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SPOT DIAMETER METHOD OF QUANTITATIVE AUTORADIOGRAPHY OF RU^{106} PARTICLES IN LUNG TISSUE

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SPOT DIAMETER METHOD OF QUANTITATIVE AUTORADIOGRAPHY OF RU^{106} PARTICLES IN LUNG TISSUE

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SPOT DIAMETER METHOD OF QUANTITATIVE AUTORADIOGRAPHY OF RU^{106} PARTICLES IN LUNG TISSUE

When Ru^{106} particles in lung are autoradiographed a quantitative straight line function exists between the sum of the spot diameters or a single spot diameter and the total activity per slide.

In the study of the effects of radioactive particulates on tissue the quantity of the radioactive material in the individual particles is of importance in describing the dosimetry and in obtaining any quantitative causation relationship. As this investigation is usually carried out by the administration of large numbers of such particles, it is desirable to be able to measure the radiation dose rate from a single particle in the presence of several others.

The following quantitative study is from mouse lung tissue supplied by the Pharmacology and Experimental Therapeutics Unit of the Biology Section.

METHODS

The particles used were collected from the ventilation system of an industrial plant by impaction methods. The bulk of the radioactive material was Ru^{106}, with a small fraction of Ru^{103}. A suspension containing 47 μc in a 0.1 per cent solution of "Tween 80" was injected into the tail vein of a mouse. The lung was excised after 100 days exposure and fixed in seventy per cent ethyl alcohol. The tissue was then dehydrated in graded ethyl alcohol, cleared in benzene, imbedded in 62-64°C "Tissuemat" and blocked for cutting. Serial sections were cut at five microns thickness and the Ru^{106} content of every third section was measured on a mica

window counter. The sections used for the previously mentioned measurements were floated on distilled water and attached to an NTB autoradiographic emulsion of eleven microns thickness (including a one micron protective coat to prevent abrasions of the emulsion). Exposure was made for 72 hours at 39°C, in a light tight box to which Drierite ($CaSO_4$) had been added to control humidity. After the slides had been warmed to room temperature the paraffin was removed from the tissue preparations by processing for five minutes in xylol, five minutes in "Cellosolve", and washing for five minutes in distilled water.

The autoradiographic emulsion was developed by controlled processing at 18°C in "D-19" developer for five minutes, washing in distilled water and allowing to fix for thirty minutes in Kodak fixer. The preparations were dehydrated and subsequently covered by "Permount" and a coverslip 0.18 mm thick.

A total of 514 spots were measured in the survey of 90 autoradiographic slides containing the tissue and particles. A calibrated ocular micrometer was used for measurement of the spots. Measurements of spots were uniform and included only the darkened areas. Gray areas fringing the black were excluded.

For purposes of correlation, the activity of a single particle was taken as proportional to the diameter of the darkened area. Thus if a number of particles were present on a single slide, the total counting rate was compared with the sum of all darkened areas on the slide.

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RESULTS

The general appearance of lung tissue containing radioactive particles in a stained lung-autoradiograph preparation is illustrated in Figure 1. The autoradiographic spots should not be confused with the cell nuclei which also appear as darkened areas but are smaller and more sharply defined.

The spot diameters varied considerably and ranged in size from eight microns to 170 microns. Representative spot diameters appear in Figure 3 and have a diameter of 8, 32 and 63 microns.

The individual diameters of all of the developed spots were measured and totaled for each slide. These were then plotted in relation to the quantity of Ru^{106} per slide (Figure 2). Solid dots are single particles.

SPOT DISTRIBUTION PER AUTORADIOGRAPHIC SLIDE

Number of Spots/Autoradiograph	Frequency of Occurence
1	11 slides
2 to 5	35 slides
6 to 10	27 slides
11 or more	16 slides

A linear correlation between spot diameter and Ru^{106} content resulted in the equation

$$\mu c = 3.0 \times 10^{-6} D + 1.2 \times 10^{-5}$$

where D is the sum of the spot diameters on a given slide in microns. The standard error of estimate is given by $\sigma_y = 1.2 \times 10^{-4}$ (Figure 2).

Correlations attempted by using the sum of the squares of individual darkened areas again indicated a direct relationship between diameters and Ru^{106} content. Of the eleven slides each containing one particle, only one fell outside the 68.2 per cent limits as defined by σ_y.

