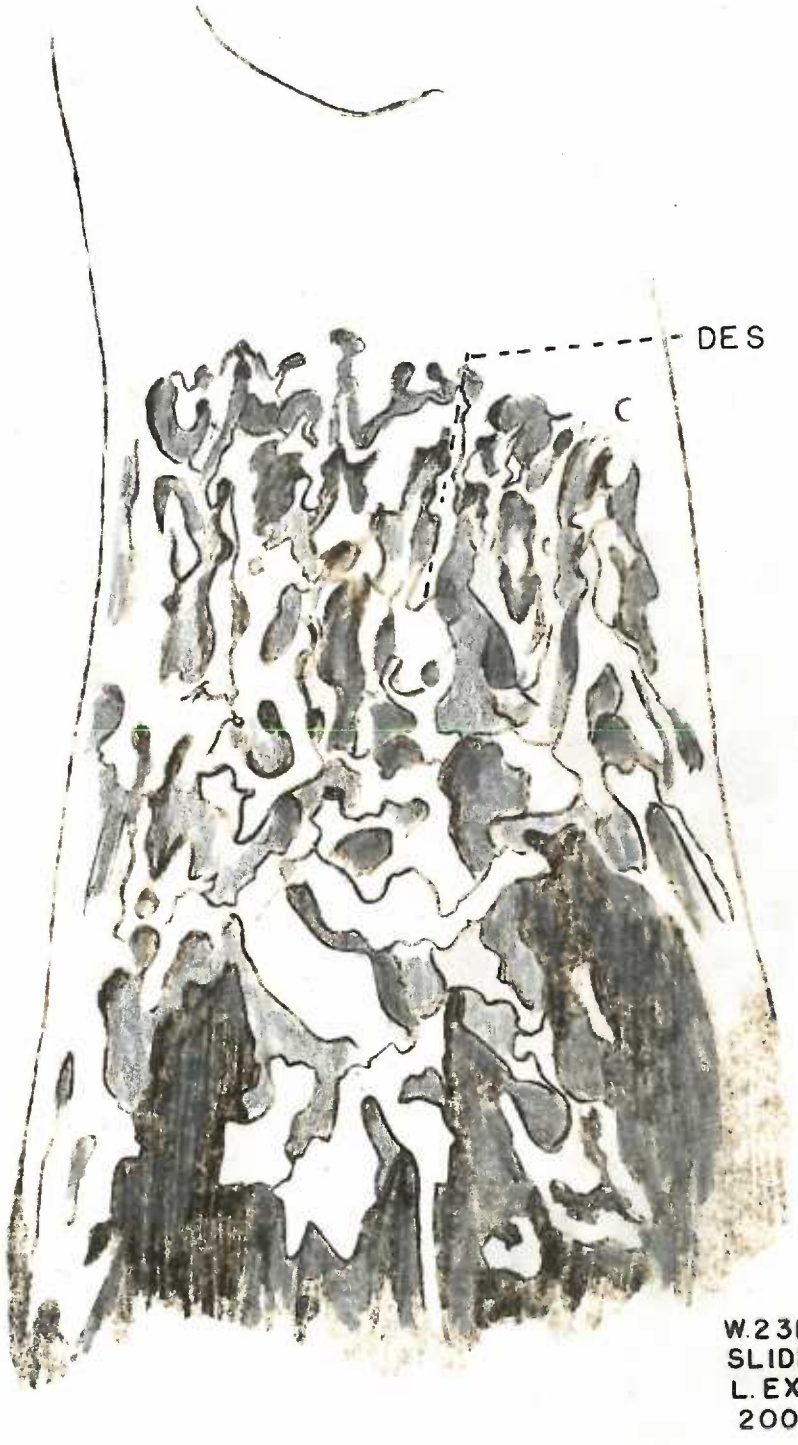


PLATE 8.

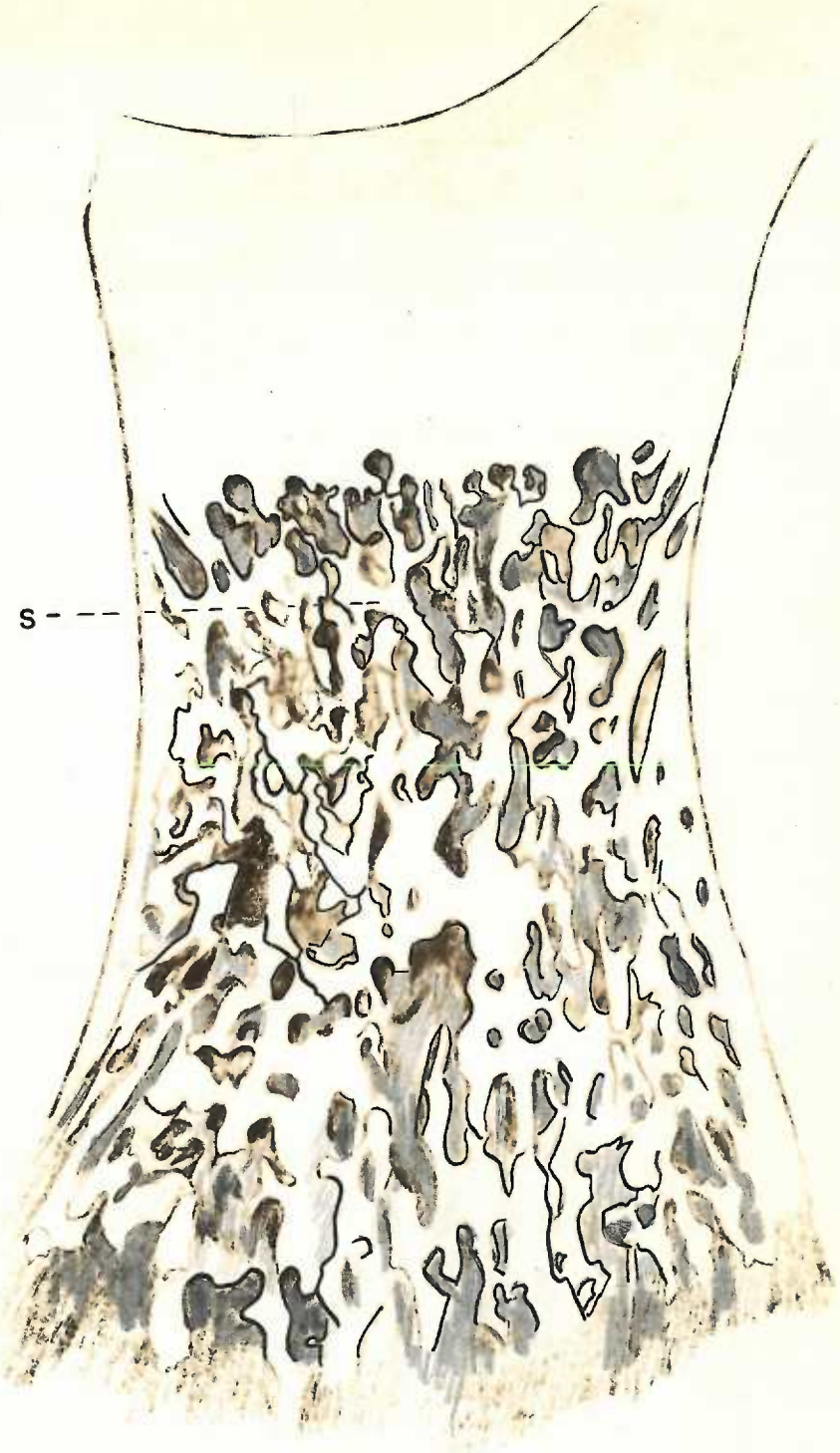
Plate 9--Microscopic reconstruction of normal scapula from foetus W 2310-1 in which part of the humerus was removed. The sections were cut transversely and were reconstructed from four different levels of the neck using 24 mm. objective and 10 per ocular lenses; x 50. MS, many spicules.

