RECEIVED BY DTIE FEB 17 1970

MASTER

ANNUAL TECHNICAL REPORT

(COO-1181-1)

AEC No. At (11-1) - 1181

March 1, 1963 - Feb. 28, 1964

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TECHNICAL REPORT

AEC No. At (11-1)-1131

(WSU Account 306-1619) COO-1181-1

"THE RESPONSE OF THE RESPIRATORY TRACT AND LUNG TO INHALED STABLE AND RADIOACTIVE ISOTOPES OF CERTAIN ELEMENTS"

TO

THE DIVISION OF BIOLOGY AND MEDICINE
ATOMIC ENERGY COMMISSION
WASHINGTON, D.C.

FROM

THE DEPARTMENT OF INDUSTRIAL MEDICINE AND HYGIENE
COLLEGE OF MEDICINE, WAYNE STATE UNIVERSITY
DETROIT, MICHIGAN

Submitted By:

Harry J. Berke

Harry J. Berke, Ph.D.
Associate Professor
Department of Industrial
Medicine and Hygiene

Arthur J. Vorwald

Arthur J. Vorwald, Ph.D., M.D.
Professor and Chairman
Department of Industrial
Medicine and Hygiene

Approved By:

Ernest D. Gardner, M.D.
Dean, College of Medicine
Wayne State University

Clin E. Thomas, A.M.
Vice-President and Treasurer
Wayne State University

Date: February 1, 1964
Detroit, Michigan

fcg

TABLE OF CONTENTS

In the past year, long term exposures of animals to radioactive aerosols have been carried out in furtherance of our efforts to delineate more clearly the sequence of events following inhalation of radioactive materials.

The distribution-excretion patterns following repetitive daily inhalation as well as retention data for the lung and whole body are being evaluated at present and an attempt will be made to correlate the body burden with the effect. The emphasis during this period has been concentrated on the pathology following the deposition of Europium$^{152-154}$ particularly as reflected in changes in pulmonary morphology and function.

In addition a single high dose of both Eu$^{152-154}$ and Scandium^{46} were administered via inhalation. The animals were used to determine the disposition of the inhaled aerosol in terms of the usual parameters - distribution, excretion, retention, but not effect.

The choice of Eu$^{152-154}$ and Sc^{46} was based on the reported findings that, although in soluble form (chloride nitrate), these were well retained in the parenchyma of the lung following inhalation. These isotopes along with Yttrium (a pure Beta emitter, also showing high lung retention) are considered useful for our objectives as stated in earlier reports. The retention half time in the lung of these isotopes is on the order of 45 days.

The results of previous experiments using high levels of $Eu^{152-154}$, Sc^{46}, and Co^{60} have been evaluated and are presented. Tissue values of activity, weight changes and the results of blood examination for both control and experimental animals are in process of evaluation.

I. INHALATION EXPOSURES OF ANIMALS TO RADIOACTIVE ISOTOPES

A. EUROPIUM$^{152-154}$ - SHORT-TERM STUDIES*

Mice and rats were exposed to aerosols containing small amounts of radioactive Europium (10^{-3} to 10^{-5} microcuries/ml of air) both in the chloride and oxide form. These studies involved either single or repetitive short (30 minute) exposures.

The isotope was also given intraperitoneally and subcutaneously in order to clarify the patterns of translocation by the various pulmonary clearance mechanisms following particulate deposition.

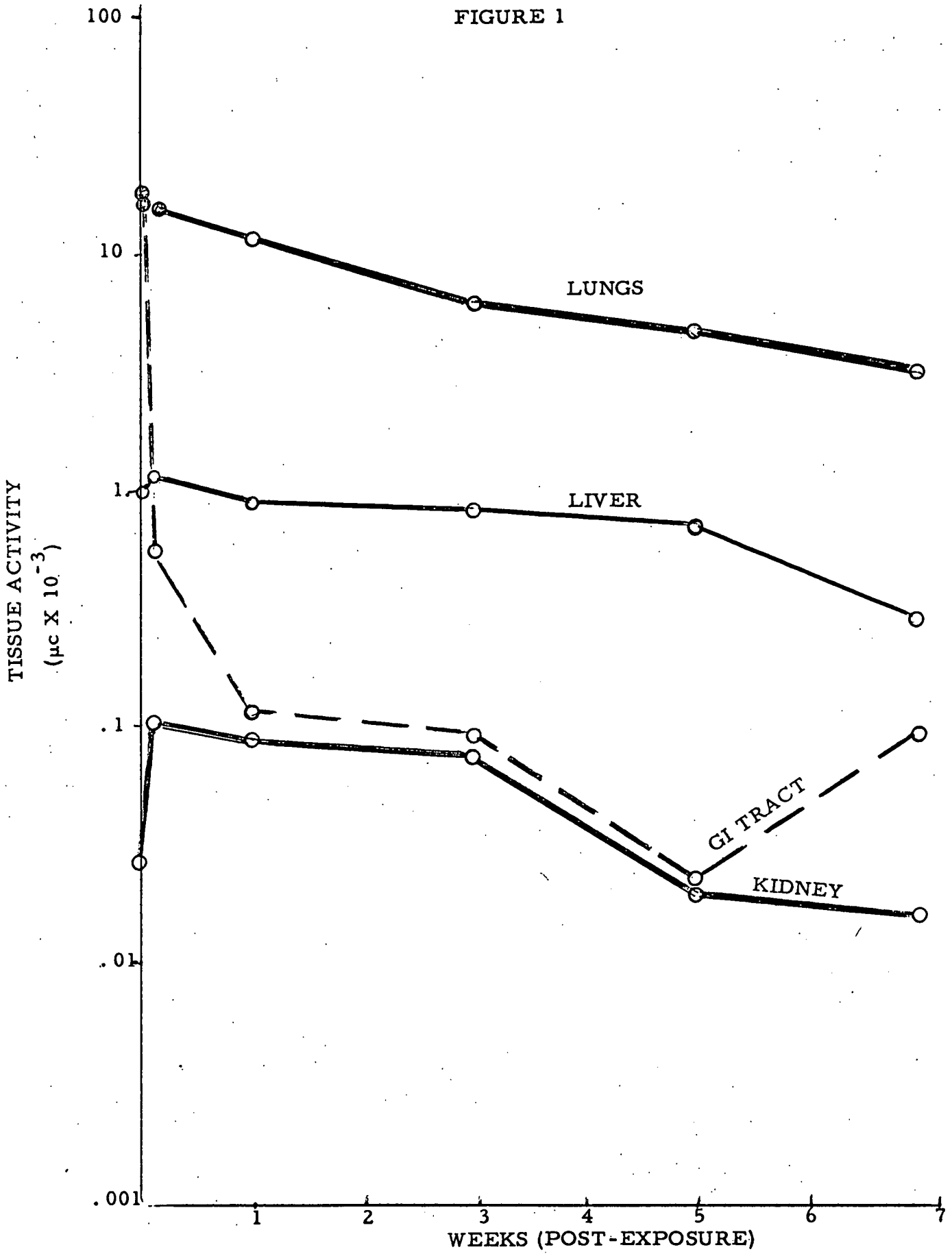
Following single exposure to the oxide, pulmonary activity declines exponentially except for the more rapid clearance rate observed during the first 24 hours following the deposition (Figure 1).

Other organs show variable but slow rates of decline. Following chronic exposure, the body burden also increased logarithmically as did the retention as determined 24 hours later (Figure 2) indicating the attainment of a steady state in

*This material has been published as a Masters Degree Thesis. Donald H. Willard, June 1963, Wayne State University.

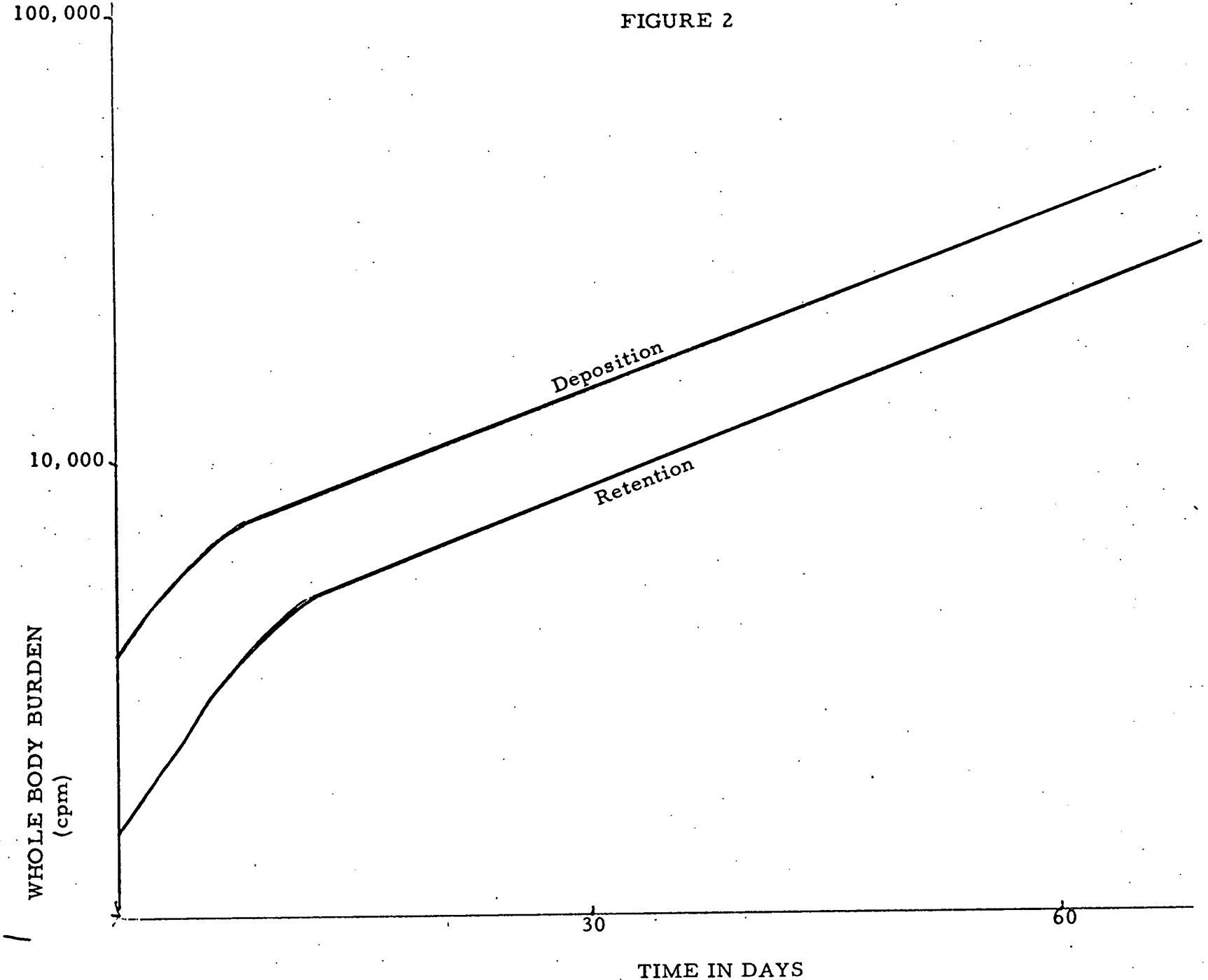
TISSUE CONTENT FOLLOWING SINGLE EXPOSURE OF MICE TO Eu^{152} OXIDE

FIGURE 1



DEPOSITION AND RETENTION FOLLOWING REPETITIVE EXPOSURES TO Eu^{152} OXIDE IN RATS

FIGURE 2

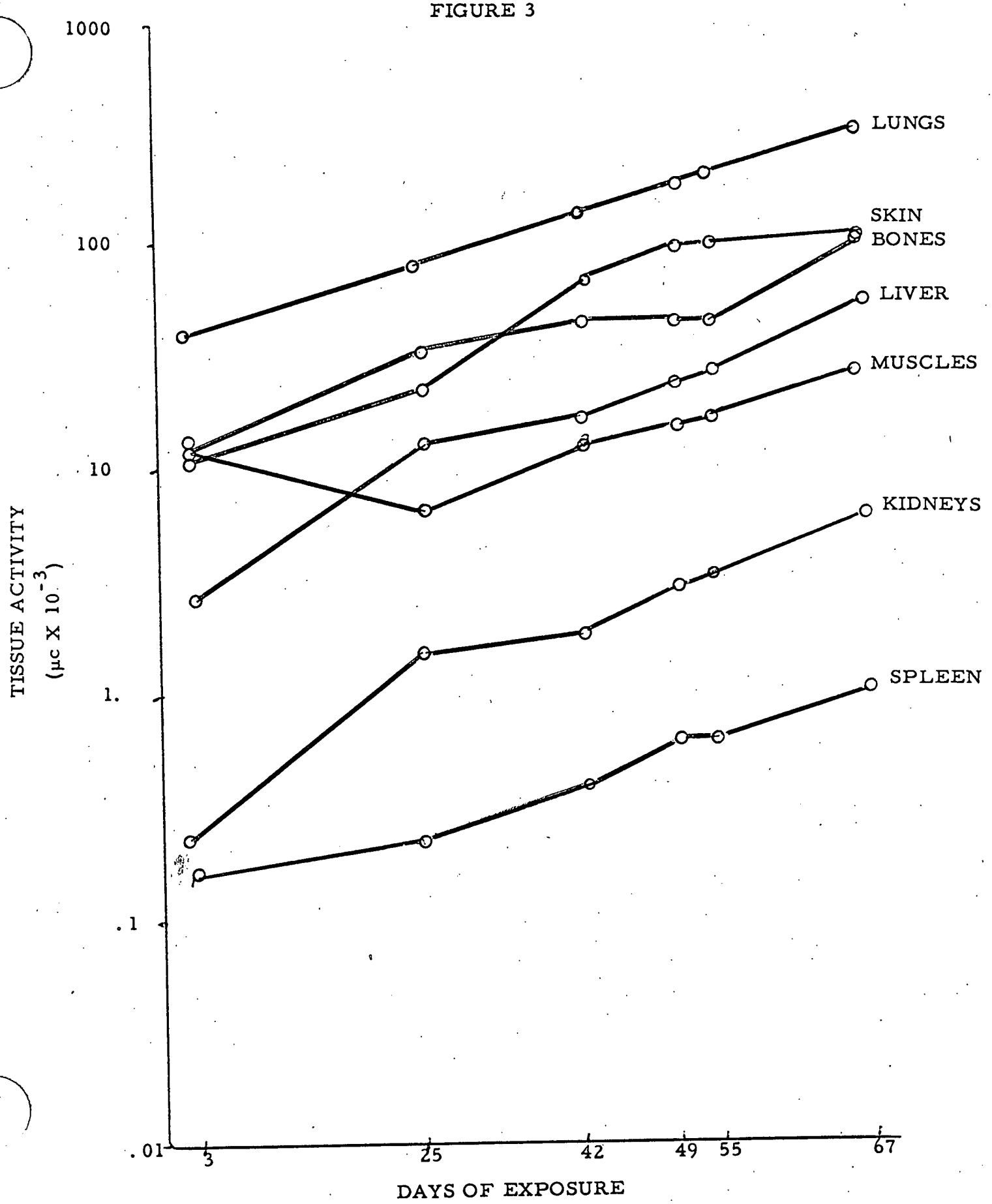


terms of accumulatory percentages of what is already present in both cases. Tissue contents of activity increased as shown in Figure 3. During the entire period of exposure, the lung activity increased exponentially while other organs except spleen showed variable rates of accumulation.

Briefly, a large percentage of the deposited isotope seems to be cleared from the lung by ciliary action. While particle sizing could not be done because of lack of facilities when this study was being done, the aerodynamic characteristics of the particulates are such that they deposit in the upper respiratory tree. Clearance from the lung is rapid for the first day following exposure and rather slow thereafter. It is important also to note that the isotope whether as the chloride or oxide is eliminated from the whole body and lung in a similar fashion.

TISSUE ACTIVITY FOLLOWING REPETITIVE EXPOSURES OF RAT TO Eu^{152} OXIDE

FIGURE 3



B. YTTRIUM^{91}

Inhalation studies using aerosols of Y^{91} chloride and oxide were conducted in a special exposure chamber which was designed especially for nose exposures of mice and rats. The 8 inch plexiglass chamber was so designed to allow exposure of 84 mice. The mice were enclosed in a 50 ml. conical centrifuge tube which has a 3/8 inch hole cut in its tapered end. The mice were held forward in the tubes by a rubber stopper.

In both the acute and chronic exposures, the generation of the aerosol was accomplished by an air-jet atomizer. The atomizer used in the acute exposure of both oxide and chloride was easily constructed. It consisted of two plastic (5/8 inch I. D.) tubes joined together at right angles to facilitate the generation of the aerosol for 30 minute intervals. For the chronic exposure a similar type of generator was used, but a constant and continuous suspension of hydrosol was delivered to the generator for atomization.

Chronic whole-body exposures were carried out in a 1 cu. meter chamber. The rats were enclosed in a metal screen holding cage directly underneath the generator. No restraint was used to keep animals from licking themselves or receiving material deposited on the cages or food. The excess aerosol produced was filtered through an electrostatic

precipitator packed cotton, activated charcoal and then through a Cambridge absolute filter before discharging to the outside environment. Samples for determination of the radioactive aerosol concentrations were collected by millipore filters. Calculations of μc/cc air were determined by counting the filters in a proportional counter and correcting the results.

The Yttrium chloride used in the acute exposures was that received from Oak Ridge National Laboratories. The isotope was diluted with distilled water and atomized into the chamber.

The Yttrium oxide was made by precipitation of the Yttrium with oxalic acid. The oxalate was converted to the oxide by heating in a muffle furnace to 600° C. Ultrasonic generations were used to help suspend the hydrosol and to break up aggregates.

Serial sacrifices were performed by cervical dislocation. Each organ was separated and digested with nitric acid. Counting was performed by plating aliquotes on stainless steel planchets and counting the planchets in a Tracer Lab thin window proportional counter. Assay values were corrected for radiological decay.

Results

Yttrium Chloride

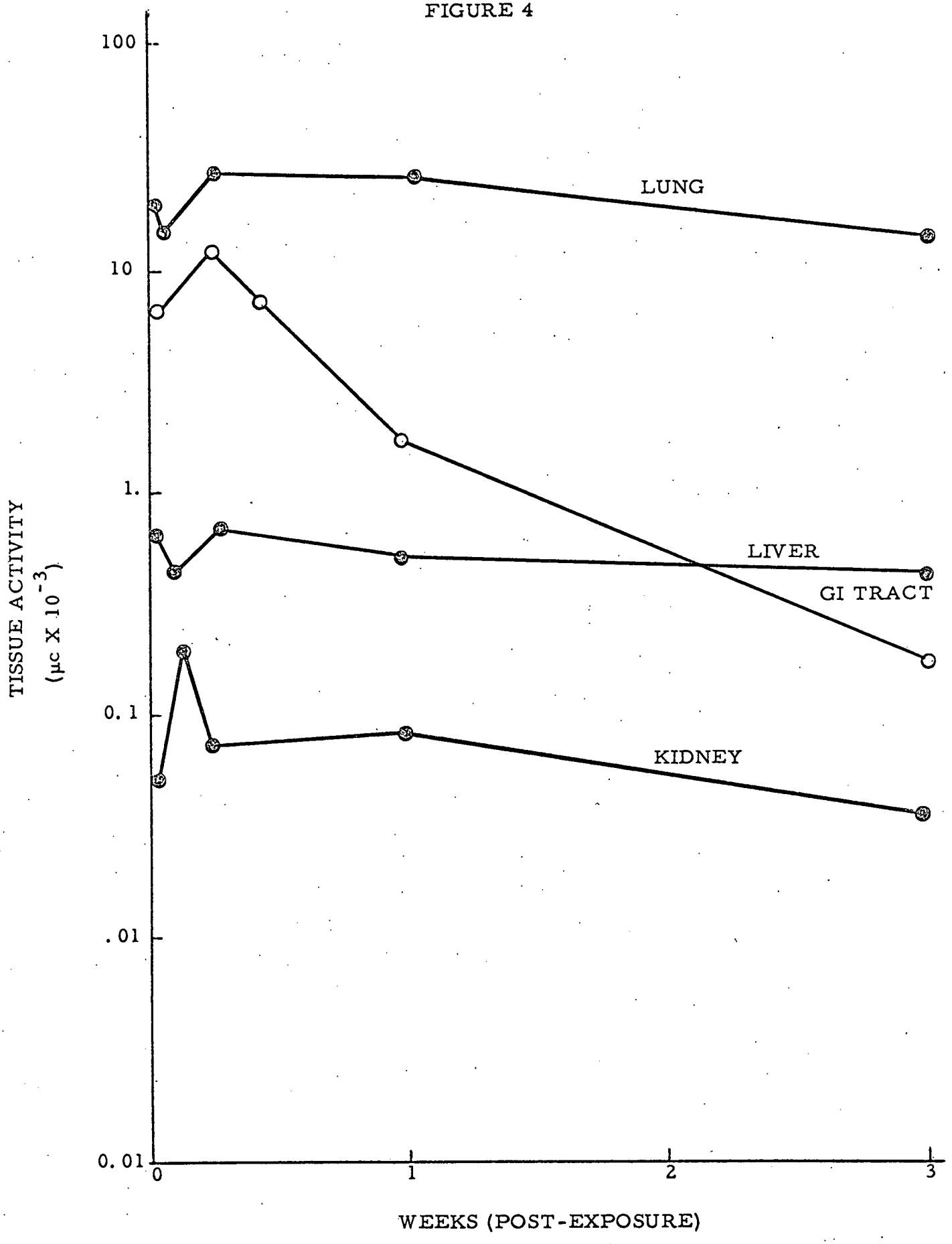
Immediately and at predetermined times after the cessation

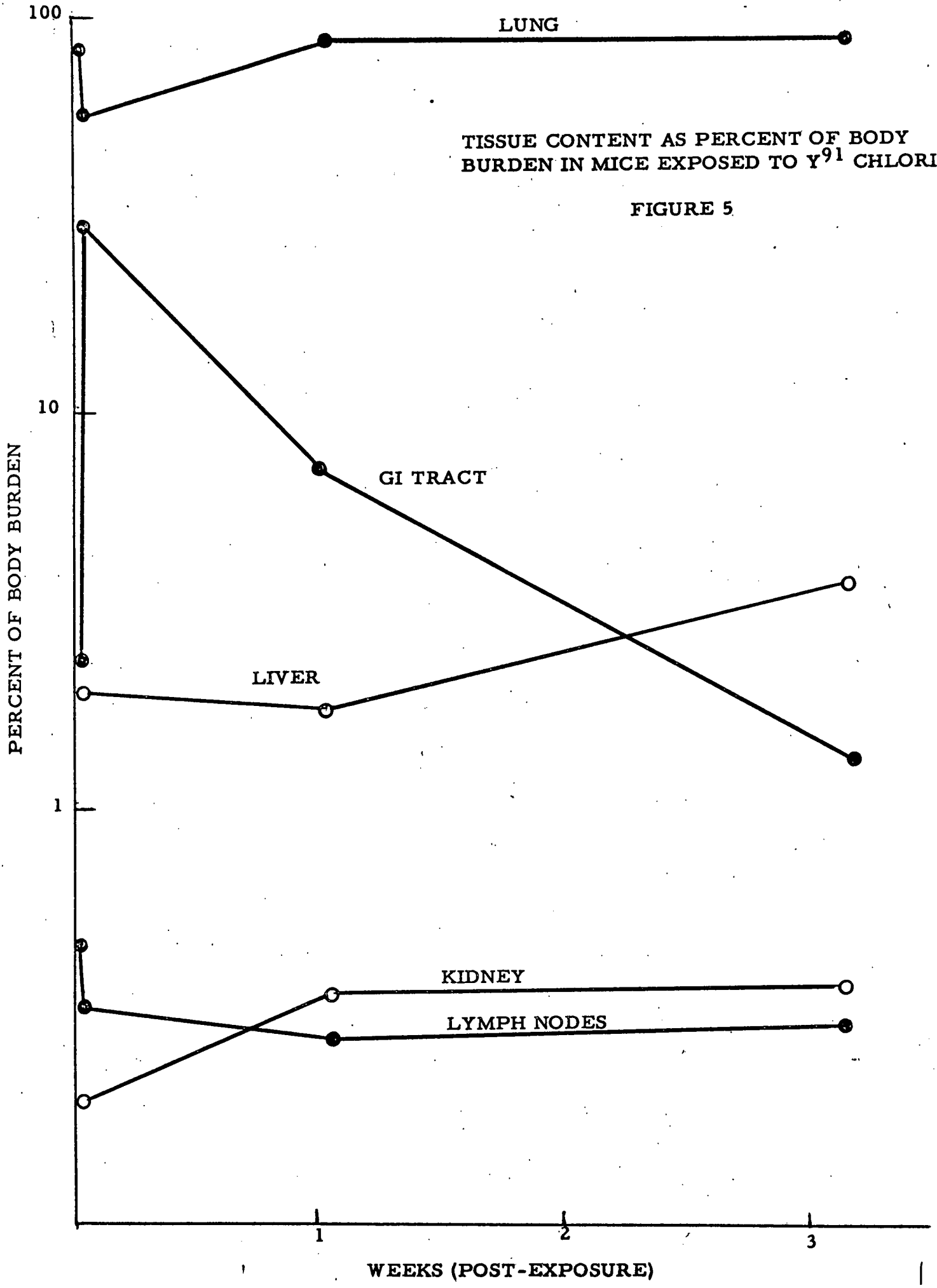
of inhalation, a single 30 minute nose exposure, mice were killed for radiochemical determination of the aerosol retention. If we assume that during a 24 hour post inhalation period, the aerosol deposited on the ciliated bronchi is largely eliminated by ciliary activity then the radioactivity that remains must be in the alveolar space. The retention data plotted as a function of time are given in Figure 4. In order to determine the elimination of the retained aerosol, groups of mice were killed after one to several weeks. In each case the lungs were analyzed individually and mean values were obtained from the radioactive analytical results. The rapid early lung clearance period, is followed by a more gradual decrease over the period of the first 3 weeks, reflecting biological redistribution for Y^{91} chloride from the various body compartments with differing half lives.

