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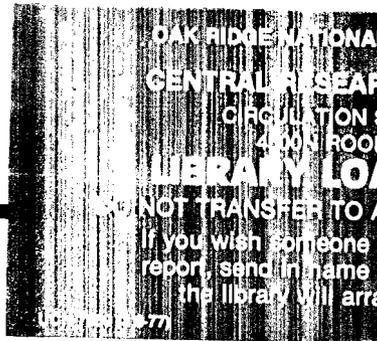
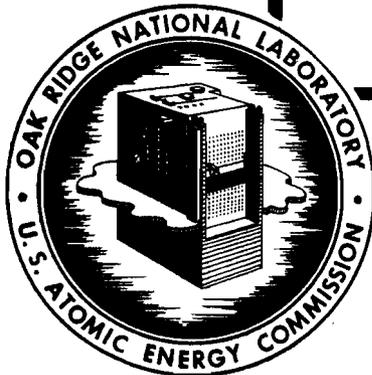


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ORNL 1048
Health Physics
Waste Disposal
90

PREPARATION OF BIOLOGICAL SAMPLES
AND CORRECTION OF DATA

Venus I. Knobf



OAK RIDGE NATIONAL LABORATORY
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Health Physics
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Date Issued

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PREPARATION OF BIOLOGICAL SAMPLES AND CORRECTION OF DATA

ABSTRACT

Numerous problems were encountered in the laboratory studies of fish taken from the Clinch River and White Oak Lake. Acid digesting and ashing of flesh of fish taken from White Oak Lake resulted in a small loss of activity but this treatment of bone and scale from the same fish produced no detectable loss. Ashing of samples reduced self-absorption and, thus usually permitted a more accurate counting measurement. Nitric acid digestion without the addition of sulfuric acid appeared preferably since the samples treated with both acids had higher ashed weights and were also more hygroscopic.

The internal organs and gills of fish from White Oak Lake often contained volatile radioactive isotopes. The inconsistency of results from aluminum absorption and decay studies indicated the presence of a diversity of short-lived, high energy isotopes. This discouraged attempting to correct for decay and self-absorption.

PREPARATION OF BIOLOGICAL SAMPLES AND CORRECTION OF DATA

INTRODUCTION

The studies of fish from White Oak Lake and the Clinch River system⁽¹⁾ called attention to numerous problems, indicating the need for improving the methods of preparing biological samples and of applying necessary corrections to the counting data. Some improvements were developed as the work progressed but additional experimentation seemed warranted. Shortly before starting the survey of fish in White Oak Lake in May 1949 and continuing to the present time, studies have been made which answered some of the questions raised. This work was not exhaustive because of time limitations and the number of problems involved.

All samples were prepared and dried in counting dishes and an end window GM tube counter was used for all counting. The following problems were considered:

1. Preparation of samples for counting
 - A. Sample geometry (contour of surface of sample)
 - B. Deliquescence of samples
 - C. The effect of digestive acids on the ash content
2. Volatilization of radioactive isotopes during preparation

3. Self absorption in samples
4. Future preparation and counting of samples.

Preparation of Samples for Counting

Several methods of sample preparation were tried and evaluated as a means of expediting the processing and of overcoming some of the physical factors which interfered with the reliability of results.

Flesh from a contaminated fish was homogenized with water in a Waring blender and four series of eight samples each (a total of 32 samples) were prepared in small porcelain dishes. All samples were dried slowly, care being taken during the drying period to keep them spread evenly and firmly over the bottom of the dishes. An infra-red lamp was placed on a ringstand at a height of two feet and the samples were kept approximately two feet from the base of the stand outside the area of the most intense light and heat. Without such precautions the samples shrank and buckled and uniform spread of samples could not be obtained. After all samples appeared to be dry, they were kept in the drying oven (at 95°C) for about eighteen hours.

All samples were weighed after drying and then counted. Following this procedure the four sets of samples were treated differently. The first group of eight was muffled at 450°C without

acid treatment; the second series was treated with nitric acid and muffled; the third was treated with nitric acid plus sulfuric acid and then muffled. The fourth series was not ashed and served to provide unashed controls.

The ashed samples were cooled in a desiccator after removal from the muffle furnace, re-weighed as soon as they were cool, and kept in a desiccator after weighing. Only a few samples at a time were taken to the counting room and care was taken not to expose any samples to the atmosphere unnecessarily. The last counts on the 24 ashed samples were made within five days of the first counts on the unashed samples. By recounting the controls the loss from decay was found to be insignificant.

From these experiments, it was found that the addition of sulfuric acid slightly decreased the time of preparation and consequently reduced the lapse of time between the catch and the counting of the samples. However, the weight of the ash was increased (Table I) and the samples seemed to be more deliquescent. There was no evidence that volatile elements, (such as ruthenium and iodine) were more fully retained by the addition of sulfuric acid. Any iodine present would have been lost by muffling with or without acid pre-digestion.

Table I

Comparison of Average Percentages of Ash Obtained from Three Separate Series of Eight Samples in Each
of Flesh, Bone, and Scale Following Treatment in Three Different Ways

Treatment	Per Cent Ash		
	Flesh	Bone	Scale
Dried and Muffled (average of 8 samples)	5.13	61.2	44.2
Dried, HNO ₃ digested and muffled (average of 8 samples)	5.32	61.2	46.2
Dried, HNO ₃ + H ₂ SO ₄ digested, and muffled (average of 8 samples)	5.67	79.7	54.8

On the basis of data (Table I) there appears to be no significant differences between the ashed content of the dried and muffled samples and those treated with nitric acid before ashing in the muffle furnace. The greater amount of ash content apparent in the samples of bone and scale treated with sulfuric acid might be explained by the reaction produced by calcium and sulfuric acid. The comparative amount of sulfuric acid and of bone or scale would determine the extent of the reaction and the per cent of excess ash.

Samples were prepared with extreme care by allowing only partial combustion of samples before complete ashing. This was accomplished by inserting a sample into the furnace and quickly withdrawing and re-inserting. The pre-digested and dried samples did not have the same tendency to explode in the muffle furnace with the consequent loss of sample, which occurred with undigested samples, (presumably because of the entrapped water vapor). The addition of acid also increased the cohesiveness of the sample, a desirable quality in uncovered samples requiring subsequent handling.

