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# A METHOD FOR CHRONIC NOSE-ONLY EXPOSURES OF LABORATORY ANIMALS TO INHALED FIBROUS AEROSOLS*

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## ABSTRACT

The U.S. Thermal Insulation Manufacturer's Association (TIMA) is sponsoring a study at our laboratory to determine any biological effects when rats and hamsters inhale man-made mineral fibers (MMMFs). MMMF's to be tested include glass fibers, mineral wool, and ceramic fibers, with crocidolite asbestos serving as a positive control aerosol material. A prime objective of this study is to expose animals to high airborne concentrations of long thin fibers ($\leq$ 3 μm diam x > 10 μm in length). Animal exposures are currently being conducted with a 0.45 μm mean diameter glass microfiber material and the standard UICC crocidolite. Aerosols are produced from bulk materials using a modified Timbrell type fibrous aerosol generator and a controlled density infusion plug packing procedure.

For this endeavor, a specialized method of restraining rats and hamsters for inhalation exposure was developed providing for aerosol exposure only to the nose and a small fraction of the animal's head. This method eliminates external contamination and prevents animals from burying their noses in their fur to filter out aerosolized particles. Stainless steel chambers have been modified by placing two metal insert panels in place of doors, each containing 45 insert ports for Syrian hamsters or 32 for rats. Animals are loaded into tapered polycarbonate holding tubes and the tubes placed in the panel inserts for exposure. Body weights, rectal temperatures, clinical chemistry profiles, complete blood counts, and plasma corticosterone levels clearly indicate that this technique does not produce measurable stress in the animals.

## INTRODUCTION

In our program to assess the potential long-term effects in laboratory animals of inhaled man-made mineral fibers

(MMMF's), an inhalation exposure system was needed that would: 1) be able to expose relatively large numbers of Osborne-Mendel (O-M) rats and Syrian golden hamsters 6 hours a day, 5 days a week for periods up to 24 months; 2) minimize trauma and stress to the animals; 3) minimize potential exposure to the aerosolized materials by personnel handling the animals; and 4) have small chamber volumes so that only minimal amounts of aerosol need be generated while the quality and distribution of the aerosol would be optimal.

Whole-body exposure systems were not considered because, in our experience: 1) animals often pile-up together or hide their faces in their axillary spaces, using body hair as a filter, reducing the amount and quality of aerosol actually inhaled; 2) the aerosolized material is deposited cutaneously, resulting in increased gastro-intestinal tract deposition from grooming and the potential for personnel working with the animals to be exposed during handling; 3) some have large chamber volumes that require large amounts of aerosol to be generated; 4) some require many chambers to expose large numbers of animals; and 5) loading and unloading animals can cause undue trauma to the animals, including injury and even amputation of limbs.

Described in this presentation are an MMMF aerosol generation system and a method we developed for long-term nose-only exposure of laboratory rats and hamsters to fibrous aerosols.

## METHODS

### Inhalation Exposure Chamber and Animal Restraining Tubes.

Chambers originally designed for whole-body inhalation exposures (Hinners et al, 1968) were purchased from Unifab Corp., Kalamazoo, MI. The two glass doors, internal shelving and cages were removed and two inserts placed into the openings created by removal of the doors. These inserts were made of 5 mm thick dural (6061-T6, Kaiser Aluminum Corp., Los Angeles, CA). The portions extending into the chambers (Figure 1) measured 43.5 cm high x 56.5 cm wide and 22.0 cm deep. Trilaminar plates consisting of 1/4 inch thick pieces of Silastic® (Dow Corning Corp., Midland, MI) laminated between two plates of 5 mm thick dural are held in place on the internally extended chamber inserts by 7 cm long suitcase hinges (Figure 1). These trilaminar plates contain multiple circular ports which hold the animal restraining tubes in place. The holes have a 5.2 cm diameter for the tubes used with rats and a 4.7 cm diameter for those used with hamsters. Thus, one plate and insert panel will hold either 32 rats or 45 hamsters. The trilaminar plates are held together by 1/8 inch-32 socket-head screws