Plate 10--Right experimental from foetus W 2310-1 at same magnification. Sections cut longitudinally. S, few spicules.

SLIDE 5

SLIDE 4

PLATE 9.



W-2310-1
SLIDE
R.EXP.
200μ

PLATE 10

Plate 11--Microscopic reconstructions of right control scapula sectioned transversely from Foetus BH 6290 in which most of the left forelimb was removed at 16 days; using 24 mm. objective and 10 per ocular lenses; x 50. BS, many spicules.

Plate 12--Experimental left scapula of same foetus sectioned transversely and reconstructed at approximately same levels as those of plate 11. Same magnification. M, large marrow spaces.

SLIDE 3
100μ

SLIDE 4
100μ

SLIDE 4
100μ

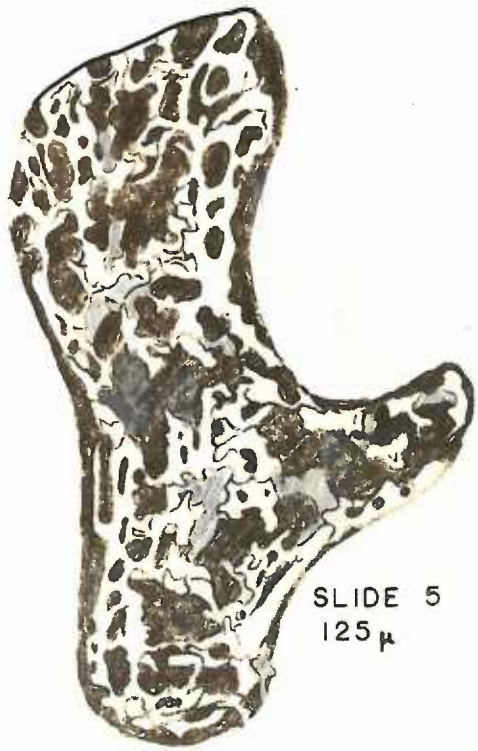
SLIDE 4
150μ

B.H. 6290
C.R.

PLATE II.

SLIDE - 6.
125μ

SLIDE 6
125 μ



SLIDE 5
125 μ

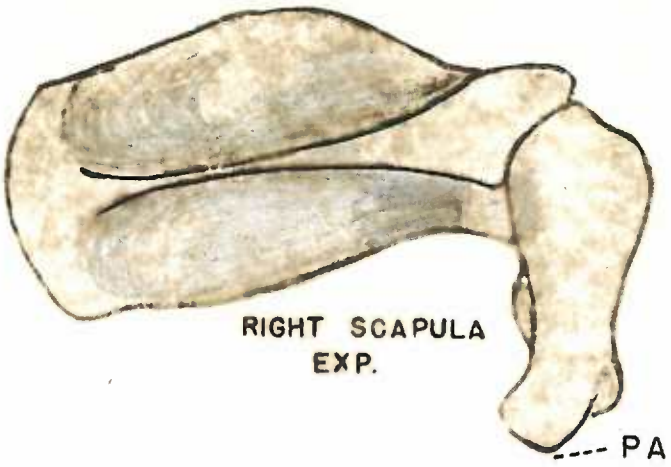
B.H. 6290
L. EXP.

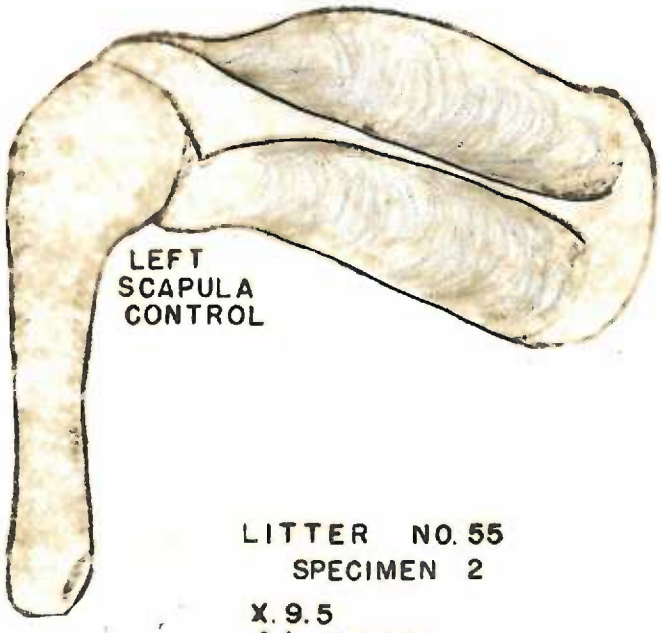
PLATE 12

Plate 13--Gross enlargement of scapulae from opossum foetus #55-2 with the right forelimb amputated leaving a portion of the humerus 8 days after birth and killed at 31 days; using 64 mm. objective and 6 per ocular lenses; x 55. PA, point of amputation.