The distribution of Y^{91} remaining in tissues after inhalation is shown in Figure 5 and is expressed as the percent of the total body burden at time of sacrifice. The lungs contained 90% of the total body burden at all sacrifice dates except the two hour and 24 hour time period. The gastrointestinal tract had a high percent of the Y^{91} body burden at these times, reflecting the passage of Y^{91} from the upper respiratory tract to be excreted in the feces. The remaining activity

TISSUE DISTRIBUTION AFTER SINGLE
(30 MINUTE) EXPOSURE TO Y^{91} CHLORIDE

FIGURE 4





TISSUE CONTENT AS PERCENT OF BODY BURDEN IN MICE EXPOSED TO Y^{91} CHLORIDE

FIGURE 5

being almost entirely distributed in the liver, kidney, and lymph. No bone or muscle were taken for this phase of the experiment.

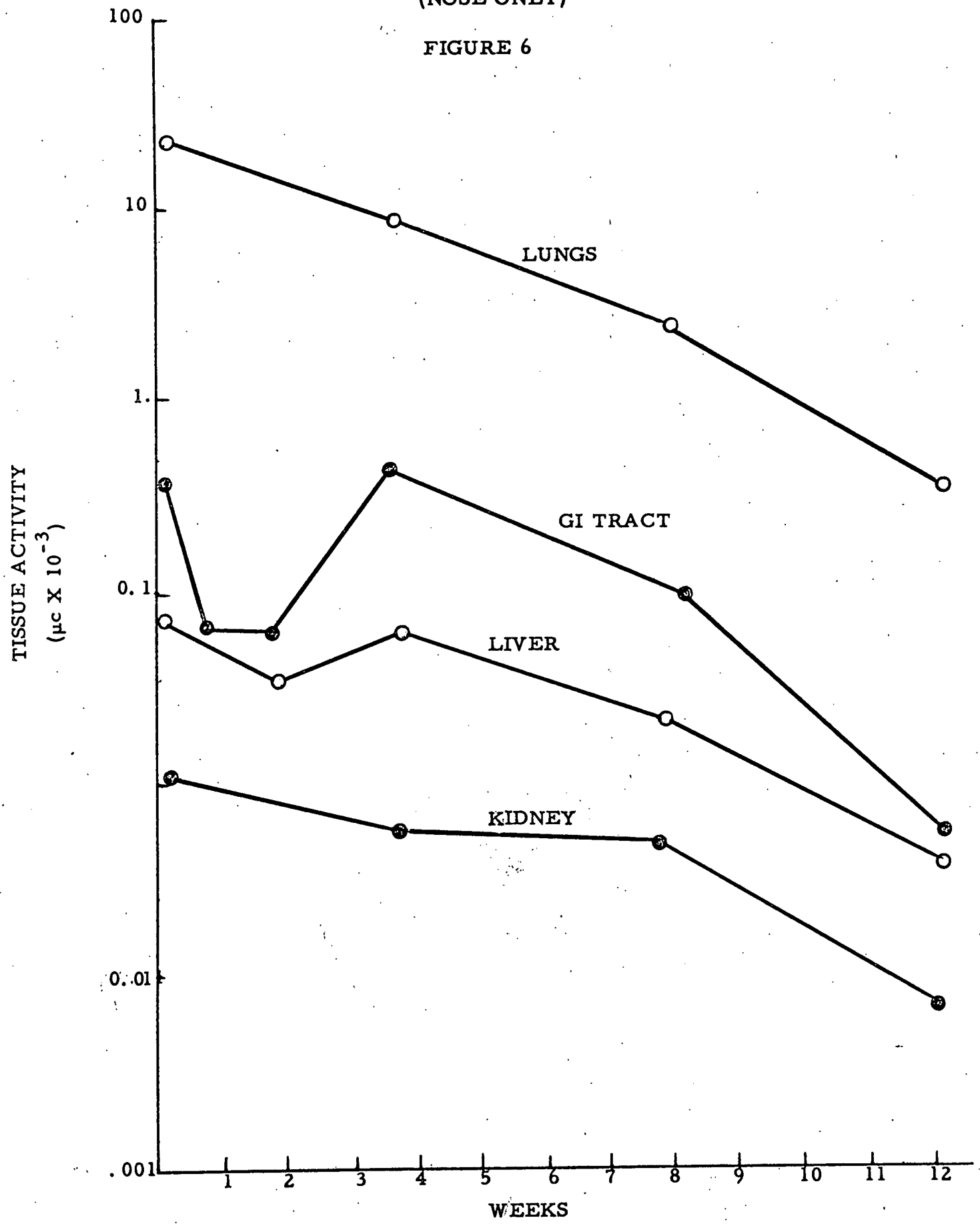
Oxide Inhalations

A sacrifice schedule similar to that used in the Y^{91} chloride study was followed after inhalation of Y^{91} oxide except for continued sacrifices up to 12 weeks. The mean tissue values for 6 mice are plotted as a function of time in Figure 6. Only representative tissues are shown, although all tissues of the mice killed were analyzed for radioactivity. The distribution of radioactive Yttrium following the oxide inhalation was in large part divided between lung, liver, and the gastrointestinal tract. The lower concentration of Y^{91} activity in the kidney may reflect the extent of solubility of the oxide in body fluids.

Almost immediately after exposure, some of the inhaled Y^{91} oxide was absorbed from the lung as shown by the level of activity in other tissues. Once the initial absorption occurred, only small additional absorption occurred. The amount of activity in the lung continued to decrease with time after exposure. There seems to be no increase in other tissues except the gastro-intestinal tract which increased at the fourth week.

TISSUE CONTENT AFTER SINGLE EXPOSURE TO Y^{91} OXIDE IN MICE (NOSE ONLY)

FIGURE 6



Comparisons of the curve for lung and the curves presented for the other organs indicate similar biological half lives.

A group of 44 Sprague Dawley rats were given chronic (7 hours daily) Y^{91} oxide exposures for 12 weeks. The concentration of aerosol in the chamber was 4×10^{-8} μc/cc air. Each sacrifice of 4 animals were replaced with new animals of the same age group. All remaining animals, after the chronic exposure sacrifices, will be observed for a maximum of 2 years. Only the data for the turnover and tissue distribution up to 12 weeks of chronic exposure will be reported here. The results of tissue distribution for chronic exposure are represented in Figure 7.

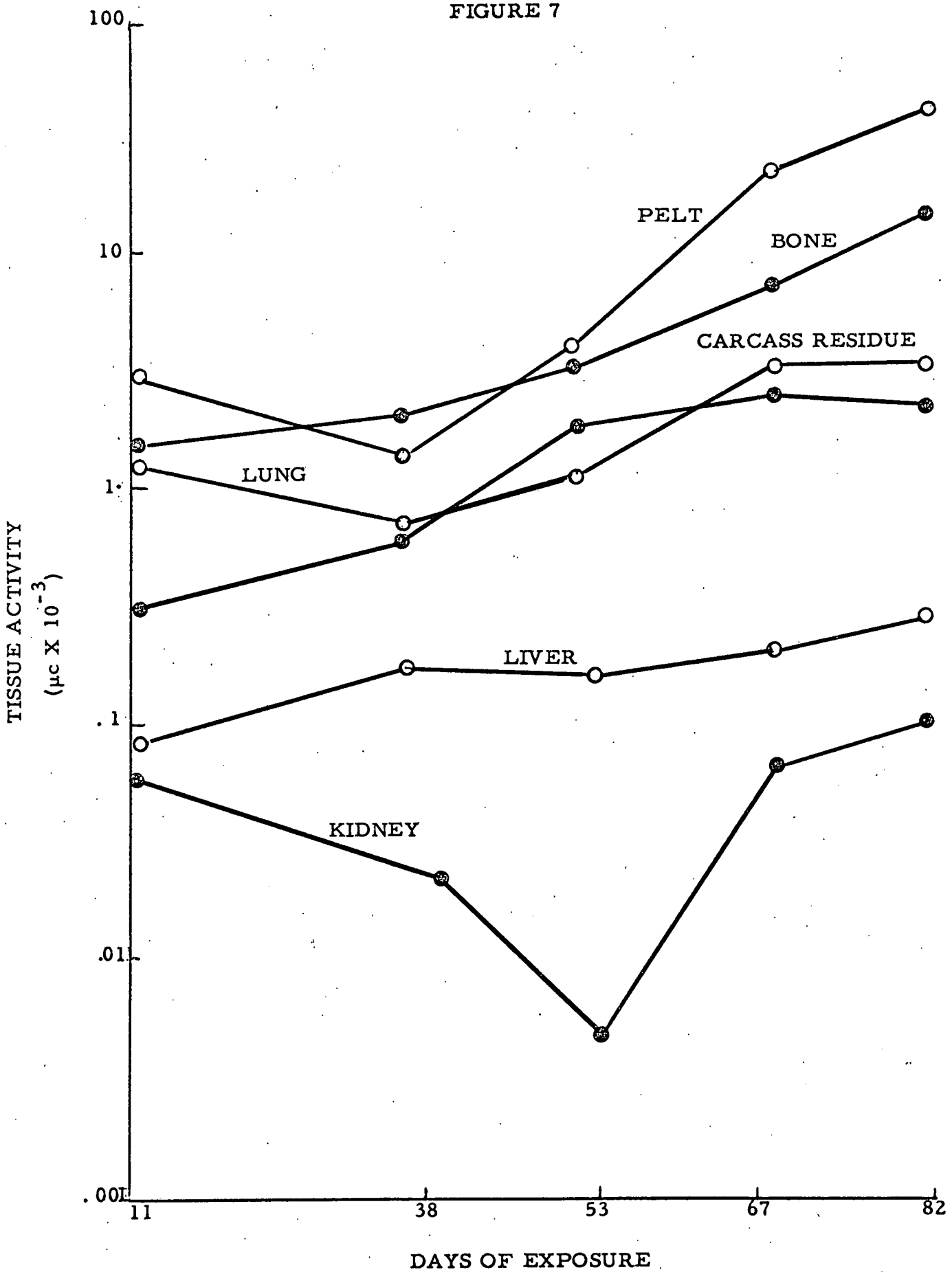
Concentration of activity in the lung increased for the first 50 days of exposure, and then reached a plateau over the remaining test period.

Skin, bone, and muscle activity were higher than expected and showed burdens even higher than lung. High skin values could reflect the contamination on the pelt because no measures were taken to keep the animals from licking the fur or preventing the accumulation on the skin. Liver was the only internal organ which showed a high concentration.

When defining the critical organ in any inhalation or toxicity experiment it is assumed the organ with the highest

TISSUE DISTRIBUTION FOLLOWING MULTIPLE EXPOSURES TO Y^{91} OXIDE (4×10^{-8} μc/ml)

FIGURE 7



concentration per gram of tissue is the critical organ. Calculation of the data from chronic studies on this basis indicates that the lung is the critical organ.

A group of mice were given an I. P. injection of Y^{91} chloride to study the transport of the chloride from the peritoneal cavity. The average concentration per organ is given in Figure 8. The bone and muscle are the highest in activity. The high burden in muscle may be due to adsorption of the chloride on the peritoneum. Since the chloride may form a hydroxide at the site of injection, the high bone concentrations observed indicates that Y^{91} is a bone seeker.

The servical lymph nodes increase as a percent of the body burden;they do also in concentration (activity/gram). This movement of Y^{91} to lymph node shows translocation not only to bone but to other organs outside the visceral cavity.

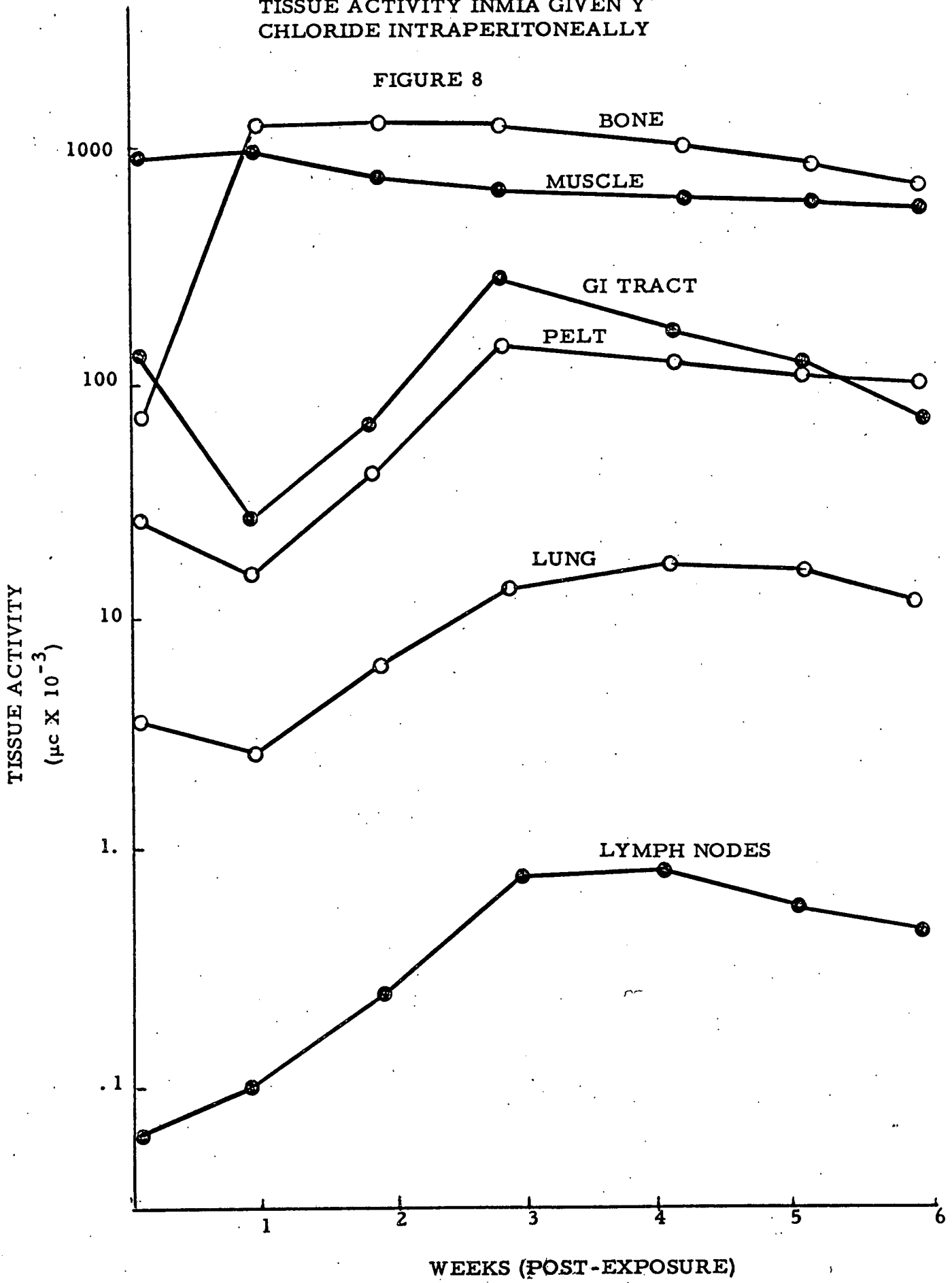
Summary

Biological data were obtained on the deposition, retention and translocation of the inhaled isotope of Y^{91}. The hydrosol used in atomizing was also used in I. P. and subcutanous injections to provide data on the uptake of tissues as compared with inhalations.

Data from either chronic or acute inhalation studies and confirming this with I. P. injection shows that Y^{91} is a bone seeker.

TISSUE ACTIVITY INMIA GIVEN Y^{91} CHLORIDE INTRAPERITONEALLY

FIGURE 8



A great variety of aerosols have been used in animal experimentation, and great differences cannot be fully explained. They are not caused by differences in solubility alone. It is generally accepted that particle size is of great importance. The Y^{91} chloride used for the exposures is considered very soluble in water. The distribution and excretion data showing the behavior of Yttrium in the body cannot be made solely on the basis of the solubility of the chloride used.

Two systems of exposing animals to Y^{91} isotope has been accomplished. Data from serial sacrifices show that accumulation in skin is a major contamination when whole-body exposures are investigated. Y^{91} chloride remained in the lung approximately the same time as the oxide. It is suggested that the chloride must form an insoluble complex when the aerosol reaches the lungs. Lung is still the critical organ even though the data is calculated on a per gram basis.

Retention of inhaled Y^{91} chloride in the lungs was similar to that found from inhalation of Y^{91} oxide. The biological half life of Y^{91} chloride was 23 days while the biological half life of Y^{91} oxide was 19 days. From a comparison of the biological half lives, a logical explanation can be made. The similarity of the half lives may be explained by the conversion of the chloride to an insoluble state upon deposition in the lung.

C. SCANDIUM

Inhalation data using Scandium^{46} as the chlorides have been reported for mice exposed in the nose only manner for a short time period (experiment 670). Similar studies using the rat have been completed and the tissue distributions etc., are being evaluated at present (experiment 811).

Partial data on repetitive exposures have also been reported for the rat inhaling Sc^{46} chloride (nose only) for a period of 6 months (experiment 679). Observation of these animals, now in the post-exposure period in respect to long term effects; viz, hematology, life span, pathology, etc., will be reported as soon as these have been obtained.

Long term, whole body exposures (6 months) are also being carried out using rats and designated as experiment 758. Data now available for this study is given below.

One hundred rats (Sprague-Dawley albinos - Charles River), 12 weeks old were exposed to an aerosol of Scandium chloride whose activity was 5.7×10^{-6} μc/ml for 7 hours/day, 5 days/week

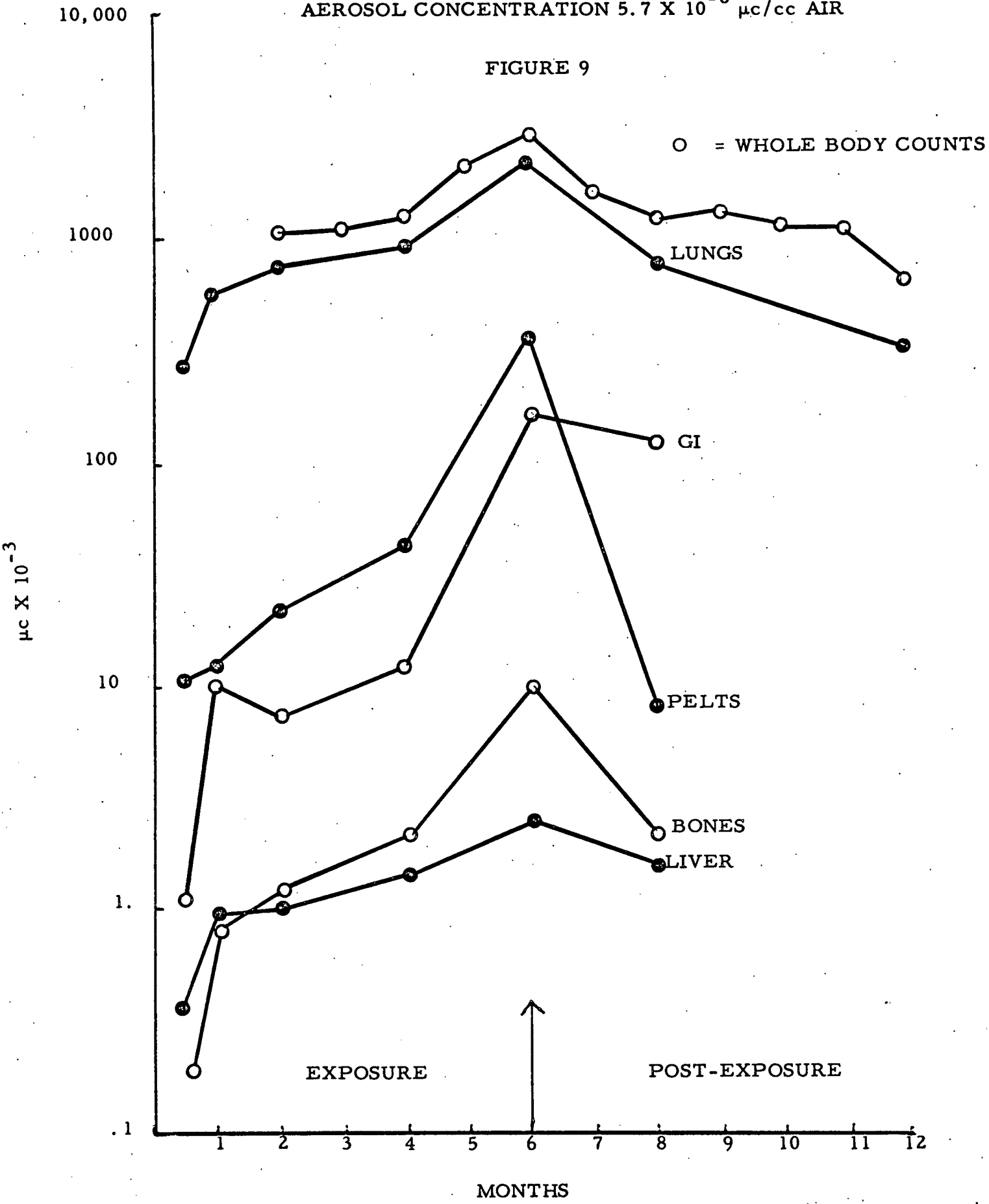
for a total period of 24 weeks. All animals were weighed monthly, a few serially sacrificed for determinations of tissue activities, hematological and pathological observations made periodically according to protocol. The animals were also assayed whole body in a Packard Armac counter every second week.

Figure 9 shows the activities of some selected body tissues during and for a few months after exposure. The increase in lung burden parallels the whole body counts closely after the first two months of exposure, appears to stabilize and then again shows increasing activities at about the 6 month period. The low liver values probably reflect the ionic nature of absorbed Scandium. Bone tissue, while accumulating the isotope during exposure, loses it quickly in the post-exposure period thus far observed. This may indicate residence in bone marrow or spongy bone tissue where vascularity is high with consequent rapid mobilization via the blood.