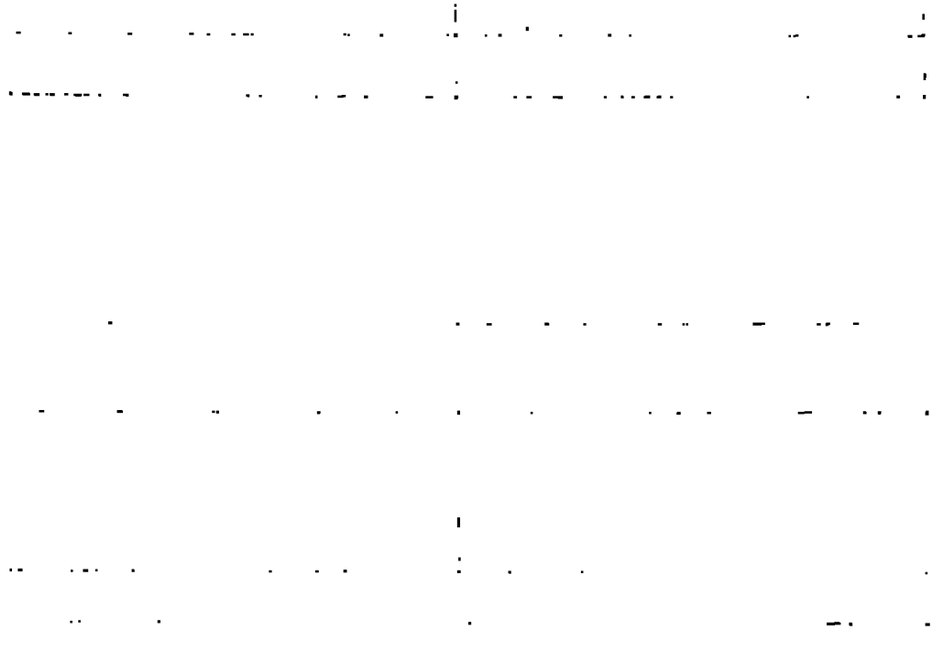

Figure 1. Chamber insert panel with plate containing 45 ports for polycarbonate hamster holding tubes. Trilaminar plate with ports is removable and is held in place with suitcase hinges as illustrated.

located between each tube port opening. They are tightened so that the Silastic® is forced slightly into the port openings, forming occlusive seals when the animal restraining tubes are in place. The Los Alamos National Laboratory Plastics Shop made the animal restraining tubes of polycarbonate by an extrusion process. Their dimensions are as follows:

RATS (used with female O-M and female and male Fischer-344s)
- Inside Diameter: 4.8 cm
- Thickness: 2.5 mm
- Length: 22.5 cm
- Nasal Orifice: 1.7 cm diameter
- Taper: 6°° beginning 3.0 cm from nasal end

SYRIAN HAMSTERS
- Inside Diameter: 4.4 cm
- Thickness: 2.0 mm
- Length: 17.0 cm
- Nasal Orifice: 1.5 cm diameter
- Taper: 6°° beginning 2.5 cm from nasal end

To maintain hermetic integrity and prevent the aerosols from getting around the animals and leaking out into the room, a polyethylene cap with a centrally located 1.0 cm diameter hold, which allows the rats' tails to protrude, is

used to cover the end of the tube. A seal is then obtained and the rat's tail supported by placing one of the hamster tubes whose tapered end has been left sealed in the cap, as demonstrated in Figure 2. Tubes used to contain the Syrian hamsters are sealed with polyethylene caps.

After each 6 hour exposure, the tubes and caps are washed thoroughly in a disinfectant detergent and rinsed.

Figure 2. Insert panel in place in exposure chamber. The ports are filled with polycarbonate restraining tubes, each holding a female Osborne-Mendel rat as ddemonstrated at left.

## AEROSOL PRODUCTION

The device used to generate our fibrous aerosols is schematically illustrated in Figure 3. This generator, described in detail elsewhere (Ortiz et al, 1977) was modeled after one developed by Timbrell et al (1968b). The operating principle of this device is based on the controlled feeding of a fixed density, fibrous plug compact, into a rotating blade assembly to produce the aerosol. Fibers are shaved from the end of the advancing plug by the blades and a stream of air exhausts the airborne fibers from the generator chamber.

Figure 4 illustrates the aerosol generator exposure chamber hookup arrangement. The aerosol leaves the generator, travels through an intermediate dilution device where

