IMMOBILIZATION/REMOBILIZATION AND THE REGULATION OF MUSCLE MASS (NAG 2-211)

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A. INTRODUCTORY REMARKS

1. Administrative: This proposal was funded as of November 1st, 1982. However, because of a variety of factors unclear to the principal investigator, funds were not released until November 28th, 1982. In addition, because of local affirmative action procedures, it is virtually impossible to hire personnel in less than 6 weeks. These procedures include prior committee approval of the approach to the search; a designated open posting period; and subsequent committee approval of the procedures used in the personnel search. The original budget provided a salary for one relatively senior technician. However, because of the backed-up salary money and the highly organized nature of the work the first year, two laboratory aides were hired. These laboratory aides work under very close supervision of the PI and a Postdoctoral Fellow who is funded by the ALS Society, Dr. D. C. DuBois. At present, because of lack of funds, the two laboratory aide positions will terminate on September 1st, 1983. At that time we will begin to search for a technician to participate in the work planned during year two of the proposal.

2. Experimental Approach: This is a new project, the objective of which is to study the role of glucocorticoids in disuse atrophy of skeletal muscle. This project uses the extensor digitorum longus (EDL) and soleus muscles from small animals. Small animals are required because of the organ culture work proposed in subsequent years. Our first goal was to define the relationship between animal body weight and the wet and dry weights of the soleus and EDL muscles. Next, we re-examined our procedures for tissue homogenization, fractionation, protein determination and DNA determination. Initially we had proposed to carry out each of the several different aspects of protein determinations and DNA determination on separate series of muscles. Our goal in this reexamination was to develop a sequence of procedures and buffers which would allow us to carry out all analyses on one small muscle. This would yield a considerable increase in analytical strength associated with paired statistics.

   Finally, we extensively re-examined the proposed casting procedure which was to be used for immobilization. Initially we proposed to use the plastic-embedded plaster procedure developed at Montreal by Philip Gardner. This procedure was developed for adult animals (~250 grams) and therefore required adaption to small animal (~50 grams).

B. SUMMARY OF RESULTS

This section contains a very brief summary of our results. In the next section we will provide the data to support these statements:

1. We have clearly delineated the change in the size of both the soleus and extensor digitorum longus muscles as a function of animal weight within the range of 50 grams to 120 grams. This includes a complete analysis of muscle water content as a function of muscle size (ratio of dry weight/wet weight).

2. We have compared the diphenylamine colorimetric procedure (Burton) for DNA determination with a variety of fluorescent assays which have greater sensitivity. Based on this analysis we have adapted an approach for DNA determination in muscle using the Hoescht fluorescent procedure.

3. We have modified our disruption, fractionation, and analytical procedures so that we can obtain from a single muscle DNA content as well as the following protein determinations: 1) total; 2) total non-collagen; 3) collagen; 4) soluble; 5) total myofibrillar; 6) myofibrillar non-collagen; 7) collagen in myofibrillar fraction. All of these are measured protein values with the exception of the collagen contents, which are derived values. Together these values provide an overall picture of the protein distribution of a muscle. In addition, together they allow us to check validity by determining if the total is equal to the sum of the parts. Furthermore, because the DNA is determined in the same muscle, we can use paired statistics to analyse the relationship between DNA and the various protein fractions.

4. We have rejected the Gardner plastic-impregnated plaster approach to casting. Although the procedure was practicable, the casts are too heavy. We felt that the weight of the cast would invalidate contralateral controls.

5. We have developed a new casting procedure which uses a combined plastic medium and porous-hypoallergenic surgical tape. The result is a cast which is stronger than the plastic-impregnated plaster and approximately one tenth the weight. In addition, using the new approach we have developed several different cast forms which can be put in place quite rapidly. This will allow us to develop the type of large data bases never before possible with non-invasive casting methods.

6. We have analysed rather extensively one cast form which slightly stretches the muscles in the front of the leg (e.g. EDL) and shortens the muscles in the back of the leg (e.g. soleus). The result is an extensive decrease in the size of the soleus and a less extensive decrease in the size of the EDL.

7. We have carried out some remobilization experiments from the cast described in 6. above.

8. We have begun analysis on a second cast form which strongly stretches the muscles in the back of the leg (soleus) and shortens the muscles in the front of the leg (extensor digitorum longus). The result is a significantly smaller decrease in the atrophy of the soleus and a significantly increased atrophy of the EDL.

9. We have begun development of a third cast form which greatly stretches the muscles in the front of the leg (EDL) and greatly shortens the muscles in the back of the leg (soleus).

C. METHODS AND RESULTS

The data presented in this section is organized with numberation corresponding to the organization of the previous section, B. Summary of Results.

1. Specific Aim 4 of this project requires that we use small intact muscles which are amenable to organ culture. Therefore, it was necessary for us to construct this project around small growing rats. We obtain male Sprague-Dawley rats as weanlings from a local supplier (Blue Spruce Farm). The animals arrive at approximately 50 grams body weight. Figure 1 illustrates the change in animal body weight as a function of days after arrival. Figure 2 expresses these same data as the fractional increase in weight (percent original body weight) as a function of time. The data show that the animals double their weight in 11 days.

Initially, we proposed 4 weeks of immobilization and 4 weeks of remobilization. Since we are restricted for the purposes of organ culture to muscles from animals under one hundred grams, these data indicate that we must shorten the entire experimental period to 10 days. Data presented subsequently on casting show that 3-6 days of immobilization and 3-6 days of remobilization is clearly practicable.

Given the data on the body growth, we next examined the growth of both the soleus and EDL muscles. Those data are presented in Figures 3 and 4 respectively. These data establish a base line against which to compare the results with experimental animals.

There has always been a question concerning the water content of muscle. We therefore asked if muscle relative water content changes as the muscle increases in size. The results presented in Figures 5 and 6 clearly show that relative muscle water content is constant regardless of the size of the muscle.

2. During our preliminary experiments we found that it required almost an entire muscle to obtain triplicate determination of DNA using the Burton diphenylamine procedure (Biochem. 62: 315, 1956). We therefore examined both the Giles and Meyers modification (Nature 206: 93, 1965) and the Richards modification (Anal. Biochem. 57: 309, 1974) as possible alternatives. The 30-50 percent increase in sensitivity was not sufficient to allow us a total analysis using only part of a muscle. We also examined the tissue processing modifications using precipitation of Munro and Fleck (Analyst 9: 78, 1966) and Ortov and Orlova (Biochem. USSR 26: 834, 1961). In short, no colorimetric assay is sufficiently sensitive to allow DNA determination and protein determination on a single small muscle. We next examined flurimetric approaches to DNA analysis. The DABA procedure as described by Thomas and Farquher (Anal Biochem 89: 35, 1978) although more sensitive than colorimetric procedures was ruled out because of lipid interference. The EtBr procedure

as described by Bentle et al., (Anal. Biochem. 116: 5, 1981) is also more sensitive than colorimetric procedures. However, RNA also signals in this procedure. This procedure was rejected because we did not wish to worry about changes in the relative amounts of RNA and DNA.

We finally settled on the Hoescht procedure as described by Labaraca and Paigen (Anal. Biochem 102: 344, 1980). This procedure has virtually no RNA interference, and is extremely sensitive. Several comparative analyses with the Burton on identical muscle samples yielded identical results. Figure 7 is a representative example of the standard curve obtained with the Hoescht procedure.

3. Utilizing the highly sensitive Hoescht assay we developed a procedure to determine both DNA and all protein measurements in the same muscle. That procedure is described in Figure 8. Using this procedure we have developed control data for the following: 1) total protein; 2) total non-collagen protein; 3) total collagen; 4) soluble protein; 5) total myofibrillar protein; 6) myofibrillar non-collagen protein; 7) collagen in the myofibrillar fraction and 8) DNA. The data for these protein fractions as a function of increasing muscle weight are presented in Tables 1 and 2. The data for DNA are presented in Figures 9 and 10. The results presented in Figures 9 and 10 show that there is an increase in the DNA content of both the soleus and EDL muscles as the muscle increases in size.

4. Initially we proposed to use the Gardner plastic-impregnated plaster casting procedure. However, the weight of the cast in larger animals (~250 grams) is approximately 80-100 grams. Because of surface to volume considerations, the cast for small animals is still approximately 50 grams. Fifty grams is the weight of the animals that we are starting with. In short, casting a small animal virtually immobilizes the entire animal. We therefore rejected this procedure and began developing our own approach.

5. Three factors were critical to our development of the new casting procedures. The first was a concerted effort to study the structure of the animal. For this we made plaster molds of rats in a variety of positions. We then filled these molds with plastic resin and obtained plastic models of rats in different positions. With these molds we began to simply play with different ways of wrapping up the hind limb of a rat using the least amount of material. The result of this effort was the second critical factor to the development of this procedure: the key to casting small animals is not to use smaller and smaller bits of material but to use single piece patterns which mold up the leg. In essence, rather than wrapping, such as is done with humans, you construct a pattern of a single layer boot which molds up the leg holding it in the desired position. The last factor was introducing the use of a plastic casting material made by 3M Company. This material co-molds extremely well with hypo-allergenic porous surgical tape. Together these materials can be used to form an extremely strong, lightweight cast which is literally molded to the contours of the animal's leg.

6. Using this approach we developed a cast for the leg (Form A) which slightly stretches the muscles in the front of the leg and shortens the muscles in the back of the leg. Because of the difficulty in describing these procedures in prose, we have provided (Figure 11) a series of photographs which illustrate the formation of this cast. In addition, in Figure 12 we provide a few x-ray pictures of animal legs with the Form A cast in place. In all we have casted close to 100 animals using Form A.

At present, we have data on the affect of Form A casts on the wet and dry weight of the muscles as well as on animal weight.

Figures 13a and 13b illustrate the effect of Form A casts on the fast fiber EDL muscle and slow fiber soleus muscle respectively. These data show that both the EDL and soleus muscles from the casted leg are significantly smaller than the contralateral leg. However, the effect on the slow fiber soleus (which is located in the back of the leg) is substantially greater than the effect on the fast fiber EDL (which is located in the front of the leg). Figures 14a and 14b provide comparable data on dry weight. Figures 15 and 16 illustrate the change in the contralateral control and casted EDL muscles as a function of body weight. Figures 17 and 18 present comparable data for the soleus. Our preliminary conclusion from these data is that both contralateral control muscles (EDL and soleus) are comparable to normals while the casted EDL is significantly reduced in size and the casted soleus is extraordinarily reduced in size. A summary of those data are provided in Table 3.

It is entirely possible that procedures such as this may cause muscle edema or otherwise alter the water content of the muscle. To examine this question we analysed the dry weight/wet weight ratio of these muscles as a function of muscle wet weight. Those data are presented in Figures 19-22. Those data indicate that casted muscles are comparable to contralateral controls which are comparable to normals (Figures 5, 6). Finally we asked if casting influences the normal increase in body weight of these animals which occurs with age. The results are presented in Figures 23 and 24. Comparable data for normal animals were presented in Figures 1 and 2. Comparison of the two sets of figures suggest that casting may slightly reduce the normal weight gain of the animal. At present a statistical answer to this question is unclear. However, as our data base increases, a clear answer to this question will be possible.

These data clearly pose the question of the difference between the lack of growth and atrophy. One could interpret the data as meaning that casting retards the normal growth of the EDL and completely blocks growth of the soleus. In all experiments casts were removed and replaced every three days to prevent inhibition of bone growth. A preliminary analysis of x-ray films indicates that the length of the bones is normal. Therefore, for this interpretation to be valid the conclusion must be that the casting impedes muscle growth but not bone growth. One could also interpret these data as indicating that disuse atrophy is counterbalancing growth hypertrophy. The difficulty with either interpretation is that no one understands the difference between work

induced hypertrophy and growth hypertrophy. However, part of the answer may be derived from the analysis of the DNA and the distribution of protein in the various compartments of muscles from casted animals relative to muscles from normal animals. For example, Figures 9 and 10 show that DNA increases in normal muscles with growth. It will therefore be very important when we find out if DNA continues to increase in the casted muscle even though the increase in protein mass is retarded. Finally the stretch factor seems to be central to understanding this mechanism. In Section 8 below we present data on a second cast (Form B) in which the leg is immobilized but stretched in a different way. The result is that the impact on both the soleus and EDL muscles are different from Form A.

7. We have also conducted a few remobilization experiments. At present all we can say is that the muscle begins to increase in weight following remobilization. Those data are presented in Table 4. Of particular interest in our future experiments will be determining if a remobilized muscle increases in weight faster than a normal growing muscle. This result should also greatly aid us in broaching the distinction between work induced hypertrophy and growth.

8. In an effort to address the question of stretch effects we developed a second cast (Form B). Form B greatly stretches the muscles in the back of the leg and shortens the muscles in the front of the leg. A few x-ray photographs of this cast form are presented in Figure 25. Our preliminary results on muscle weights are presented in Table 5. These data indicate that when the leg is immobilized in Form B there is a reduction in the atrophy of the soleus and an increase in the atrophy of the EDL. The comparison of the results obtained with Forms A and B should greatly enhance our ability to understand the effect of stretch on muscle mass.

9. Finally, in our pursuit of an understanding of the relationship between the effect of use/disuse, stretch, and growth on muscle mass, we have begun work on a third cast form (Form C, Figure 26. Form C is intermediate between Form A and Form B. We anticipate using all three casts. The intent is to use x-ray photographs to measure joint angles. This will allow us to introduce joint angle of immobilization as an independent variable. We decided to introduce a third intermediate cast form so that our complete analysis would not be relegated to drawing a straight line between two points.