This study is in its early stages. Further observations will be reported as obtained.

SCANDIUM^{46} CONTENT OF RAT TISSUE
MULTIPLE INHALATION $Sc^{46}Cl_3$ - WHOLE BODY EXPOSURE
WHOLE BODY COUNTS AND TISSUE ACTIVITY IN
MICROCURIE PER TISSUE
AEROSOL CONCENTRATION 5.7×10^{-6} μc/cc AIR

FIGURE 9



D. COBALT^{60}

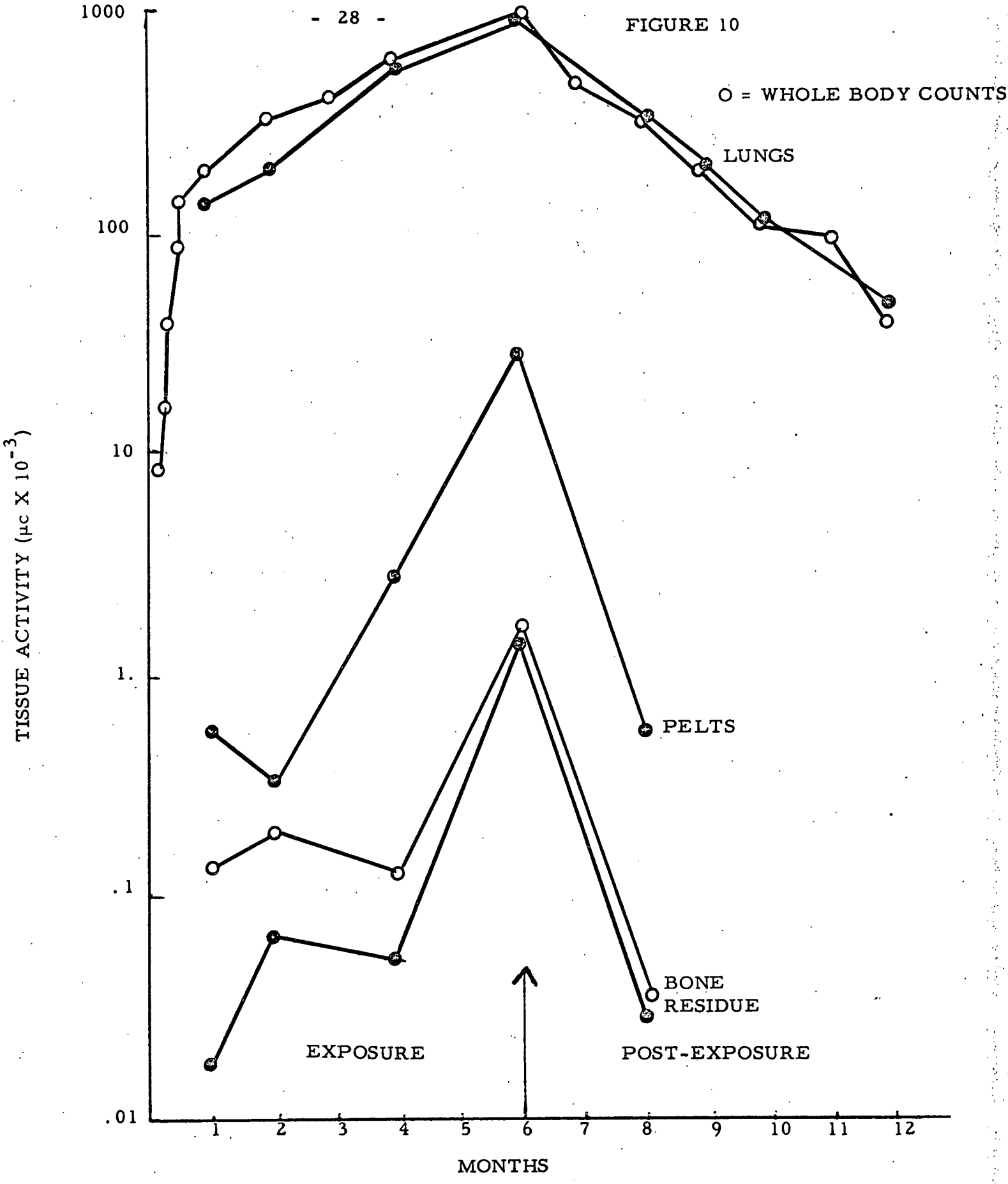
A study using Co^{60} chloride aerosols given to mice has been reported previously (experiment 697). Further studies had been initiated in which Co^{60} in oxide form has been aerosolized and mice again exposed (nose only), for short time periods (30 minutes) in order to compare the disposition in the body of this form into that obtained, under identical conditions, with the chloride over a post-exposure period of about 6 weeks (experiment 794, 795). The level of aerosol activities were 150 MPC_a (1×10^{-6} µc/ml of air) and 650 MPC_a (4.3×10^{-6} µc/ml air).

Using the same Cobalt^{60} Oxide aerosol but at an air concentration of about 1,000 MPC_a (7.8×10^{-6} µc/ml), a long term study using 100 Sprague Dawley rats were initiated using nose only exposures, of 30 minute duration daily for a period of 6 months. The body burdens were determined daily for one month and then monthly. Tissue activities were obtained by serial sacrifice of animals during and after the exposure period. In addition, these animals are being observed for blood and pathological effects.

The time course of tissue activity changes is shown in Figure 10. Again as in the case of Scandium^{46} whole body counts reflect in large part the lung burden during exposure and indeed in the post-exposure period shown. The pelt is unexpectedly high in activity which may be caused by faulty nose exposure technique or high levels of blood Cobalt^{60}. The latter unfortunately was not determined. Better control of these factors in future experiments is on our agenda. Cobalt builds up in bone which contains as much of the isotope as the carcass residue (remainder after removal of organs) representing much more tissue in terms of weight. However, the decline in bone following exposure indicates a rather slight affinity for bone tissue as was the case for Scandium. Further observations are being made in this respect.

FIGURE 10

COBALT^{60} CONTENT OF RAT TISSUE
MULTIPLE INHALATION Co^{60} OXIDE - NOSE EXPOSURE
WHOLE BODY COUNTS
AEROSOL CONCENTRATION 7.8 X 10^{-6} µc/cc AIR



E. PHOSPHORUS^{32}

The use of radiophosphate as a tracer in many biological studies and its rather well documented biological disposition when administered parenterally suggested its use in aerosol form as phosphorus acid which presumably would easily penetrate the pulmonary parenchyma into the circulation following inhalation.

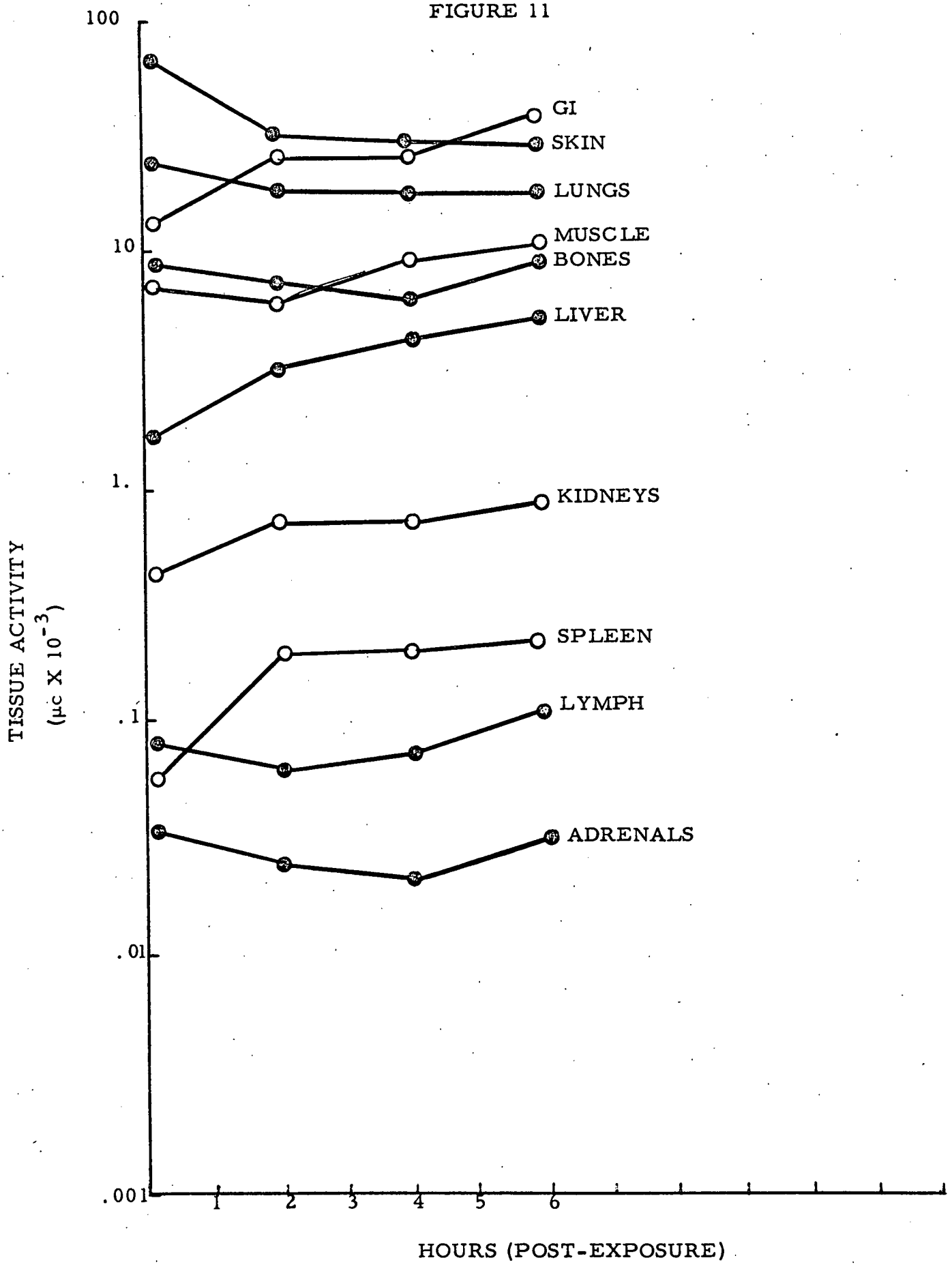
Morgan has reported* that 10% of a P^{32} dose reaches the bone where it is retained with an effective half life of 13 days. P^{32} penetration into red cells and other soft tissues with incorporation into organic phosphates occurs soon after intake.

Mice were exposed, nose only, to a P^{32} aerosol for about 40 minutes. The aerosol activity determined by millipore membrane sampling throughout the exposure period was 3.6×10^{-5} μc P^{32}/ml of air. Figure 11 and 12 show the immediate (hours) and longer term (weeks) tissue distribution of the radionuclide. All values were corrected for physical decay.

*Morgan, K. Z., J. Phys. & Colloid Chem., 51, 1003 (1947).

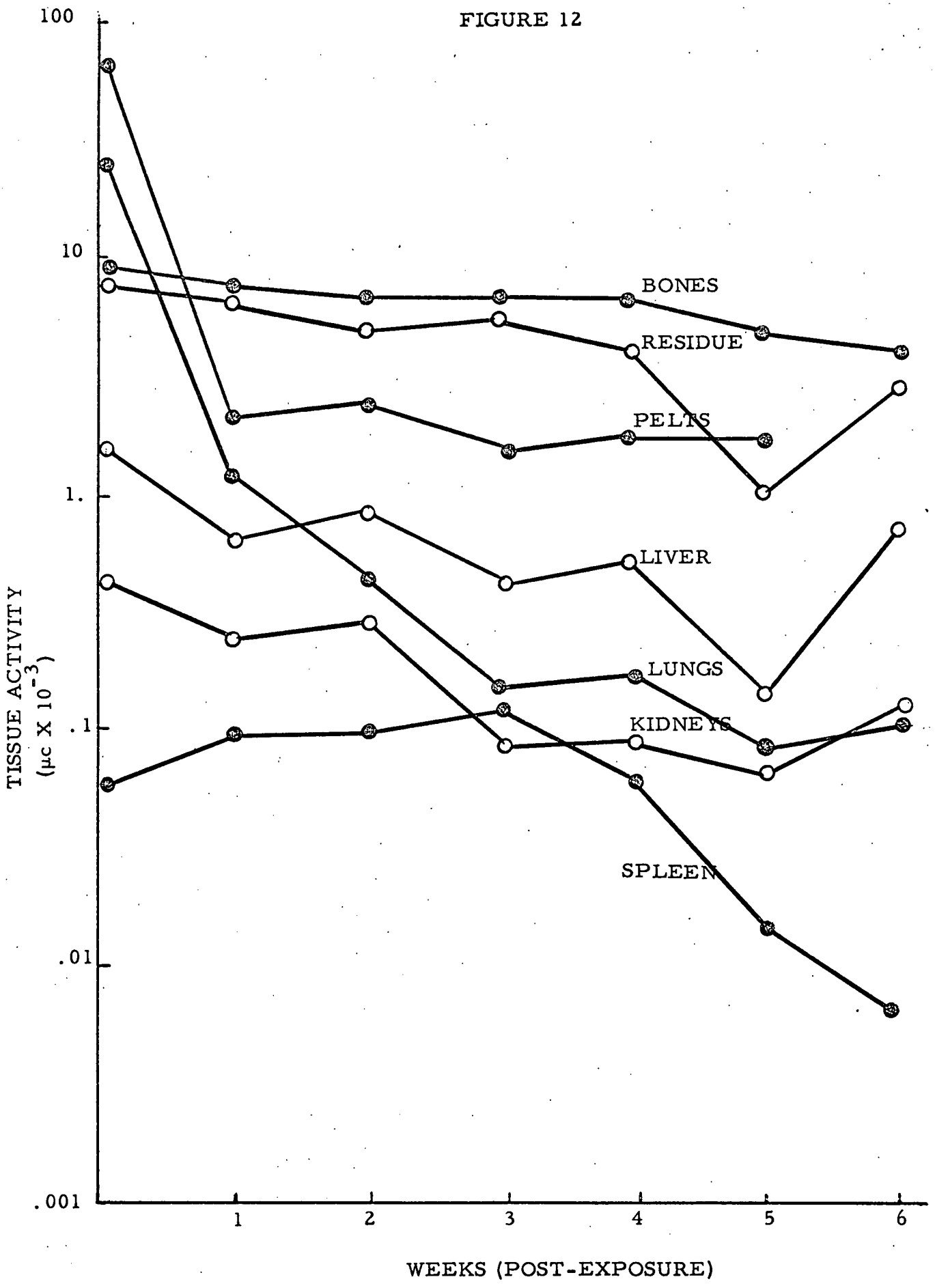
PHOSPHORUS CONTENT IN MOUSE TISSUE FOLLOWING SINGLE EXPOSURE P^{32} (H_3PO_4) - NOSE ONLY AEROSOL CONCENTRATION 3.6×10^{-5} μc/cc AIR

FIGURE 11



P^{32} CONTENT IN MOUSE TISSUE FOLLOWING SINGLE EXPOSURE TO $H_3PO^{32}_4$ AEROSOLS 3.6×10^{-5} μc/ml AIR

FIGURE 12



For short periods following exposure (Figure 11), the activity accumulates in all organs in varying amounts except lung, skin (pelt), and bones. It is especially noteworthy that lung clearance during the first 6 hours following exposure is relatively low for a presumably soluble material. Precipitation and fixation of H_3PO_4 by metals present on lung surfaces (calcium, magnesium, etc.) may be involved. The spleen and liver appear to increase most rapidly in activity. Over a period of 6 weeks, the bone shows little decline in activity compared to the lung. While the biological half time is of the order of 56 days assuming exponential kinetics for bone clearance, the effective half time of about 10 days is very similar to that reported by Morgan, mentioned previously. The lung clearance, rapid initially, stabilizes in about 5-6 weeks probably by incorporation of P^{32} into lung tissue with slow turnover relation to the initial rate of transport out of lung. The spleen maintains its burden for a time but then loses activity more rapidly than all other tissues observed.

Further studies along these lines in other species may well prove valuable in elucidating P^{32} incorporation rates into organs via inhalation.

F. EUROPIUM$^{152-154}$ - LONG TERM STUDIES

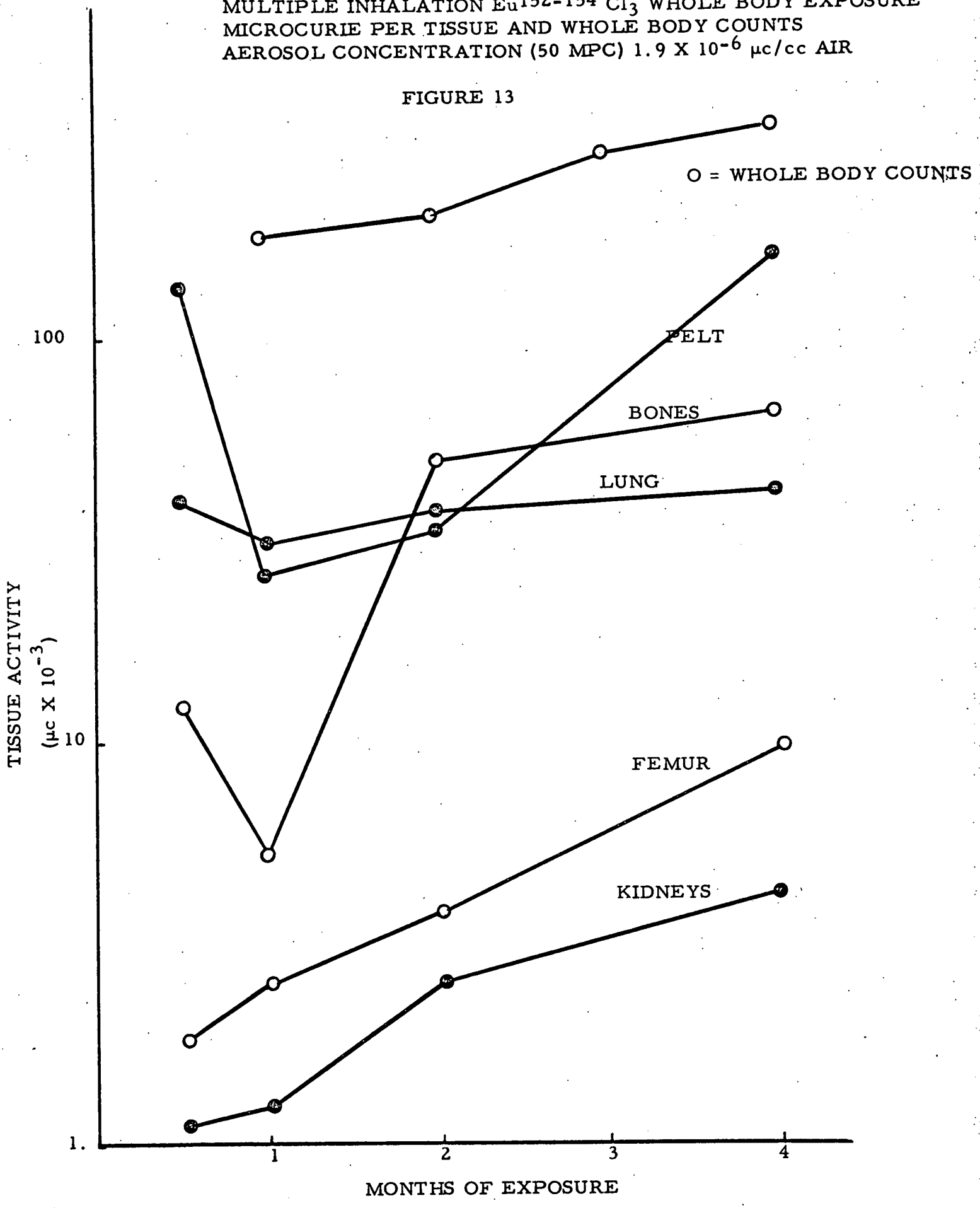
With the objective of obtaining further information on the effects of multiple exposures of rats to radioactive Europium, experiments were devised using different levels of aerosol concentration. In addition, a group of Sprague Dawley rats were exposed to stable Europium so that the weight concentration of the nuclide in the aerosols, stable and radioactive, would be equivalent. In order to achieve complete similarity of treatment for control and experimental animals, the control rats were exposed to the stable aerosol for equal time periods (7 hours/day, 5 days/week for a period of 6 months) in identical inhalation chambers. A group of animals were also set aside in our animal housing quarters and were never placed inside an inhalation chamber throughout the observation period.

Animals were exposed (whole body) to 50 MPC_a of $Eu^{152-154}$ chloride (experiment 849), 500 MPC_a (experiment 848) and to stable Europium, about 1 μg/ml air (experiment 850). In addition one group of rats were exposed to 50 MPC_a continuously (24 hours/day). The latter is designated as experiment 850. The average air concentrations appear in Figures 13, 14, and 15 along with tissue contents.