Plates 14 and 15--Microscopic reconstructions of left control scapula from same foetus using 24 mm. objective and 10 per ocular lenses; x 50. DCS, direction of control spicules.

Plates 16 and 17--Reconstructions of right experimental at same magnification as control. DES, parallel direction of experimental spicules.





LITTER NO. 55
SPECIMEN 2

X. 9.5
64. MM. OBJ.
6. PER. OC.

61.5" ABOVE TABLE.

PLATE 13

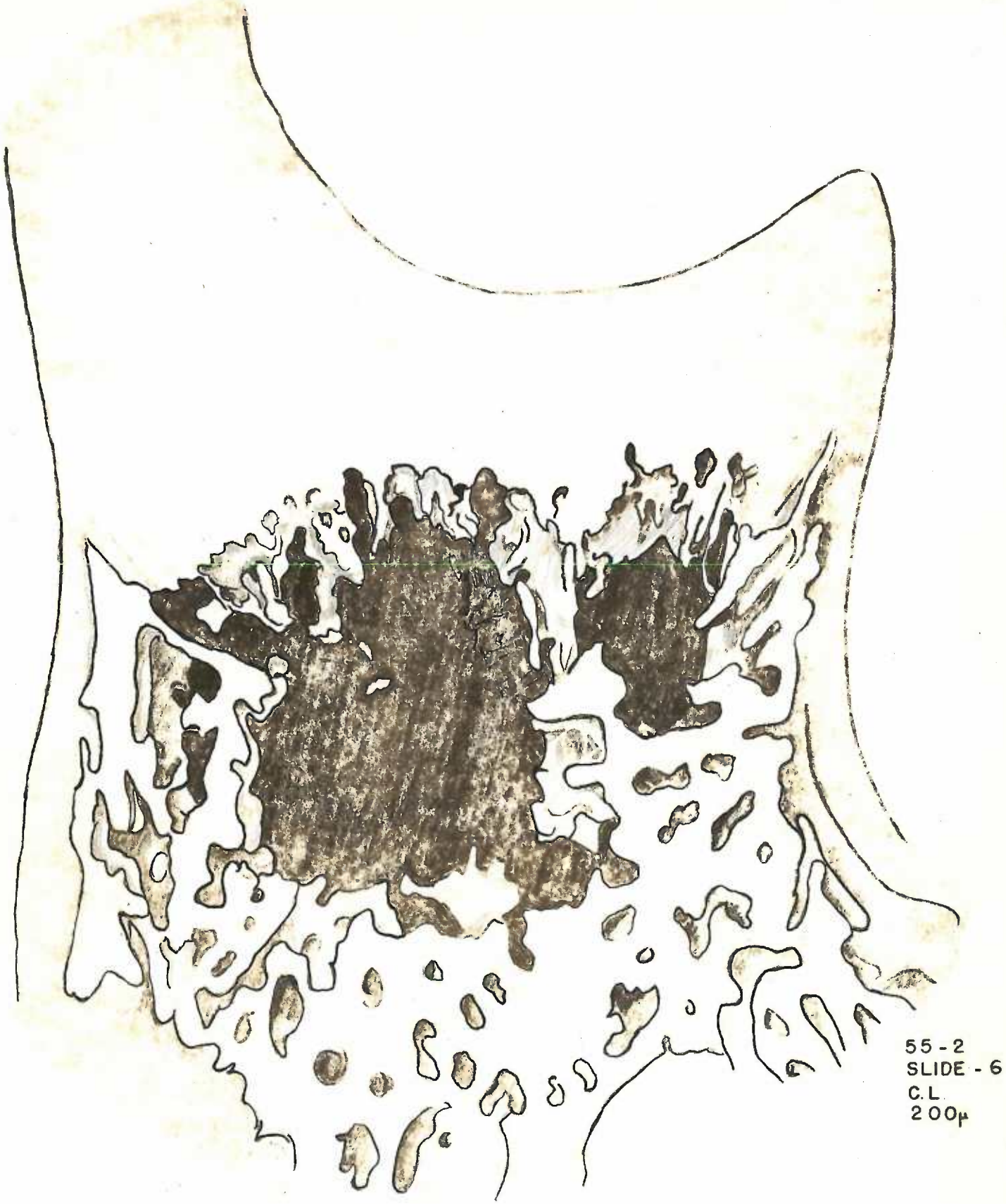


PLATE 14.