D. DISCUSSION

In the preceding pages we have described rather rapid progress on Specific Aim 1. All procedures have been reduced to "cookbook" form and we anticipate completing Specific Aim 1 by early summer. To a great extent, the rapid progress has been the result of hiring two entry-level technical aides. They were both intensively drilled in the "cookbook" procedures and now they are simply progressing through the outlined experiments, each one repeating the other. At present

the only technical requirement of the PI is to cast the animals. As indicated, we have altered proposed procedures in several respects. First, we have modified procedures such that wet weight and all biochemical analyses can be conducted on a single muscle. This change allows us to use paired statistics which enables us to reduce the number of animals necessary for statistical significance. The changes have been carefully checked with respect to proposed procedures to be certain that we are obtaining the same number but with higher sensitivity.

The second major change is in the casting procedure. The procedure we have evolved seems to provide a degree of control of stretch and atrophy never before available in non-invasive immobilization methods. Because of the mission of this project, we have expanded our analysis to follow up our initial observation on the tremendous impact of stretch on the result. In addition, because of the extraordinary light weight of these casts it appears that we have created a situation in which the contralateral control is entirely valid. Although we have not completely decided this question, by the end of Specific Aim 1 this question should be clearly resolved. Finally it appears that we have interested Professor Zobel of this university in conducting a histological analysis and perhaps Professor Norman Robbins of Case Western in carrying out electrophysiological analyses of these muscles. We would also like to ourselves explore the possibility of embedding electrodes in the cast for high frequency electrical stimulation of the immobilized muscle.

We also anticipate completing Specific Aim 2 before the end of the grant year. We have had considerable experience in steroid treatment of animals. At present the only unknown in Specific Aim 2 is the steroid dosage appropriate for small animals. This will be answered by a simple dose response titration experiment which will be carried out in the next month or so. If contralateral controls turn out to be valid then Specific Aim 2 will progress much more rapidly than originally anticipated.

Because personnel funds will be depleted by the beginning of September, it is probable that Specific Aim 3 will not be started until the beginning of year 02 (Nov. 1). However, it should be pointed out that Specific Aim 3, the steroid receptor analysis, rests entirely on procedures which were developed and are well established in this laboratory.

Once Specific Aim 3 is in progress, the PI will begin setting up the organ culture methodology necessary for measuring the anabolic and catabolic indexes which are central to Specific Aim 4. It is hoped that the report one year from now will contain preliminary data on those procedures. At present the only impediment to progress is resources. This project would greatly benefit from a permanent second technical assistant, a bit more supply money, and funds to purchase a fluorimeter (currently we use a shared department instrument).

TABLE 1

PROTEIN DISTRIBUTION OF NORMAL MUSCLES EXPRESSED AS MICROGRAMS PROTEIN PER MILLIGRAM WET WEIGHT

A. Extensor Digitorum Longus

Muscle Weight (mgs)	Total Protein	Myofibr. Protein	Soluble Protein	Derived * Total Protein	- % - Derived/ Measured	Total Non-Collagen
21.43 ± 1.53	144.67 ± 6.71	102.81 ± 6.78	46.19 ± 3.03	149.00 ± 7.52	103.04 ± 3.09	139.66 ± 6.68
40.53 ± 2.50	161.05 ± 6.93	111.73 ± 7.71	46.53 ± 2.74	158.27 ± 6.99	98.37 ± 4.65	143.75 ± 5.95

* Total Collagen	% Total Collagen	Myofibr. Non-Collagen	* Myofibr. Collagen	- % - Myofibr. Collagen
4.98 ± 2.97	3.43 ± 2.08	95.61 ± 6.21	7.20 ± 2.42	6.97 ± 2.14
17.30 ± 3.25	10.72 ± 1.82	103.08 ± 5.80	9.38 ± 5.63	8.18 ± 4.46

* Indicate Derived Values

TABLE 1 (cont'd)

PROTEIN DISTRIBUTION OF NORMAL MUSCLES EXPRESSED AS MICROGRAMS PROTEIN PER MILLIGRAM WET WEIGHT

B. Soleus

Muscle Wet Weight (mgs)	Total Protein	Myofibr. Protein	Soluble Protein	Derived * Total Protein	- % - Derived/ Measured	Total Non-Collagen
18.95 ± 2.35	136.66 ± 5.77	92.44 ± 5.56	49.00 ± 3.17	141.44 ± 6.91	103.51 ± 3.08	131.86 ± 5.99
38.93 ± 3.89	147.55 ± 19.09	103.34 ± 12.51	45.44 ± 5.53	148.80 ± 17.72	101.07 ± 4.88	129.10 ± 15.70

* Total Collagen	% Total Collagen	Myofibr. Non-Collagen	* Myofibr. Collagen	- % - Myofibr. Collagen
4.80 ± 4.54	3.48 ± 3.26	84.07 ± 3.85	8.38 ± 2.32	8.97 ± 2.07
18.45 ± 6.52	12.36 ± 3.61	92.70 ± 11.92	10.65 ± 5.95	10.27 ± 5.31

TABLE 2

PROTEIN DISTRIBUTION OF NORMAL MUSCLES
EXPRESSED AS MILLIGRAMS PROTEIN PER MUSCLE

A. Extensor Digitorum Longus

Muscle Wet Weight (mgs)	Total Protein	Myofibr. Protein	Soluble Protein	Derived * Total Protein	- % - Derived/ Measured	Total Non-Collagen
21.43 ± 1.53	3.097 ± .244	2.208 ± .228	0.988 ± .071	3.196 ± .267	103.20 ± 3.07	2.990 ± .229
40.50 ± 2.50	6.532 ± .554	4.623 ± .494	1.884 ± .146	6.507 ± .581	99.73 ± 3.96	5.826 ± .455

Total Collagen	% Total Collagen	Myofibr. Non-Collagen	Myofibr. Collagen	- % - Myofibr. Collagen
.110 ± .070	3.41 ± 2.09	2.048 ± .197	0.160 ± .060	7.15 ± 2.28
0.706 ± .152	10.75 ± 1.84	4.290 ± .598	0.422 ± .224	8.19 ± 4.46

* Indicate Derived Values

TABLE 2 (cont'd)

PROTEIN DISTRIBUTION OF NORMAL MUSCLES
EXPRESSED AS MILLIGRAMS PROTEIN PER MUSCLE

B. Soleus

Muscle Wet Weight (mgs)	Total Protein	Myofibr. Protein	Soluble Protein	Derived * Total Protein	- % - Derived/ Measured	Total Non-Collagen
18.95 ± 2.35	2.587 ± .312	1.753 ± .245	0.927 ± .112	2.679 ± .343	103.51 ± 3.08	2.493 ± .274
38.93 ± 3.89	5.708 ± .665	3.996 ± .404	1.757 ± .176	5.753 ± .566	101.09 ± 4.88	4.991 ± .487

Total Collagen	% Total Collagen	Myofibr. Non-Collagen	Myofibr. Collagen	- % - Myofibr. Collagen
0.090 ± .100	3.48 ± 3.25	1.593 ± .207	0.160 ± .050	8.98 ± 2.07
0.717 ± .267	12.33 ± 3.63	3.585 ± .404	0.412 ± .299	10.27 ± 5.31

TABLE 3

MUSCLE WEIGHTS (% CONTRALATERAL CONTROL)

AS A FUNCTION OF DAYS CASTED (TYPE A CASTS,

A. WET WEIGHTS

Days Casted	n	Extensor Digitorum Longus	Soleus
1	5	[a] 108.25 ± 8.09	[a] 97.61 ± 2.55
2	10	[a] 98.45 ± 8.78	[a] 89.88 ±11.44
3	10	[a] 99.54 ± 8.59	[d] 76.98 ± 8.52
6	8	[d] 86.17 ± 6.90	[d] 59.79 ±14.56
9	15	[d] 84.79 ± 6.69	[d] 50.80 ± 8.83

B. DRY WEIGHTS

Days Casted	n	Extensor Digitorum Longus	Soleus
1	5	[a] 104.18 ± 7.82	[a] 98.65 ± 10.10
2	10	[b] 93.11 ± 9.20	[b] 87.17 ± 17.17
3	10	[a] 90.30 ± 6.02	[d] 72.21 ± 6.74
6	8	[c] 78.97 ± 7.98	[d] 62.02 ± 14.23
9	15	[d] 83.46 ± 8.83	[d] 51.98 ± 12.12

a Not significantly different from control

b Different from control P .02

c Different from control P .01

d Different from control P .001

TABLE 4

MUSCLE WEIGHTS (% CONTRALATERAL CONTROL) FOLLOWING

3 DAYS CASTED (TYPE "A") AND 6 DAYS REMOBILIZED

	Wet Weight	Dry Weight
Soleus	* 80.88 ± 11.21	* 82.14 ± 5.50
Extensor Digitorum Longus	98.44 ± 6.99	98.19 ± 3.17

*Different from control P .01 Paired t-test

TABLE 5

MUSCLE WEIGHTS (% CONTRALATERAL CONTROL) FOLLOWING

6 DAYS CASTING IN TYPE "B" CASTS

	Wet Weight	Dry Weight
Soleus	*75.25 ± 5.75	* 72.98 ± 7.62
Extensor Digitorum Longus	*74.82 ± 6.07	* 76.97 ± 4.91

*Different from control P .001 (paired t-test)