The chambers were sampled periodically for concentration and for purposes of particle sizing. The latter measurement

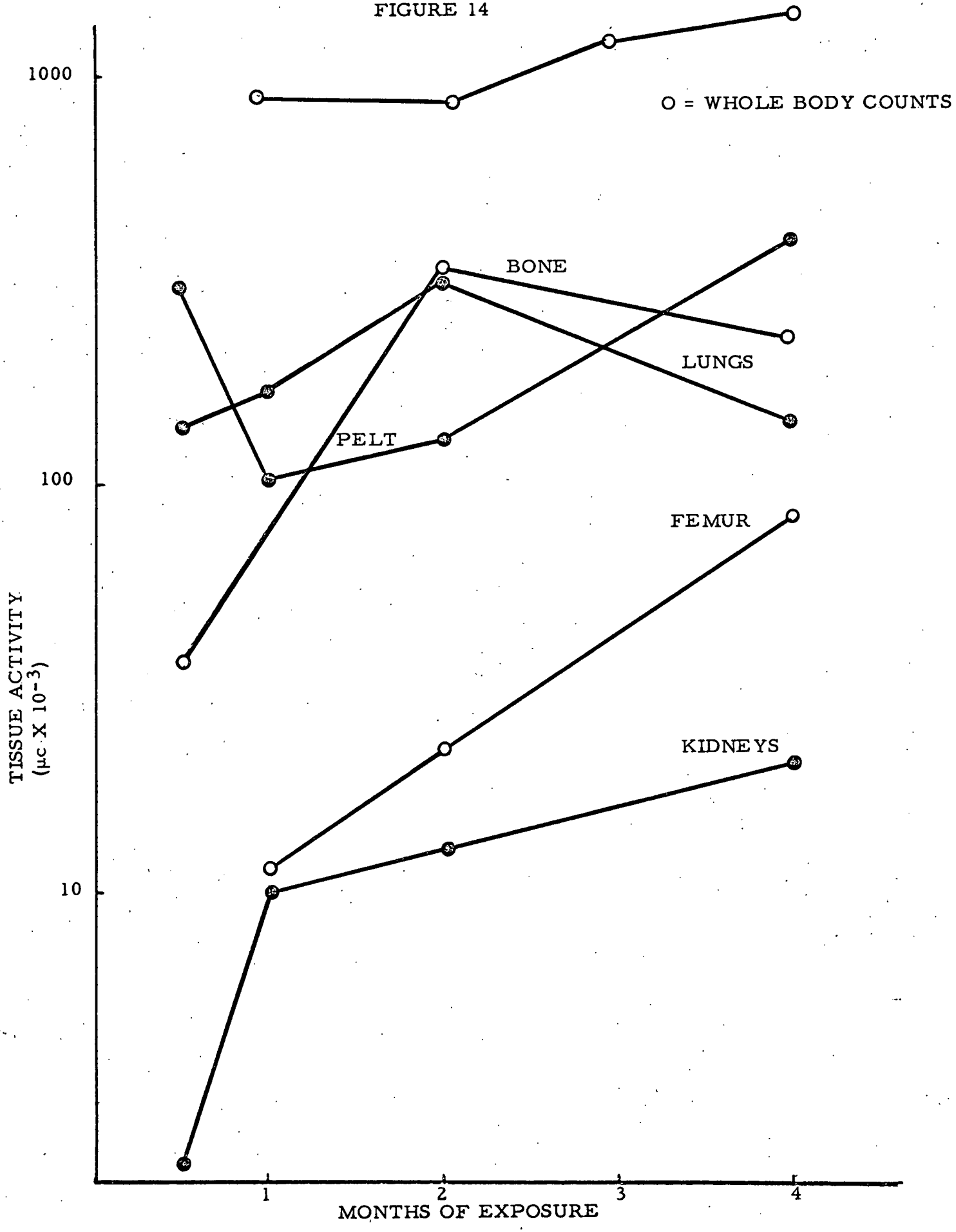
EUROPIUM$^{152-154}$ CONTENT IN RAT TISSUE
MULTIPLE INHALATION $Eu^{152-154}Cl_3$ WHOLE BODY EXPOSURE
MICROCURIE PER TISSUE AND WHOLE BODY COUNTS
AEROSOL CONCENTRATION (50 MPC) 1.9 X 10^{-6} µc/cc AIR

FIGURE 13



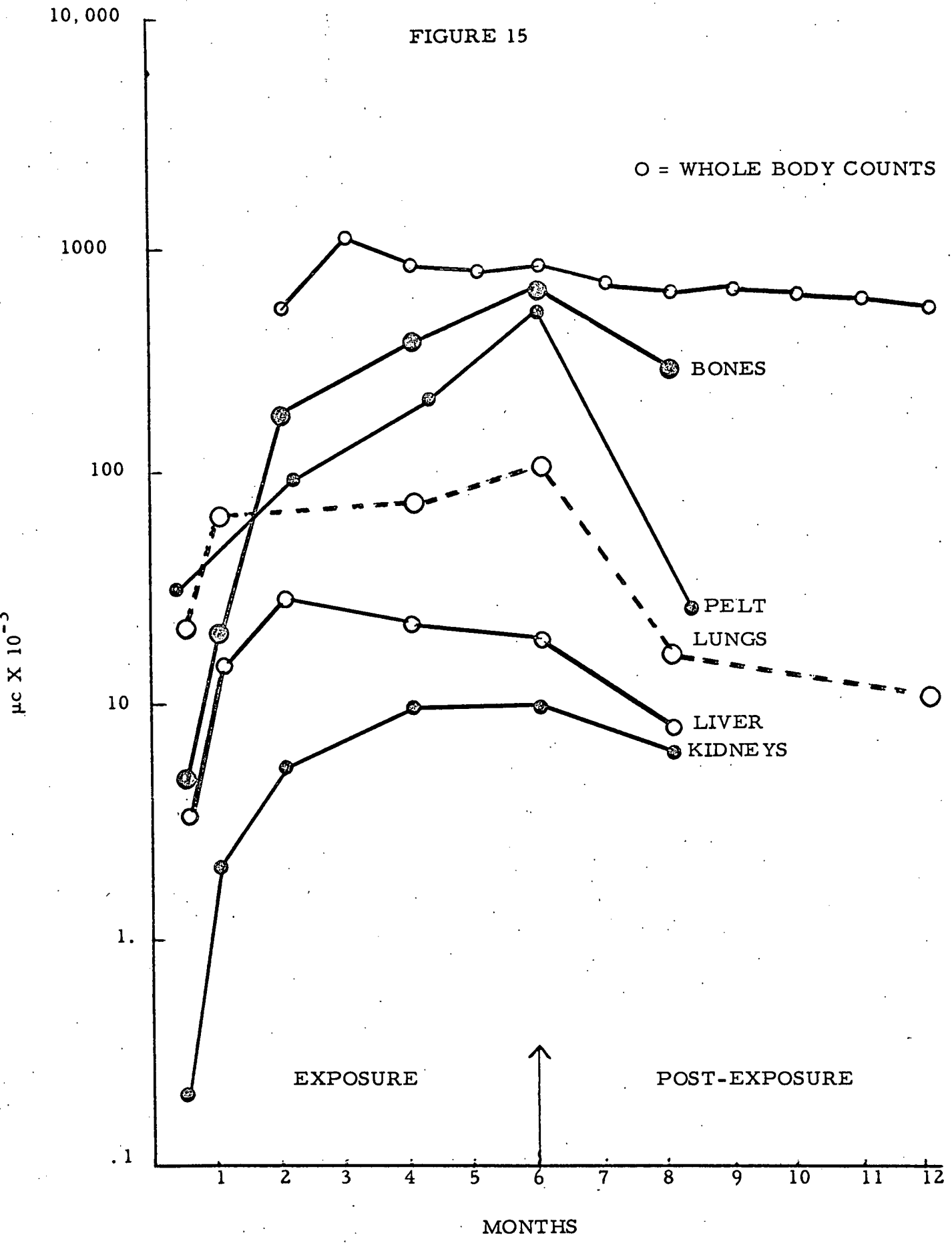
EUROPIUM CONTENT IN RAT TISSUE
MULTIPLE INHALATION $Eu^{152-154}$ Cl_3 WHOLE BODY
MICROCURIE PER TISSUE AND WHOLE BODY COUNTS
AEROSOL CONCENTRATION (500 MPC) 3.1×10^{-5} μc/cc AIR

FIGURE 14



EUROPIUM$^{152-154}$ CONTENT IN RAT TISSUE
MULTIPLE INHALATION $Eu^{152-154}Cl_3$ WHOLE BODY EXPOSURE
MICROCURIE PER TISSUE AND WHOLE BODY COUNTS
AEROSOL CONCENTRATION 2.7×10^{-6} µc/cc AIR

FIGURE 15



was in large part not very successful for various reasons with the exception of a few samplings (see section on aerosol characterization).

Serial sacrifice schedules were set up at 2 weeks to 22 month intervals. Weights and mortality were closely observed. Data on hematology, pathology, lung function testing and electrophoretic studies of serum as well as life span and tumor incidence data have been and are continuing to be collected in the post-exposure period.

The animals inhaling $Eu^{152-154}$ chloride were assayed in vivo for body burden of activity individually in a Packard Armac counter by insertion into a paper can and whole body counting. Tissues after dissection and weighing, were assayed in a well type sodium iodide (thallium activated) crystal with a Packard scaler (model 410A). Spleen, salivary glands, lungs, kidneys, adrenal, gonads, liver, trachea, stomach, intestines, pelt, bone and carcass residue were assayed. When tissues were too bulky, they were wet ashed, transferred to a test tube and then placed in the well crystal for counting.

The tissue activities in each of two experiments are shown in Figure 13 and 14. Figure 15 indicates the same data of a previous experiment not yet reported, in which a different aerosol concentration was used.

At the 3.1×10^{-5} μc/ml air level (Figure 14, 500 MPC_a), the whole body burden at the end of the month exposure is still not equilibrated with the excretory process but is increasing exponentially. The lung activity, however, reaches a plateau 2 months after the initial exposure as is true in the other studies (Figure 13 and 15). The increases in whole body burden may, however, be an artifact of continuing fur contamination. If so, the rate of decontamination of the animal pelt is surely a lengthy process.

The bone content as reflected in the activity found in the femur increases exponentially and at a greater rate than that of the whole body. There is little doubt about the affinity of Europium for bone tissue as shown in all three studies. The site of bone deposition will be a point of continuing research.

It is anticipated that with the collection of more data a clearer picture of the tissue distribution will emerge.

II. ANCILLARY RESEARCH

A. AEROSOL CHARACTERIZATION

1. A great deal of effort is being directed at present, as in the past, to the sampling and physical characterization of our aerosols, radioactive as well as stable, in terms of the usual statistical parameters reflecting particulate distributions.

In the past year, an electron microscope became available on a temporary basis for this work and personnel have been trained, making it possible to attack with greater precision the problem of the evaluation of our experimental aerosols. A Zeiss particle size analyzer TGZ-3 has been purchased and is now in use.

The sampling equipment available to us, a cascade impactor, proved useless for our purposes. It has however, been possible by use of the oscillating thermal precipitator to collect particulates directly on electron microscope grids and to obtain the mean particle diameters and geometric standard deviations for some of the nuclides currently being aerosolized. For example, Figure 16 is a photograph of a Europium chloride aerosol at a magnification of 51,400 X (5.14 [152-154]

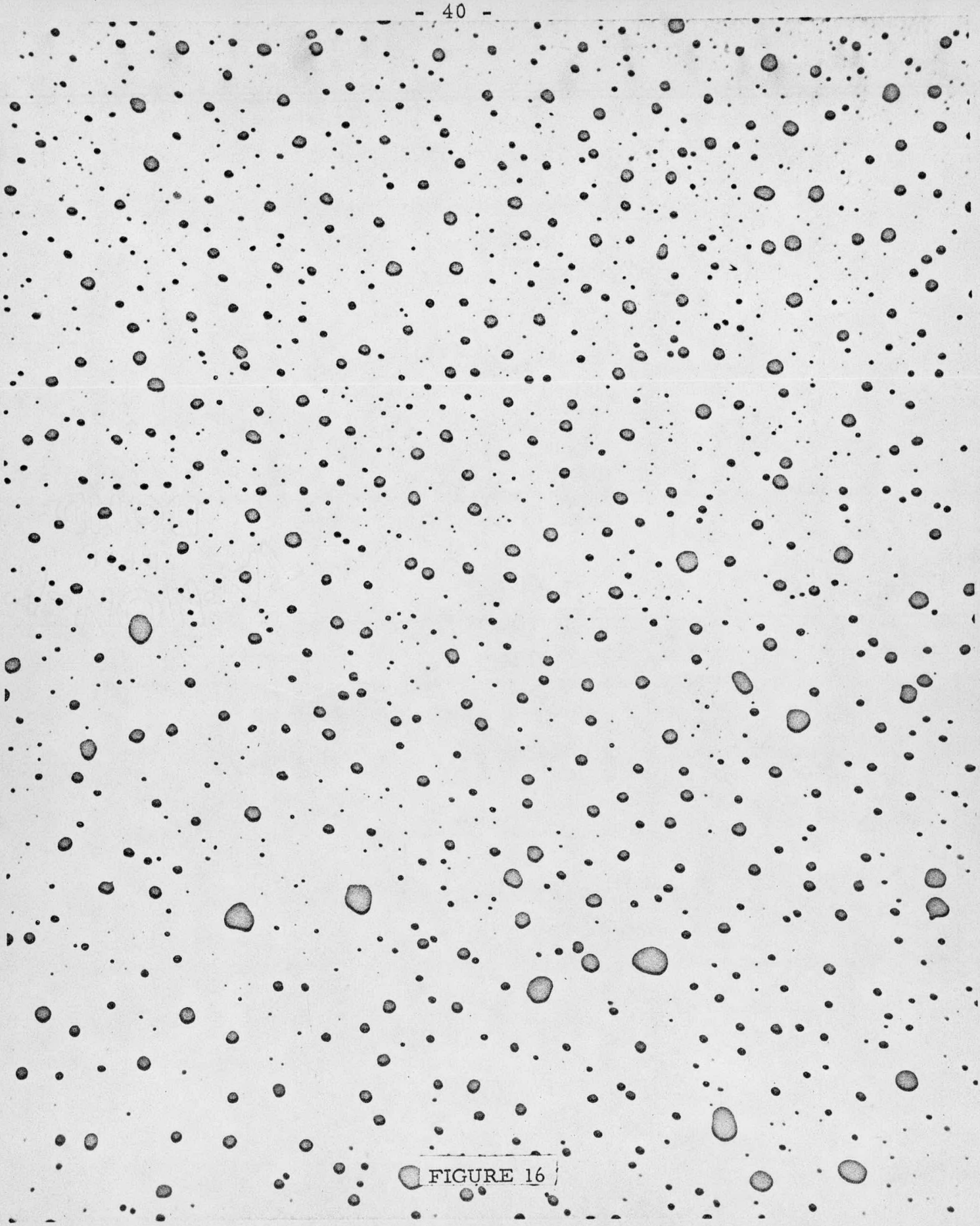


FIGURE 16

cm = 1 μ). The distribution is shown in the log-probit plot (Figure 17). Since no carrier was used and attempts were being made to get down to aerosol activities in the MPC_a range, it is not surprising that the mean particle size is very small (0.017 micron). Of 1000 particles counted, about 1% (the rest if present are too small to see) are below 0.01 microns. The geometric standard deviation is what one anticipates in an air jet generated aerosol. For fuller characterization, it is patently necessary to use further techniques such as shadowing, etc., to validate the observations obtained thus far.

A $Cobalt^{60}$ Oxide suspension is shown in Figure 18 and its distribution in Figure 19. While the median size is submicronic, 0.105 microns, (carrier Cobalt was added in the preparation of the suspension), the almost monodisperse nature of the aerosol is not only surprising but may, if reproducible, be of immense significance in current inhalation studies being carried on here. The aerodynamic behavior of the agglomerates shown in the photograph (Figure 18) if indeed present in air and not an artifact of sampling would of course be an

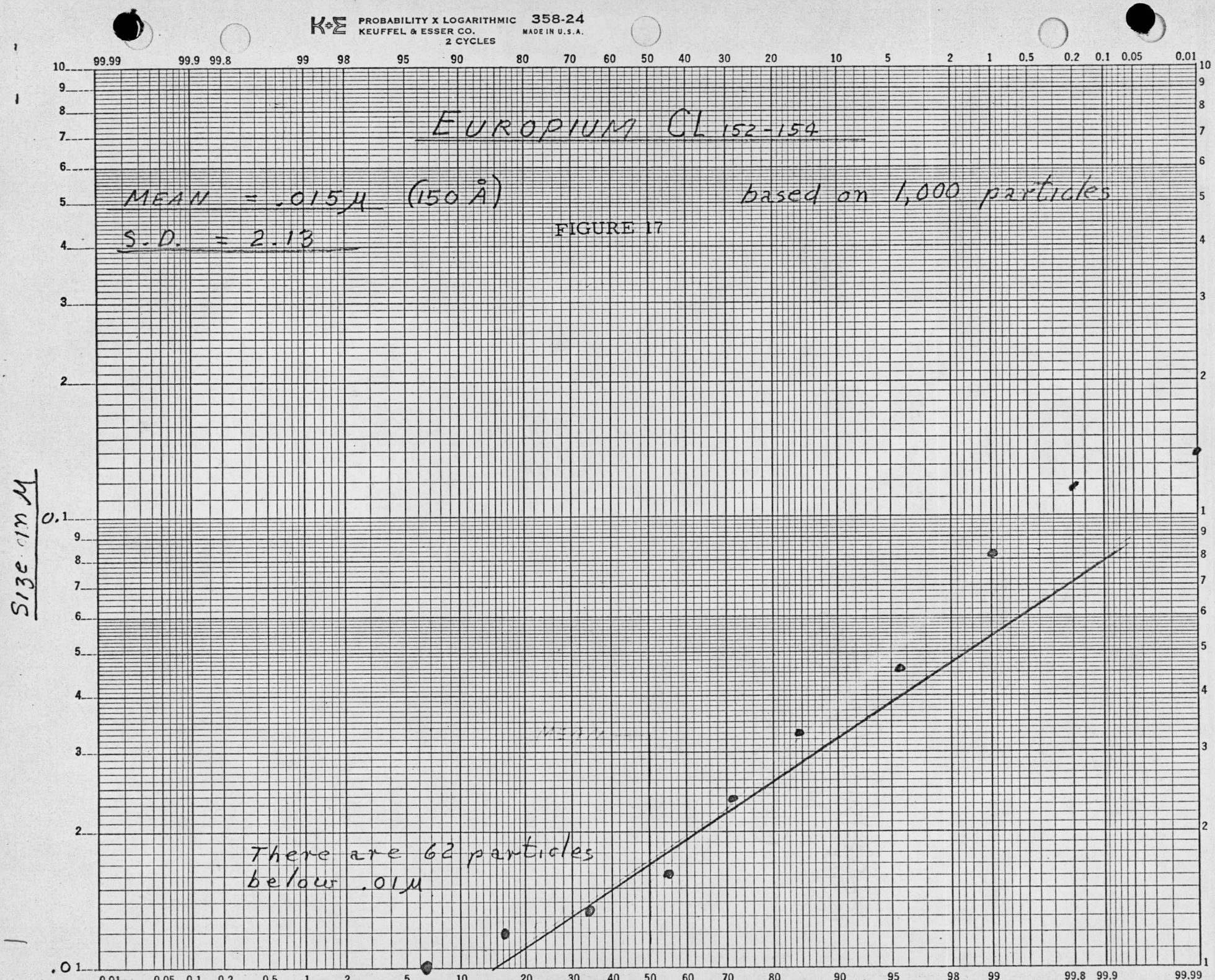

K&E PROBABILITY X LOGARITHMIC 358-24
KEUFFEL & ESSER CO. MADE IN U.S.A.
2 CYCLES
EUROPIUM CL 152-154
MEAN = .015μ (150 Å)
S.D. = 2.13
based on 1,000 particles
SIZE IN μ
There are 62 particles below .01μ
99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99

FIGURE 17

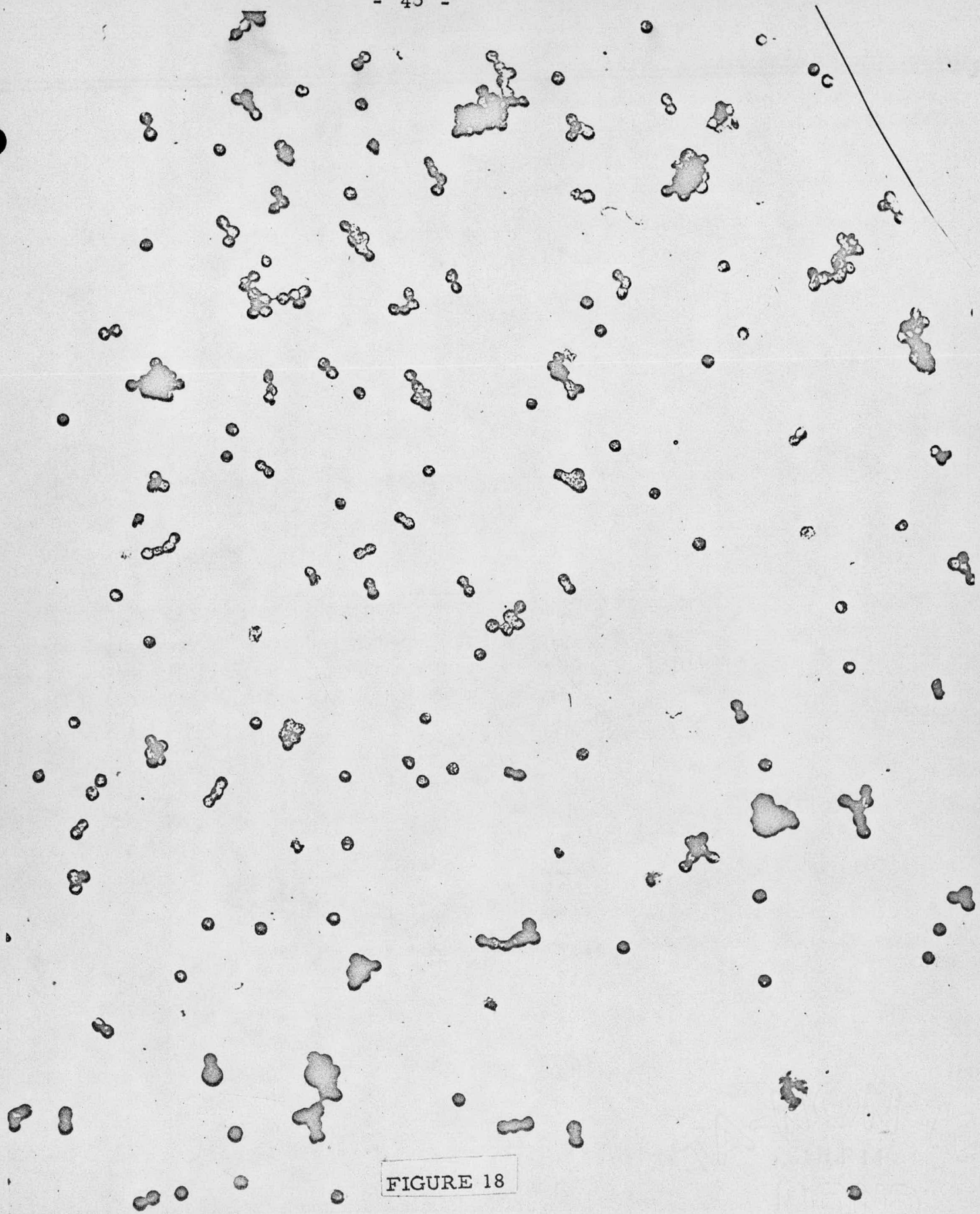
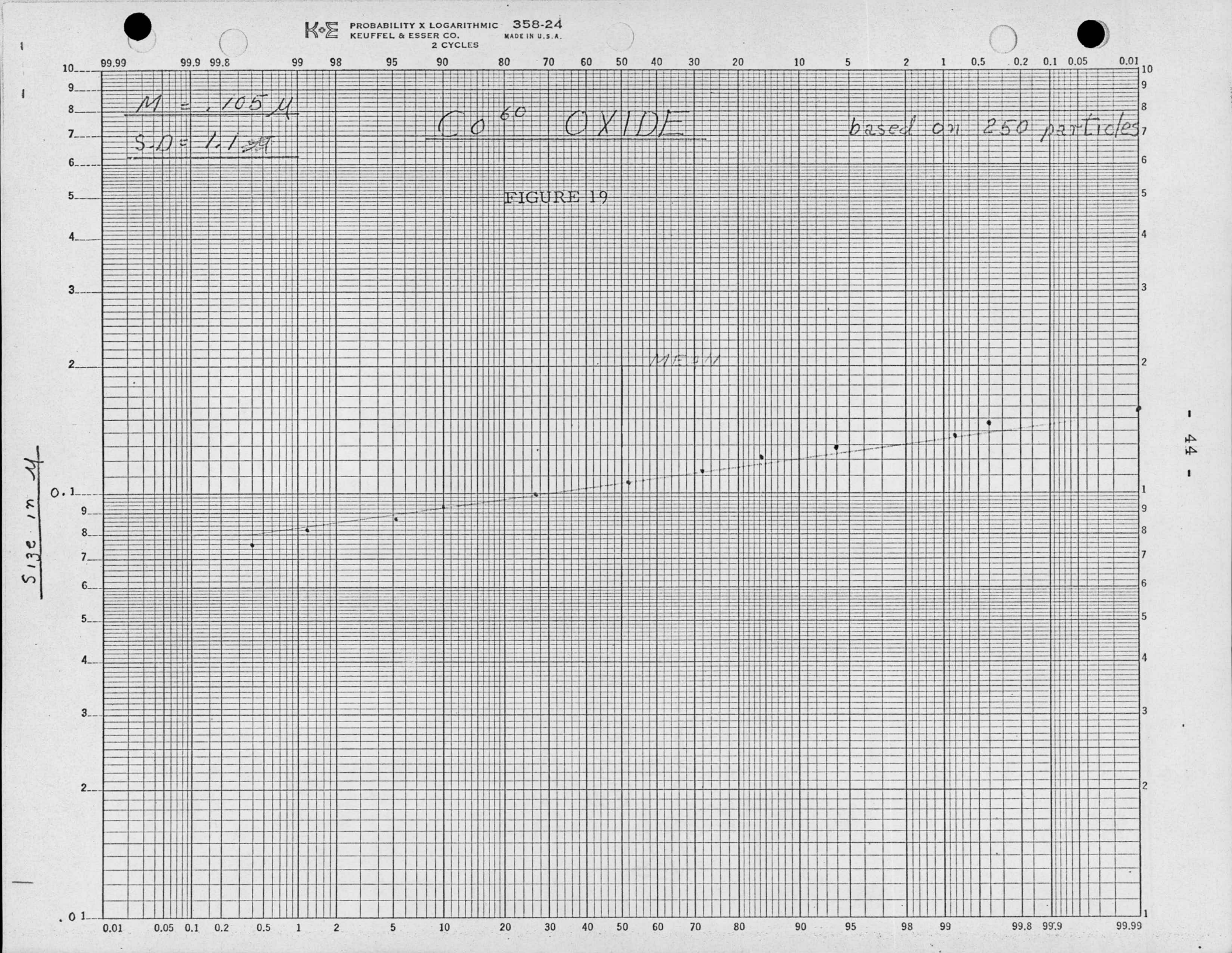


FIGURE 18


K+E PROBABILITY X LOGARITHMIC 358-24
KEUFFEL & ESSER CO. MADE IN U.S.A.
2 CYCLES
99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
10 9 8 7 6 5 4 3 2 0.1 9 8 7 6 5 4 3 2 0.01
M = .105 μ
S.D = 1.1 μ
Co 60 OXIDE
based on 250 particles
FIGURE 19
MEAN
SIZE IN μ
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99

important consideration in the interpretation of inhalation data. Again, much more must be done in this area of the study.