DISCUSSION

This technique provides a method of determining the activity of a single radioactive particle in a tissue specimen when measurements of autoradiographs can be made. Such a method may assist in the establishment of quantitative relationships between the size of the spots and dose rate in areas where exposure occurs. Development of a portion of the autoradiographs at temperatures other than $18^{o}C$ would invalidate the quantitation because spots may be enlarged beyond the normal by development at a higher temperature.

Direct examination of the film spots is possible by limiting the magnification to one in which there is a minimum apparent fading of the outer portion of the spot image. Subsequent tissue samples containing Ru^{106} particles, when developed under the same standardized conditions, can be autoradiographed and the Ru^{106} content assigned for any particular spot diameter.

ACKNOWLEDGMENT

Acknowledgment is made to A. C. Case and personnel of the Radiochemistry Laboratory who supplied counting rate data of the samples, and to Dr. R. W. Wager for making available the experimental lung tissue.

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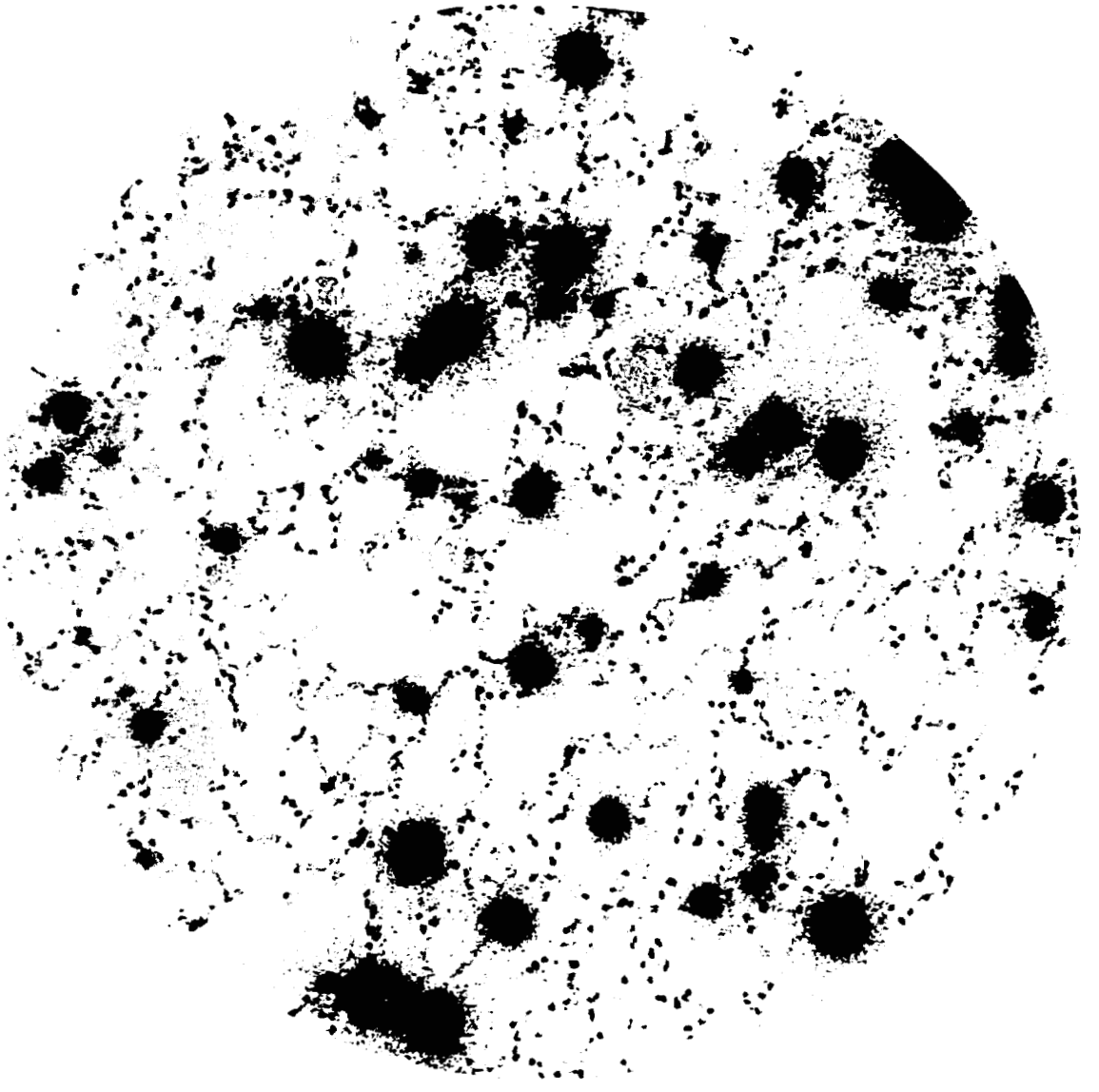