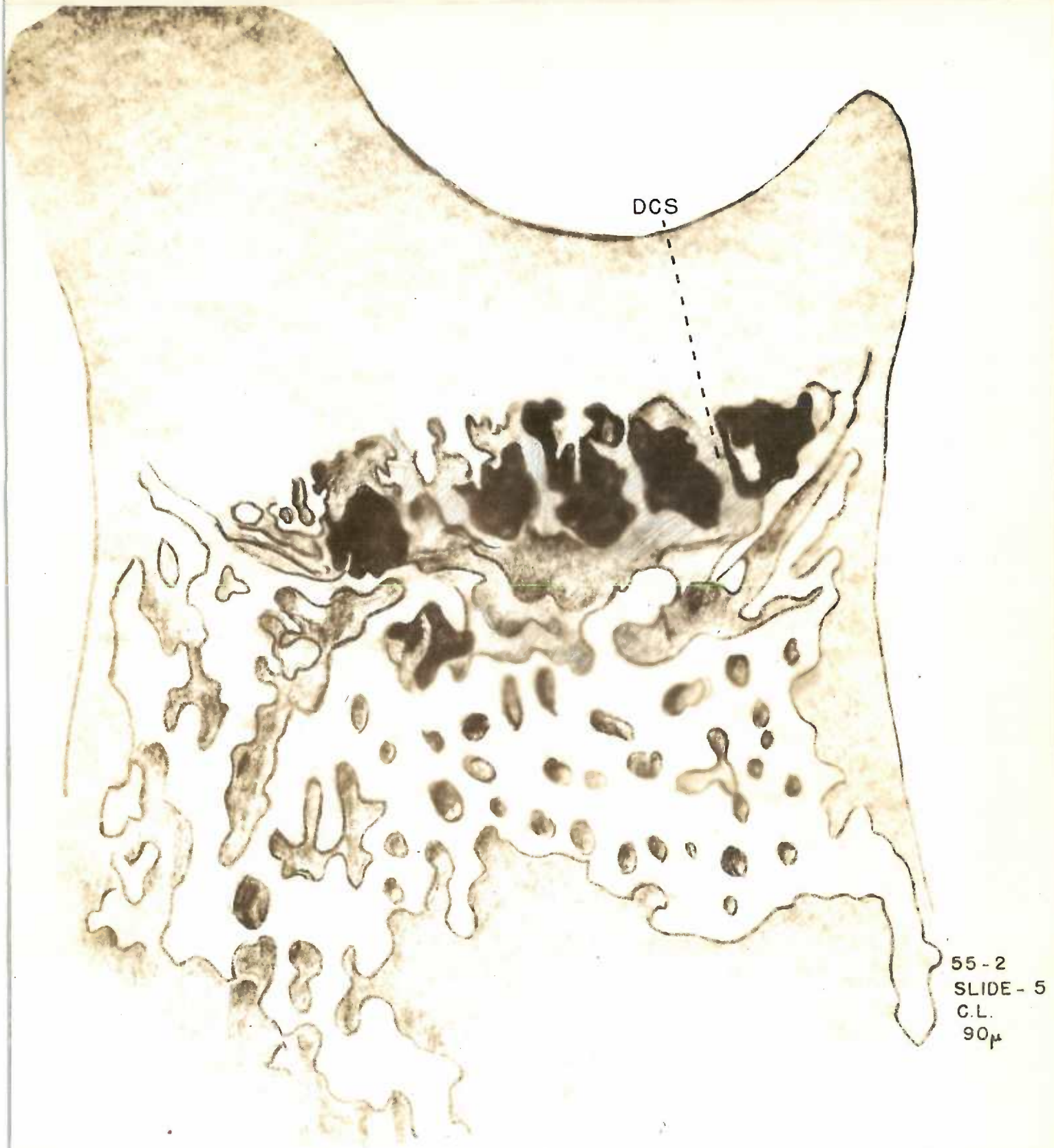


PLATE 15.