BODY WEIGHT OF NORMAL MALE SPRAGUE-DAWLEY RATS
VERSUS DAYS AFTER ARRIVAL

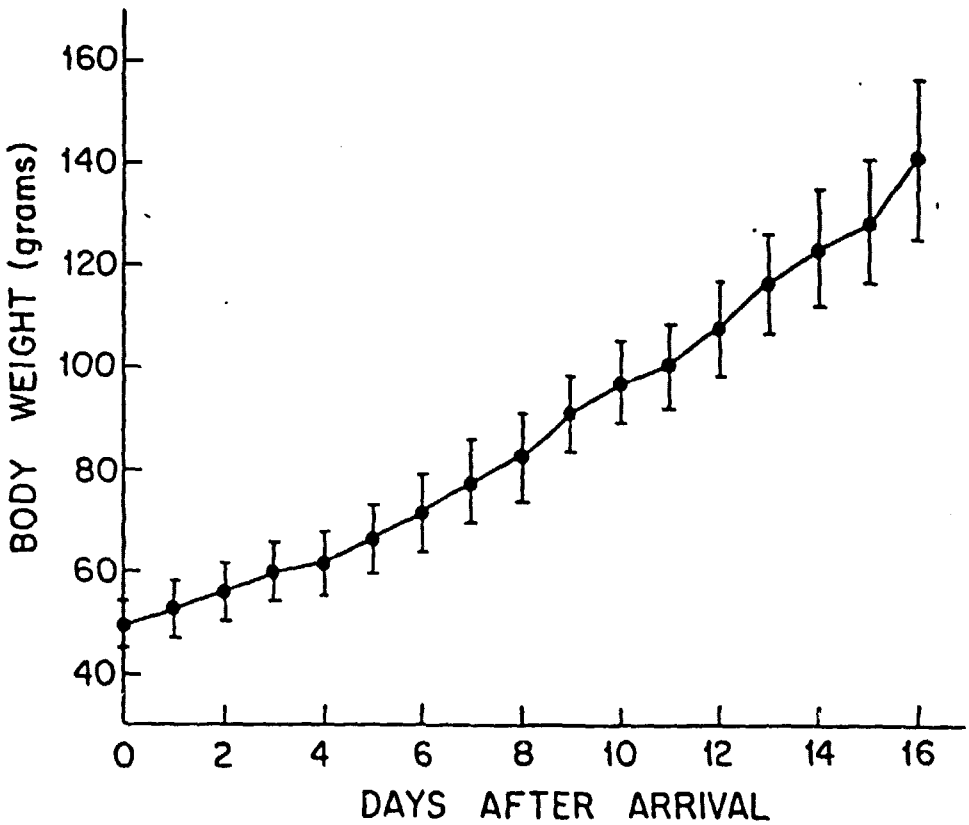


FIGURE 1

BODY WEIGHTS (EXPRESSED AS % ORIGINAL BODY WEIGHT)
OF NORMAL MALE SPRAGUE-DAWLEY RATS VERSUS
DAYS AFTER ARRIVAL

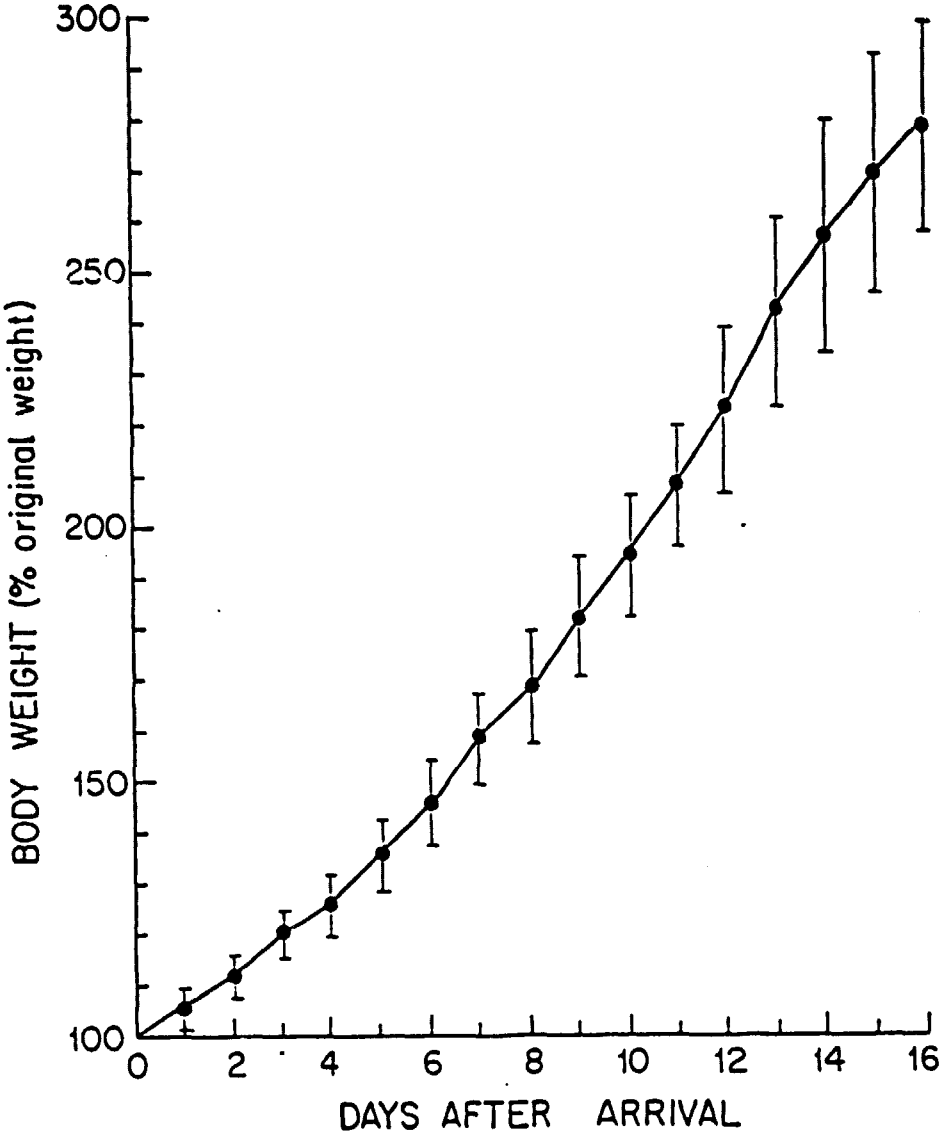


FIGURE 2

BODY WEIGHT OF NORMAL MALE SPRAGUE-DAWLEY RATS
VERSUS WET WEIGHT OF SOLEI MUSCLES

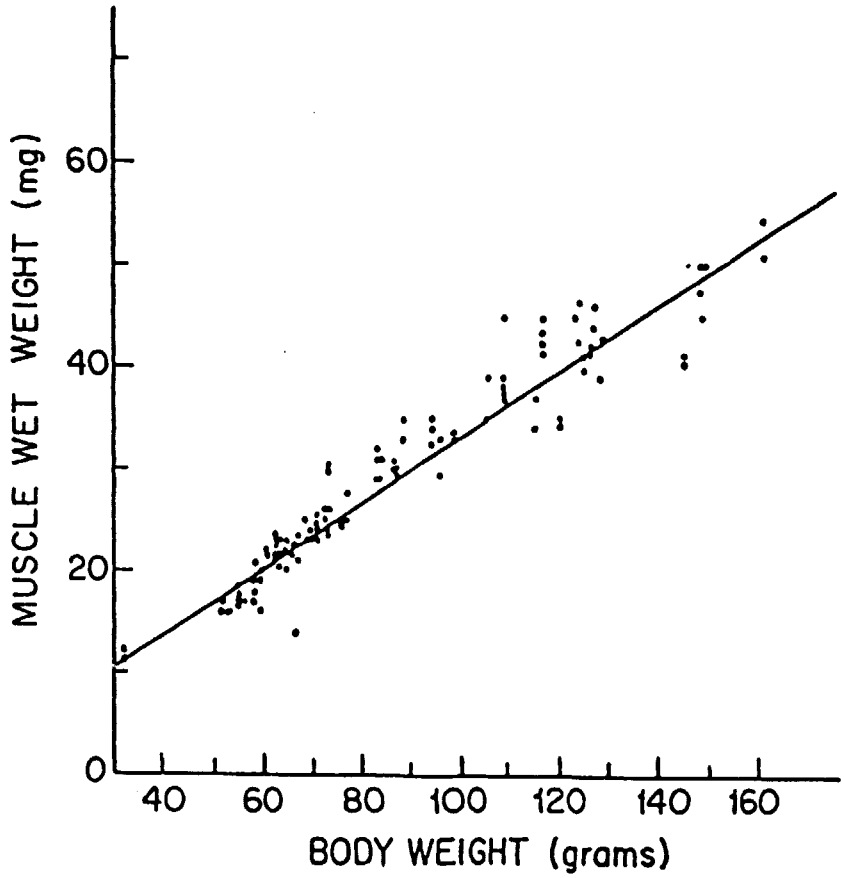


FIGURE 3

BODY WEIGHT OF NORMAL MALE SPRAGUE-DAWLEY RATS
VERSUS WET WEIGHT OF EXTENSOR
DIGITORUM LONGUS MUSCLES

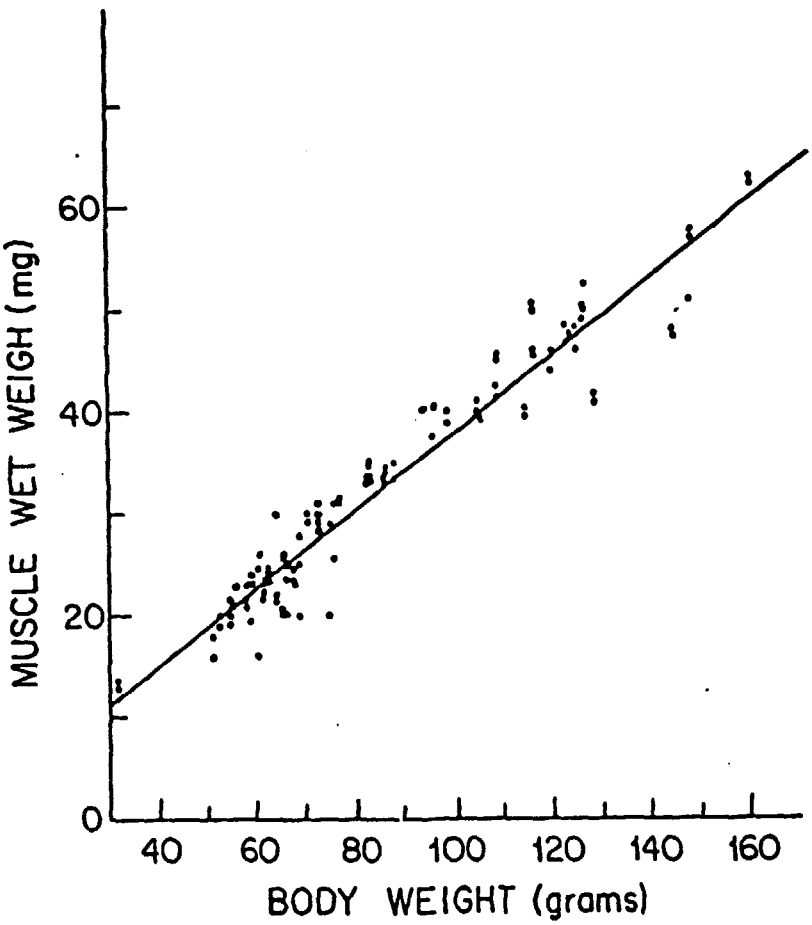


FIGURE 4

RATIO OF DRY WEIGHT TO WET WEIGHT OF NORMAL EXTENSOR DIGITORUM LONGUS MUSCLES VERSUS MUSCLE WET WEIGHT