The importance of cooling the samples in a desiccator and weighing immediately was investigated. A number of samples were left out of the desiccator overnight and then weighed. Different weight increases were noted, the greatest increases being in the samples

treated with sulfuric acid. All of the extra weight was not lost by drying in the oven at 100°C for several hours but was driven off by about ten minutes of exposure in the muffle furnace at 300°C.

Inasmuch as the addition of sulfuric acid was perhaps more deleterious than advantageous, and since nitric acid digests bone and scale readily, it is recommended that only nitric acid be used in future work where digestion or ashing seems advisable.

Volatilization of Radioactive

Isotopes During Preparation

It was expected that the radioactive iodine which might be present in fish flesh from White Oak Lake would be lost by muffling but it was unknown whether ruthenium would be lost by ashing after acid treatment. Little ruthenium or iodine was expected to be found in the flesh, bone, and scale. Five radiochemical analyses of White Oak Lake water during the previous two years had indicated that from 12 to 37 per cent of the beta activity in White Oak Lake was contributed by ruthenium. Consequently it seemed advisable to investigate the effect of our method of processing on any radioactive ruthenium which might be present in the fish samples.

An uncontaminated fish was procured and the flesh separated from the bone. Eleven counting dishes were prepared with equal amounts of Ruthenium¹⁰⁶ pipetted into each (giving approximately 6,000 counts per minute when counted on the second shelf). Counts were taken after the dishes were dry and then six various size samples of flesh and five various size samples of bone were added to the dishes.

Acid was added from a medicine dropper in amounts sufficient to digest the tissue into a uniform mixture which could be spread evenly over the bottom of the counting dish. From one to four milliliters of nitric acid were used for flesh samples and one-half to one milliliter for bone. Too much nitric or sulfuric acid caused trouble in drying and just enough sulfuric acid to speed up digestion was used in the preparation of all samples. The amount of sulfuric acid used ranged from three to twelve drops in the flesh samples and from two to five drops in the bone. Since bone digested quickly in nitric acid little sulfuric acid was needed.