2. In the past year, also, Wayne State personnel have cooperated in various phases of aerosol characterization with other inhalation installations around the country. It is anticipated that these data will be published in the near future.

3. In order to further facilitate the acquisition of data relative to our aerosols, electrostatic point to plane precipitators are being fabricated, a power supply has been obtained, and the work is expected to accelerate in the near future.

4. Characterization of carrier aerosols of salt (sodium chloride) using an air jet aerosolizer is proceeding rapidly. These aerosols, contaminated with tracer amounts of the nuclides of interest will be used to achieve reproducibility in terms of deposition, where other methods may not be feasible. The advantages of a very soluble, easily absorbed vector of known physical character are obvious when working with aerosols.

B. WEIGHT DATA

The observation of changes in the weight of an animal caused either by depression of growth rate in the young growing animal or actual loss of weight in the mature animal is a sensitive indicator of the general health of the animal. All animals used in our research are weighed periodically (1 to 4 week intervals depending on animal age and life span). The following observations of animal weight are of interest in this regard.

Cobalt^{60}

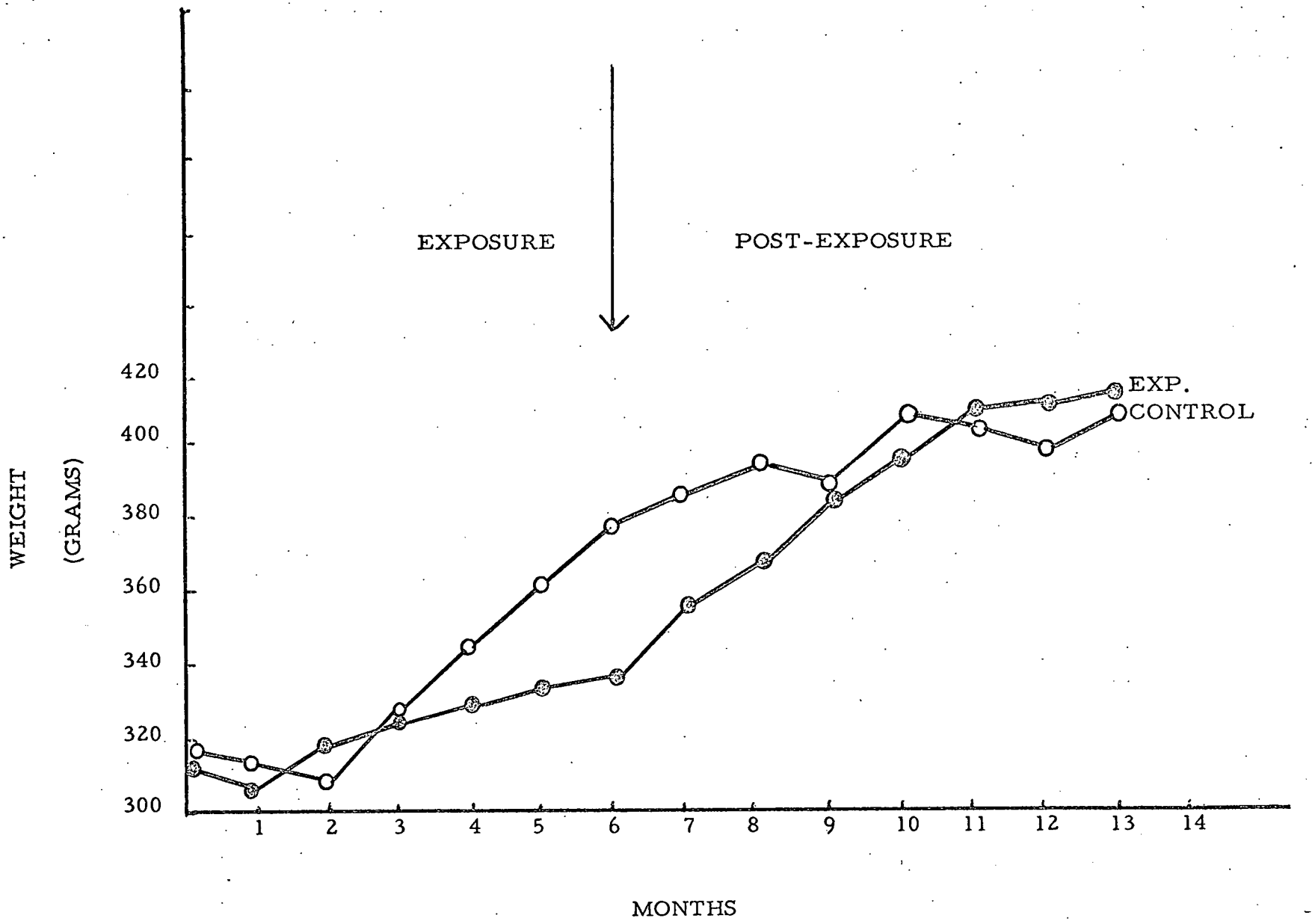
In the case of Co^{60} oxide administered in the nose only manner, previously described, there were minimal changes. No significance can be attached to this data at this time. It should be noted that air concentration of activity were as high as those shown in Figure 26 for Scandium^{46}. The importance of the effect of relative retention, deposition and radiological decay rates of the isotope involved on weight curves must obviously also be considered. (Figure 20)

Europium

In order to compensate for differences in the conditions of animal maintenance of control animals compared to those in the chamber and also to differentiate a possible chemical from the radiologic toxicity of the nuclide, experiments were planned using stable and radioactive Europium.

EFFECT OF Co^{60} ON GROWTH RATE OF RATS
MULTIPLE INHALATION Co^{60} OXIDE - NOSE EXPOSURE 30 MIN. / DAY
AEROSOL CONCENTRATION 7.8×10^{-6} μc/ml AIR

FIGURE 20



The data is shown in Figure 21 to Figure 24 and indicates the marked differences in weight gain of animals exposed continuously (24 hours/day) to rather low levels of activity (Figure 21) compared to those exposed to similar or much higher activities (Figures 22 and 23) but for only 7 hours/day (as shown in Figure 25). The differences shown between the weights of control and experimental animals which received only minimal amounts of nonradioactive Europium indicates that maintenance and housing conditions may well be a complicating factor in the interpretation of weight changes since chemical toxicity of Europium is negligible at the air concentrations used.

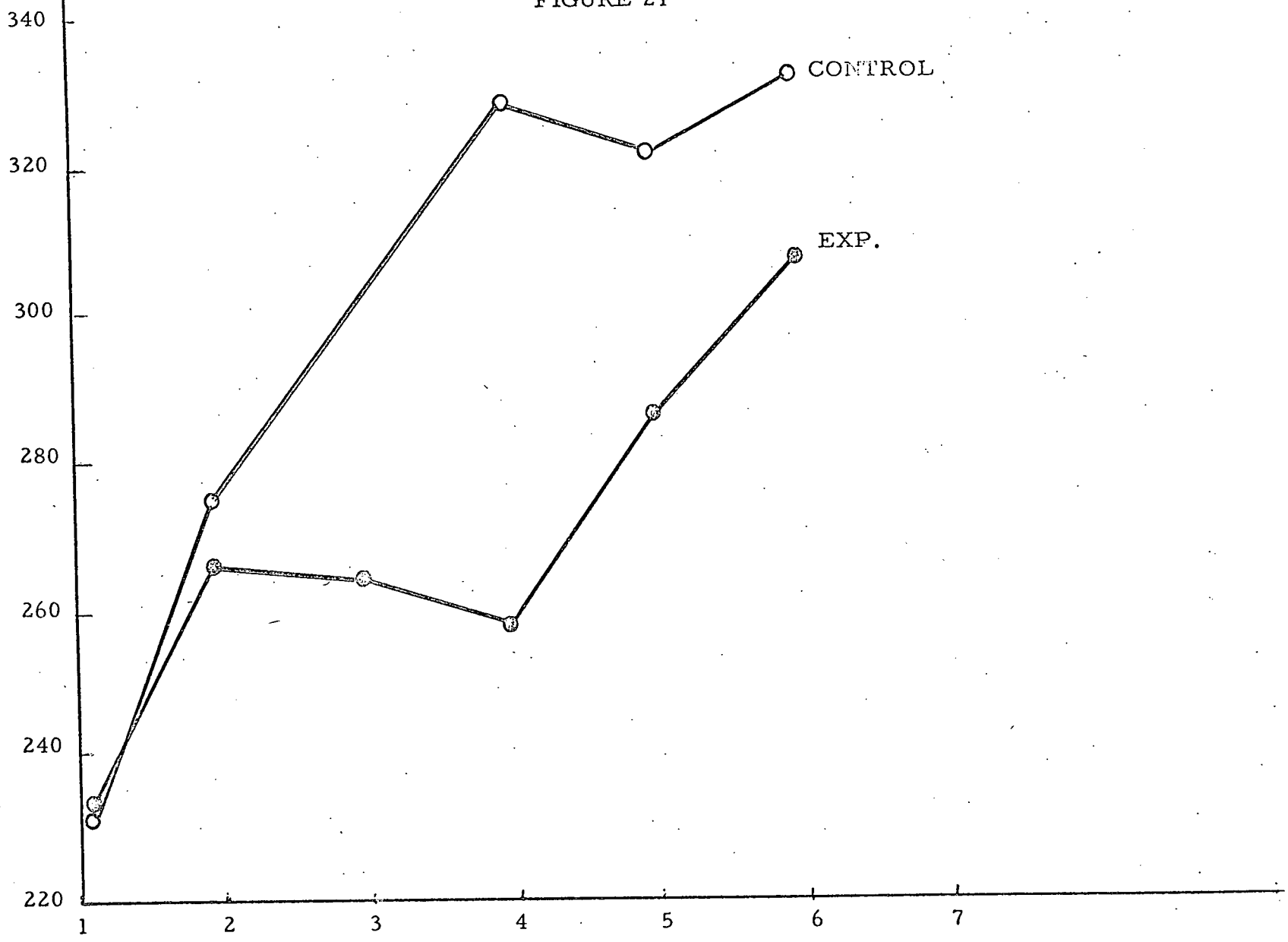
Scandium^{46}

The weight changes in mature rats (6 months old at initial exposure) during and following whole body exposure and during the post-exposure period following short repetition (nose only) exposures are shown in Figures 26 and 27, respectively.

The data indicates (Figure 26) sharp decline in average animal weight during the first month of exposure with recovery of normal growth rate but not weights for the rest of the exposure and indeed post-exposure period. It should be noted, however, that the exposed group were housed in rather crowded quarters with competition for food and water while controls were housed 3 to a cage.

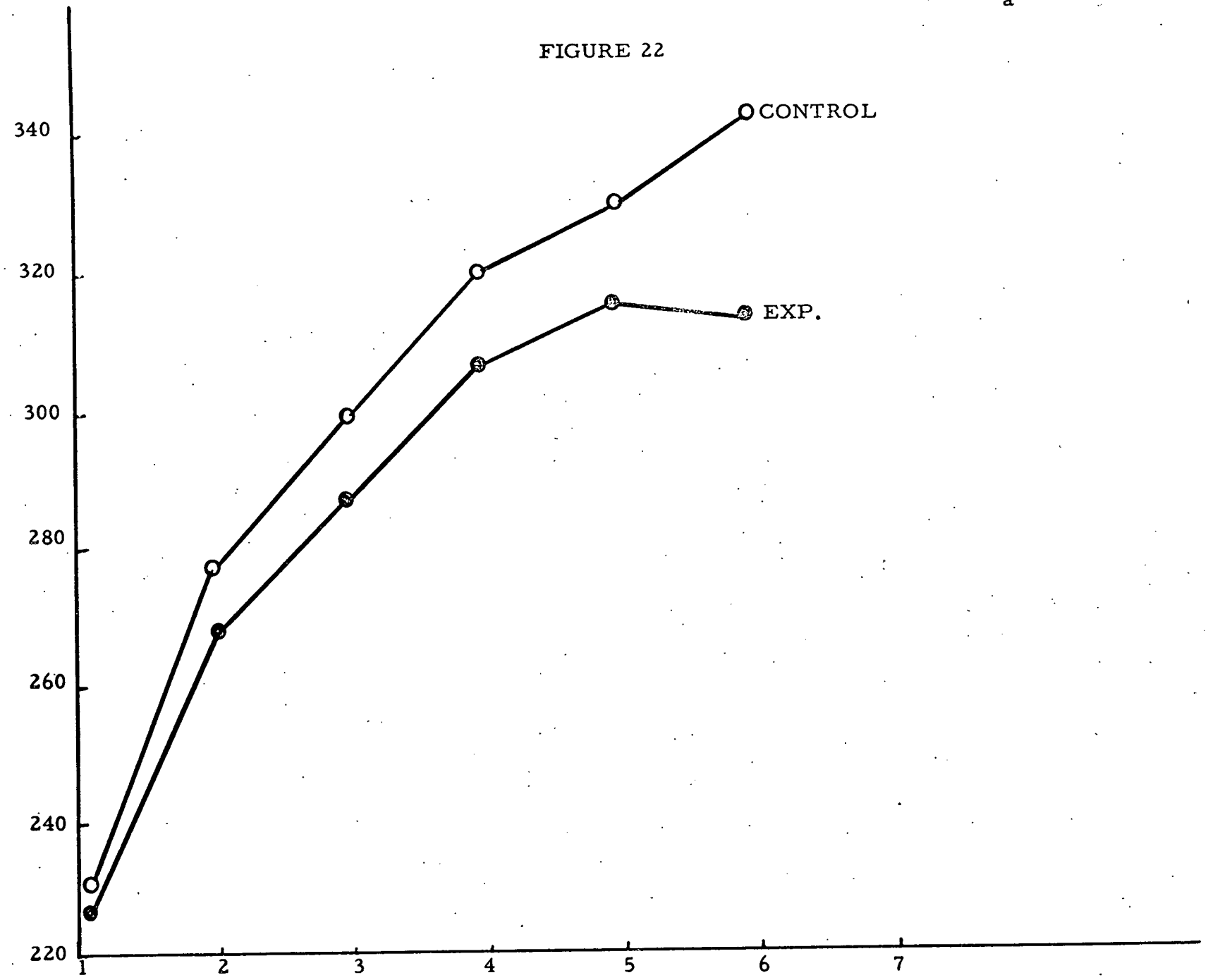
EFFECT OF Eu^{152} ON GROWTH RATE OF RATS
MULTIPLE INHALATION $Eu^{152-154}$ Cl_3 - WHOLE BODY (24 hours/day)
AEROSOL CONCENTRATION 1.9×10^{-6} μc/ml AIR (50 MPC)

FIGURE 21



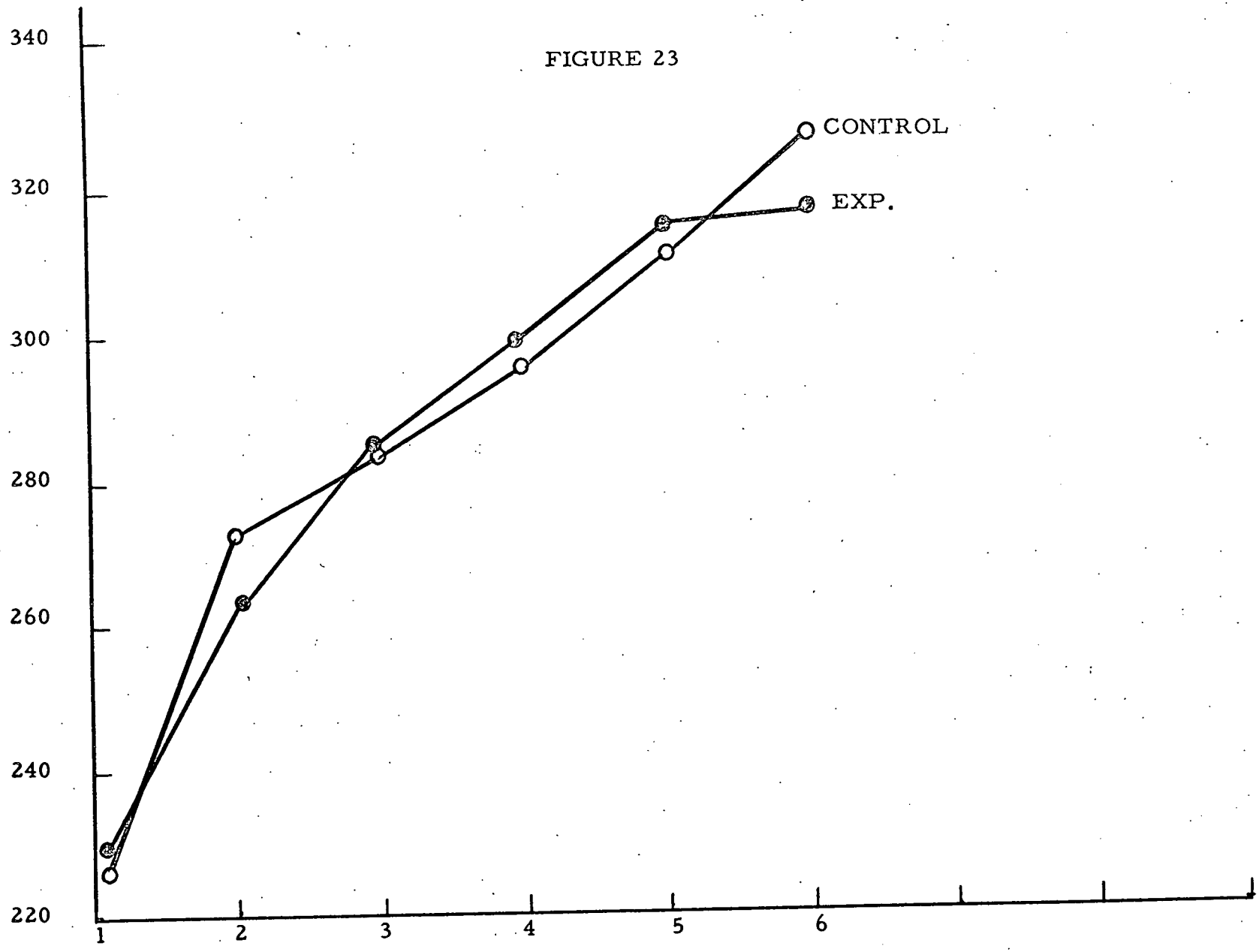
EFFECT OF Eu^{152} ON GROWTH RATE OF RATS
MULTIPLE INHALATION $Eu^{152-154}$ Cl_3 WHOLE BODY
AEROSOL CONCENTRATION 3.1×10^{-5} μc/ml AIR (500 MPC_a)

FIGURE 22



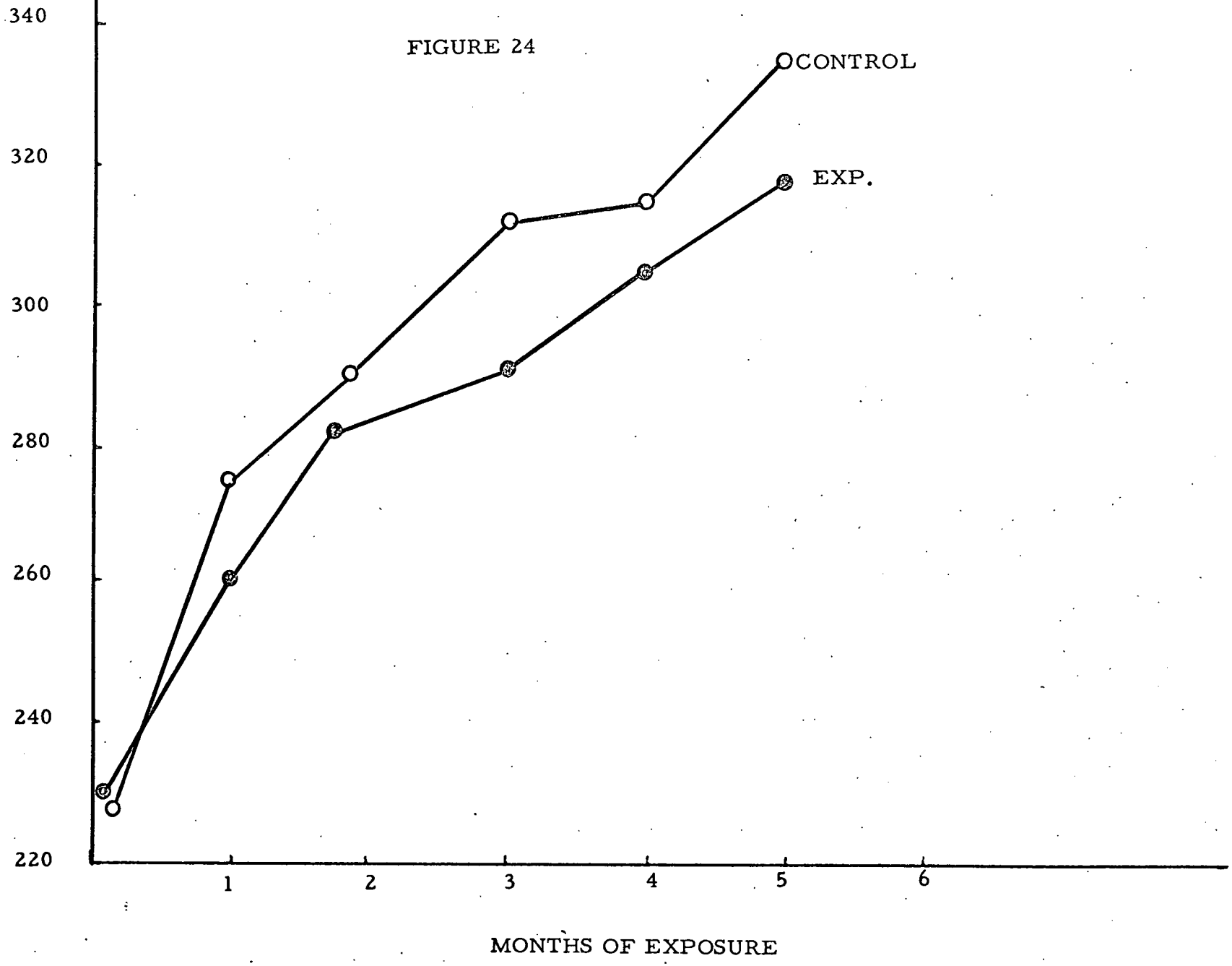
EFFECT OF Eu^{152} ON GROWTH RATE OF RATS
MULTIPLE INHALATION $Eu^{152-154}$ Cl_3 WHOLE BODY
AEROSOL CONCENTRATION 0.71×10^{-6} μc/ml AIR (50MPC)