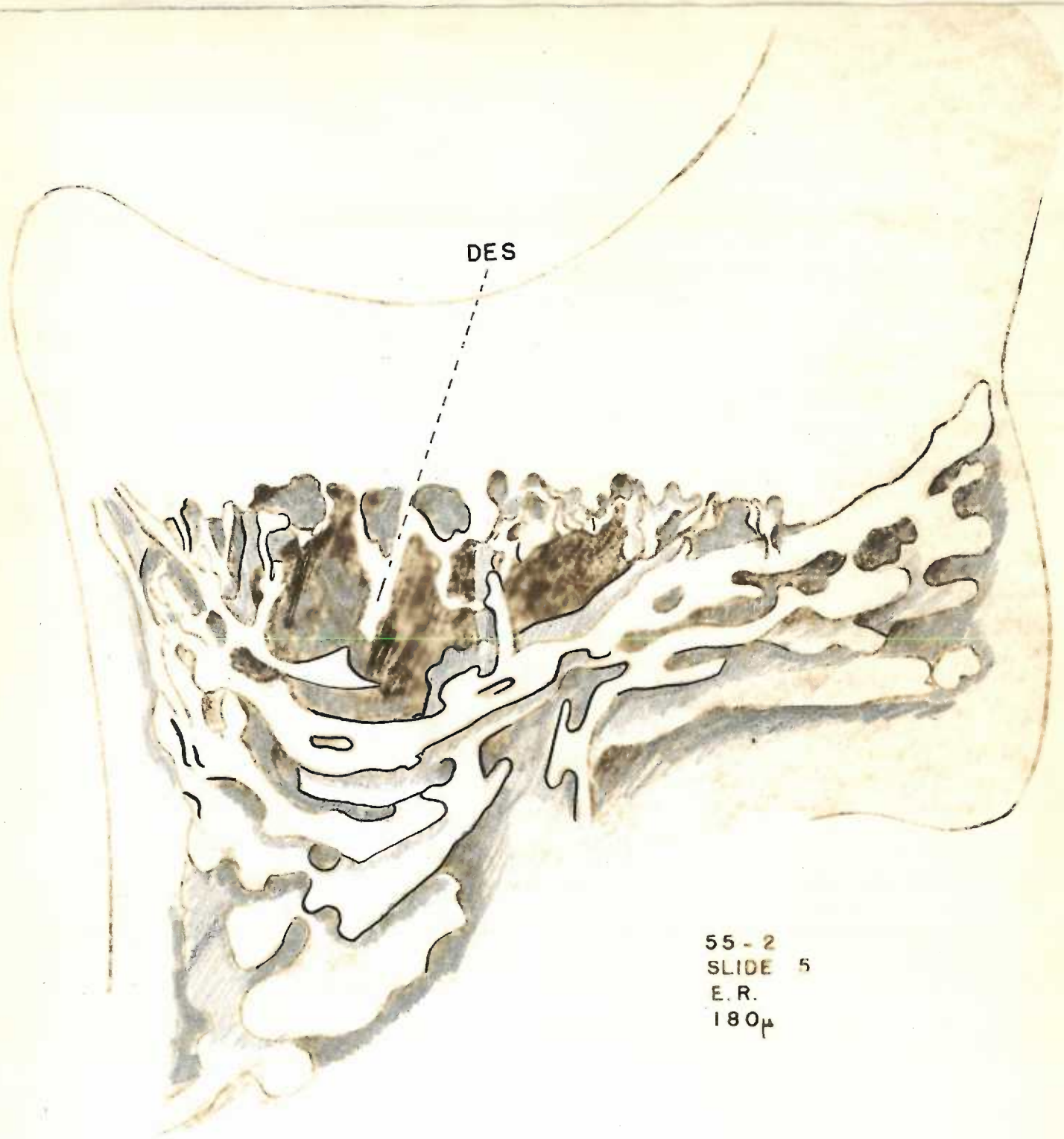
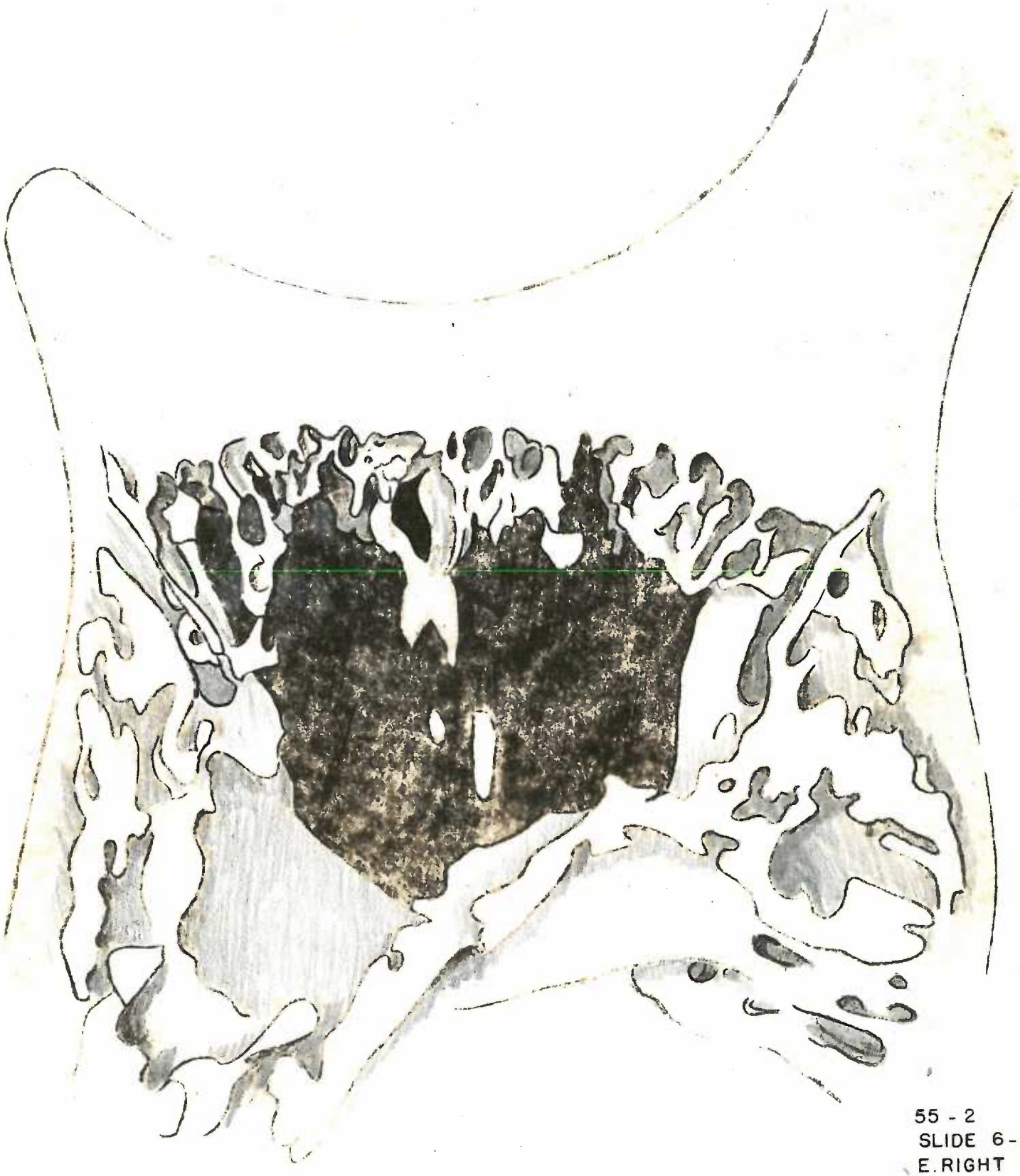


PLATE 16.



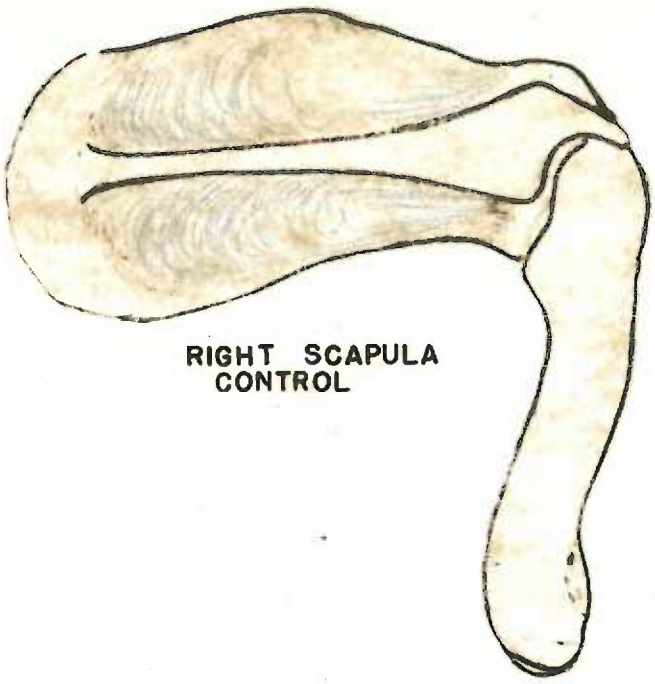
55 - 2
SLIDE 6 - 7
E. RIGHT
270μ

PLATE 17.