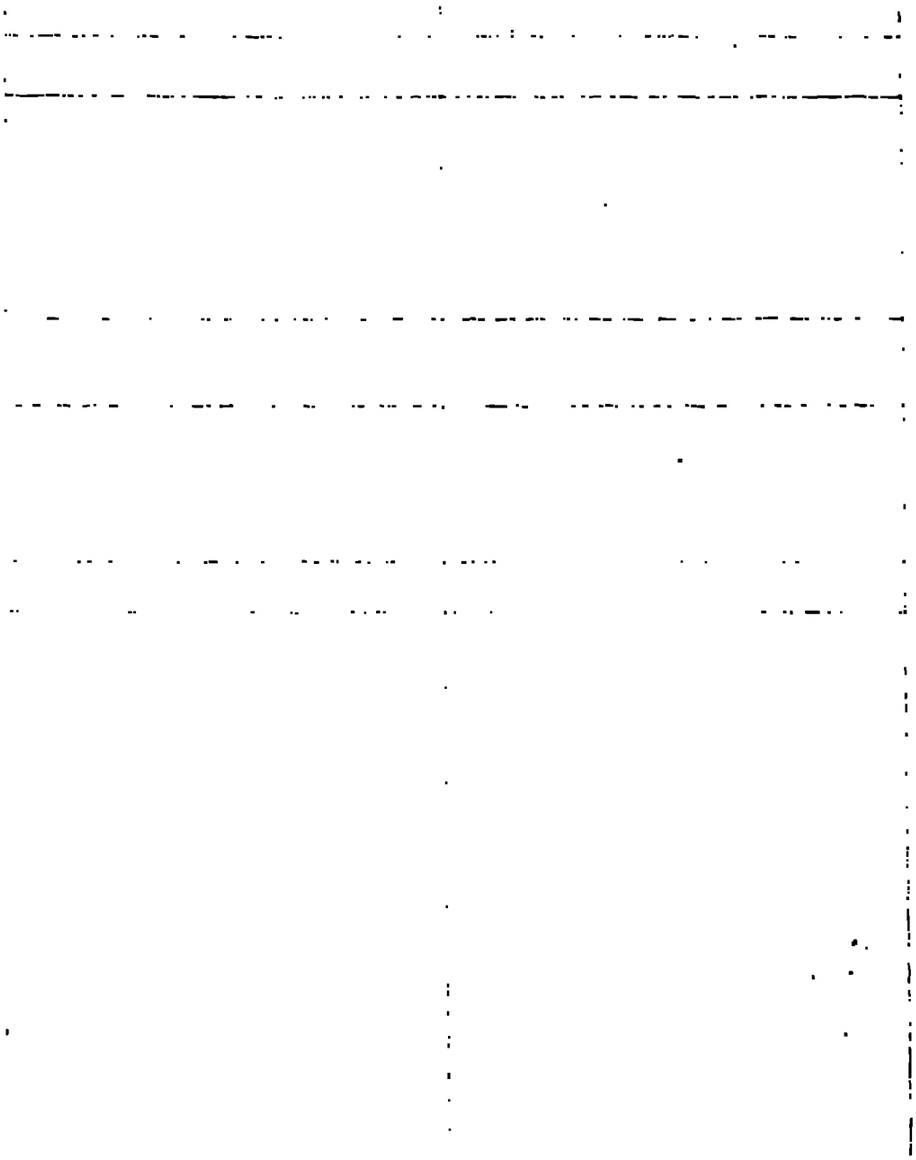

Figure 3. Schematic diagram of generator used to aerosolize fibrous materials. The plug compact is slowly advanced into the rotating blade assembly, shearing off fibers produced as an aerosol.

additional clean air is mixed with the aerosol, then the diluted aerosol is passed through a 10 mCi Krypton 85 aerosol deionization source (Thermo Systems, St. Paul, MN) and into the top of the animal exposure chamber. Aerosol flow in the chamber is from top to bottom. Total airflow rate into the exposure chamber was ~30 l/min (15 l/min primary

Figure 4. Aerosol generator-exposure chamber hookup. The aerosol fibers exit the generator (located on shelf at right) vertically, pass through the deionizing krypton column, and enter the exposure chamber through the stainless steel [illegible] at the top right of the chamber.

aerosol carrier air and 15 l/min clean dilution air). Generator feed plug infusion rate was set at 20 mm/min with rotor speed fixed at ~1000 RPM for these aerosol uniformity tests.

Figure 5 illustrates the sampling arrangement used for obtaining gravimetric filter samples. These samplers are 6.3 mm I.D. copper tubing probes, fitted with Gelman, 25 mm in-line filter holders (Gelman Instrument Co., Ann Arbor, MI), which have been adapted to shortened animal holding tubes for positive seal support (Figure 6). These adapted sampling probes are placed in vacant animal exposure ports for aerosol collection and are designed to simulate "breathing zone" samples of the rodents undergoing exposure. This aerosol monitoring arrangement also minimizes the possibility of human exposure to the challenge aerosol as both collection filter and holder are located outside the exposure chamber.

Figure 5. Two sampling probes in place in exposure chamber during sampling operation.