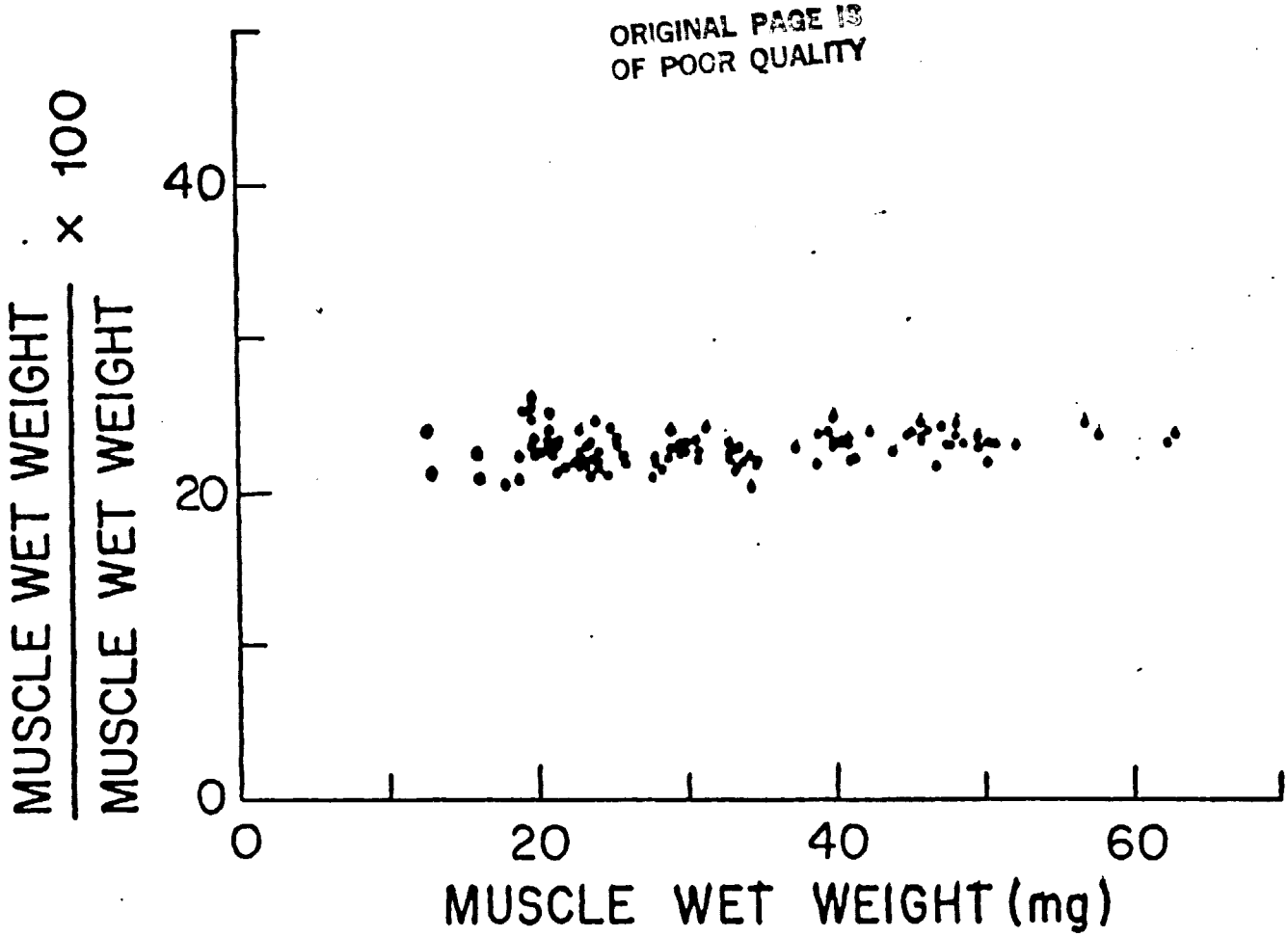


FIGURE 5

RATIO OF DRY WEIGHT TO WET WEIGHT OF NORMAL
SOLEI MUSCLES VERSUS MUSCLE WET WEIGHT

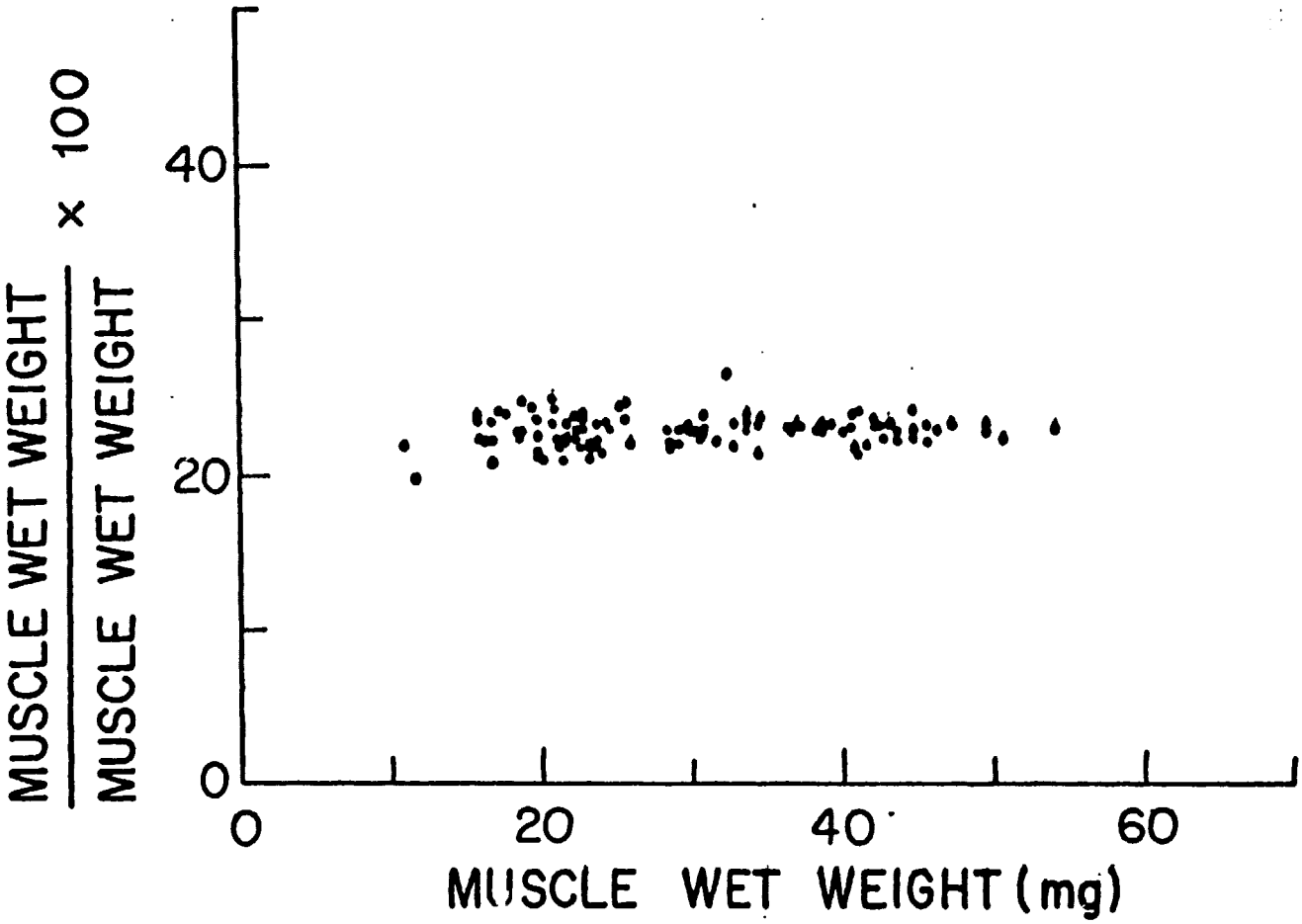


FIGURE 6

DNA-HOESCHT REACTION STANDARD CURVE

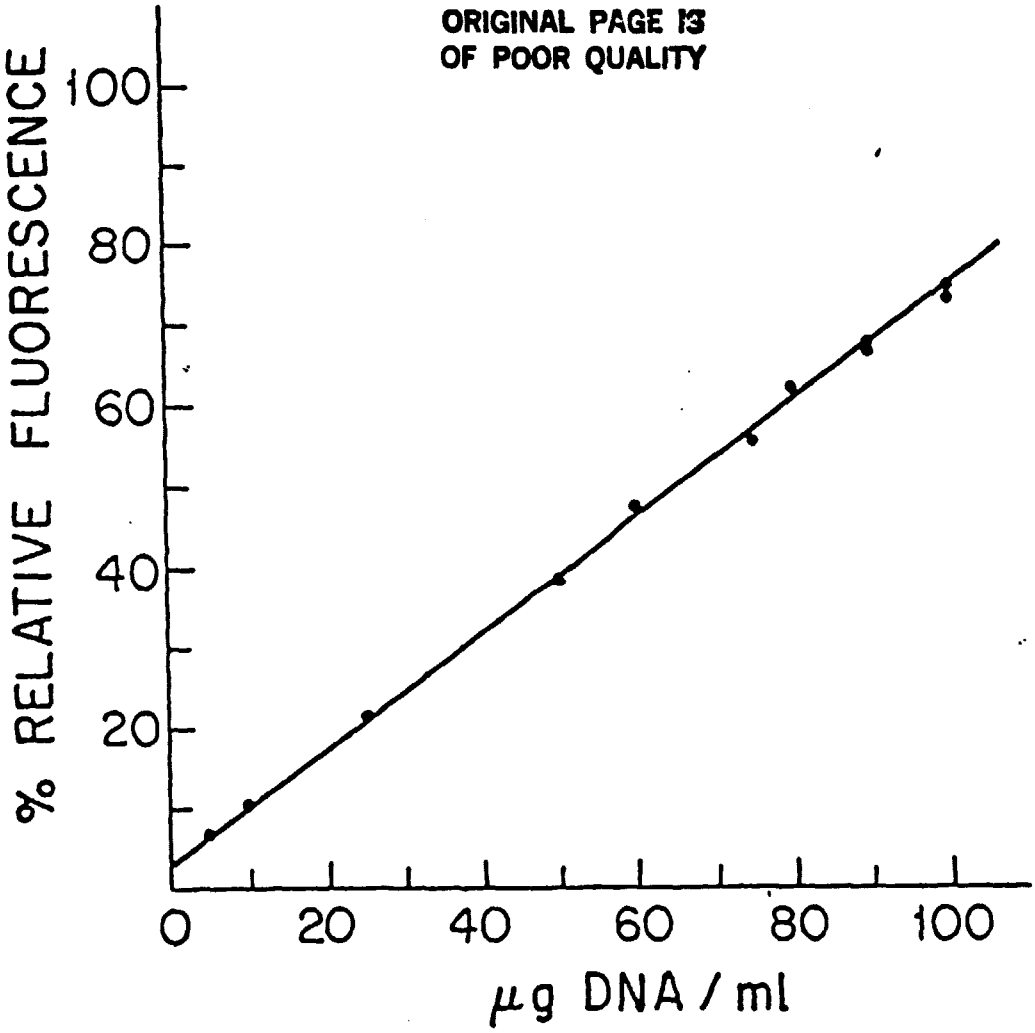
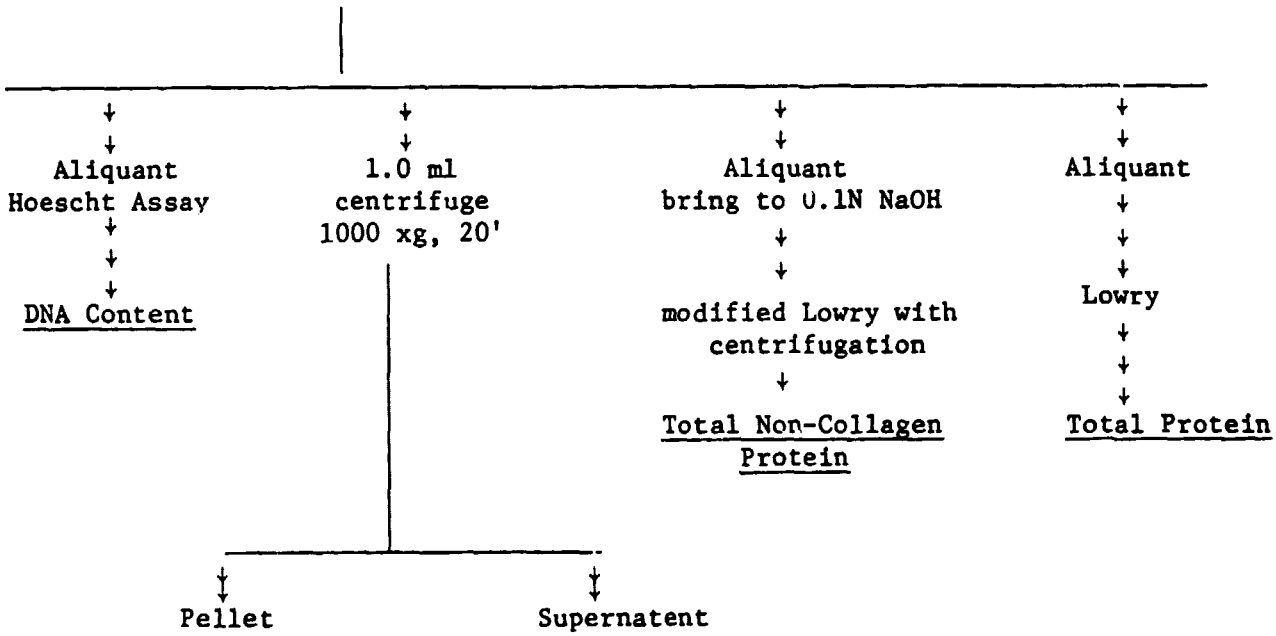


FIGURE 7

FIGURE 8

Processing of Skeletal Muscle

1. Remove muscle
2. Weigh on torsion balance — wet weight
3. Transfer to 10 ml beaker
   a.) Add cold distilled water (30 X muscle weight)
   b.) Mince with scissors
   c.) Homogenize (Duall homogenizer - 10 strokes - medium speed)
   d.) Rinse homogenizer (2 X 500 µl cold distilled water)
   e.) Combine rinse with homogenate