FIGURE 1

Photomicrograph of Stained Lung-Autoradiograph Preparation Illustrating Deposition of Ru^{106} Particles with Resultant Spot Diameter Darkenings. (X200)

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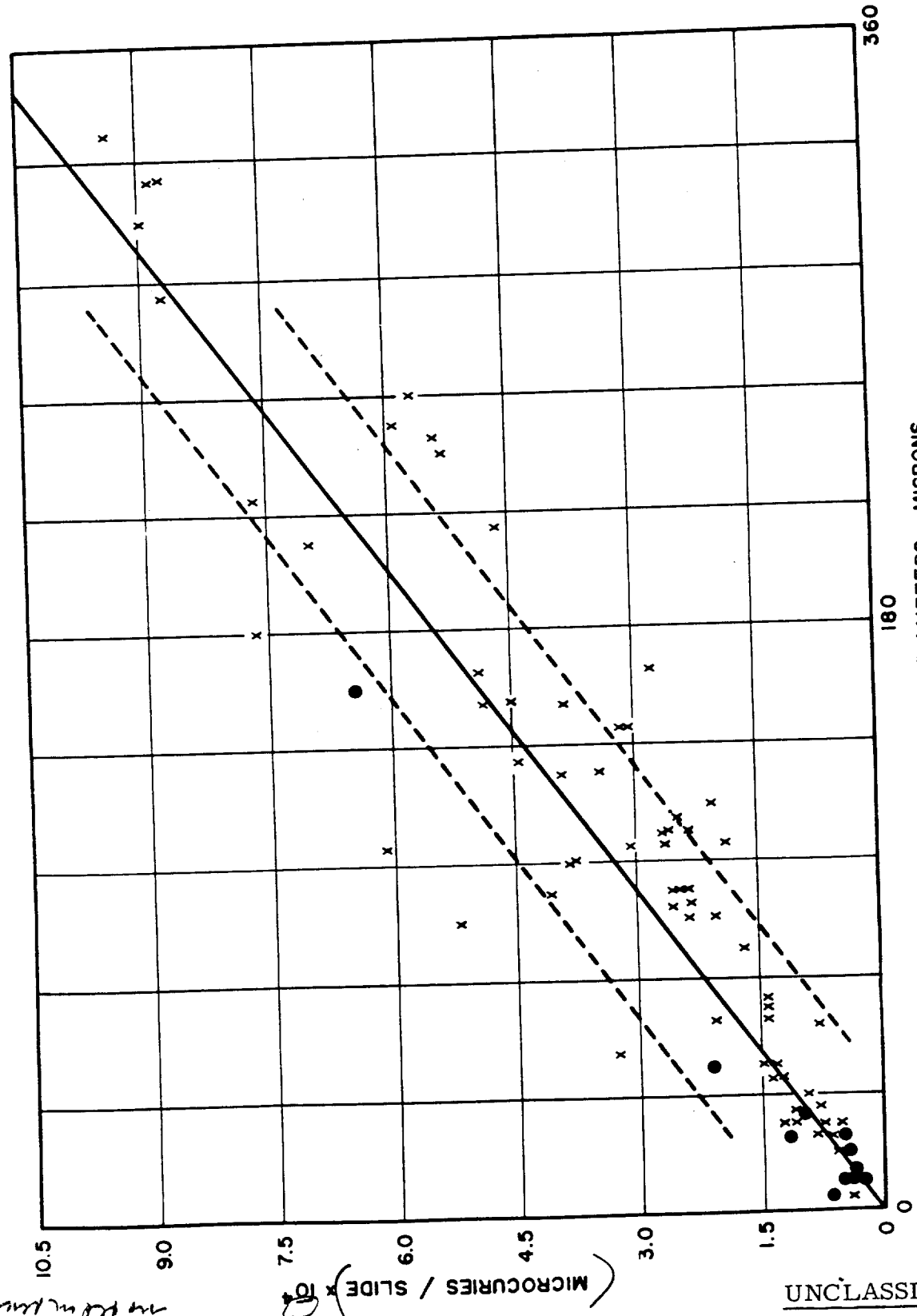