Animals

Female O-M rats were obtained from Cimm Research Laboratory Animals, Wayne, NJ, and male Syrian golden hamsters from Engle Laboratory Animals, Hammond, IN. All animals were housed in Class-100 laminar flow clean rooms (Hazleton Systems, Inc., Cornwell Heights, PA), two to a polycarbonate cage containing low-dust-factor aspen shavings. The cages were suspended on aluminum shelves and covered with spun polyester filters (Dupont #2? Spunbonded Polyester Filter, E.I. Dupont Co., Wilmington, DE). Cages were changed twice

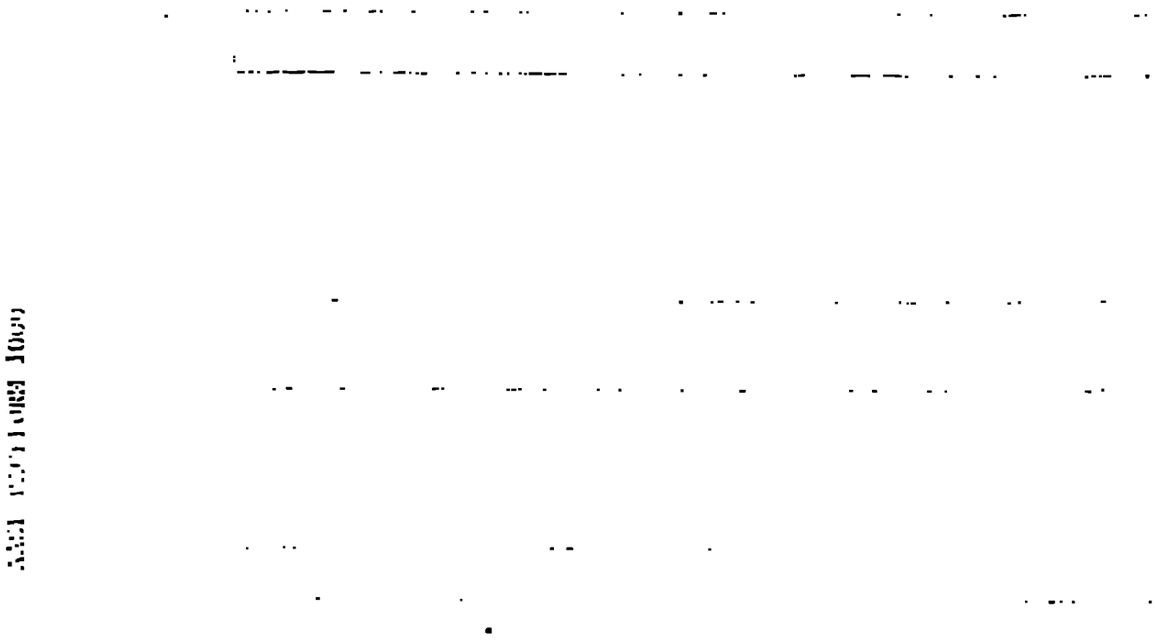

Figure 6. Polycarbonate restraining tube modified to form sampling probe.

a week. The rats were fed Teklad Rat and Mouse Diet ® and the hamsters, Teklad Hamster Diet ® (Teklad Mills, Winfield, IA). All animals were given chlorinated water ad libitum.

Stress Analysis

A study was initiated to examine any stress associated with nose-only exposures compared to whole-body exposures. One hundred days-old female O-M rats were randomized to 1 of 3 groups: 1.) caged controls that received no experimental manipulation; 2.) a group that was exposed to atmospheric air in the chamber nose only 6 hours a day, 5 days a week; and 3.) a group that was exposed to atmospheric air in the chambers in the traditional whole-body mode, 6 hours a day, 5 days a week. After either 1, 10 or 30 exposures, 10 animals each from the nose-only and whole-body groups were sacrificed by decapitation and blood samples taken for complete blood counts (CBC's), clinical chemistry profiles (SMAC-20's, New Mexico Medical Reference Laboratory, Santa Fe, NM) and plasma corticosterone assays using a modification of the method of Foster and Dinn (1974). These last assays were performed by Dr. J. Standefer, Department of Pathology, University of New Mexico Medical School, Albuquerque, NM. All experimental and control animals were treated as much alike as possible, i.e., housed in same

room, cages changed at same time, etc., so not to introduce extraneous stress in one group compared to another.

Other parameters monitored included measurements of body weights and body (rectal) temperatures.

## RESULTS

Aerosol distribution data presented are limited to that obtained against two fibrous aerosols currently being used in our ongoing study: 1.) a glass aerosol produced from a bulk material having a ~ 0.45 μm nominal fiber diameter and 2.) an asbestos aerosol produced from UICC (International Union Against Cancer) crocidolite (Timbrell et al, 1968a). Figure 7 is an example

Figure 7. Aerosol mass concentration versus time output for glass fiber aerosol.