DNA CONTENT OF NORMAL SOLEI MUSCLES (MICROGRAMS DNA/MUSCLE) VERSUS MUSCLE WET WEIGHT

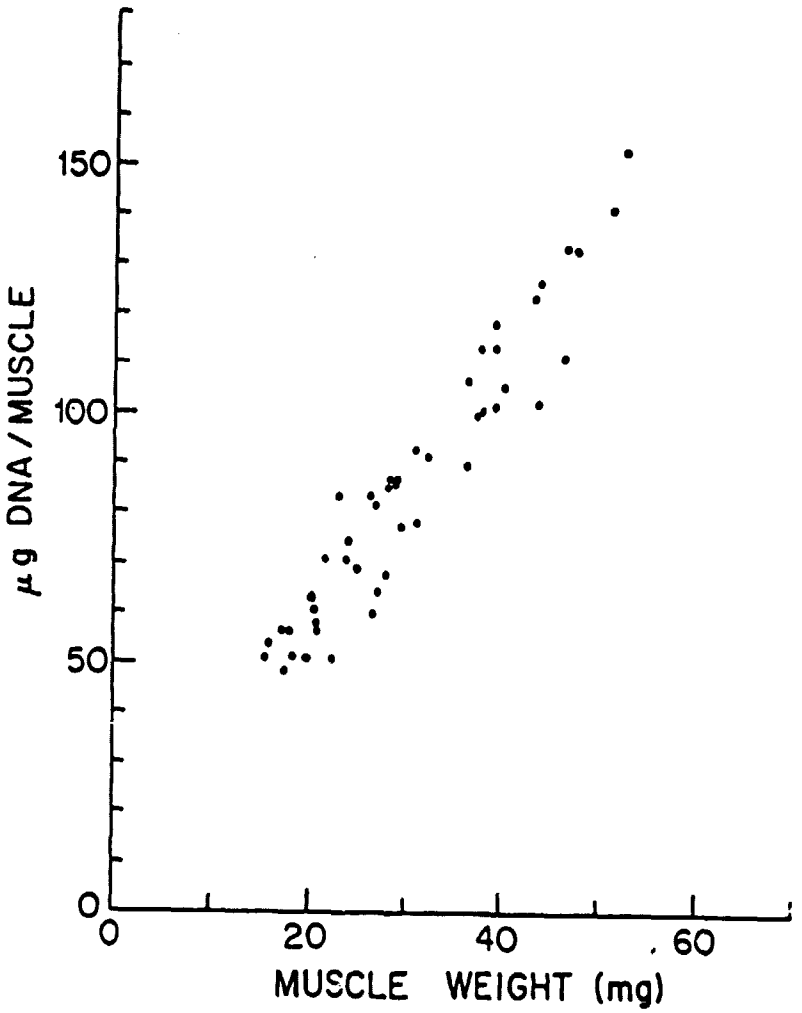


FIGURE 9

DNA CONTENT OF NORMAL EXTENSOR DIGITORUM LONGUS MUSCLES (MICROGRAMS DNA/MUSCLE) VERSUS MUSCLE WET WEIGHT

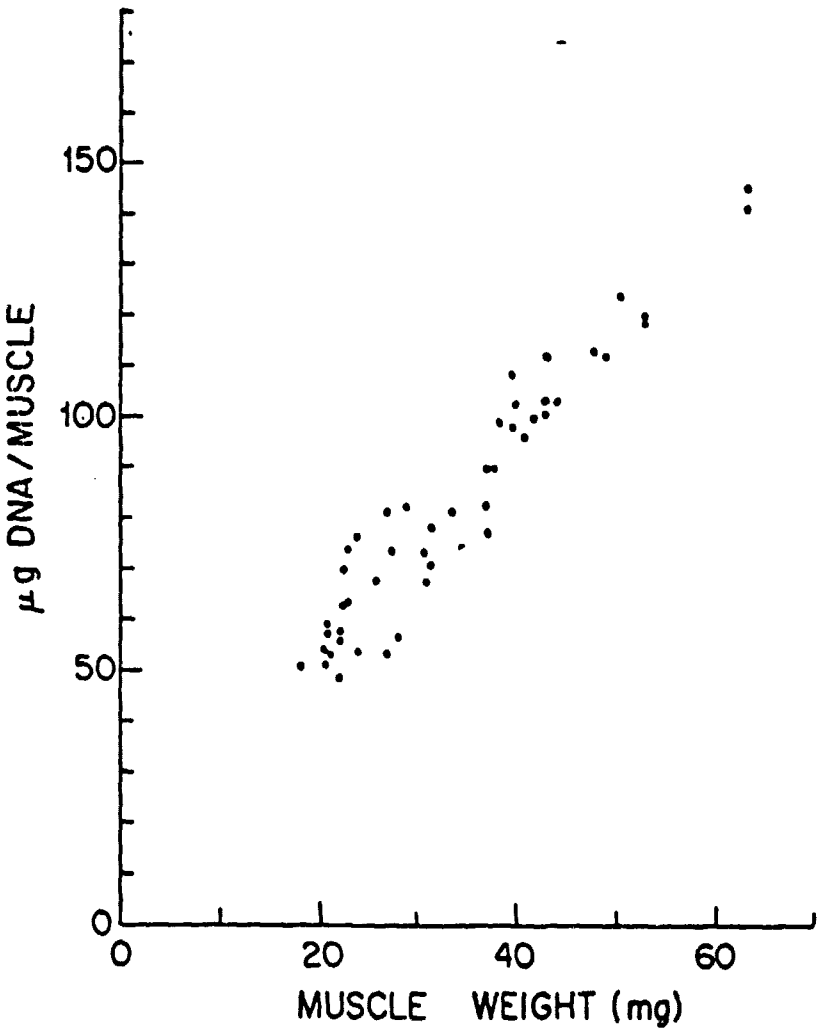


FIGURE 10

FIGURE 11

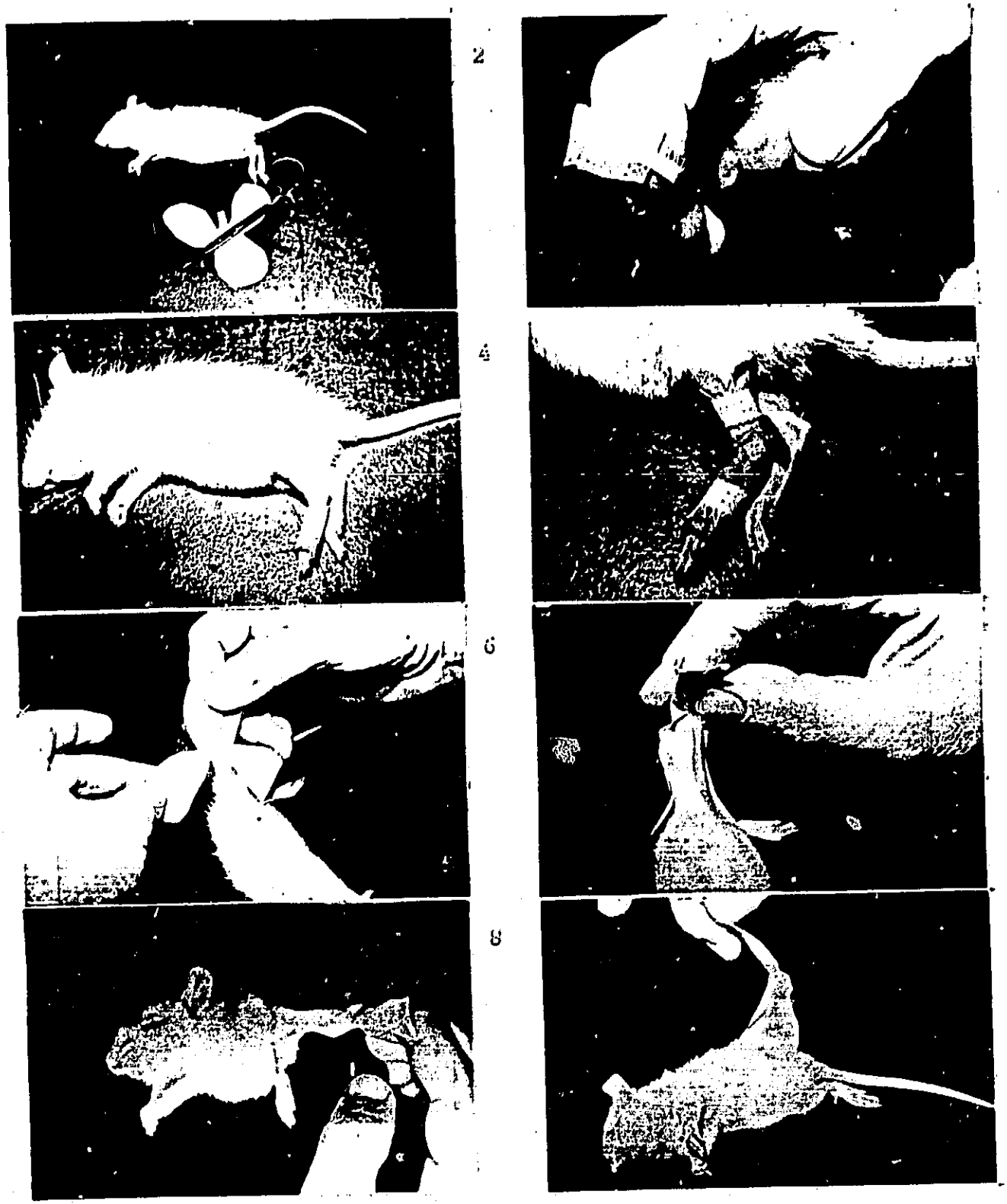


FIGURE 11 cont.

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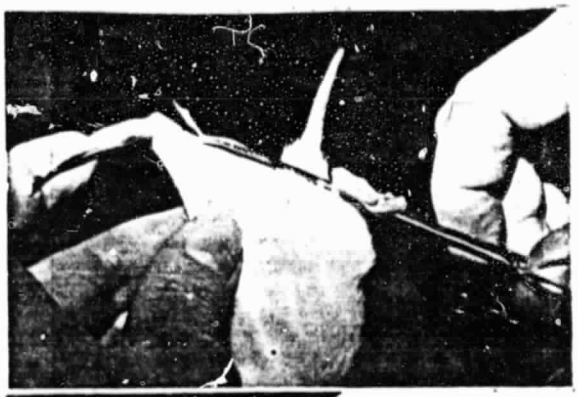

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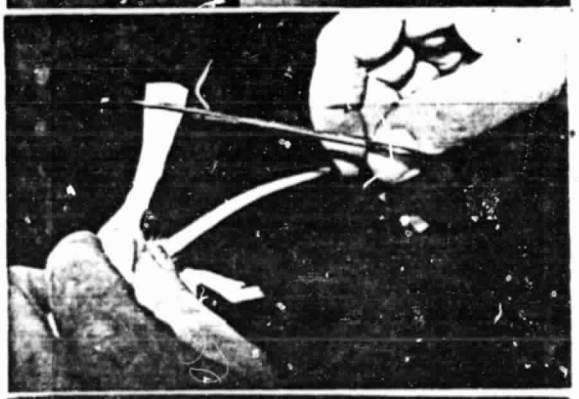

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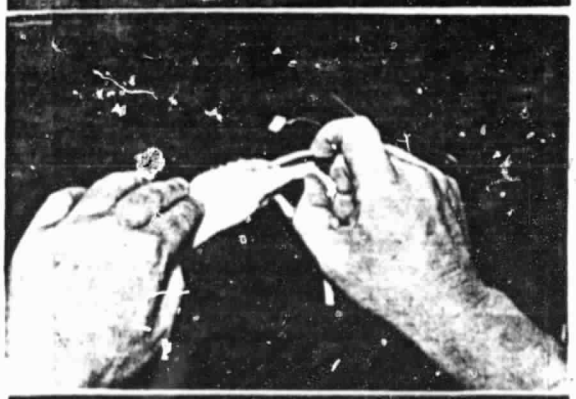

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FIGURE 11 cont.

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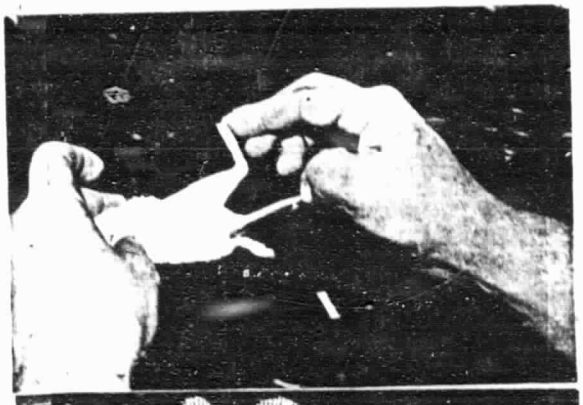

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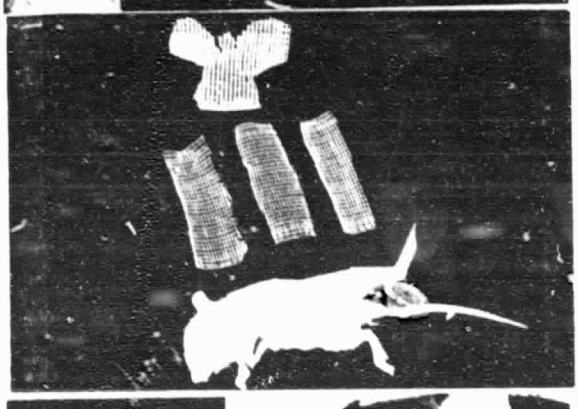

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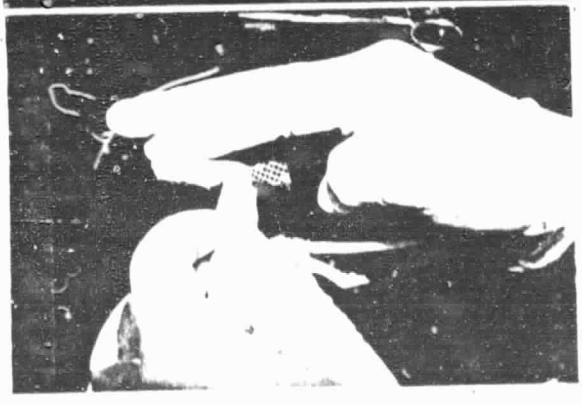

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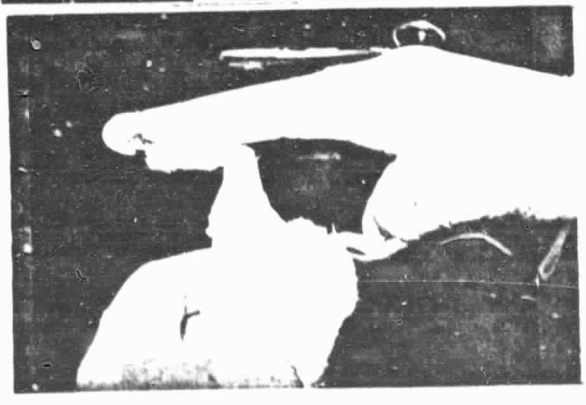
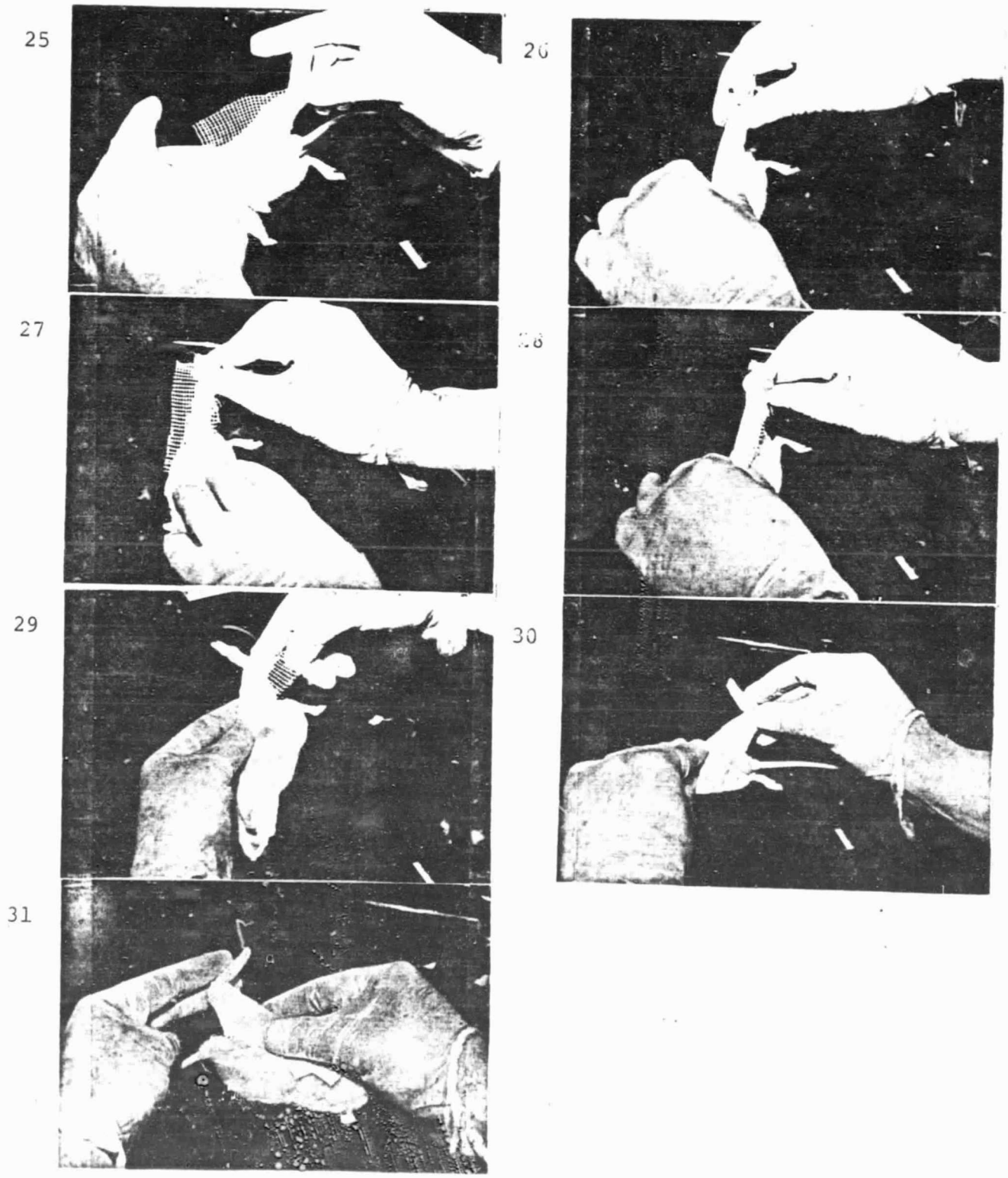


FIGURE 11 cont.