FIGURE 23



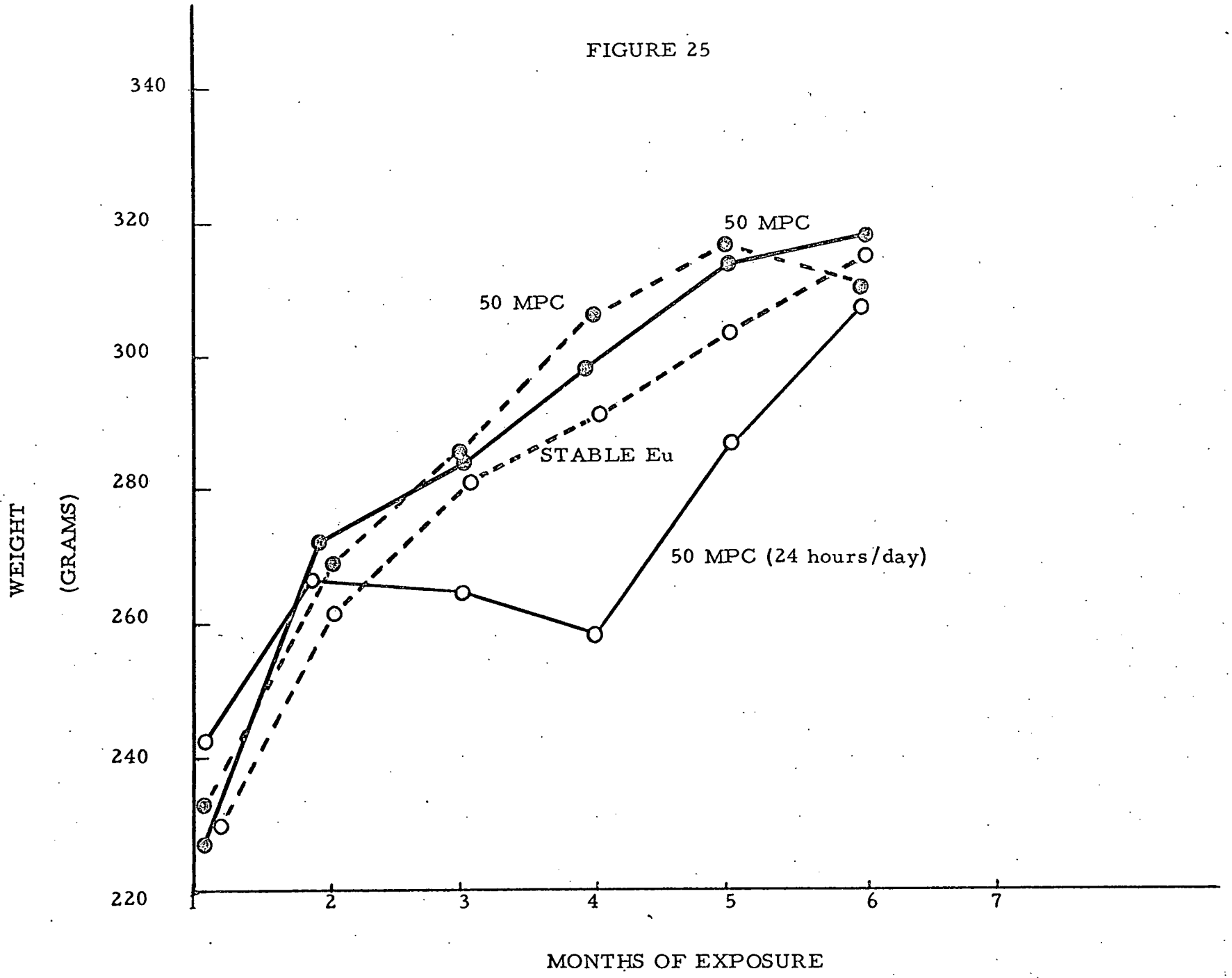
EFFECT OF Eu^{152} ON GROWTH RATE OF RATS
MULTIPLE INHALATION $EuCl_3$ WHOLE BODY
AEROSOL CONCENTRATION CALCULATED AT 1 μg/ml AIR CONTROL

FIGURE 24



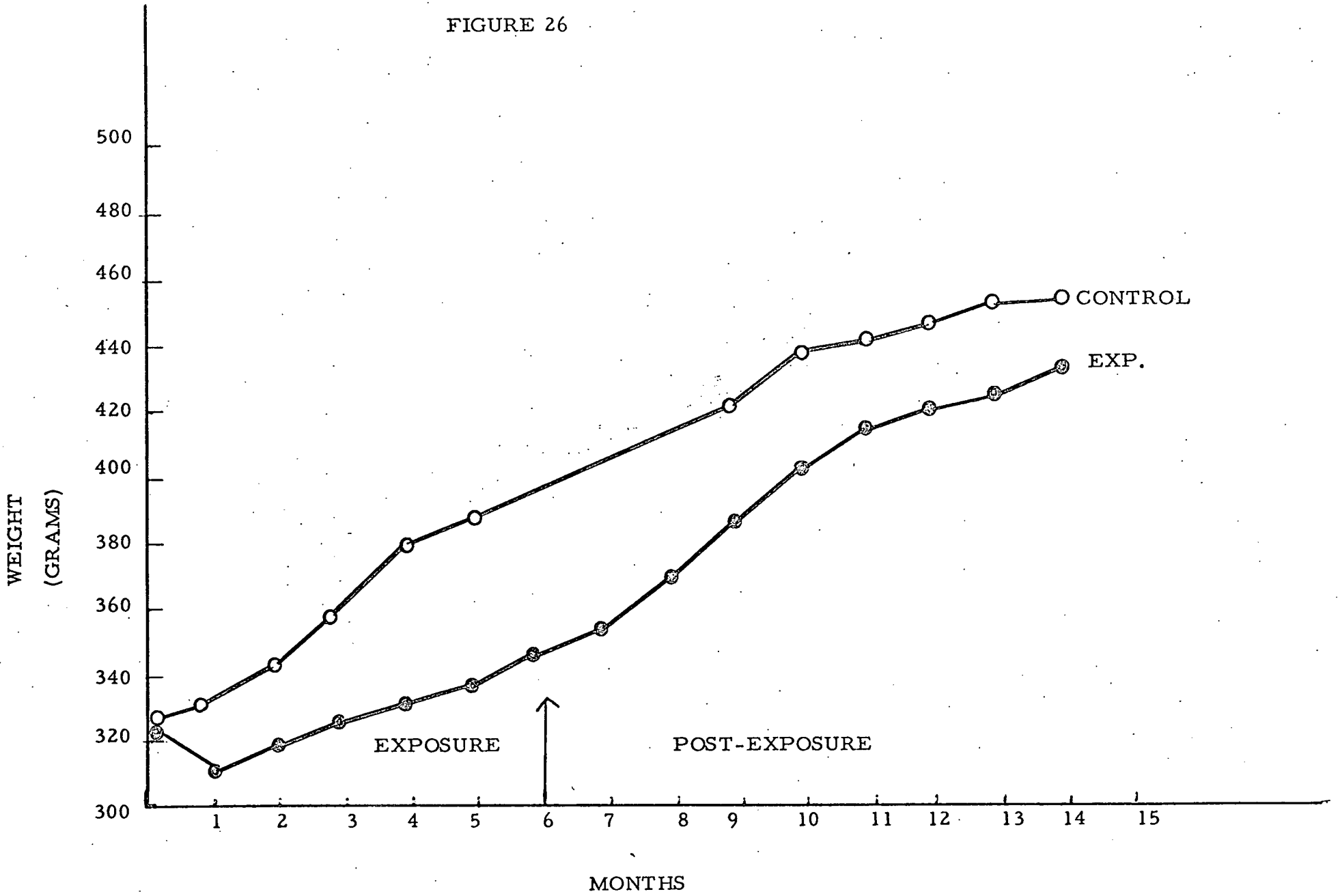
COMPARATIVE WEIGHT GAIN
MULTIPLE INHALATION - STABLE AND ISOTOPIC EUROPIUM

FIGURE 25



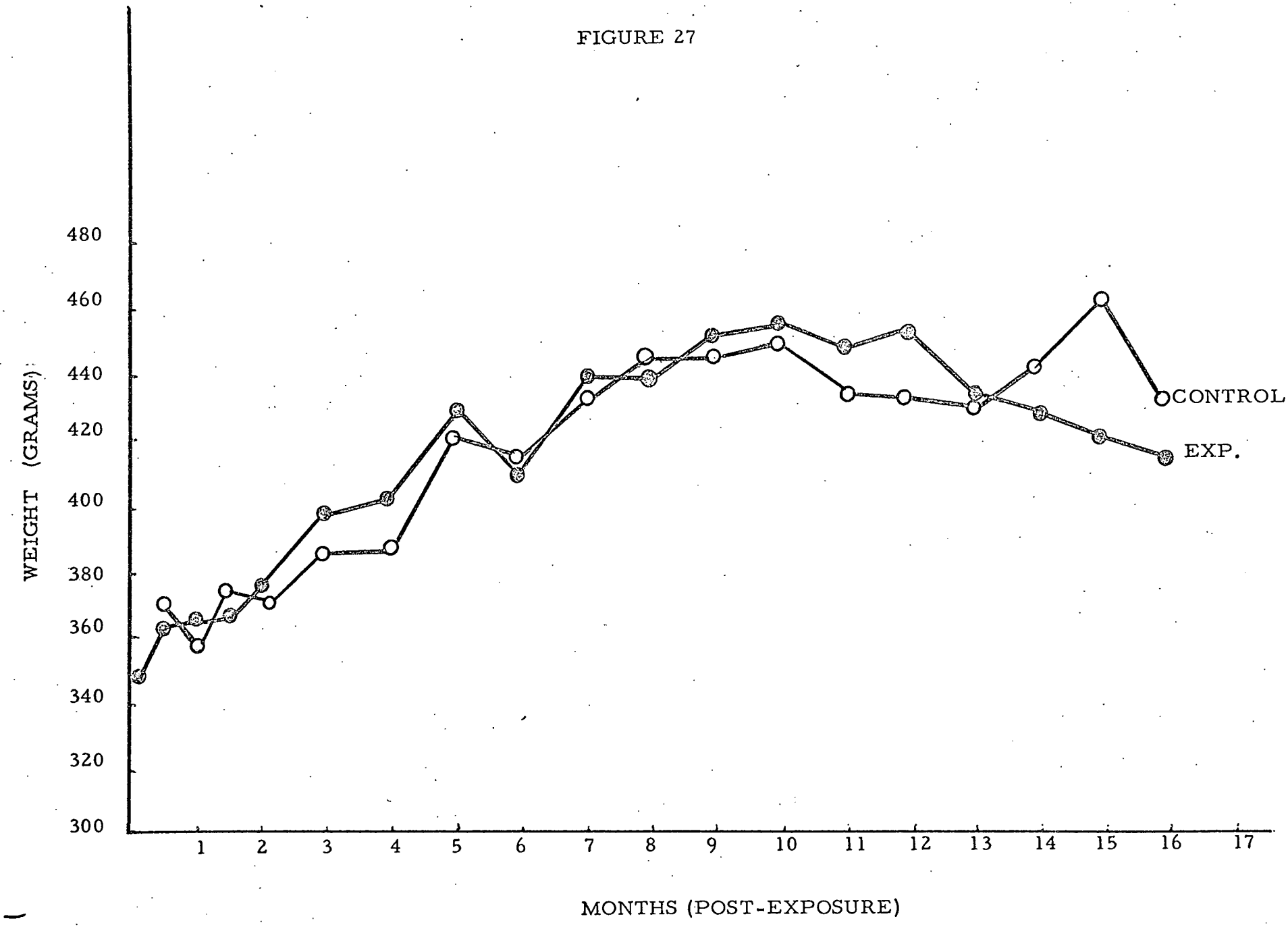
EFFECT OF Sc^{46} ON GROWTH RATE OF RATS
MULTIPLE INHALATION $Sc^{46}Cl_3$ WHOLE BODY EXPOSURE (7 hours/day)
AEROSOL CONCENTRATION 5.7×10^{-6} μc/ml AIR

FIGURE 26



EFFECT OF Sc^{46} ON GROWTH RATE OF RATS
MULTIPLE INHALATION Sc^{46} Cl_3 NOSE ONLY EXPOSURE (30 MIN/DAY)
AEROSOL CONCENTRATION 1.0×10^{-5} μc/ml AIR

FIGURE 27



With an aerosol containing twice the activity but of shorter daily duration of exposure (Figure 27), the post-exposure differences in weight were negligibly small.

It is impossible at this point to be definitive in regard to the validity of the weight differences discussed. If there is a long term effect in terms of weight changes caused by the isotope activity it may be more clearly defined in the future.

C. BIOCHEMISTRY

Developmental work for the biochemical evaluations of specimens derived from exposed and control animals of the isotope inhalation experiments has been under way during the past calendar year. The projected program includes both survival testing through a periodic bleeding schedule, and tissue studies on sacrificed animals.

Study of the proteinaceous component of plasma and of various tissue homogenates and extracts received first consideration. Electrophoresis on filter paper according to the Durrum[1] technique, and on starch block according to Kunkel and Slater[2] was studied, as well as the hanging curtain method of Grassman and Hannig[3]. The common shortcoming of these methods is the unsatisfactory resolution of fine structure in the electropherogram, as even with most careful conduct it is not possible to obtain differentiation beyond the five main bands of albumins, α_1, α_2, β, and γ globulins, and fibrinogen. A more informative method was needed for the indication of subtle effects of a subclinical nature.

In 1959, Smithies[4] introduced the method of vertical electrophoresis in a partially hydrolyzed starch gel, with marked improvement in the resolution of protein bands. As many as 25 distinct fractions of human serum could thus be obtained, most of which have been fully characterized, and

identified with such minor but nonetheless important serum factors as ceruloplasmin transferrin, haptoglobins, cryoglobulins, and others. A comprehensive review of these recent studies will soon be available[5]. Immunoelectrophoretical evaluation of starch gel bands led to the recognition of α_2-globulins as a mixture of a slow-moving and a fast-moving component, as well as to the discovery of the second human prealbumin[6, 7].

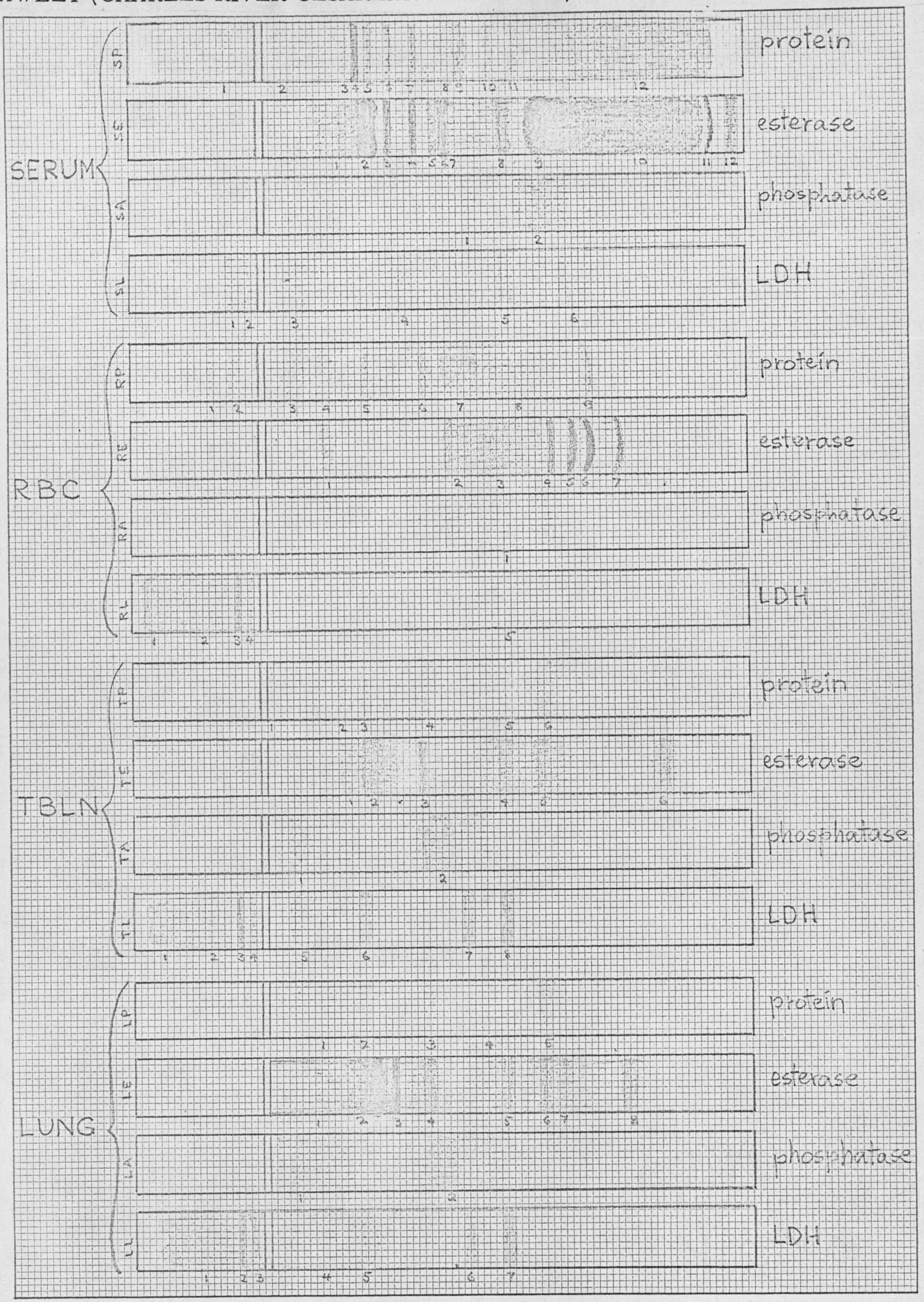
A further virtue of starch gel as an electrophoretic medium is that its diffusion characteristics for organic dyes is similar to that of fixed mammalian tissue. Thus a new area of application for histochemical enzyme stains was opened up, and the combination of such enzyme assay with starch gel electrophoresis occurred first to Markert and Hunter[8]. Successful demonstration of several enzymes in serum by this method was made by Lawrence, Melnick, and Weimer[9], and heart, liver, kidney, and brain tissue was investigated by this means by Tsao[10]. Esterase, phosphatase and dehydrogenase isozymes were discovered by this method during the past few years, and the study of such "zymograms" in different tissues of different species has become the fashionable pastime of enzymologists.

Exploitation of the method for the characterization and understanding of pathologic conditions has commenced only recently, and the use of starch gel electrophoresis as an adjunct to toxicology has been, to our knowledge, so far confined to this laboratory. Our first starch gel chamber was built in late 1962, and during the past year methodologies have been standardized for several tissues of several animal species. Special attention is given to tissues of the respiratory apparatus, and the enclosed illustration (Figure 28) shows a complete mapping of starch gel bands on the serum, erythrocyte extract, lung tissue extract, and tracheobronchial lymph node extract of the Sprague Dawley (Charles River Caesarean Delivered) rat. The tissue samples are macerated in 2 volumes of cold isotonic saline, freeze-thawed 6 times, processed through a Potter-Elvehjem homogenizer[11], centrifuged, and applied to the starch gel of Smithies[12]. The erythrocytes are toluene-washed prior to application.

The starch gel electrophoresis is conducted in pH = 8.4 borate buffer, at a constant current of 18 mA for 18 hours at 0 C°. The gel is prepared at a concentration of 74 g/600 ml., at 73 C°, degassed, poured into the mold, and run in a vertical position. 40 µl of sample is applied into each of the 8 slots formed in the gel. Current is conveyed to the gel by means of

MAPPING OF STARCH GEL ELECTROPHORESIS BANDS, SPRAGUE-DAWLEY (CHARLES RIVER CESAREAN DELIVERED) RAT

FIGURE 28



20 X 20 TO THE INCH 358-10
KEUFFEL & ESSER CO. MADE IN U.S.A.

filter paper wicks moistened with pH = 8.1 borate buffer. The gels are sliced longitudinally after the completion of each run. Since each sample is run on2 gels, there are 4 cuts resulting from each specimen; these are stained for protein, esterase, acid phosphatase, and lactic dehydrogenase.

Protein stain. 30 min. 25 C° in 0.1% Buffalo Black in 10% methanol. Wash with acetic acid:methanol:water = 1:5:5.

Esterase stain. 60 min. 37 C° in 0.1% Blue RR Salt, in 10% tris-HCl containing 0.02% naphthylacetate and 1% acetone. Wash with acetic acid:methanol:water = 1:5:5.

Acid phosphatase stain. 180 min. 37 C° in 1.3% sodium acetate, 0.1% magnesium chloride, and 0.1% naphthyl sodium phosphate in pH = 5.0 acetate buffer. Wash with acetic acid: methanol:water = 1:5:5.

Lactic dehydrogenase stain. 90 min. 37 C° in a solution containing 0.0005 M each of nicotine adenyl dinucleotide and nitro blue tetrazolium, 20 μg/ml phenazine methyl sulfate, 0.005 M potassium cyanide, 0.05 M tris-HCl, and 0.02 M sodium lactate. Wash with acetic acid:methanol:water = 1:5:5.

Figure 28 shows the distribution of bands on different tissue specimens with different stains. Mapping of additional organs, especially liver, kidney, brain, and spleen, are now in preparation. Staining methods for succinic dehydrogenase and alkaline phosphatase are also being developed.

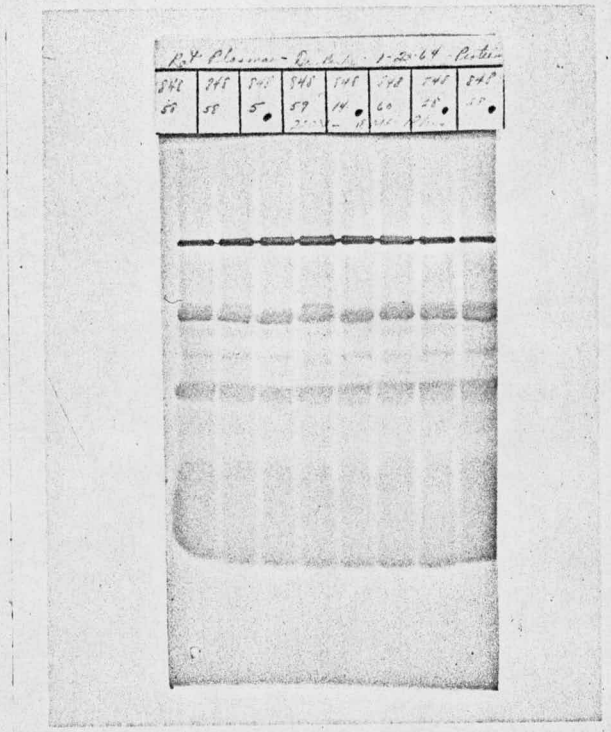
The band nomenclature code seen on the figure is provisional, as not all of the nearly 100 bands are firmly identified with known biochemical fractions. Studies in this direction are also in progress.

Reproducibility of the patterns is generally excellent, with considerable individual variation showing between animals of identical breeding background. The correlation of these variations with the susceptibility to different pathologies, and the alteration of patterns in the course of toxicological exposures is the ultimate goal of these studies. The method also allows distribution, transport, and biochemical association studies of stable and radioactive agents through chemical analysis of gel cuts, and through radioautography.