illustrating aerosol mass output as a function of generator operating time obtained during a single aerosol generation consistency test. Each mass concentration measurement reported is the average result obtained from five separate gross filter samples simultaneously collected from the aerosol exposure chamber. For this particular test, the generator system was allowed to operate undisturbed for 1 hour for equilibration purposes prior to initiating sampling. Thereafter, aerosol mass concentration was determined by collecting gross filter samples on pre-weighed Gelman IM-300 membrane filters. Five simultaneous samples were collected from opposite sides (3 samples on one side, 2 on opposite) of the chamber every hour for 15-min sampling intervals at a flow rate of 1.0 l/min. There were no animals in the chamber during this test. Total generator running time for this test was 10 hours of uninterrupted

operation. A total of 10 separate sets of filter samples were collected during this test (5 samples per set). The maximum port to port variation observed during this test was ±17% as measured simultaneously from five separate ports for each point. The average coefficient of variation for simultaneous samples taken at 50 different sampling locations within the containment chamber was ±10%. The fibrous aerosol mass concentration variation observed during this test ranged from a minimum of 1.8 mg/m³ to a maximum of 2.4 mg/m³ with the mean concentration being 2.0 ± 0.2 mg/m³. These data demonstrate that aerosol mass concentrations in the chamber, at port level, were relatively constant and uniform throughout this glass microfiber aerosol consistency test

Figures 8 and 9 are optical photomicrographs of collected glass fiber aerosol and illustrate the polydisperse (multidiameter, multilength) characteristics of this fibrous material currently being used for animal exposure studies. Figures 10 and 11 are Scanning Electron Micrographs (SEM) of the same collected aerosol illustrating a more detailed microscopic view of this glass microfiber exposure aerosol. These SEM's appear to enhance the proportion of short fibers contained in the aerosol as compared to photomicrographs obtained at similar magnifications using traditional phase contrast optical microscopy. This phenomenon is related to enhanced contrast and imaging properties of all collected

Figure 8. Optical photomicrograph of glass fiber aerosol (1000 X).

Figure 9. Optical photomicrographs of glass fiber aerosol (1000 X).

Figure 10. Scanning electron photomicrograph of glass fiber aerosol (1000 X). Note enhancement of "short" fibers in this micrograph compared to optical photomicrographs in Figures 8 and 9.

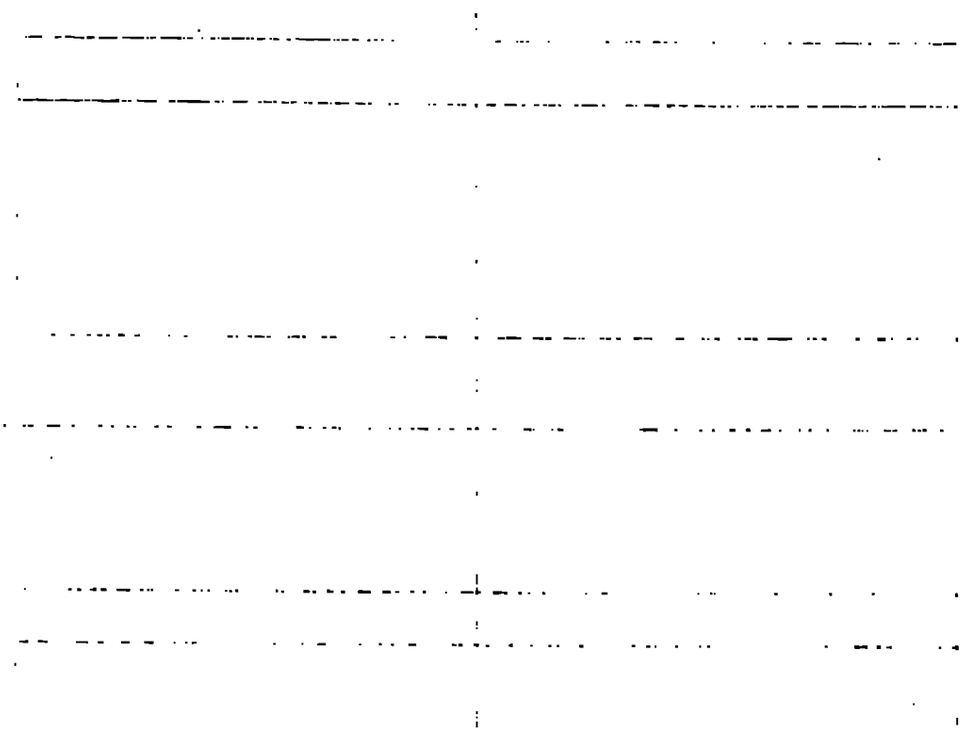

Figure 11. Scanning electron photomicrograph of glass fiber aerosol (1000 X). Note enhancement of "short" fibers in these micrographs compared to optical photomicrographs in Figure 8 and 9.

particulates when viewed by SEM versus optical microscopy, as samples for SEM are collected on smooth surfaced, 0.22 μm pore Nucleopore® filters (Nucleopore Corp., Pleasanton, CA). All fiber sizing data being accumulated in our study are being done via SEM.