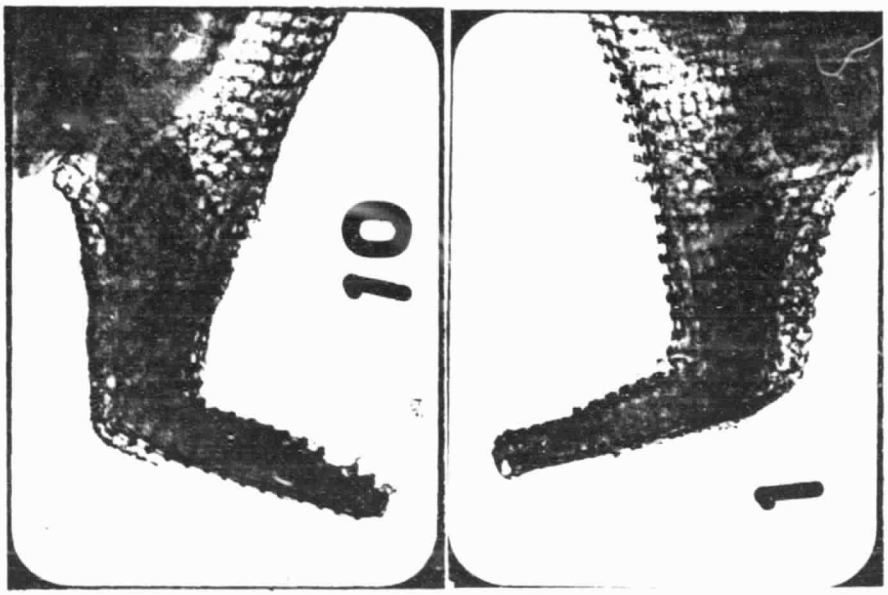


FIGURE 12

WET WEIGHT (% CONTRALATERAL CONTROL) OF MUSCLES
AS A FUNCTION OF DAYS CASTED

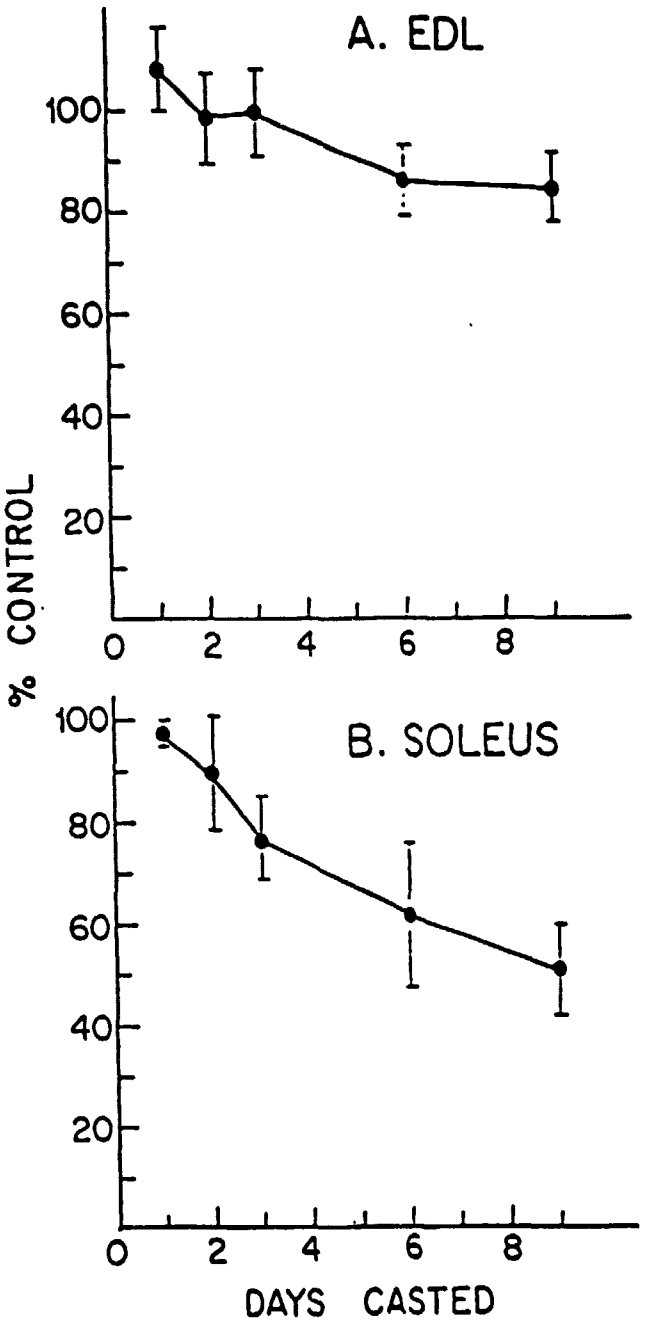


FIGURE 13

DRY WEIGHT (% CONTRALATERAL CONTROL) OF MUSCLES
AS A FUNCTION OF DAYS CASTED

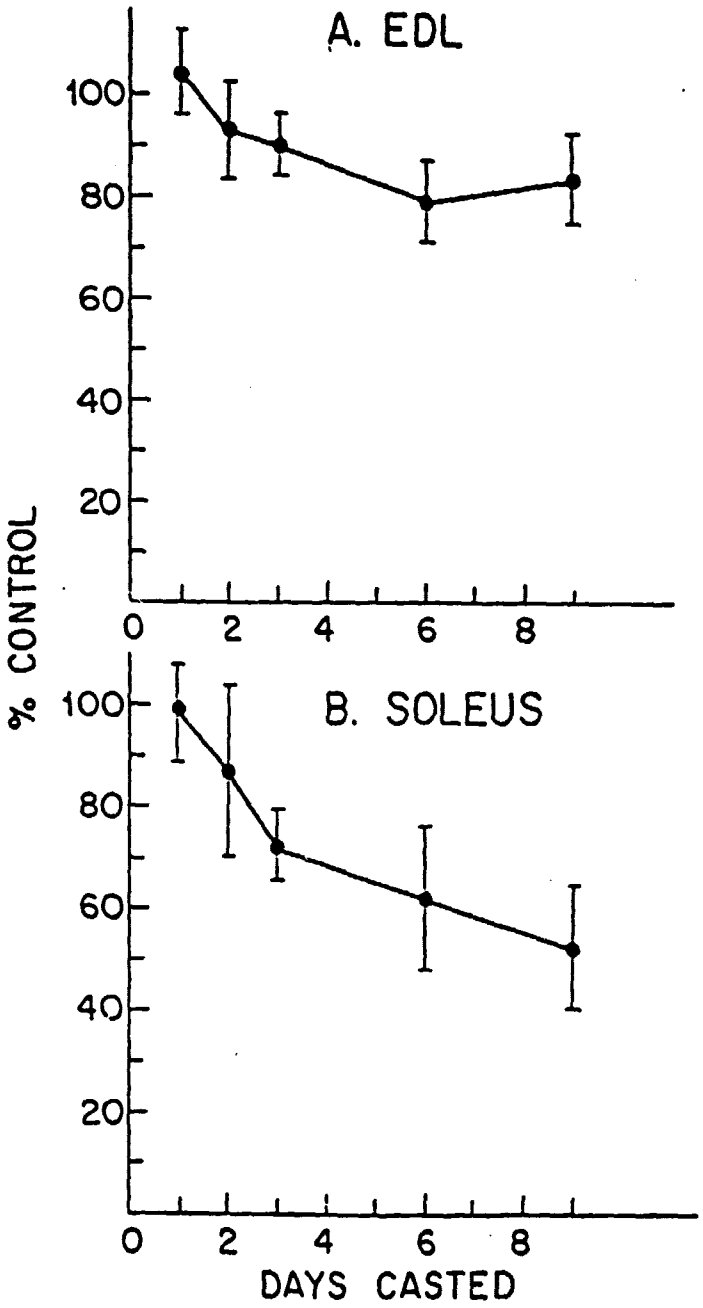


FIGURE 14

WET WEIGHT OF CONTRALATERAL EXTENSOR DIGITORUM LONGUS MUSCLES VERSUS BODY WEIGHT

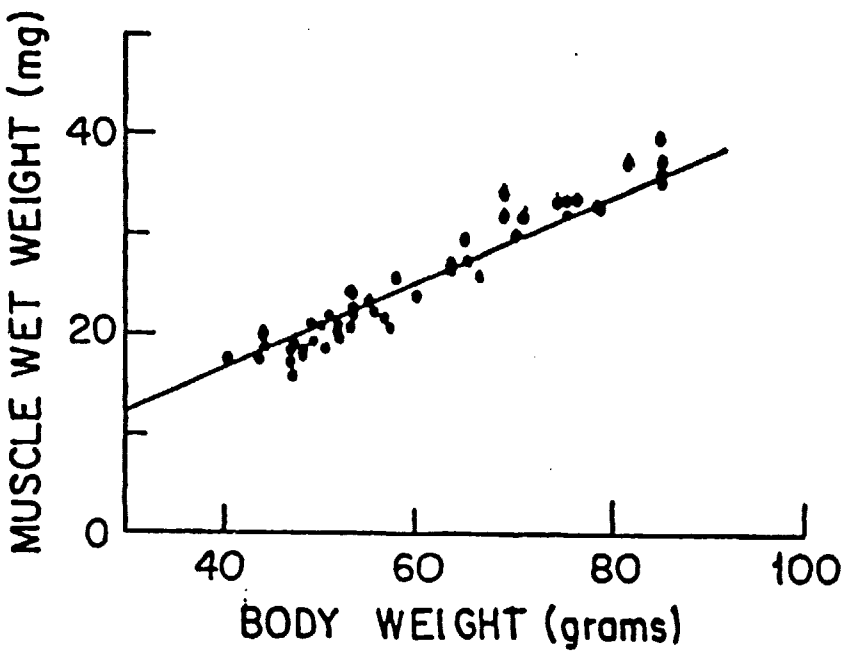


FIGURE 15

WET WEIGHT OF CONTRALATERAL SOLEI MUSCLES
VERSUS BODY WEIGHT

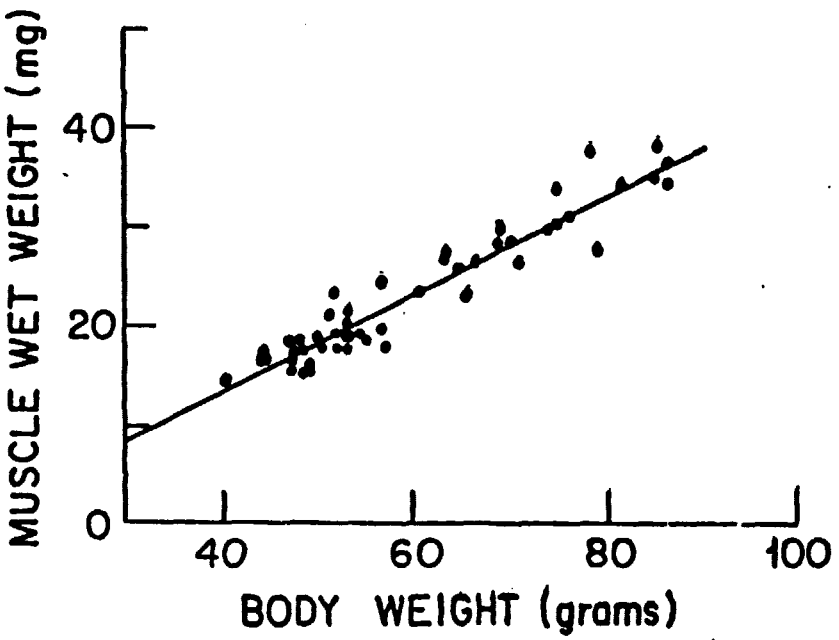


FIGURE 16