A small amount of ruthenium was lost by muffling the samples longer than two hours at 425°C but much more, in varying amounts, was lost by muffling at 550°C for one-half hour (Table II). The flesh samples treated with nitric and sulfuric acids seemed to show a

Table II

Results of Muffling Ruthenium and Tissue Samples

Flesh Samples			
Net wet weight in grams	Treatment	% of Activity after muffling at 425°C 2-1/2 hours	% of Activity left after muffling at 550°C 30 minutes
1.468	No premuffling treatment	95	30
1.496	HNO ₃ digested	94	15
1.495	HNO ₃ + H ₂ SO ₄	95	49
2.914	HNO ₃ + H ₂ SO ₄	94	66
4.700	HNO ₃ + H ₂ SO ₄	99	69
8.263	HNO ₃ + H ₂ SO ₄	100	80

Bone Samples			
		40 Minutes	30 Minutes
0.0368	No premuffling treatment	85	68
0.0268	HNO ₃ digested	93	89
0.0337	HNO ₃ + H ₂ SO ₄	97	89
0.0684	HNO ₃ + H ₂ SO ₄	98	74
0.1277	HNO ₃ + H ₂ SO ₄	100	37

direct relationship between the weight of tissue and the amount of activity left (49% to 80%) after muffling at 550°C. (The amount of sulfuric acid used had been increased with the size of the sample). The sample which had no acid added still had 30% of its activity left and the one with nitric acid added had 15% left after muffling at 550°C. (HNO_3 is an oxidizing agent; RuO_4 is very volatile at 100°C and RuSO_4 is not very volatile.)

The bone samples treated with nitric and sulfuric acids had a greater loss of activity as the sample size increased. Because of the ease of digesting bone only small amounts of acids were used. This fact as well as the presence of large amounts of calcium in the bone and the rapidity of reactions of sulfuric acid and calcium should be kept in mind when comparing these results to the results obtained with flesh samples. The reason for the large amount of activity left in the bone sample that did not have acid added and also in the one to which only nitric acid was added is not apparent.

Further work should be done on this problem if the amount of ruthenium found in future fish samples justifies it. More complete chemical analyses have been planned for different biological specimens

which should assist in determining the necessity for more experimentation with ruthenium.

Cesium

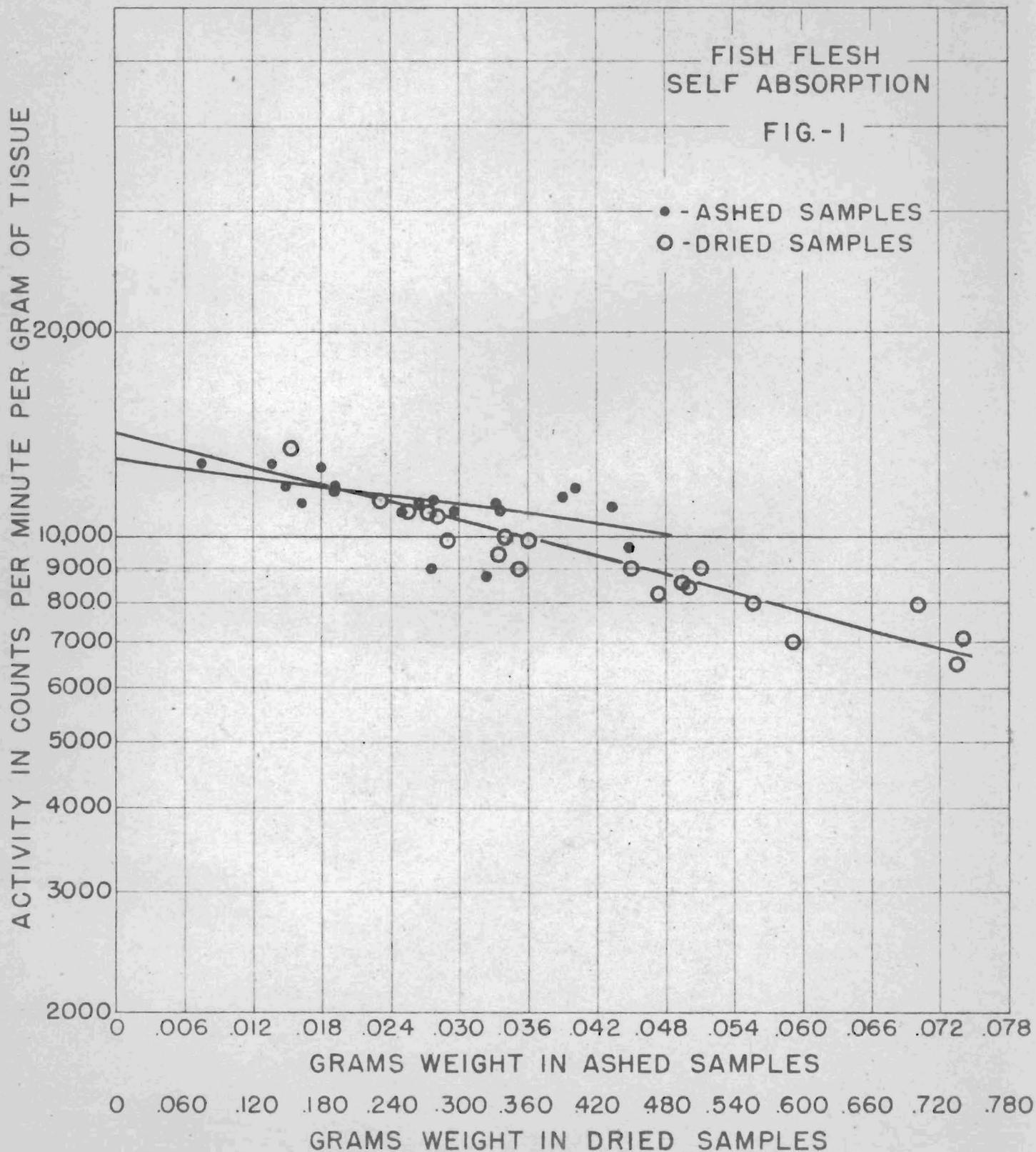
Since there was considerable Cesium¹³⁷ in the flesh of fish the possibilities of losing cesium were considered. Four samples of flesh were prepared, one without any acid, one with nitric acid, and two with different amounts of nitric and sulfuric acids. They were muffled at 500°C for over an hour and there were no losses of activity.