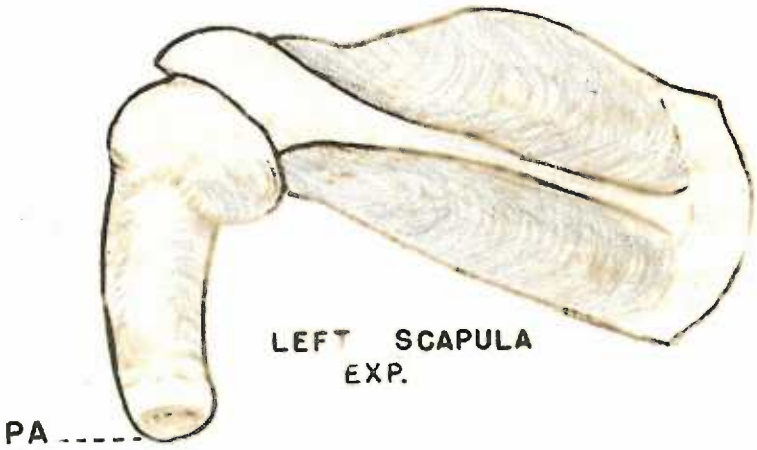
Plate 18--Cross outline of opossum scapulae from foetus #56-1 with amputation of part of the humerus 8 days after birth and killed at 31 days; using 64 mm. objective and 6 per ocular lenses; x 9.5. PA, point of amputation.

Plates 19 and 20--Microscopic reconstructions of right normal scapula of same foetus using 24 mm. objective and 10 per ocular lenses; x 50. SS, smaller spicules.

Plates 21 and 22--Reconstructions of left experimental at same magnification as control. M, large marrow spaces.



RIGHT SCAPULA
CONTROL



LEFT SCAPULA
EXP.

PA -----

LITTER NO. 55 SPECIMEN 1.

X. 9.5

64. M.M. OBJ.

6. PER. OC.

61.5" ABOVE TABLE.

PLATE 18.

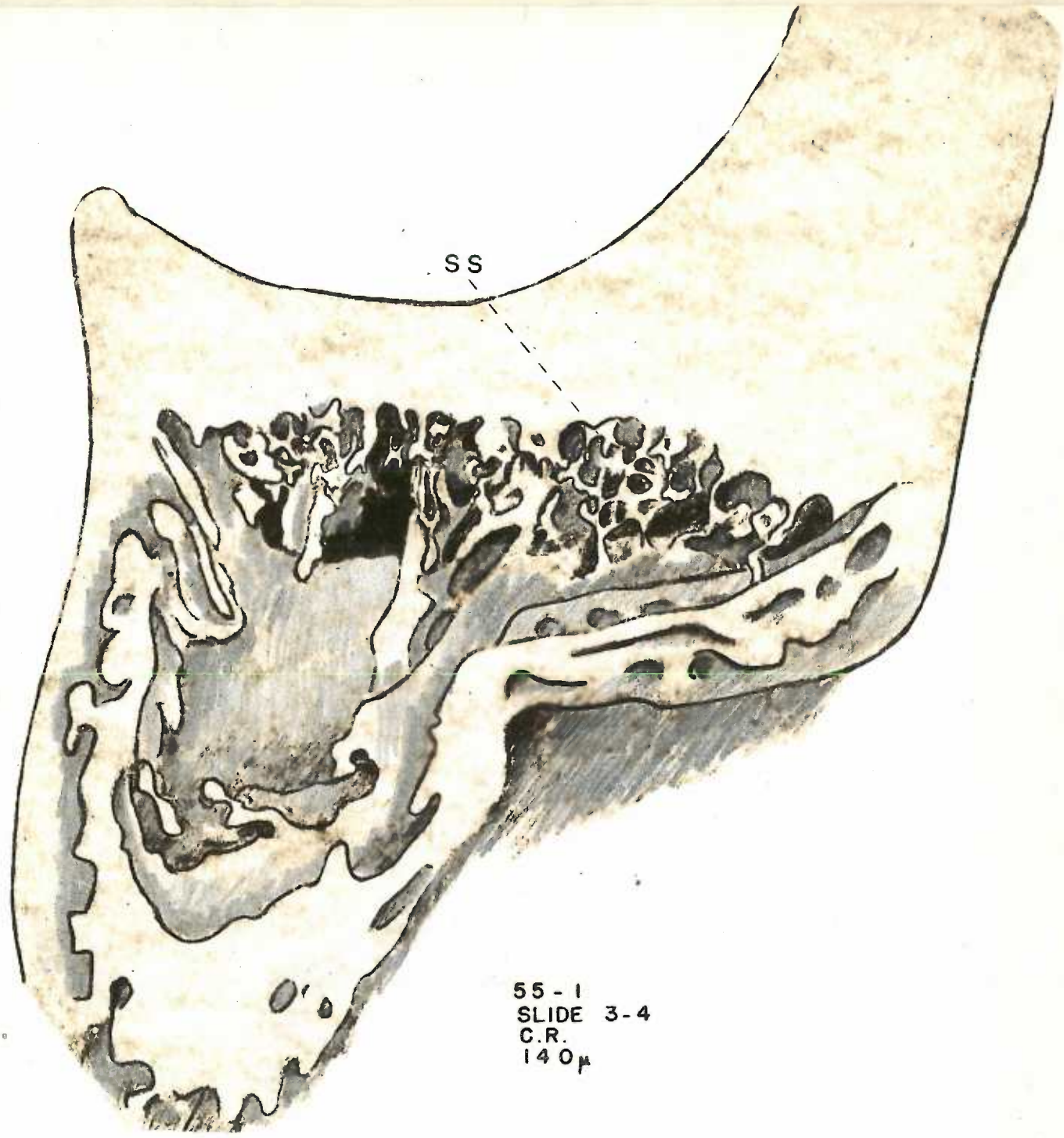


PLATE 19.