Scanning electron micrographs illustrating our UICC crocidolite exposure aerosol appear as Figures 12 and 13. Similar aerosol mass concentration data obtained against this material is summarized in Figure 14. Again, each plotted point is the mean gravimetric value obtained from five simultaneous samples taken from said modified exposure chamber. The sampling interval was once every hour with 20 minute samples simultaneously collected from opposite chamber sides at a flow rate of 2.5 l/min. The maximum port-to-port variation observed for any of the 5 simultaneous samples taken per set for this aerosol was ±12%. The mean aerosol mass concentration was 6.0 ± 0.4 mg/m³ for the entire 9 hr test period. The coefficient of variation for all 40 samples taken from 40 different ports was ±7%.

Fisher-344 and O-M rats and Syrian hamsters have been exposed to several particulate aerosols using this system, 6 hours a day, 5 days a week, for periods up to 24 months. No apparent clinical differences were observed between sham

Figure 12. Scanning electron photomicrograph (2000 X) of crocidolite asbestos aerosol.

Figure 13. Scanning electron photomicrograph (4000 X) of crocidolite asbestos aerosol.

Figure 14. Aerosol mass output time for crocidolite asbestos aerosol.

control animals exposed nose-only and their caged control counterparts. Sham control rats and hamsters routinely outlive caged unmanipulated controls--some groups have up to 50% longer mean life-spans. The longer life-spans may be a result of the nose-only exposed animals being handled at least twice a day when being loaded and unloaded from the tubes.

Body weights for sham control and caged control female O-M rats and male Syrian hamsters up to 14 months of exposure, 6 hours a day, 5 days a week, are given in Table I. No significant differences emerged in body weights between sham controls and caged controls at either 5, 8, 11, or 14 mo. is into the exposure regimen.

Body (rectal) temperatures did not increase in either rats or hamsters while they were in the restraining tubes in the chambers as long as the number of air changes in the chambers was kept greater than 10/hour. When the number of air changes was lower than 6, hyperthermia inversely proportional to the number of changes resulted.

In the stress assessment study, no significant differences were seen between nose-only versus whole-body after 1, 10 or 30 exposures (6 hours a day, 5 days a week), or unmanipulated caged controls, measuring the following parameters: body weights, complete blood counts (white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and differential white blood cell count) and clinical chemistry profiles (glucose, blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, sodium chloride, carbon dioxide, electrolyte balance, blood urea nitrogen: creatinine ratio, cholesterol, triglycerides, total bilirubin, direct bilirubin, serum

TABLE I
BODY WEIGHTS (g) ± S.D.

| Female O-M Rats | Cage Controls | Sham Controls |
|---|---|---|
| Initiation | 272 ± 9 | 242 ± 12 |
| 5 Months | 281 ± 9 | 289 ± 12 |
| 8 Months | 285 ± 11 | 290 ± 17 |
| 11 Months | 331 ± 13 | 338 ± 22 |
| 14 Months | 333 ± 17 | 340 ± 20 |
| **Male Syrian Hamsters** | | |
| Initiation | 137 ± 22 | 129 ± 10 |
| 5 Months | 150 ± 12 | 147 ± 9 |
| 8 Months | 150 ± 17 | 153 ± 13 |
| 11 Months | 208 ± 14 | 192 ± 18 |
| 14 Months | 209 ± 15 | 195 ± 12 |

glutamic oxaloacetic transaminase, alkaline phosphatase, lactic acid dehydrogenase, total protein, albumin and globulins).

Plasma corticosterone levels are given in Table II. These data indicate that nose-only exposure is no more stressful, or perhaps even less stressful, than whole-body exposure.

DISCUSSION/CONCLUSIONS

Aerosol distribution data obtained against two different fibrous aerosols demonstrate that both aerosol mass concentration, and aerosol mass distribution within these modified exposure chambers are relatively consistent and uniform at the animal "breathing zone" under exposure conditions currently being employed. Laboratory animals have been exposed to particulate aerosols via nose-only systems for many years. Large test tubes (Cohn et al, 1956; Willard et al, 1968; Casarett, 1964), baby feeding bottles (Djuric et al, 1962) and plastic restrainers (Snider et al, 1973) have all been used to hold animals. With these types of apparatus, the animals in their holders were then placed in aerosol chambers for the exposures. More recent techniques have used special restraining tubes that were plugged into aerosol chambers so that only the noses of the animals came in contact with the aerosol (Evans et al, 1973; Raabe et al, 1973; Wehner et al, 1977; Thomas and Smith, 1979; Phalen et al, 1980). However, all of these systems were used only for

TABLE II
PLASMA CORTICOSTERONE LEVELS
(ug/dl) I S.E.

| | Number of Exposures[A] | | | |
|---|---|---|---|---|
| | 0 | 1 | 10 | 30 |
| Untreated Cage controls (30 animals) | 54 ± 4 | - | -- | -- |
| Nose-only (10 animals/point) | - | 49 ± 5 | 48 ± 4 | 56 ± 6 |
| Whole-body (10 animals/point) | - | 49 ± 7 | 74 ± 3[B] | 64 ± 5[C] |