WET WEIGHT OF CASTED EXTENSOR DIGITORUM LONGUS MUSCLES VERSUS BODY WEIGHT

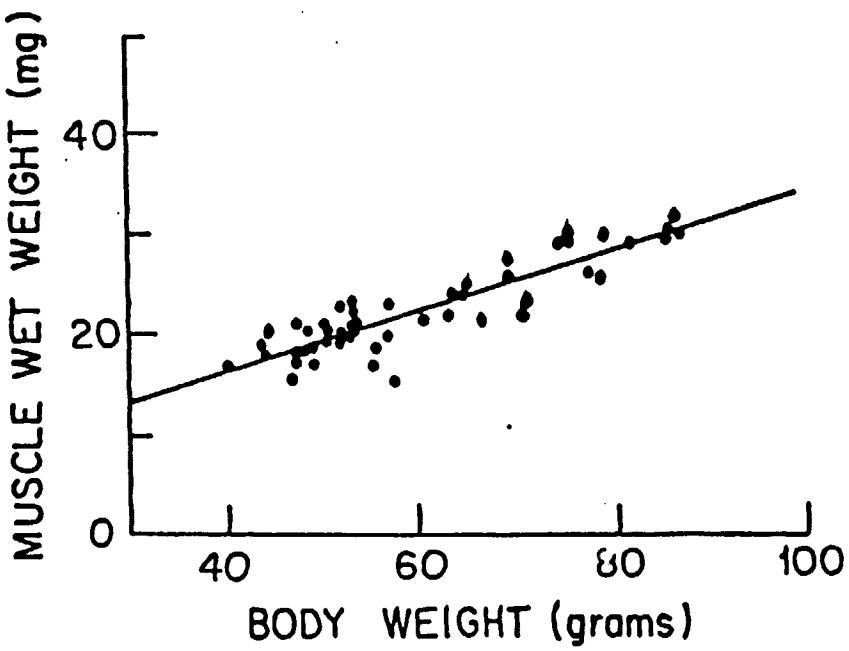


FIGURE 17

WET WEIGHT OF CASTED SOLEI MUSCLES VERSUS
BODY WEIGHT

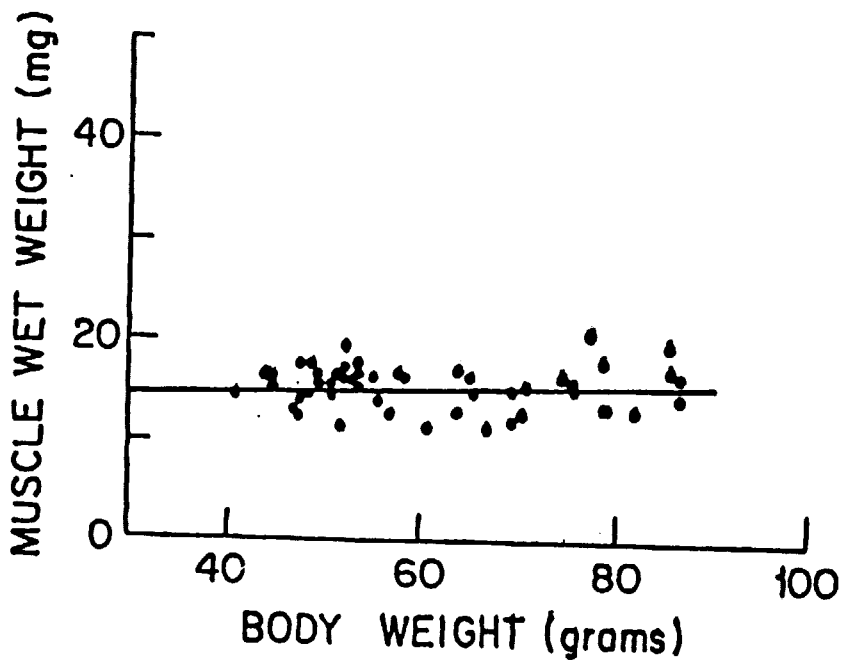


FIGURE 18

RATIO OF DRY WEIGHT TO WET WEIGHT OF CONTRALATERAL EXTENSOR DIGITORUM LONGUS MUSCLES VERSUS MUSCLE WET WEIGHT

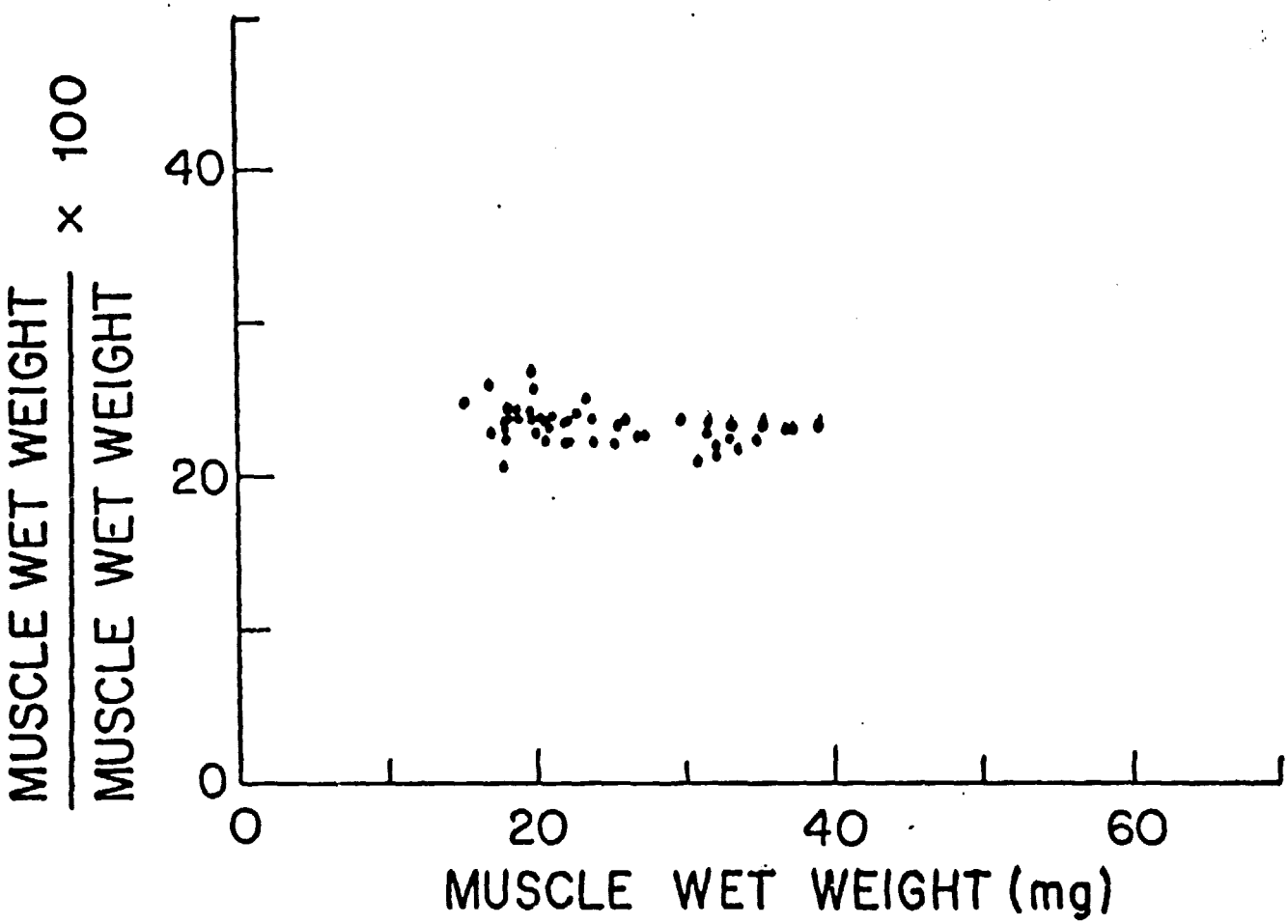


FIGURE 19

RATIO OF DRY WEIGHT TO WET WEIGHT OF CONTRA-
LATERAL SOLEI MUSCLES VERSUS MUSCLE
WET WEIGHT

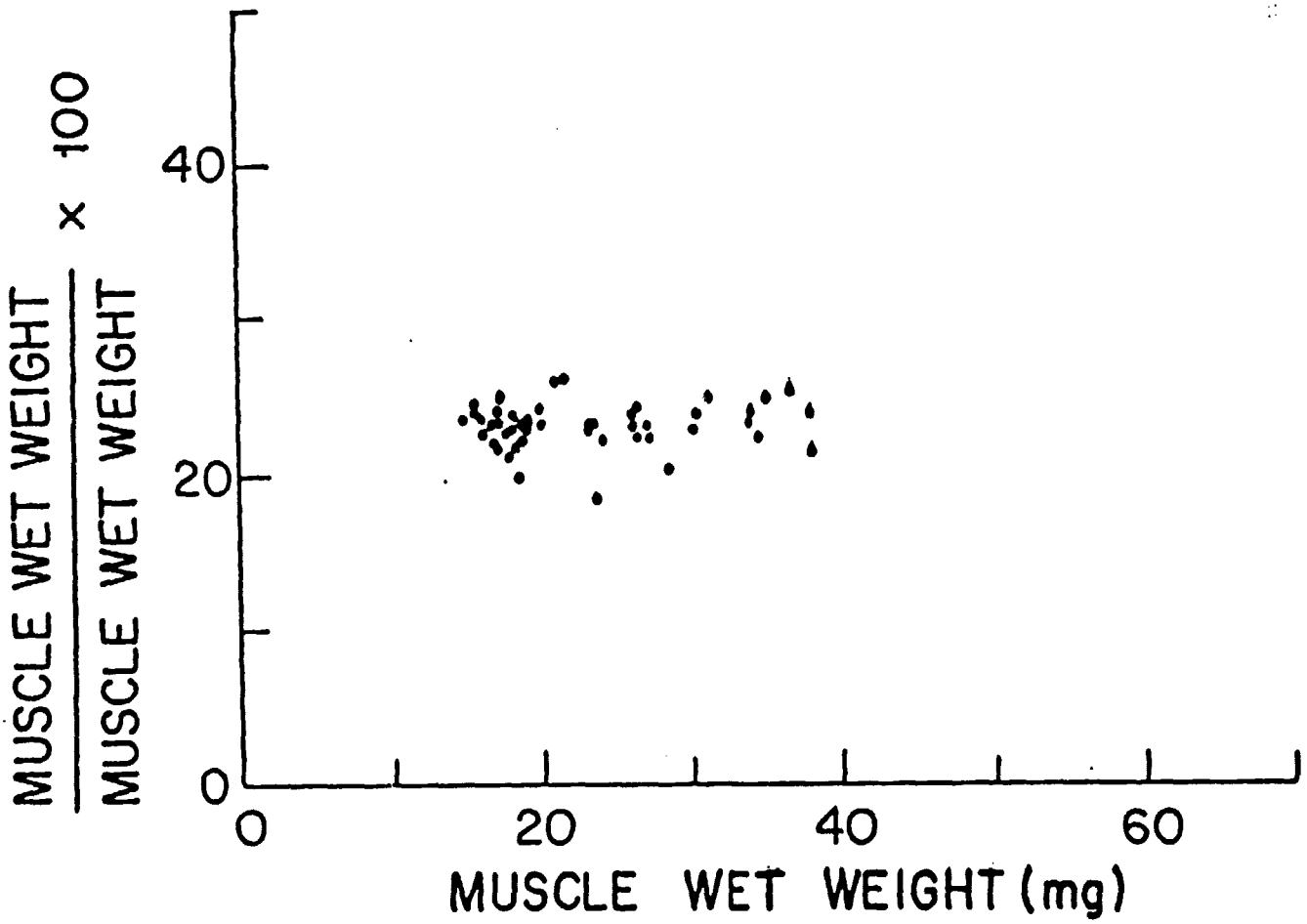


FIGURE 20

RATIO OF DRY WEIGHT TO WET WEIGHT OF CASTED EXTENSOR DIGITORUM LONGUS MUSCLES VERSUS MUSCLE WET WEIGHT