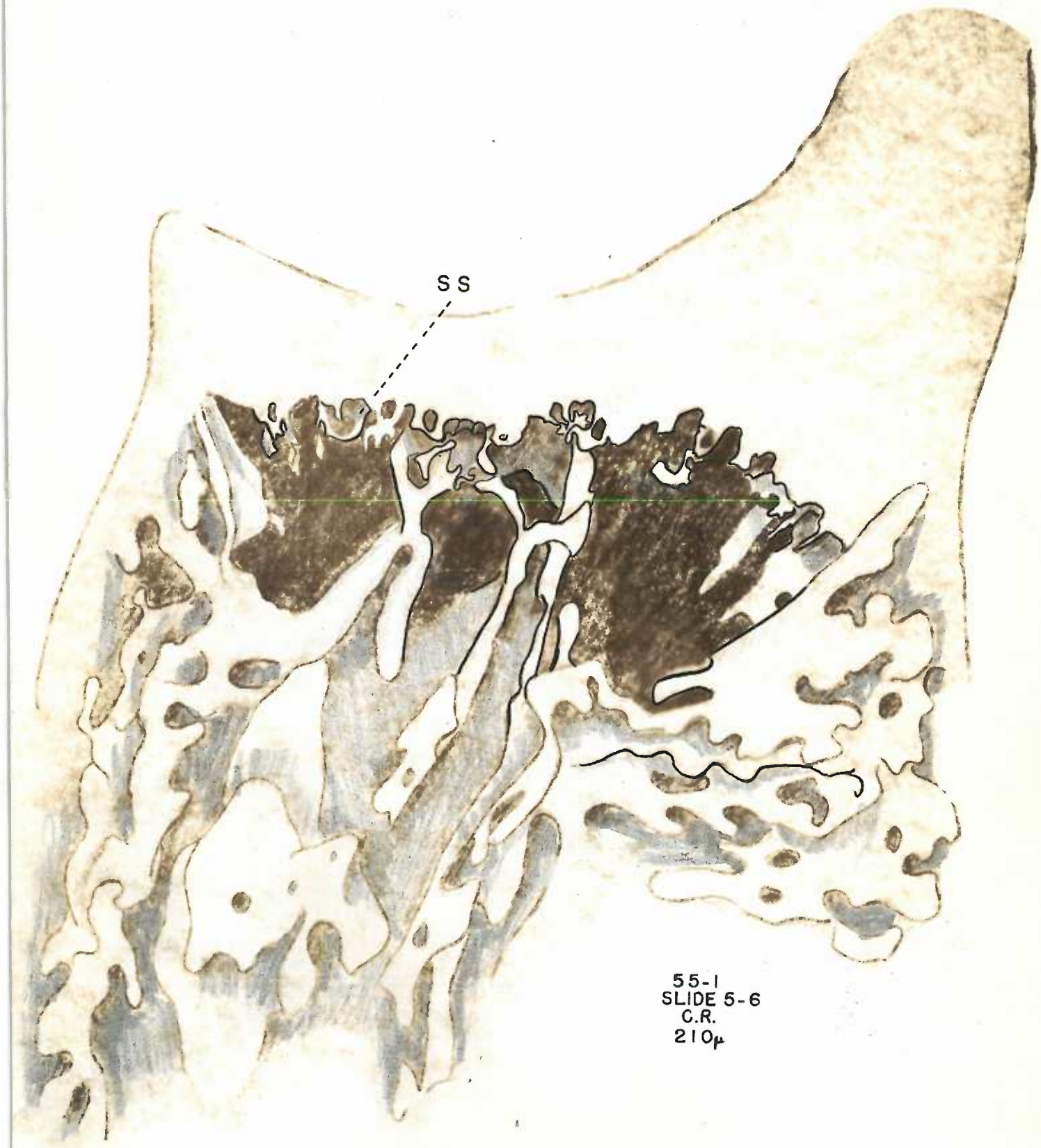


PLATE 20

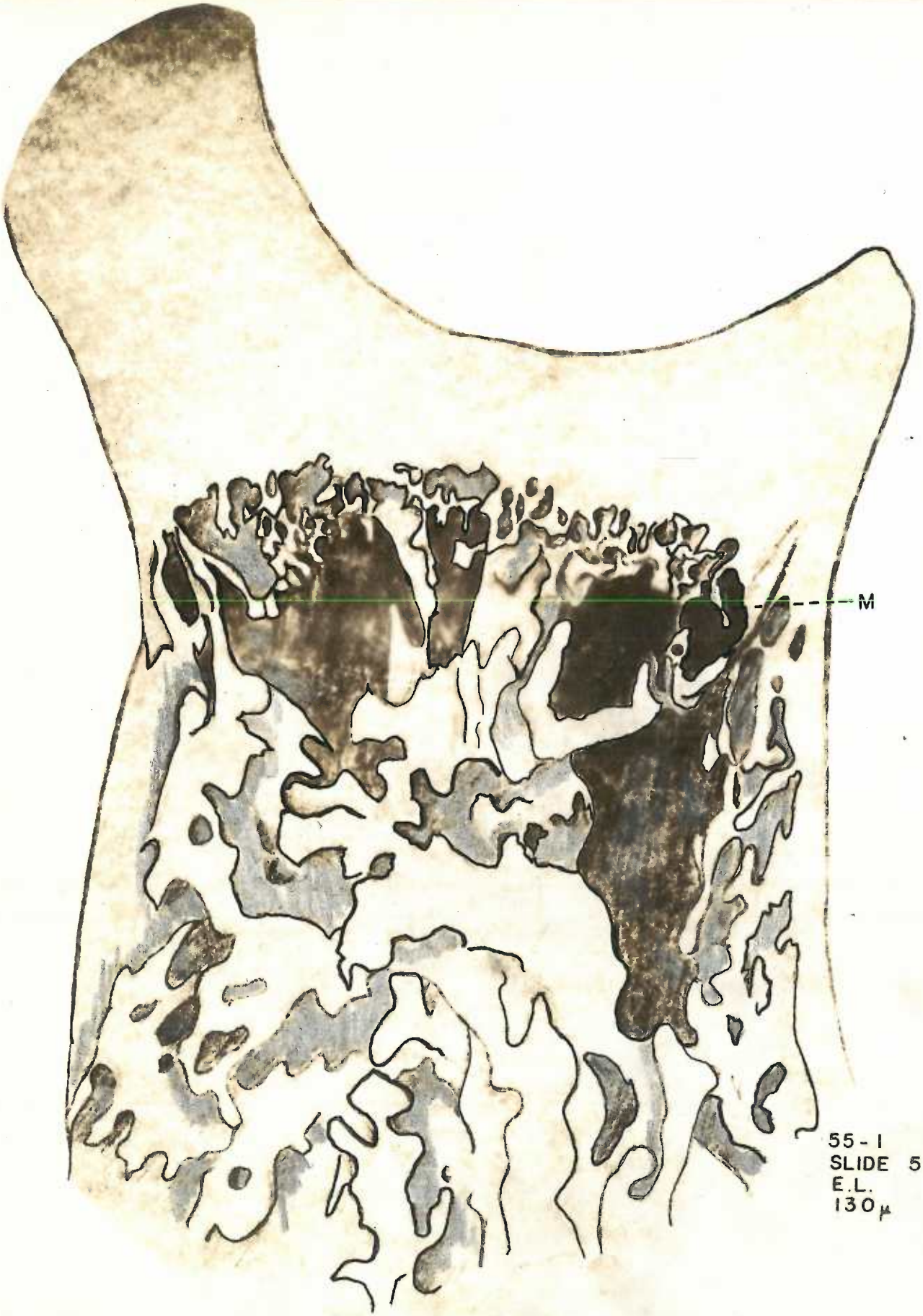