[A] 6 hrs a day, 5 days a week
[B] $P<0.001$ vs Nose only (10 exposures) or untreated cage controls
[C] $P<0.005$ vs untreated cage controls

a single or a relatively few exposures. Problems preventing long-term use that arose included over-restraining the animals in their tubes by pushing them forward with forceful plunger devices, resulting in excessive anxiety and stress and death in some cases. This is not a problem with our method because the animals are not forced forward; instead, they appear to be quite comfortable and extend their noses into the chamber environments as demonstrated in Figure 15. Another problem previously was that the animals chewed through the plastic tubes requiring either that the tubes be changed more frequently or that the nasal end of the tubes be made of a more durable, expensive material, such as machined aluminum. This was overcome in our approach by using polycarbonate tubes, which have useable life-spans of over one year. A third drawback in some systems was that the angle of the nasal end taper was too acute causing traumatic keratitis and panthalmitis with long-term use.

The described nose-only method in our laboratory has proven itself to be a very satisfactory means of exposing relatively large numbers of laboratory rats and hamsters in a limited space for 6 hours a day, 5 days a week for long periods. Analysis of CBC, clinical chemistry profile and adrenal cortical function data has shown that this nose-only exposure system is no more stressful or perhaps even less stressful than some whole-body exposure systems.

Figure 15. View of inside of exposure chamber with male Fischer-344 rats contained in polycarbonate tubes.