Because of the extensive preliminary developmental work, and because of the high level of radioactivity in the tissues of some of the exposed animals until now, actual testing of radioactively exposed animals has commenced only recently. The following protocol refers to rats no. 58, 59, 60 (controls), and 5, 14, 78 (exposed) of X 848 (Europium^{152}). The gels are shown on Figures 29, 30, 31, and 32; for easy recognition the strips belonging to the exposed animals are marked with an ink dot on the gels. Marginal positions are duplicate runs. The following comments apply:

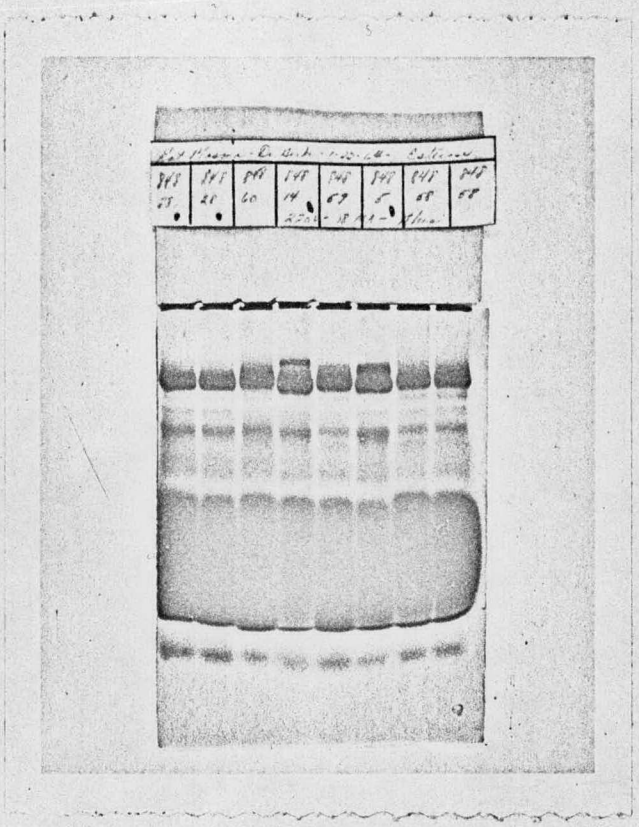
EUROPIUM^{152} EXPOSURE OF RATS: PROTEIN STAIN

FIGURE 29



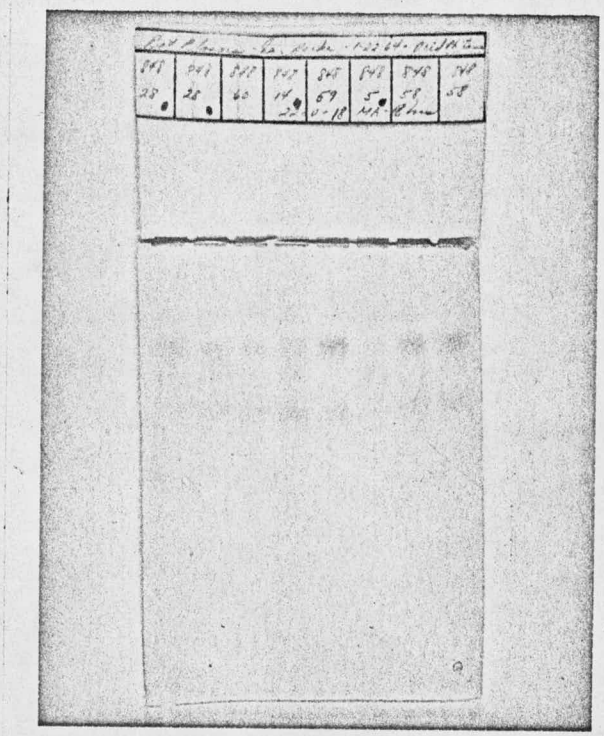
EUROPIUM^{152} EXPOSURE OF RATS: ESTERASE STAIN

FIGURE 30



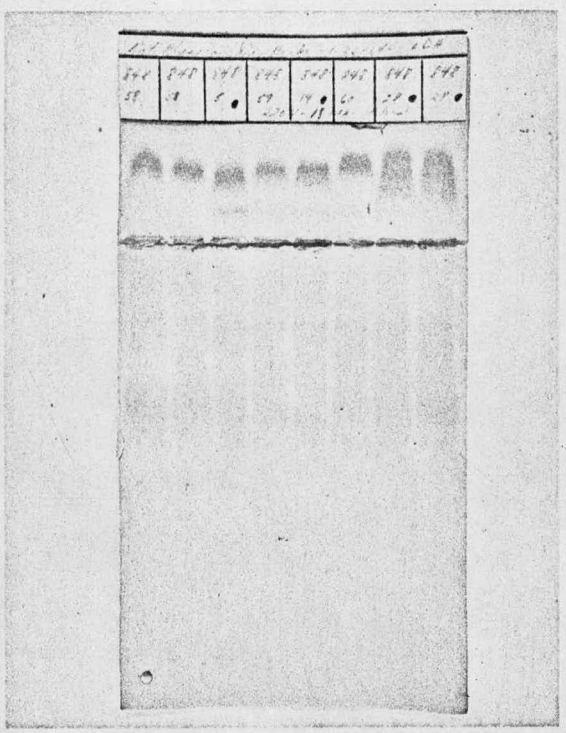
EUROPIUM ^{152} EXPOSURE OF RATS: ACID PHOSPHATASE STAIN

FIGURE 31



EUROPIUM^{152} EXPOSURE OF RATS: LACTIC DEHYDROGENASE STAIN

FIGURE 32



SP-1. (-globulin) noticeably weaker in exposed animals.

SP-2. No change.

SP-3. (A haptoglobin often seen if specimen is hemolyzed) Appears in all control and one out of three exposed animals.

SP-4 to 12. No change.

SE-1. (A slow-migrating esterase in the -globulin region) Appears in extra strength in two out of three exposed animals.

SE-2. (Strongest esterase band in the globulin region) Slightly retarded in all control specimens.

SE-3, 4, 5. No change.

SE-6. (Faint esterase apparently associated with the fast γ-globulin) Very faint in two out of three control specimens.

SE-7 to 10. No change.

SE-11. (Esterase band in front of albumins) Variable but nonrelated to exposure.

SE-12. No change.

SA-1&2. No change correlated with exposure.

SL-1&2. (Lactic dehydrogenase in the -globulin region) Marked increase in exposed animals.

SL-3 to 6. No change (these bands came out very pale in all animals for apparently procedural reasons).

It is premature to draw conclusions on such limited sample material, but the changes seen in the -globulin region are noteworthy. A general decrease in protein concentration and marked increase in lactic dehydrogenase activity is seen in this fraction of all exposed animals, which correlates well with the known antibody-inhibiting and tumorogenic properties of radiation. The phenomenon will be further investigated.

Radioautographic analysis of the starch gels is now in progress. It will indicate the areas of Eu^{152} accumulation, and thus association with one or the other protein fraction, and may account for some of the differences seen in the enzymes of the -globulin region. Significance of these observations will be assessed as the study progresses.

Bibliography

1. Durrum, E. L., J. Am. Chem. Soc. 72, 2943 (1950).
2. Kunkel, H. G. and Slater, R. J., Proc. Soc. Exp. Biol. Med. 80, 42 (1952).
3. Grassman, W., and Hannig, K., Naturwissenschaften 37, 397 (1950).
4. Smithies, O., Biochem. J. 71, 585 (1959).
5. "Symposium on Gel Electrophoresis" New York Academy of Sciences Conference Monograph; in press (1964).
6. Kuenchel, F., Dent. Z. Ges. Gerichtl. Med. 51, 464 (1961).
7. Scheiffarth, F., Goetz, H., and Knopff, H., Acta Hematol. 26, 169 (1961).
8. Markert, C. L., and Hunter, R. L., J. Histochem. Cytochem. 7, 42 (1959).
9. Lawrence, S. H., Melnick, T. J., and Weimer, H. E. Proc. Soc. Exp. Biol. Med. 105, 575 (1960).
10. Tsao, M. U., Arch. Biochem. Biophys. 90, 234 (1960).
11. Potter, V. R., and Elvehjem, C. A., J. Biol. Chem. 114, 495 (1936).
12. Smithies, O., Biochem. J. 61, 629 (1955).

D. PULMONARY PATHOLOGY

The coding system for tabulating pulmonary pathology is as shown in the accompanying table. It is basically the same as used in previous reports.

The findings in control animals is listed beneath the findings in exposed animals for ease of comparison. No significant differences are noted except in experiment no. 757 Europium^{152}. However, in this experiment the number of animals involved is too small for statistical analysis. Furthermore, in experiment nos. 758 and 810, also Europium^{152} exposures, there is no apparent difference in the tissue findings between the exposed and nonexposed animals.

It is pertinent to note that the rat does not develop spontaneous lung tumors. Under these circumstances, tumor production in our animals would be a definite index of the tumorigenesis of our aerosols. It is not surprising at this point that minimal pathological injury is observed in view of the fact that the induction of tumors is associated with rather long time-lag periods, of the order of years. Our animals will be carefully observed during their entire life span.

CODE KEY FOR PATHOLOGY

O	No significant pathological findings.
T	Tumor.
P	Pneumonia.
p	Slight pneumonitis still permitting some evaluation of the tissue.
1	Few phagocytes containing particulate, free particulate present.
2	Numerous phagocytes containing particulate with and without clumping.
3	Early fibrosis alveolar septa (focal).
4	Mature fibrosis (focal).
5	Mild inflammatory reaction (chronic).
6	Particulate in tracheobronchial lymph nodes.
7	Particulate in lymph channels.
8	Mild emphysema.
9	Small parenchymal granulomas.
10	Large and/or confluent granulomas.
11	Granulomas with fibrosis.
12	Epithelial proliferation associated with demonstrable particulate.

EUROPIUM ^{152}

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
757	30	514	115	P, 2, 5
	29	514	115	5
	28	514	115	3, 5
	27	514	115	M. A. *
Controls				
	134	514		0
	133	514		0
758	42	514	116	1, 5
	41	514	116	5
	40	514	116	P, 2, 5
	39	514	116	P, 2
Controls				
	135	514		1, 5
	133	514		2, 5

*Multiple Abscesses

EUROPIUM ^{152}

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Minutes)	Pathology
810	54	287	50	P, 3, 5, 8
	50	287	50	5
	33	287	50	5
	32	287	50	0
	27	349	50	5
	59	349	50	5
	58	349	50	5
Controls				
	116	287		5
	114	287		5
	100	349		5
	99	349		P, 5

EUROPIUM $^{152-154}$

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
847	9	109	14	0
	15	109	14	0
	11	128	33	0
	30	128	33	0
	23	156	61	0
	48	156	61	0
	10	214	119	5
	43	214	119	
Controls	50	214		0
848	23	109	10	0
	26	109	10	0
	6	128	21	3, 5
	20	128	21	5
	22	156	39	P, 5
	37	156	39	0
	32	214	77	3, 5
	39	214	77	P, 5
Controls	57	214		3, 5

EUROPIUM OXIDE NON-RADIOACTIVE

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
850	27	128	21	H*, 5
	19	156	39	3, 5
	24	214	77	5
	48	214	77	P, 5
Control	54	214		5

*Hemorrhage

SCANDIUM ^{46}

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Minutes)	Pathology
811	36	287	38	1, 3, 5
	34	287	38	p, 5
	72	287	67	H*
	56	349	67	1, 5
	38	349	38	1, 5, H
	37	349	38	p, 5
Controls				
	121	287		p, 3, 5
	120	287		5
	104	349		M. A.**
	101	349		p, 5

*Hemorrhage
**Multiple Abscesses

SCANDIUM ^{46} CHLORIDE

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
679	21	799	111	P, 2, 5, 8
	20	799	111	P, 5
	4	859	111	M. A. *
	55	859	111	M. A.
	38	859	111	1, 5
	62	810	111	M. A.
	56	859	111	M. A.
	120	810	111	P, 1, 5, 9
	99	799	111	M. A.
	63	859	111	2, 3, 5, 10
Control				
	140	528		1, 5, 9
	136	536		M. A.
	133	536		P, 2, 5, 9
	130	528		2, 5, 10
	156	528		5
	150	528		M. A.
	147	528		P, 1, 5
	142	588		P, 2, 5, 10
	157	588		5

*Multiple Abscesses

YTTRIUM ^{91}

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
691	31	614	97	P, abscess
	16	614	97	M. A. *
	103	645	136	M. A.
	101	645	136	M. A.
	78	645	136	M. A.
	70	645	136	M. A.
Controls				
	133	645		5
	204	614		M. A.
	203	614		M. A.
	161			P, abscess

*Multiple Abscesses

$COBALT^{60}$

Exp. No.	Animal No.	Animal Age (Days)	Exposure (Days)	Pathology
768	97	442	125	4, 5
	96	442	125	5
	95	442	125	Abscess
	98	442	125	2, 3, 5
	61	563	125	2, 4, 5
	58	563	125	2, 5
	51	563	125	Abscess
	50	563	125	P, 5, 8
Control				
	149	442		5
	148	442		3, 5
	137	563		3, 5
	136	563		2, 3, 5

E. LIFE SPAN DATA

It has been reported that one of the most sensitive test of injury following the intake of radioactive substances is the life span shortening which occurs.

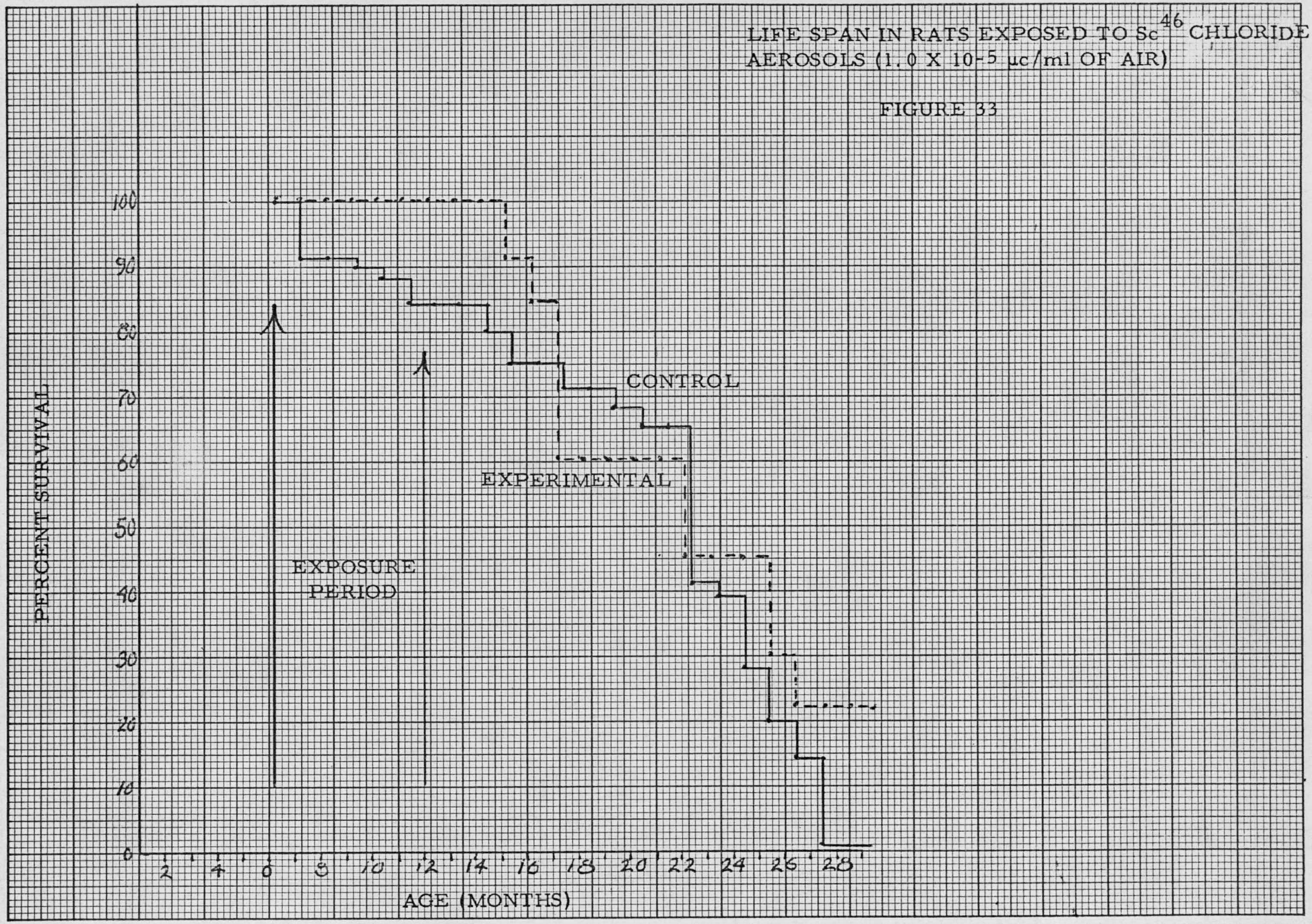
In one experimental study in which animals were exposed to different radionuclides and to graded concentrations of aerosol activity it was possible to set aside animals not involved in serial sacrifices or other procedures which might interfere with the collection of valid life span data. It is fully realized that long term exposures, where animals reside in inhalation chambers for as long as 6 months continuously (whole body exposures), cannot be validly compared to those serving as controls in animal quarters. It is nevertheless of interest to look at relative life span data collected to date for possible clues to the toxicity of the aerosols used.

These data are shown in Figures 33 to 36.

The life span of rats exposed to small amounts of Scandium^{46} (Figure 33) is not clearly different

LIFE SPAN IN RATS EXPOSED TO Sc^{46} CHLORIDE AEROSOLS (1.0×10^{-5} μc/ml OF AIR)

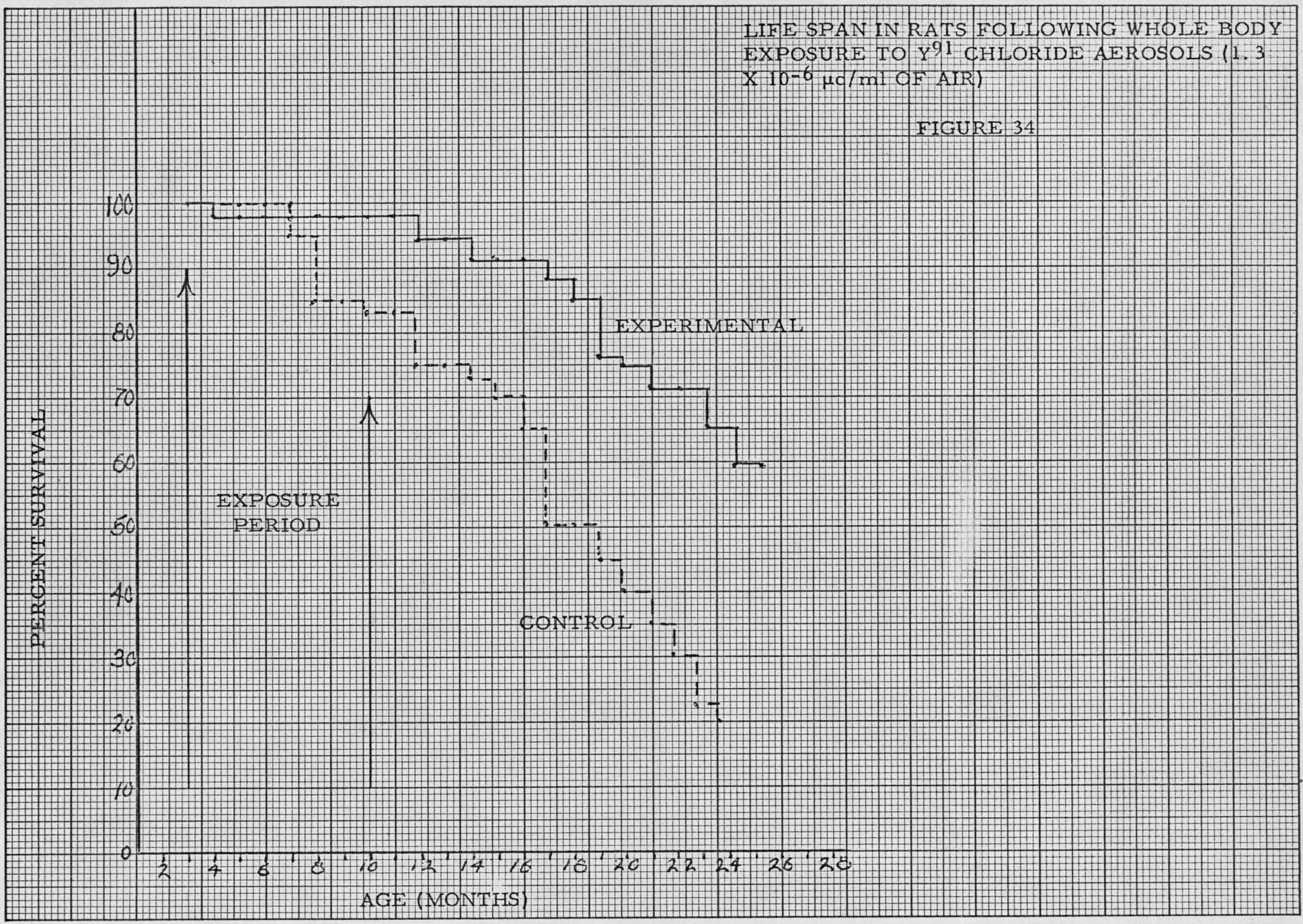
FIGURE 33



K&E 10 X 10 TO THE ½ INCH KEUFFEL & ESSER CO. MADE IN U.S.A. 358-11

LIFE SPAN IN RATS FOLLOWING WHOLE BODY EXPOSURE TO Y^{91} CHLORIDE AEROSOLS (1.3×10^{-6} μc/ml OF AIR)

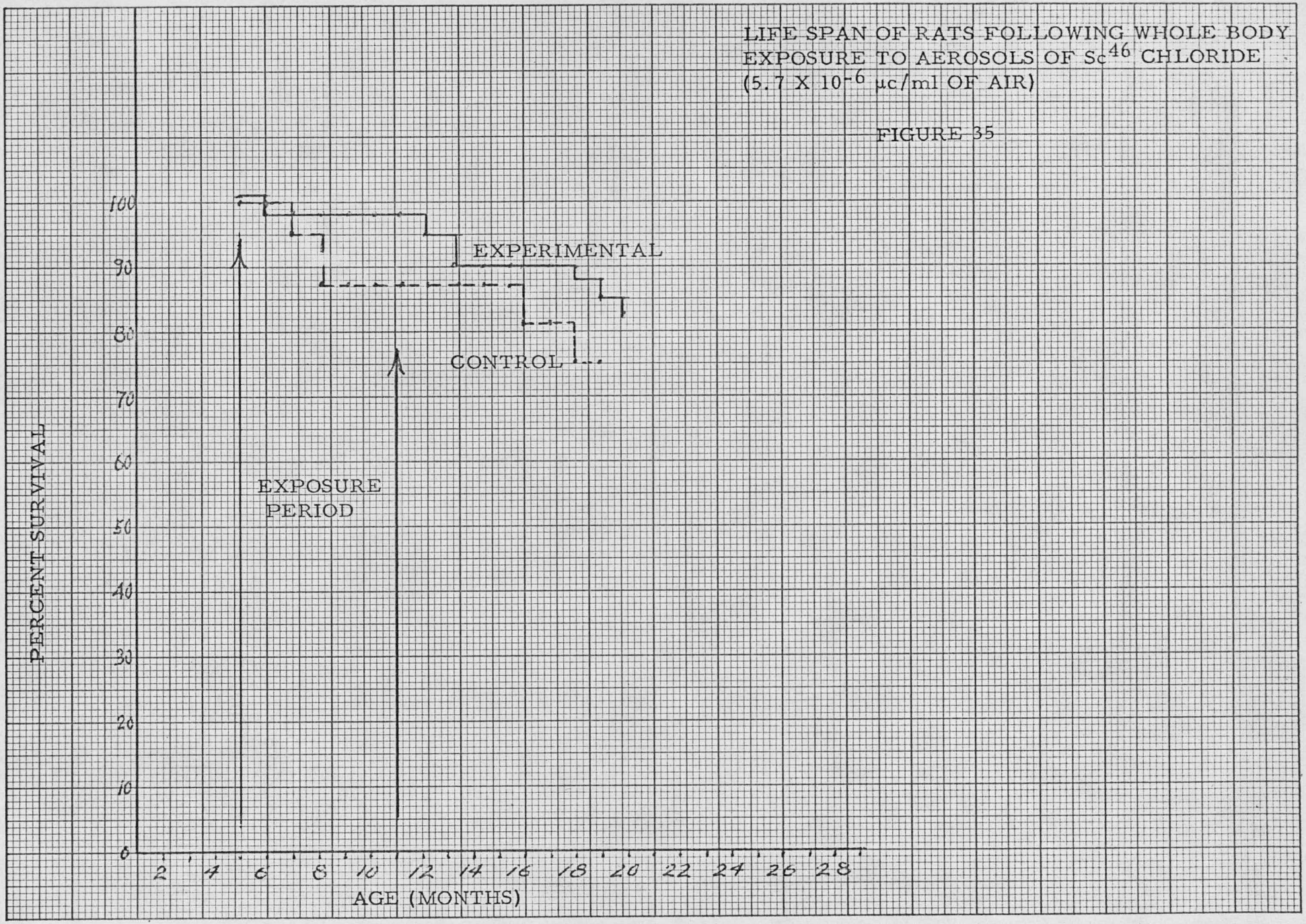
FIGURE 34



K+E 10 X 10 TO THE ½ INCH 358-11
KEUFFEL & ESSER CO. MADE IN U.S.A.