Self Absorption in Samples

The 32 flesh samples previously discussed (see page 6) were used for studying self-absorption. These samples ranged in dry weight from 152 to 1036 milligrams and in ash weight from 7.5 to 45 milligrams.

The self-absorption values for dried and ashed flesh are plotted in Figure 1. By extrapolating to zero self-absorption the activity in the ashed samples was found to be approximately 93 per cent of the activity indicated for zero absorption in the unashed samples. This difference is believed to have been caused by loss from volatilization in the muffling process.

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The calculated loss from spread of sample in the 4.2 centimeter diameter porcelain dish was 14 per cent. This value was arrived at by comparing counting results of samples of pure isotopes. (Experiments are being continued and are not included in this report.)

To procure data for corrections for self-absorption in fish flesh, points were read from Figure 1, translated to per cent, and calculated as indicated in Table III. The "efficiency of the counting determination", last column in Table III, is plotted against sample weight in Figure 2.

The observed specific activity of bone and scale in relation to the weight of the sample is shown in Figure 3. Points were read from these graphs, translated to per cent, and multiplied by 86 per cent (14% loss from spread). The resulting points were plotted in Figures 4 and 5.

The self-absorption curves for ashed and unashed samples of scales were collinear. The bone was not pulverized sufficiently to spread evenly over the surface of the counting dish and the absorption within the particles caused an apparent distortion in the upper part of this curve. Since these data were obviously in error the points were plotted but a curve was not drawn (Figure 3).

Table III

Calculations for Determining Accuracy of Counts

Obtained on Ashed Samples

Flesh

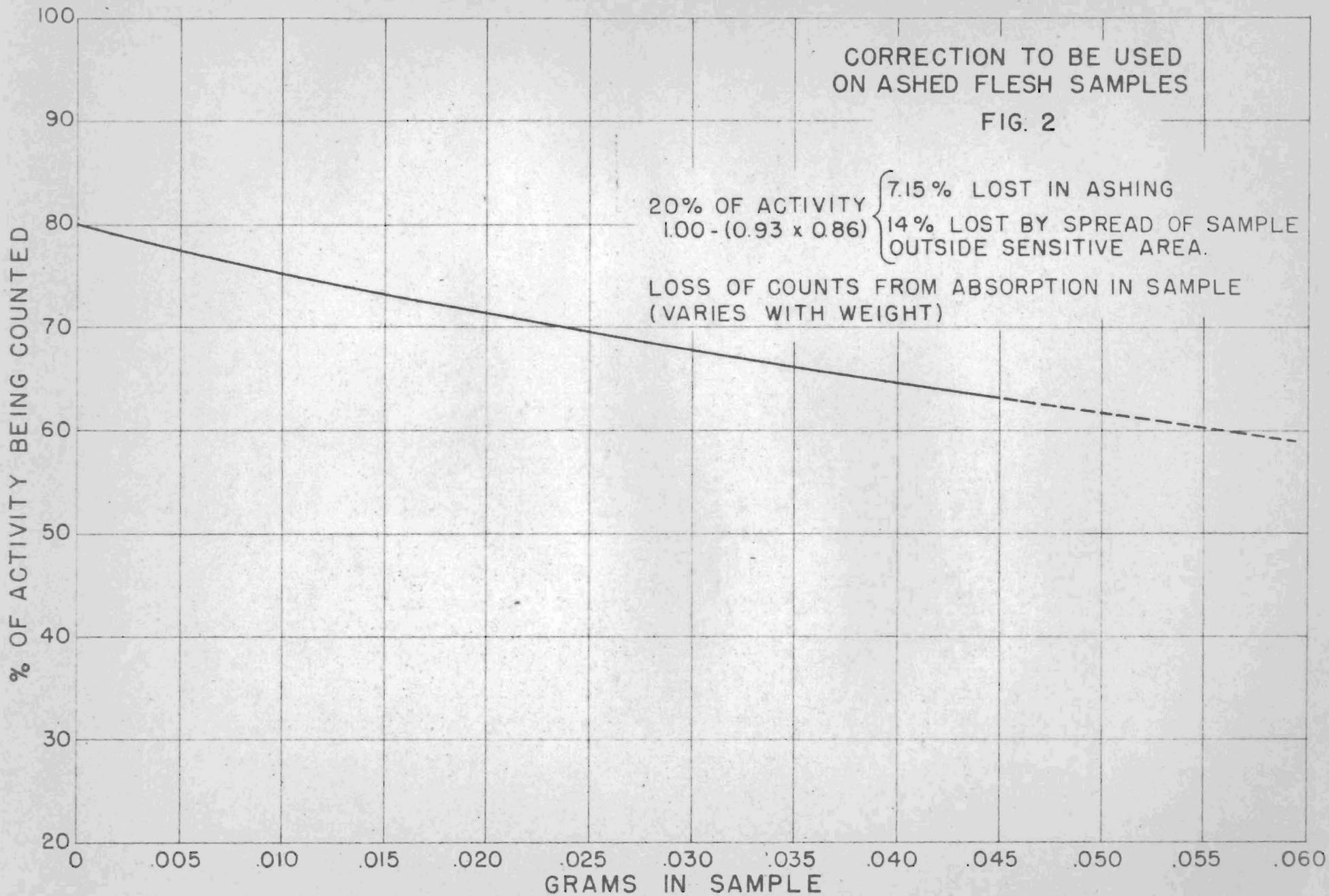
Weight of Sample gms.	Activity Measured c/m	Measured Activity in terms of %		Activity Left After Ashing %		Correction for Spread %	=	Efficiency of Counting Determination %
C	13,000	100	x	93	x	86	=	80
0.015	12,000	92	x	93	x	86	=	74
0.033	11,000	85	x	93	x	86	=	68
0.051	10,000	77	x	93	x	86	=	62