FIGURE 2. AUTORADIOGRAPH CALIBRATION

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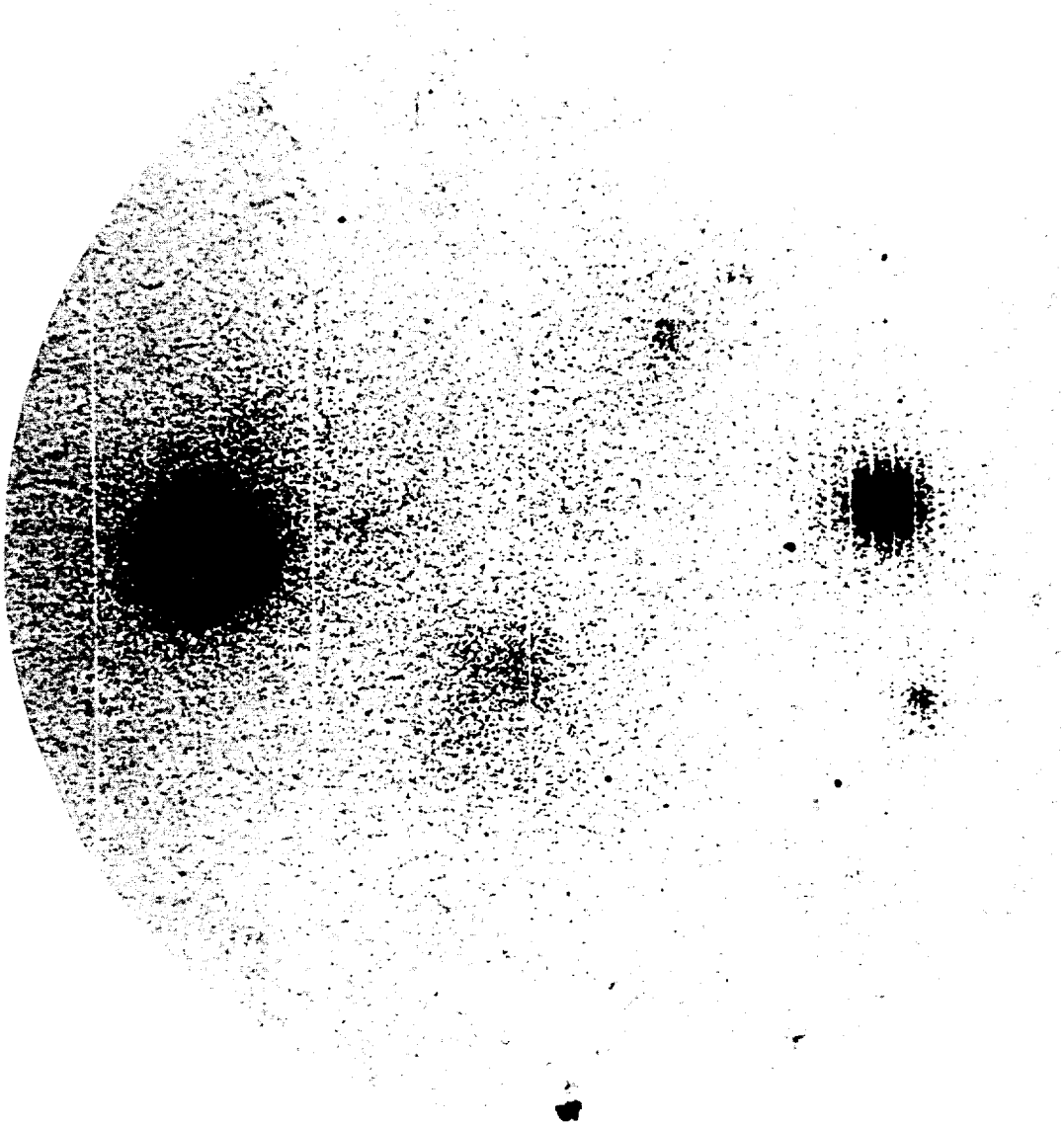

FIGURE 3

Photomicrograph of Unstained Lung-Autoradiograph Preparation of Ru^{106} Particles Used for Spot Diameter Measurements. (Original Magnification X150 Micrometer Rulings are 10 and 100 Microns)

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