LIFE SPAN OF RATS FOLLOWING WHOLE BODY EXPOSURE TO AEROSOLS OF Sc^{46} CHLORIDE (5.7×10^{-6} µc/ml OF AIR)

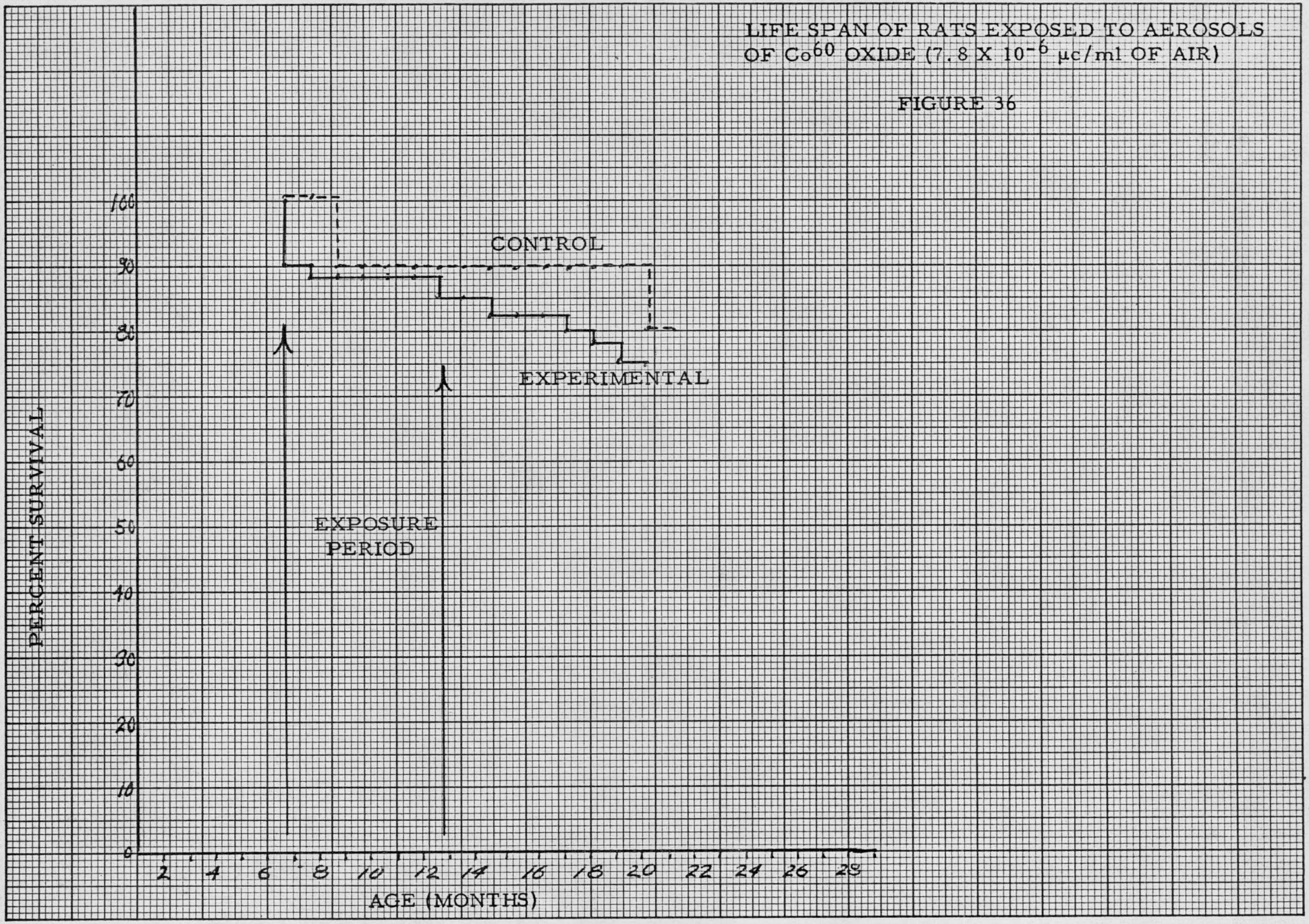
FIGURE 35



K+E 10 X 10 TO THE ½ INCH 358-11
KEUFFEL & ESSER CO. MADE IN U.S.A.

LIFE SPAN OF RATS EXPOSED TO AEROSOLS OF Co^{60} OXIDE (7.8×10^{-6} µc/ml OF AIR)

FIGURE 36



K+E 10 X 10 TO THE ½ INCH KEUFFEL & ESSER CO. MADE IN U.S.A. 358-11

from the control values while that for Yttrium^{91} chloride (Figure 34) indicates that the experimental rat's longevity is enhanced rather than shortened. The only impairment in life span appears to be caused by Cobalt^{60}, if indeed the difference is not artifactual. Both Cobalt^{60} and Sc^{46} (Figure 34, 35) studies are in initial stages, however, and the ensuing mortality may shed further light on actual life span differences. It is apparent that experiments for life span changes are subject to many difficulties including colony injections, and must be planned for much larger numbers of animals than is possible here at this time.

Nevertheless, by the application of statistical analyses, even for small numbers of animals may indicate a trend if nothing else.

F. HEMATOLOGY

All experiments to date using rats have included in their schedules, provision for the study of changes in the formed elements of blood. These included total numbers of leucocytes, erythrocytes, lymphocytes, monocytes, neutrophils (polymorphomecleocytes) and eosinophils. In addition, hematocrits and hemoglobin content were determined.

In most instances, animals showed a reversal of values in the neutrophil and lymphocyte cell counts during exposure to radionuclides. Whether these changes are attributable to the effects of the aerosol rather than extraneous conditions such as differences in maintenance of exposed rats relative to control animals is not known. Europium studies in which control rats were treated in every respect in the same manner as the others, indicated the following trends for two groups (Table I). Figure 37 and 38 compare fluctuations in various blood elements among the three experimental and the control group.

Tests of significance for the hematological data obtained are being calculated at present at the 1% and 5% level but are not yet available.

TRENDS OF FORMED BLOOD ELEMENTS IN RATS EXPOSED TO EUROPIUM

TABLE I

EXPERIMENT 847

	Exposure Days					
	-7	14	134	164	194	Trend Within Group
Neutrophils(%)*						↑
Mean	23.2	25.9	28.3	24.4	32.5	
St. Dev.	5.5	8.9	5.4	7.2	6.4	
Lymphocytes (%)						↓
Mean	73.1	72.8	69.1	72.1	63.1	
St. Dev.	6.4	9.2	5.7	7.9	12.1	
W.B.C. #Cell X10^3						↑
Mean		13.0	14.7	16.1	18.5	
St. Dev.		1.5	1.6	2.6	3.2	
Neutrophiles #Cell Count X 10^3						↑
Mean		3.3	4.2	3.9	6.1	
St. Dev.		1.2	0.9	1.2	2.0	
Lymphocytes #Cell X 10^3						↑
Mean		9.4	10.2	11.7	11.6	
St. Dev.		1.8	1.6	2.5	2.0	

*Expressed as percent of total W.B.C.

TRENDS OF FORMED BLOOD ELEMENTS IN RATS EXPOSED TO EUROPIUM

TABLE I (continued)

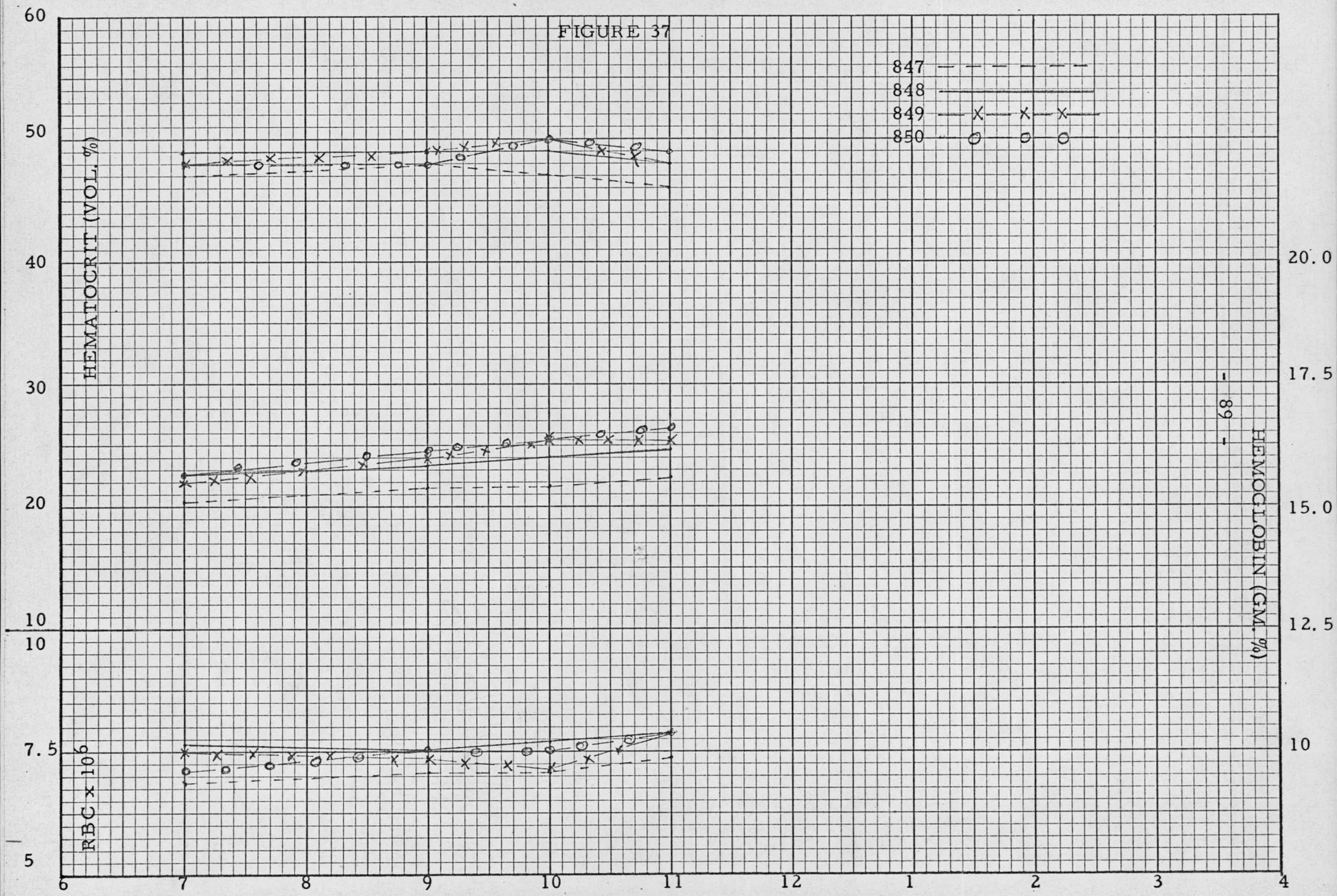
EXPERIMENT 849

	Exposure Days					
	-7	14	134	164	194	Trend Within Group
Neutrophils (%)						
Mean	21.9	27.1	28.9	27.2	28.6	↑
St. Dev.	5.1	9.0	9.7	9.4	8.9	
Lymphocytes (%)						
Mean	75.1	71.3	69.0	68.9	67.1	↓
St. Dev.	8.9	8.9	9.5	10.9	9.3	
W.B.C. #Cell X 10^3						
Mean		14.9	13.2	12.7	13.6	———
St. Dev.		3.3	3.1	2.0	3.0	
Neutrophils #Cell X 10^3						
Mean		3.9	3.7	3.5	3.8	———
St. Dev.		1.1	1.0	0.9	1.4	
Lymphocytes #Cell X 10^3						
Mean		10.9	9.2	8.6	9.0	———
St. Dev.		3.6	3.2	1.6	2.3	

K&E 10 X 10 TO THE INCH 358-5D
KEUFFEL & ESSER CO. MADE IN U.S.A.

ERYTHROPOIESIS IN ALBINO RATS EXPOSED TO EUROPIUM

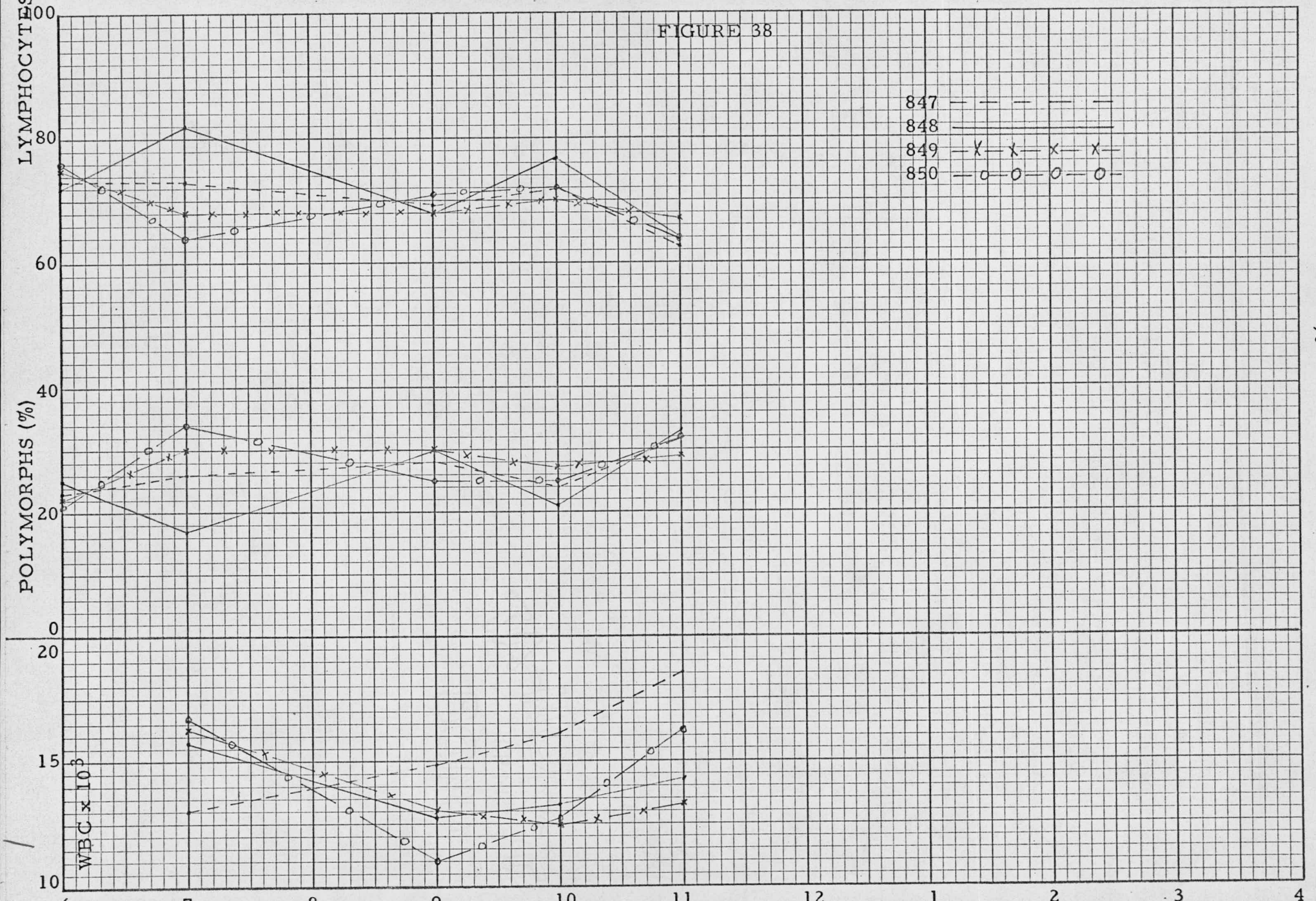
FIGURE 37



K+E 10 X 10 TO THE INCH 358-5D
KEUFFEL & ESSER CO. MADE IN U.S.A.

LEUCOPOIESIS IN ALBINO RATS EXPOSED TO EUROPIUM

FIGURE 38



There is an apparent shift in the proportions of neutrophils and lymphocytes the cause of which is not clear. Tentatively, it appears that the two groups of experimental animals (experiment 847, 849) differ significantly from each other in many instances, although they did not necessarily differ from the control groups (experiment 850). At least three more replicates are required and will be obtained to define the trends in blood changes.

All remaining animals will be tested for the rest of their life spans for purposes of defining more clearly the hematological trends over the long term. An attempt will be made to correlate these changes with the radiation dose.

G. LUNG FUNCTION

During the course of the past three years, the AEC contract has supported, in part (patented by AEC) the departmental activities concerning the design, development and application of a new instrument (patented by AEC) for pulmonary function studies. The activity was mandatory because conventional instruments and methods (spirometer, plethysmograph, interpleural puncture, etc.) did not permit easy and repeated application to an animal over a long period of time and with the desired degree of validity.

Briefly, the new respirometer employs electrical capacitance as a means to measure the pulmonary function of a test animal or human subject, in terms of air volume changes which accompany each inhalation and exhalation of the breathing cycle in a defined time period.

Although the further development of the instrument is still in progress, nevertheless a satisfying degree of reproducibility has been achieved. Accordingly, during the past year, it has been critically applied to a variety of test animals.

The results of our studies with the new instrument are exemplified in Figure 39. It depicts the tracing which represents the volume of air inhaled and exhaled in each respiratory cycle in definitely known time periods, in addition it also reveals the tracings for the concurring pressure imposed on an intraesophageal balloon, and also for the flow rate of air during each phase of the cycle. The ratio of volume to pressure yields values of pulmonary compliance. Thus, it is now possible to sequentially follow the pulmonary function in animals which have been challenged, for example with radioactive substances. We hope that such functional studies will afford a regular valid index of subtle events which have significance in terms of impairment possibly induced by inhaled pollutants having radioactivity or any other irritant potential.

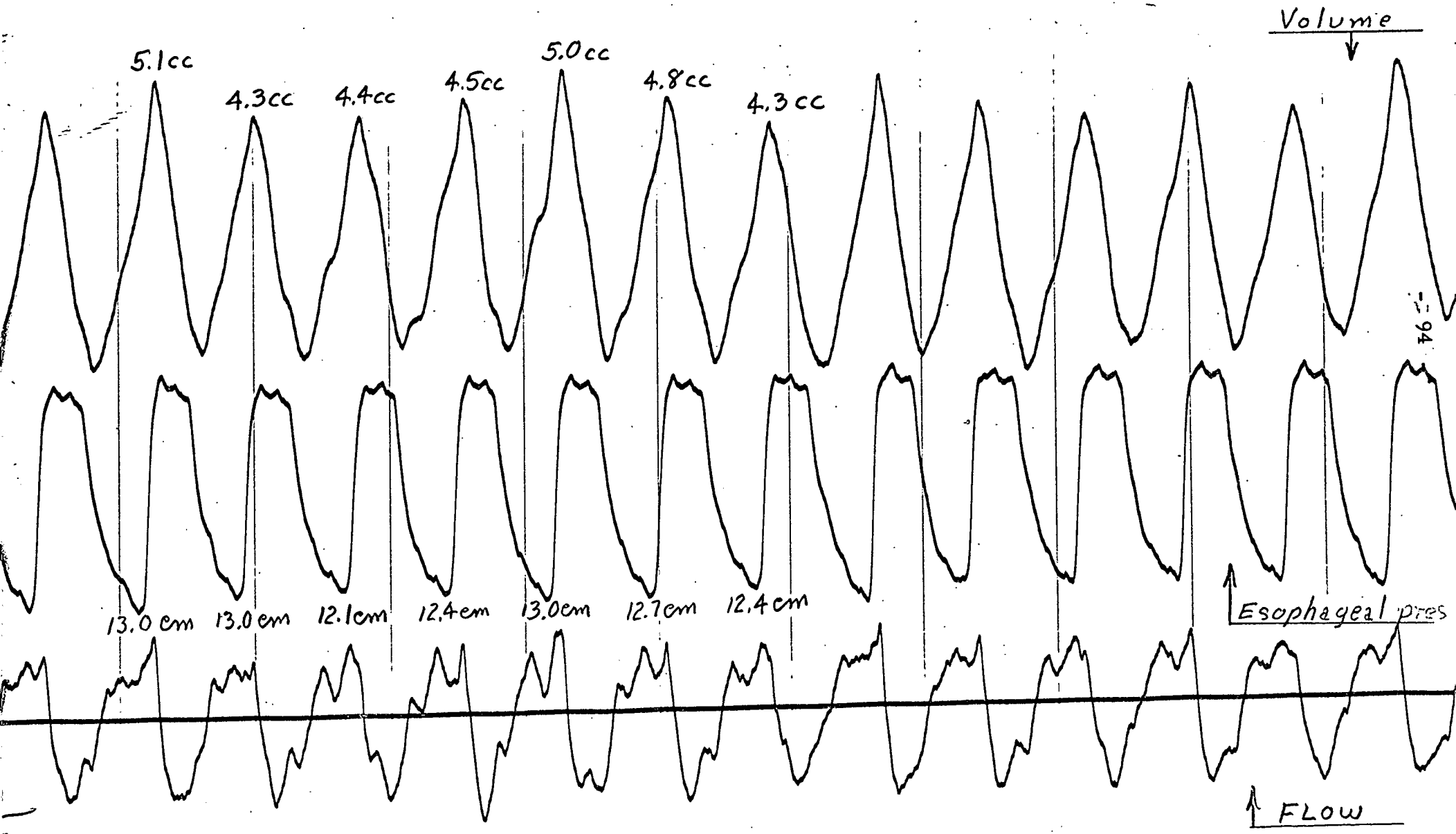
The new technique has been applied to only a few animals under study within the research program supported by AEC. We are now ready to extend our program, routinely utilizing pulmonary function values for correlation with the nature and severity of the radioactive challenge in a variety of animals.

FIGURE 39

X-845 RAT #22 JAN 7, 1964

Volume calibration: 2 Volts; 2.5 cm/V range 100 V

MEAN Volume 4.6 cc; Mean Esophageal pres. 12.7 cm. of H_2O; Mean compliance RATIO 0.37 cc. / cm of H_2O pres.



As indicated above, the new instrument and technique has been applied to a few animals within the AEC supported program. For example, the values for compliance in three control rats and three rats exposed (7 hours daily - 5 days a week) for 6 months to aerosols of Europium$^{152-154}$ in the chloride form, average 0.52 (range 0.36 - 0.76, st. dev. $\pm$ 0.32) for the control and 0.38 (range 0.19 - 0.39, st. dev. $\pm$ 0.27) for the exposed rats, whether these differences between the average values is real remains to be determined in further work along these lines. Protocol for periodic lung function studies has been established and animals from all experiments now available will be randomly selected for pulmonary function tests.

III. LONG TERM INHALATION USING THE DOG

In the past year, 12 beagle puppies have been acquired for anticipated studies of the effects of inhaled radioisotopes over a longer time span than is possible with short lived animals.

The dogs have been quarantined in special quarters, initial blood studies and electrophoretic patterns of serum have been obtained. The animals are weighed regularly.

An octagonal lucite chamber containing an inner plastic bag into which the radioactive material to be used will be introduced has been constructed. There are places for six dogs to be exposed simultaneously through specially constructed tubes attached to masks fitting snugly over the dog's muzzle. These tubes containing butterfly valves for inspiration and expiration of the aerosol are attached to the plastic bag containing the aerosol.

Some preliminary determinations of dog respiration parameters, viz., rate and volume of breathing have been obtained in a set up resembling the experimental, are for purposes of training the animals and for determining whether the feasibility of using these respiratory values during the aerosol inhalation where physiological measurements cannot at present be made.

Research is also proceeding with the objective of modifying the capacitance respirometer developed in this Department. for use in inhalation experiments such as are contemplated in the near future using the dog.