Scales

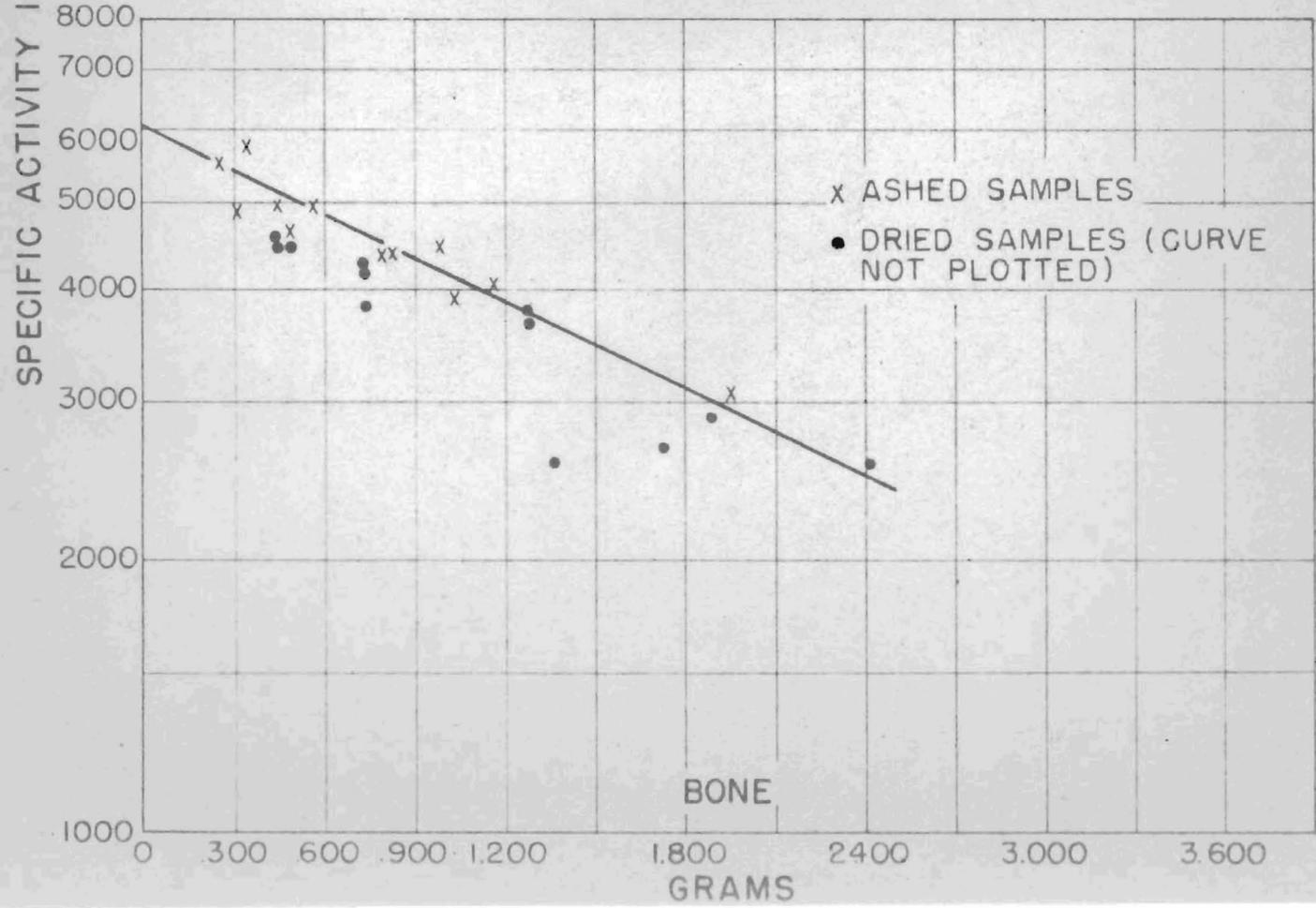
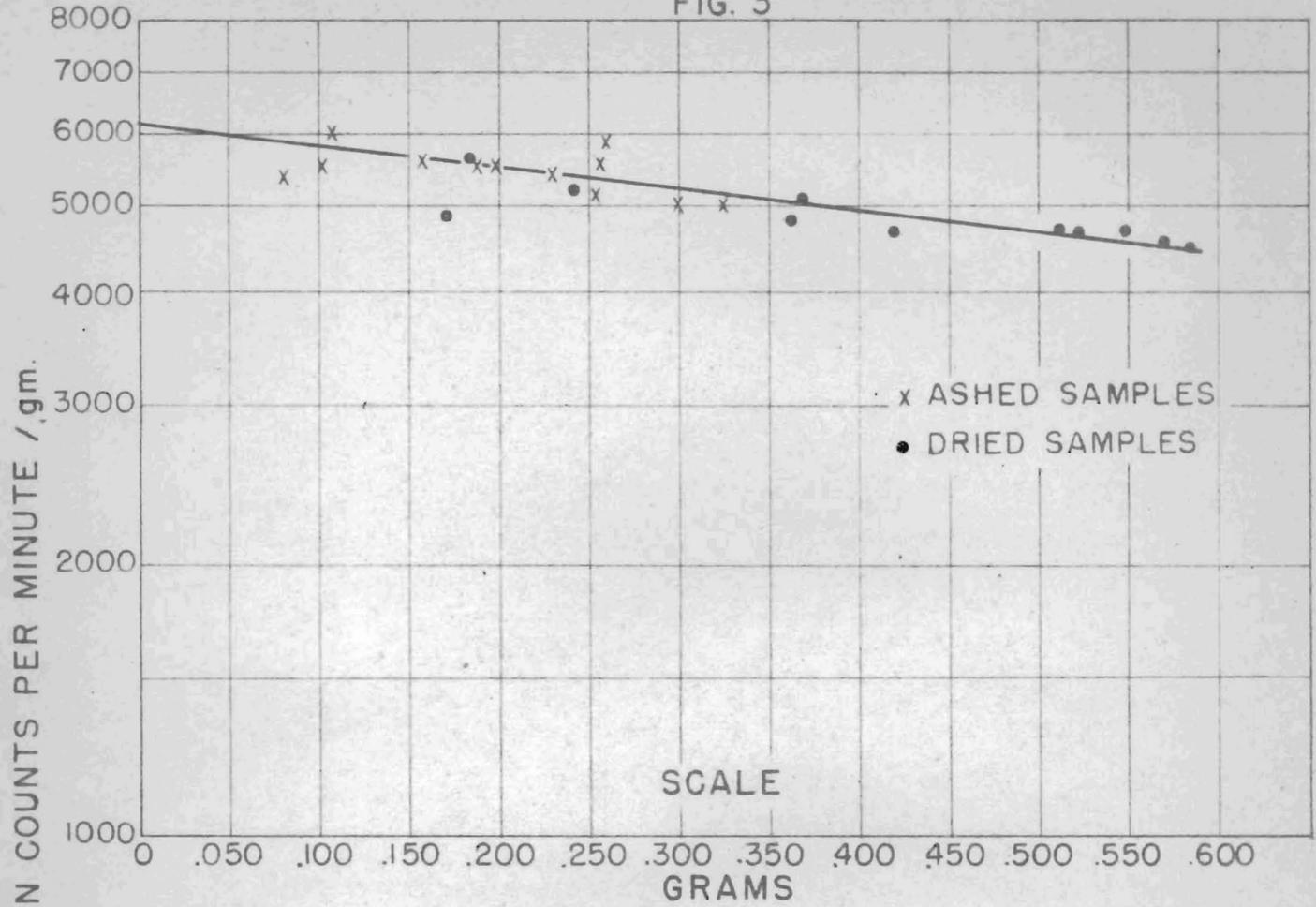
0	6,150	100	x	100	x	86	=	86
.300	5,200	84	x	100	x	86	=	73
.520	4,600	75	x	100	x	86	=	64
.570	4,460	72	x	100	x	86	=	62