PLATE 21.

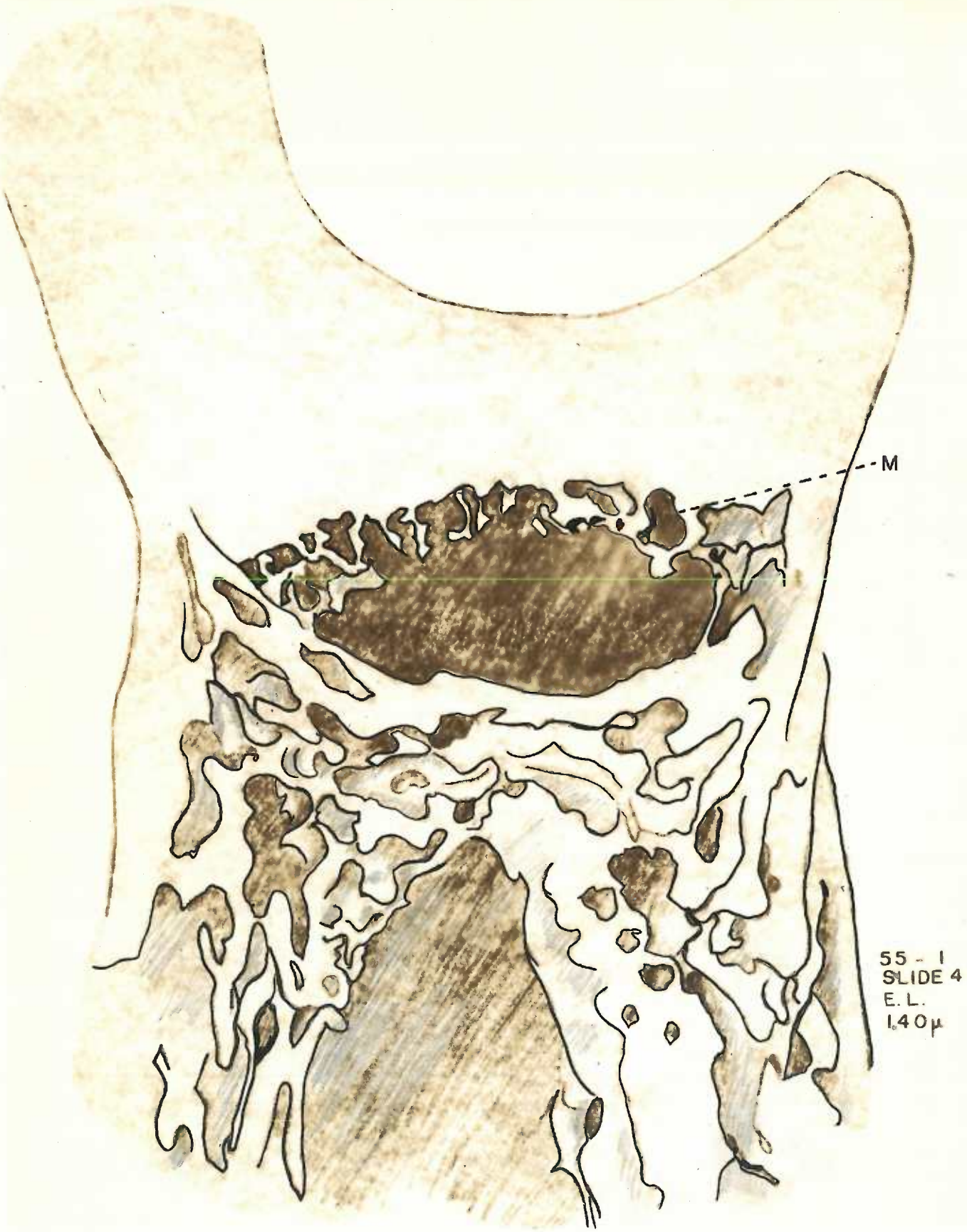


PLATE 22.

Typed by Marjory Marvin