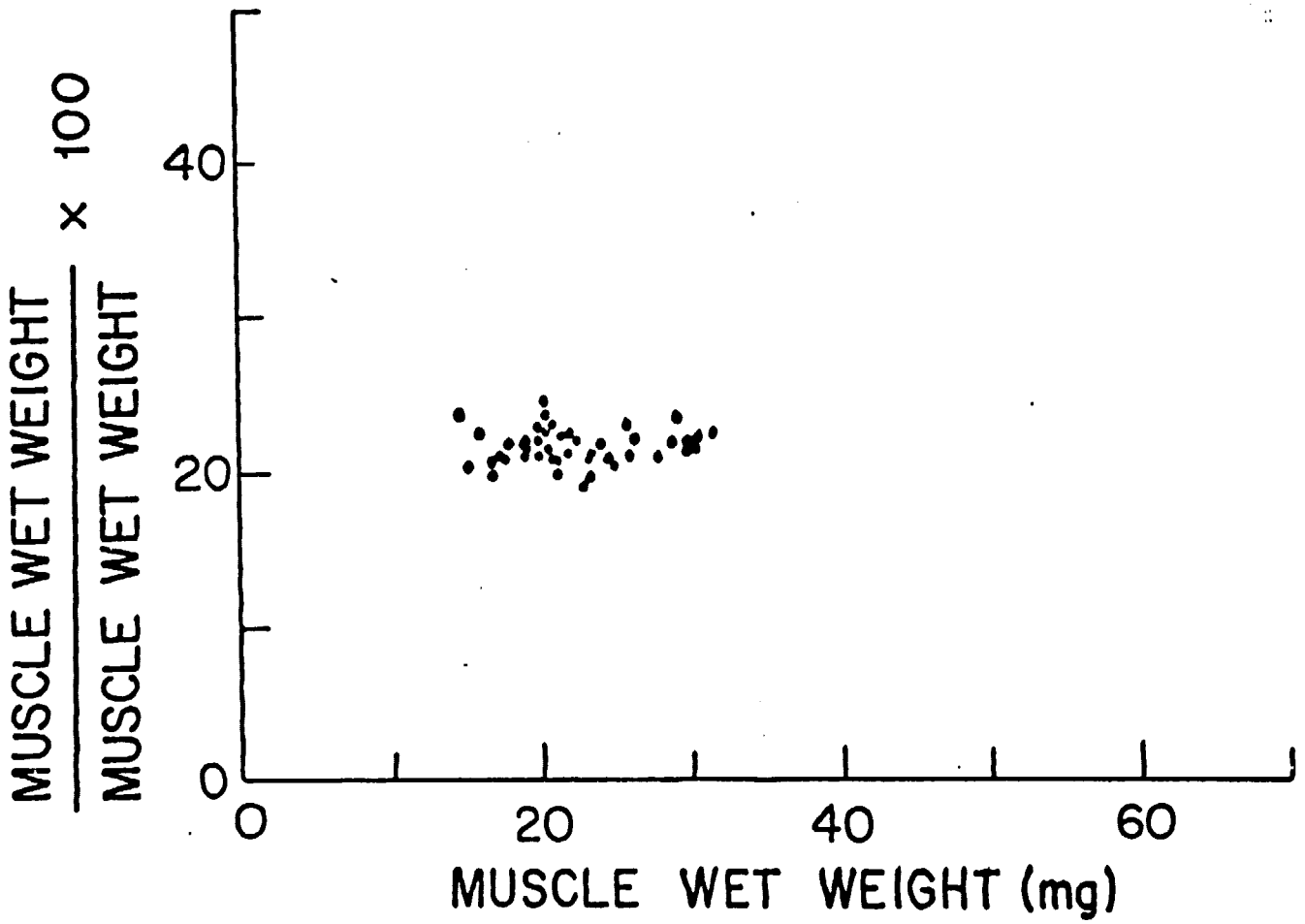


FIGURE 21

RATIO OF DRY WEIGHT TO WET WEIGHT OF CASTED SOLEI MUSCLES VERSUS MUSCLE WET WEIGHT

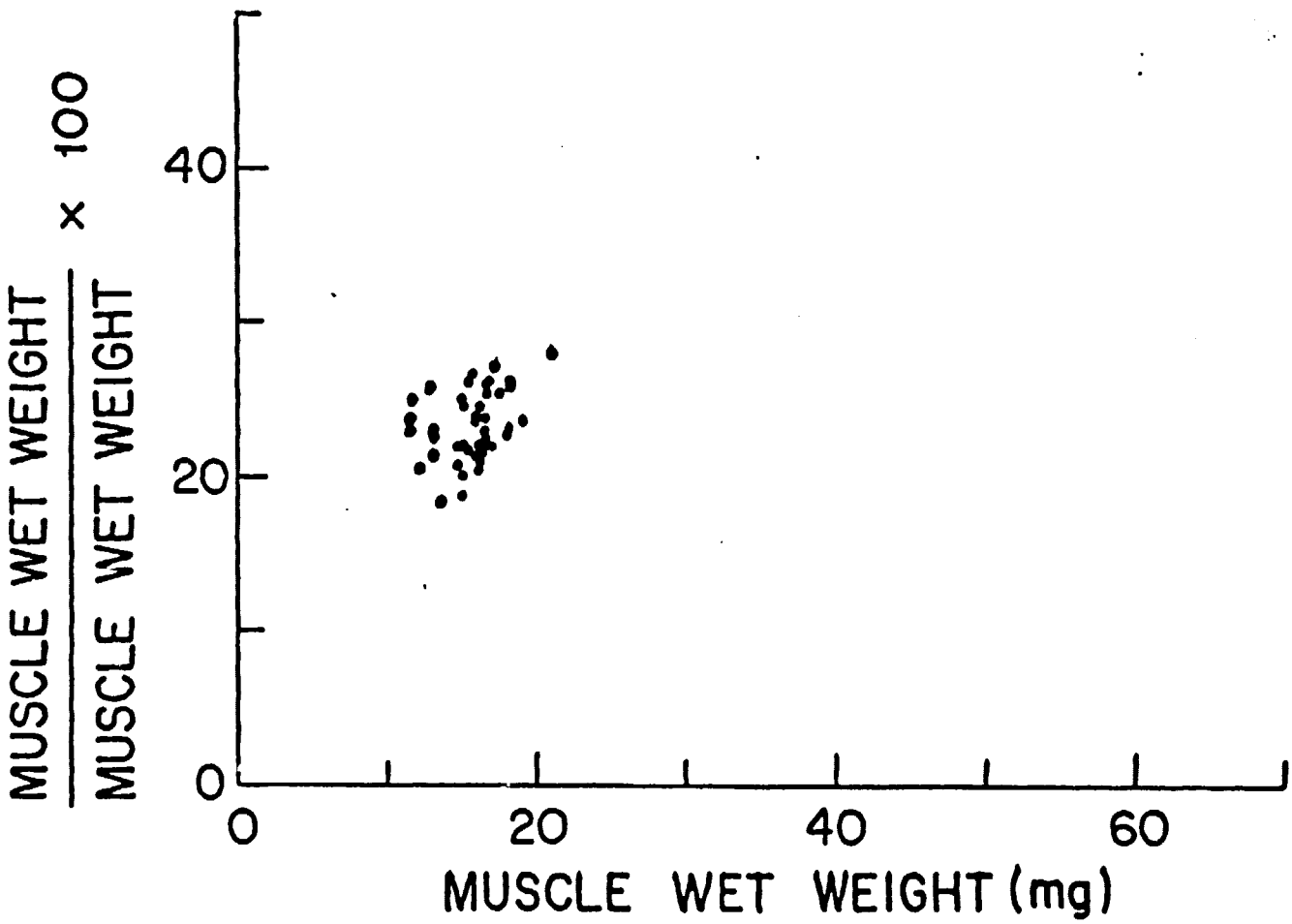


FIGURE 22

BODY WEIGHT OF CASTED MALE SPRAGUE-DAWLEY RATS
VERSUS DAYS AFTER ARRIVAL

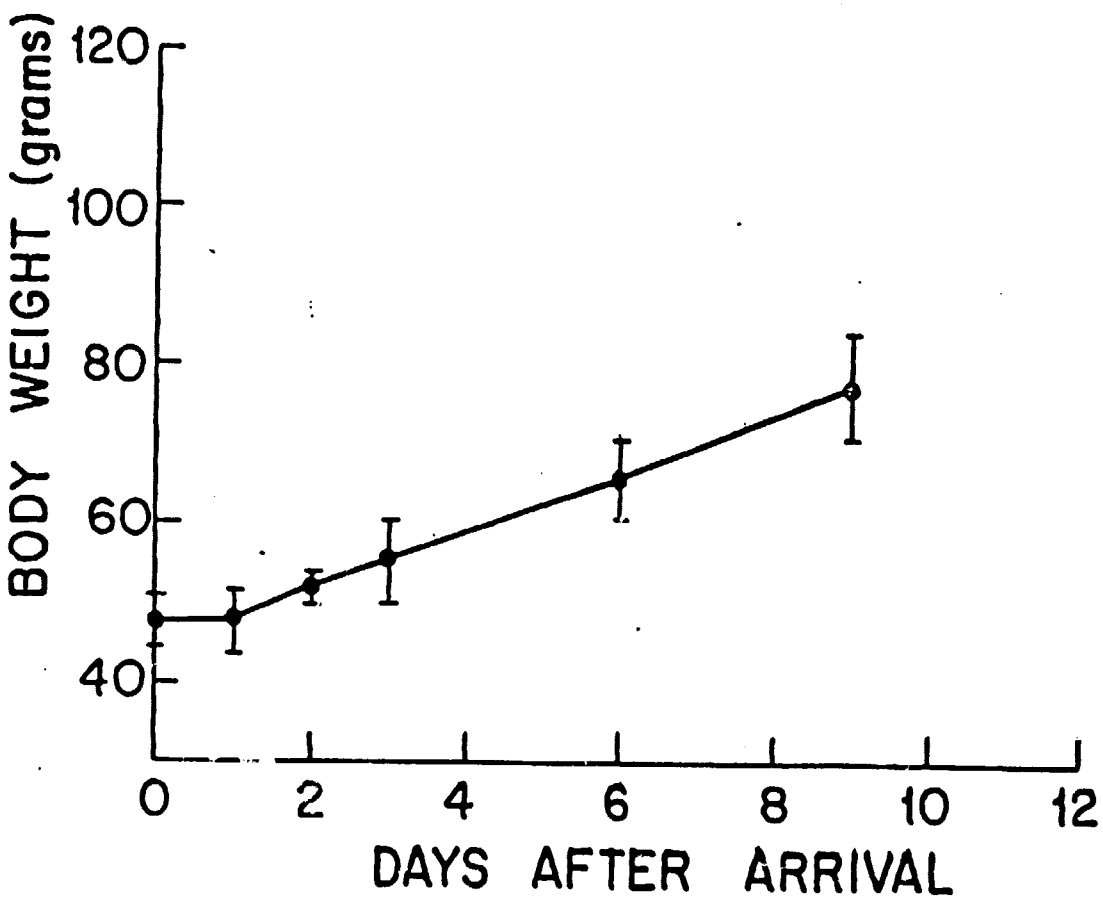


FIGURE 23

BODY WEIGHT (EXPRESSED AS % ORIGINAL BODY WEIGHT) OF CASTED MALE SPRAGUE-DAWLEY RATS VERSUS DAYS AFTER ARRIVAL

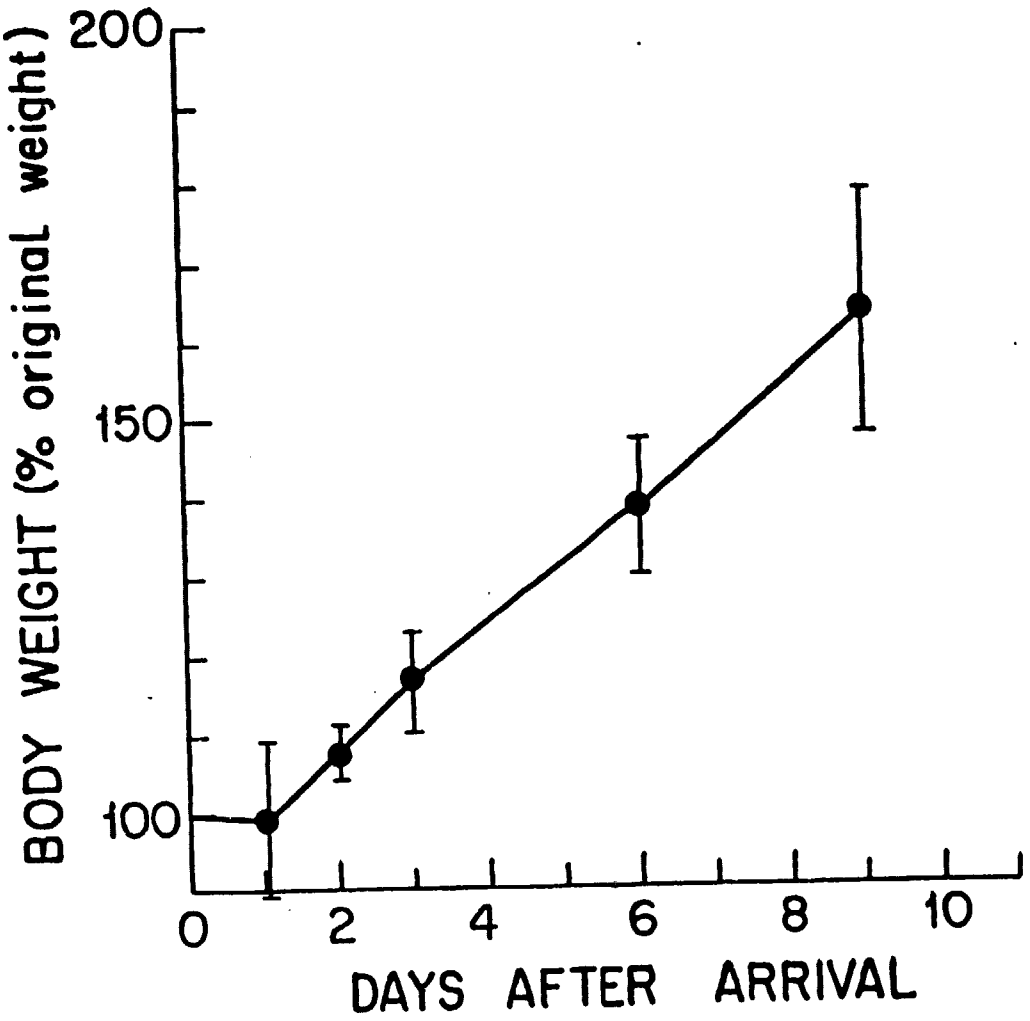


FIGURE 24

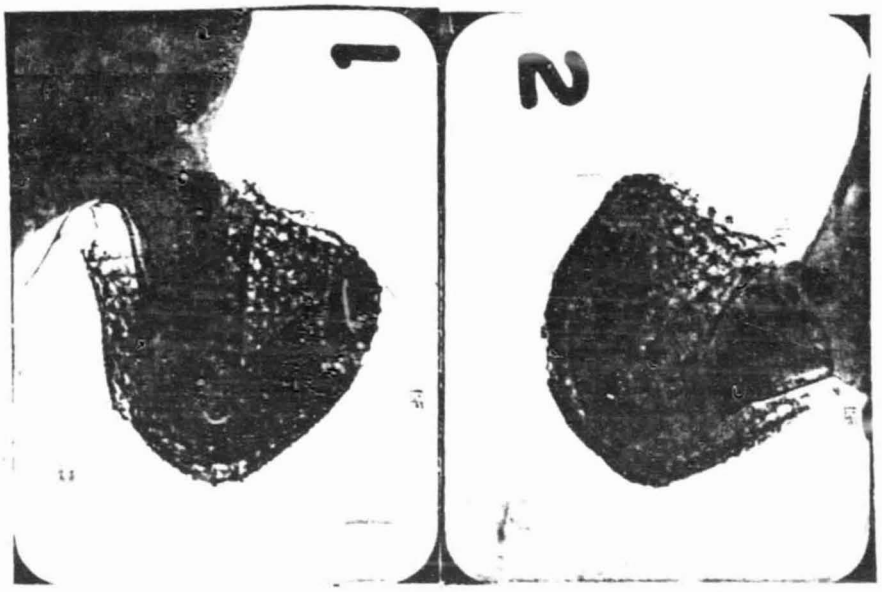


FIGURE 25

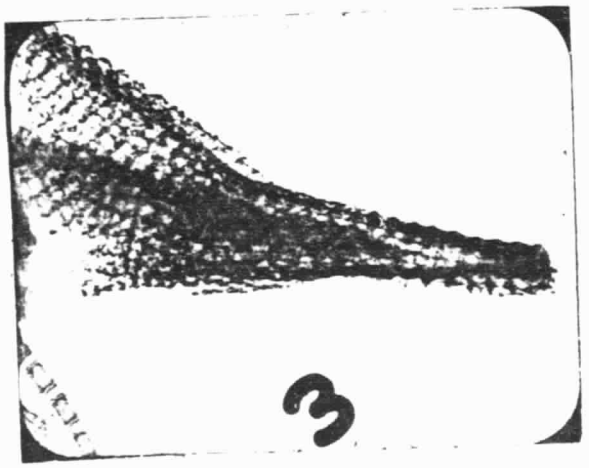


FIGURE 26