Bone

0	6,100	100	x	100	x	86	=	86
.600	4,800	79	x	100	x	86	=	68
1,800	3,100	51	x	100	x	86	=	44
2.160	2,700	44	x	100	x	86	=	38



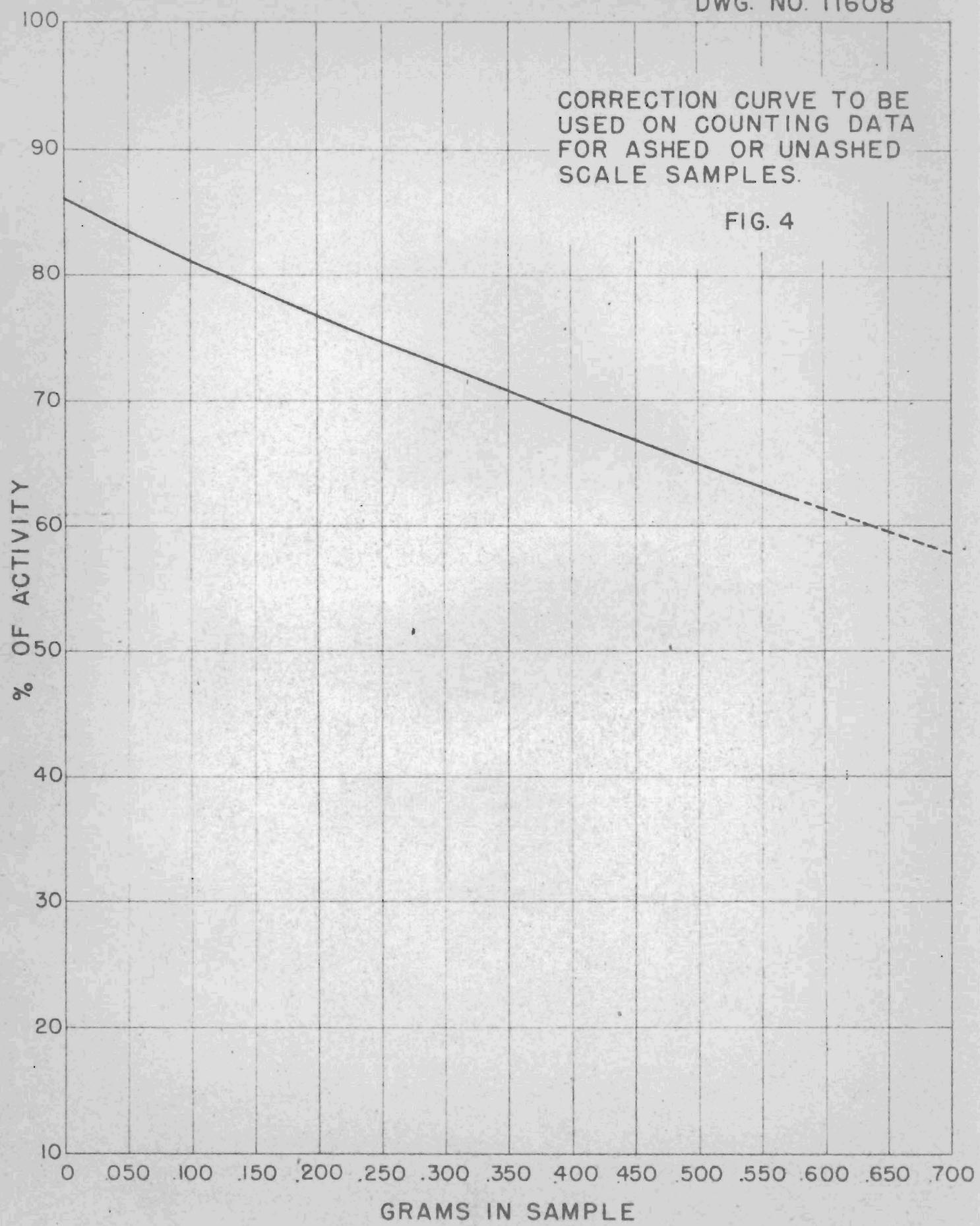
SELF ABSORPTION CURVES
FIG. 3



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CORRECTION CURVE TO BE
USED ON COUNTING DATA
FOR ASHED OR UNASHED
SCALE SAMPLES.

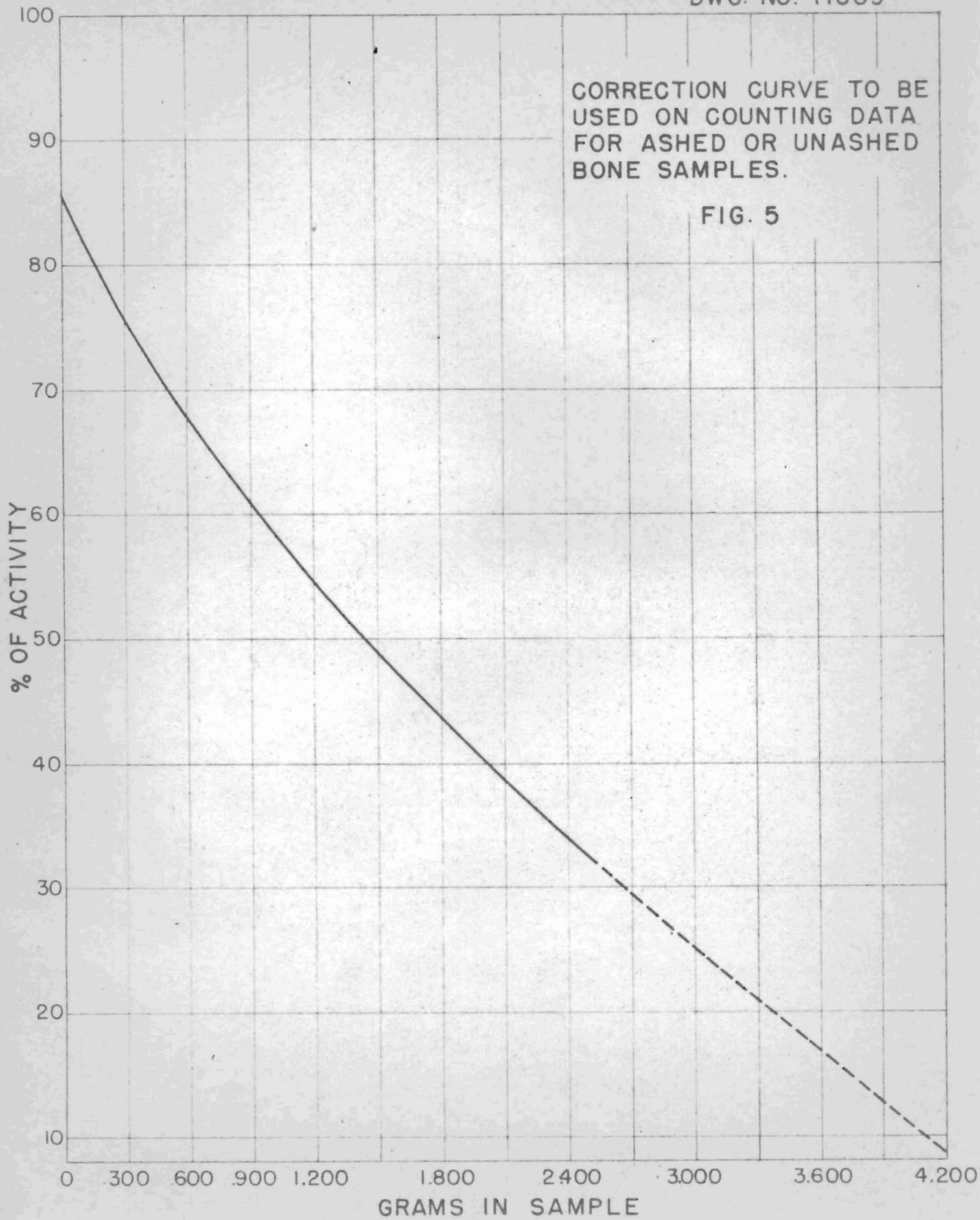
FIG. 4



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CORRECTION CURVE TO BE
USED ON COUNTING DATA
FOR ASHED OR UNASHED
BONE SAMPLES.

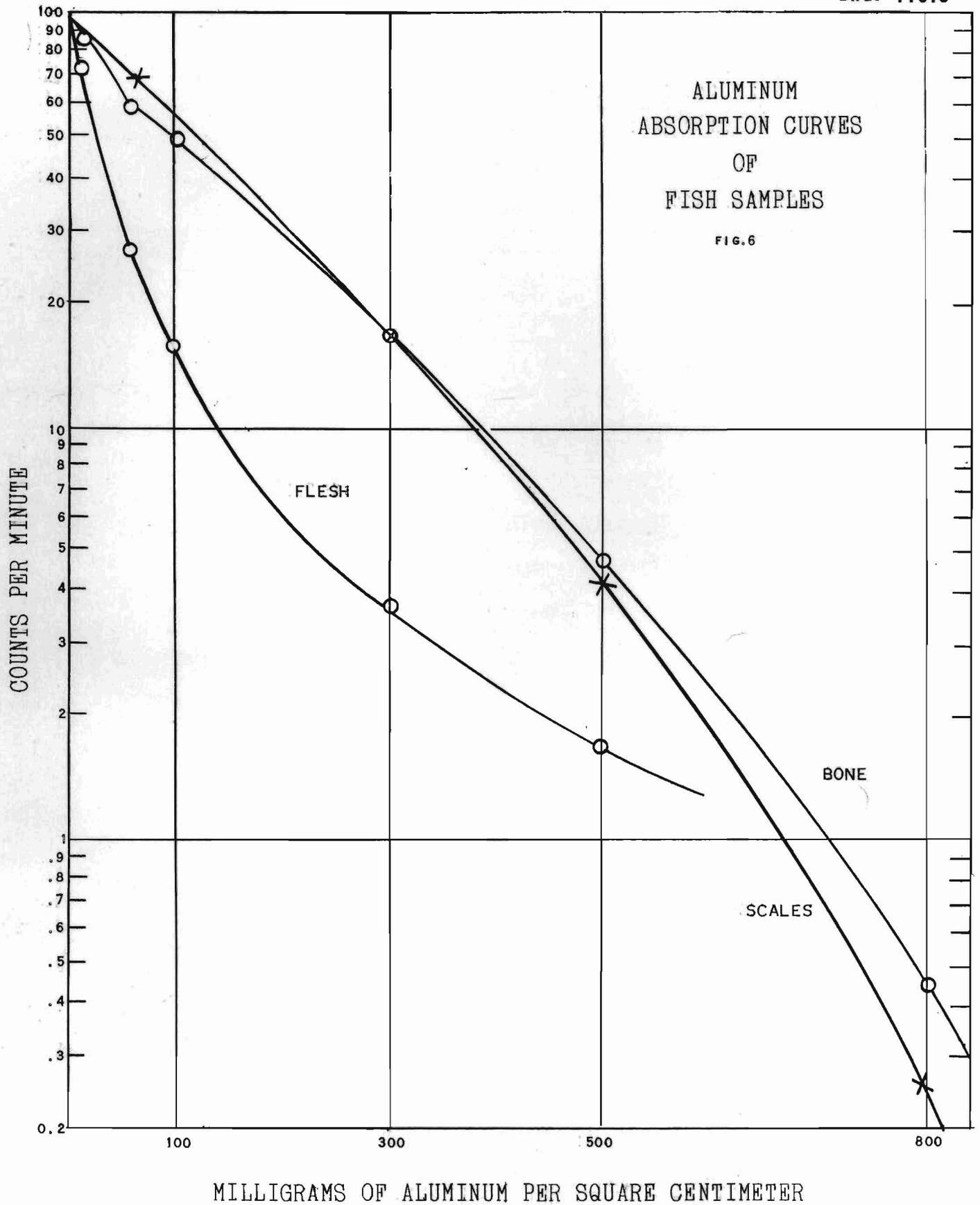
FIG. 5



There was no necessity to correct for volatilization since no volatile material was observed in these particular bone and scale samples. It is possible, however, that other fish might contain enough volatile radioactive isotopes in their bone and scale to be detectable.

Aluminum absorption curves on fish from various collection areas disclosed the presence of beta activity of a lower energy in the flesh than in the bone and scales, (Fig.6) Similar aluminum absorption curves were obtained from flesh, bone, and scale samples, respectively, of fish caught in the Clinch River and White Oak Lake. Therefore, it was believed that the same correction curves could be applied for these specific tissues from any collection area from which fish were studied.

Differences were noted in the aluminum absorption curves and the decay curves obtained for the specific internal organs of fish from the various collection areas and also in different fish from White Oak Lake. These organs also had variable amounts of volatile radioactive isotopes in a number of samples examined. Fish from White Oak Lake appear to have a diversity of comparatively short-lived hard beta emitting isotopes in most organs examined except the bone, scale, and flesh. Because of this it was difficult to determine self-absorption correction curves most applicable.



The upper part of the aluminum absorption curves for ovaries, testes, and heart usually disclosed the presence of lower energy isotopes than appeared to be present in the kidneys, liver, and gills⁽¹⁾. The few random checks for decay did not give consistent data but usually indicated shorter lived isotopes in the gills, liver and kidneys. Ovaries, testes, and heart contained shorter lived isotopes than flesh but because of the similarity of their absorption curves in the thinner aluminum absorber region (Fig. 7) the same correction curves (Figure 2) were used for correcting counting data⁽¹⁾. For the same reason the correction curves for scales (Figure 4) were used for correcting counting data obtained on kidney, liver and gill samples, (Fig. 8).

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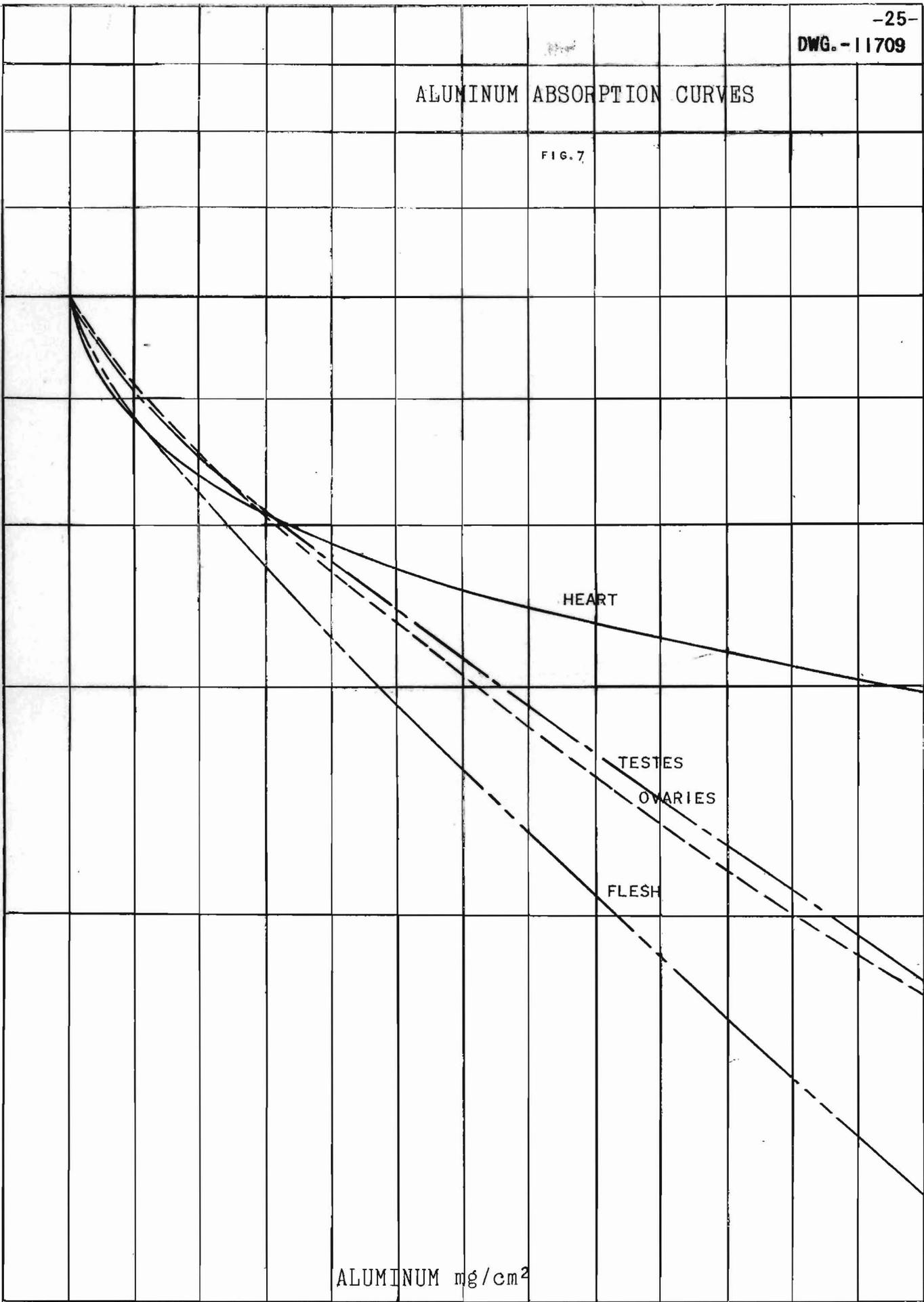
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2. Yankwich, Peter E. Correcting for Absorption of Beta Particles in Thick Samples, July 1946. CC-3567.

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ALUMINUM ABSORPTION CURVES

FIG. 7

RELATIVE COUNTS PER MINUTE

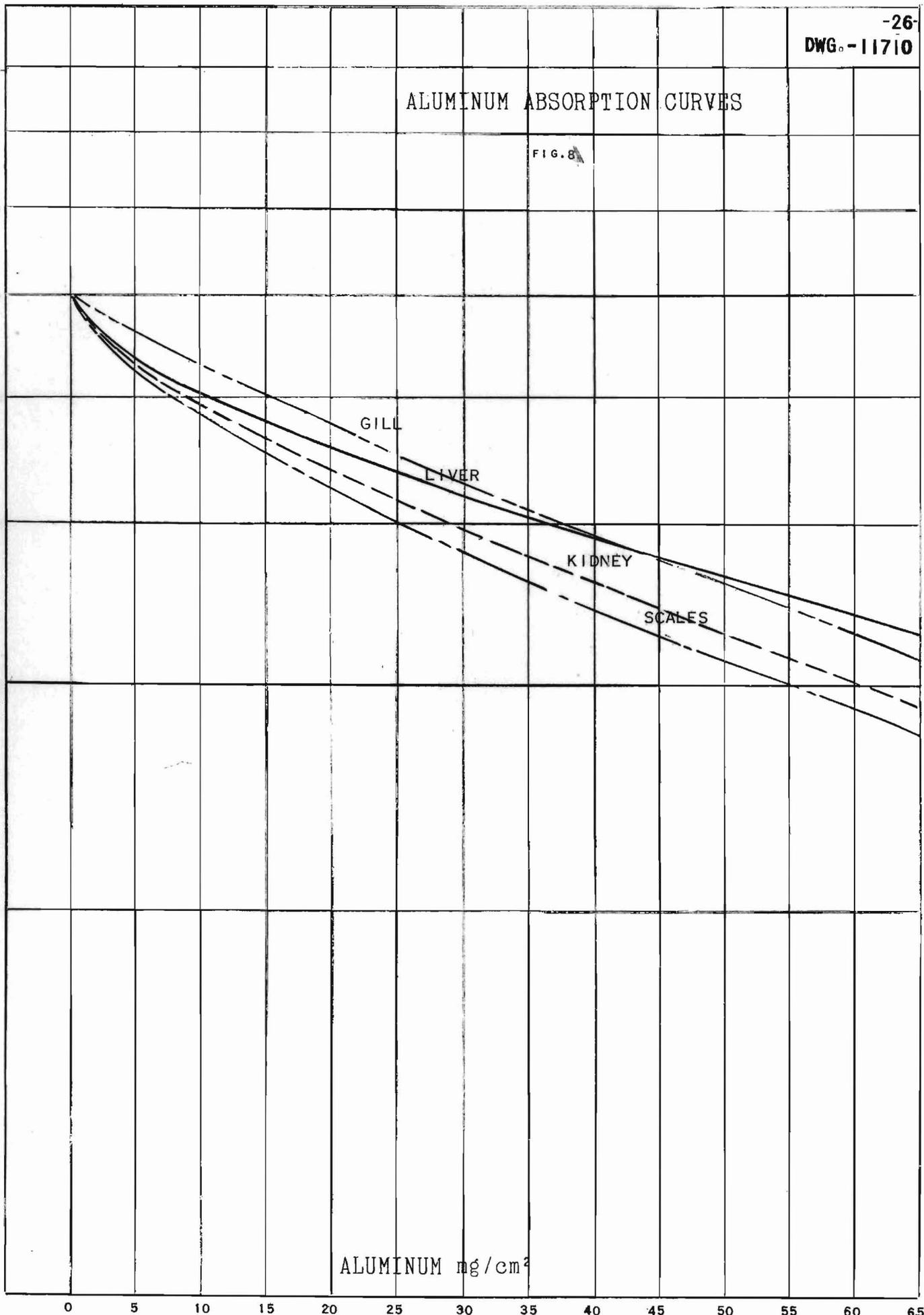


ALUMINUM mg/cm^2

ALUMINUM ABSORPTION CURVES

FIG. 8

RELATIVE COUNTS PER MINUTE



ALUMINUM mg/cm²