ACKNOWLEDGEMENTS

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Figure 2

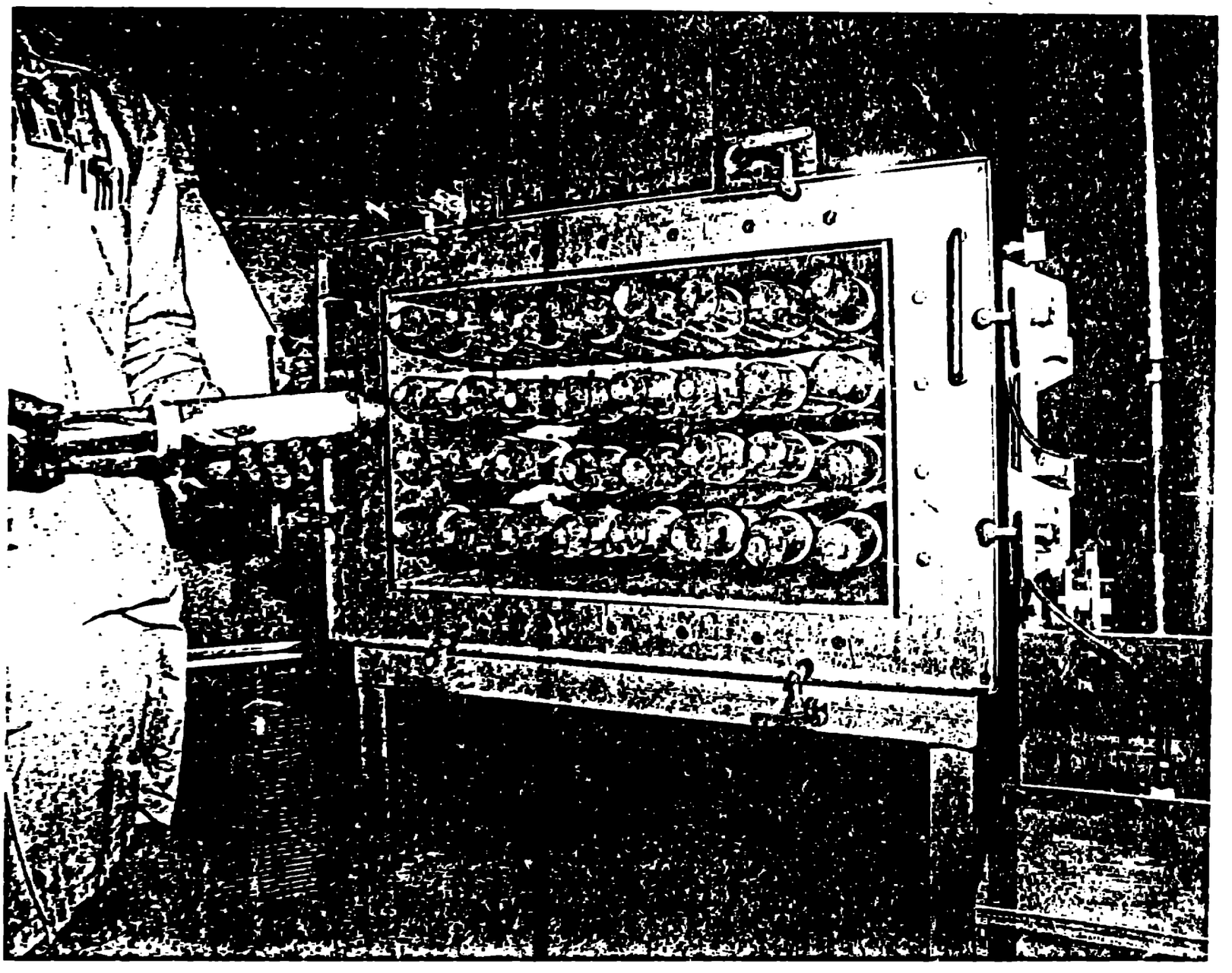
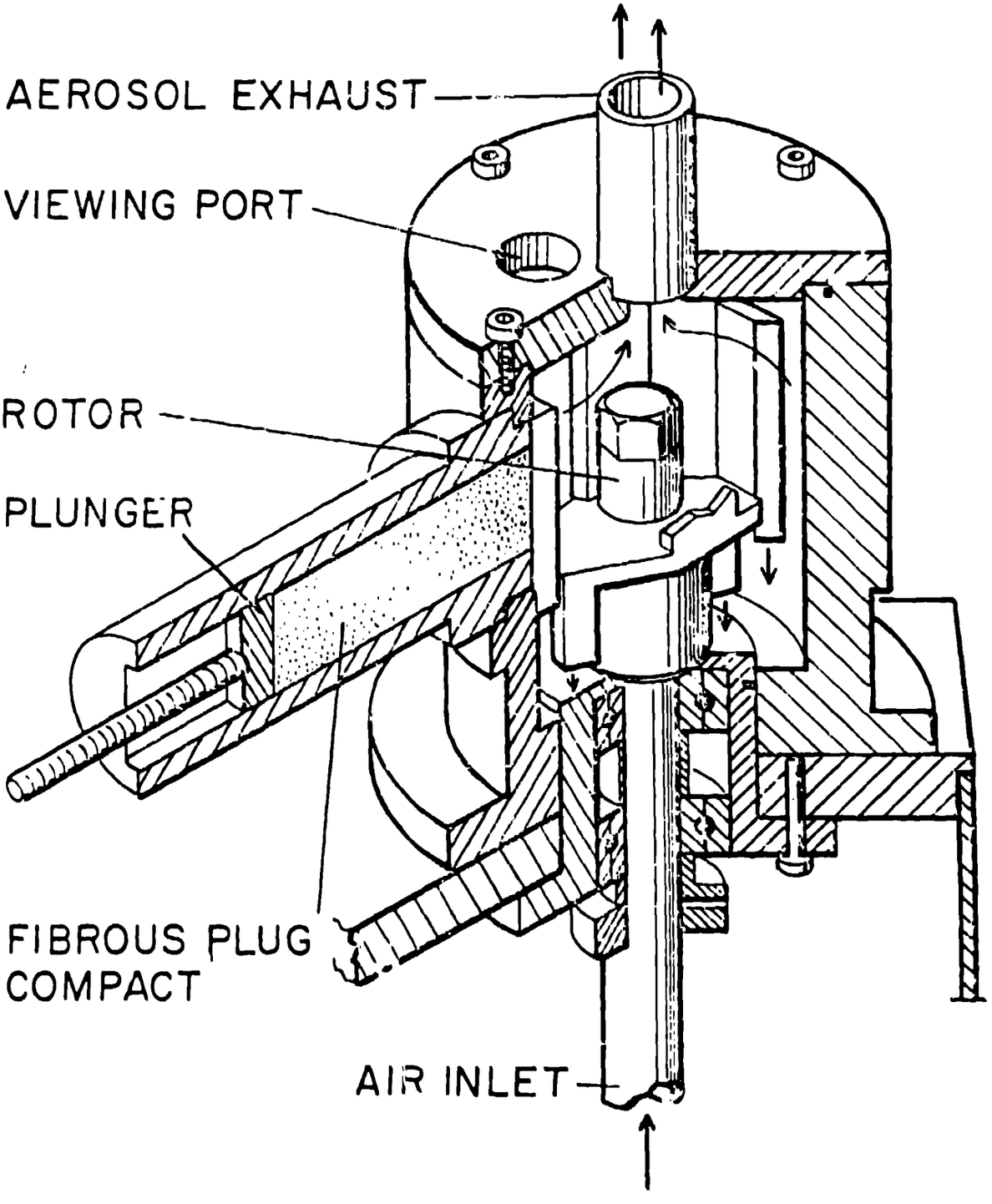


Figure 3




FIBROUS AEROSOL GENERATOR

Figure 4

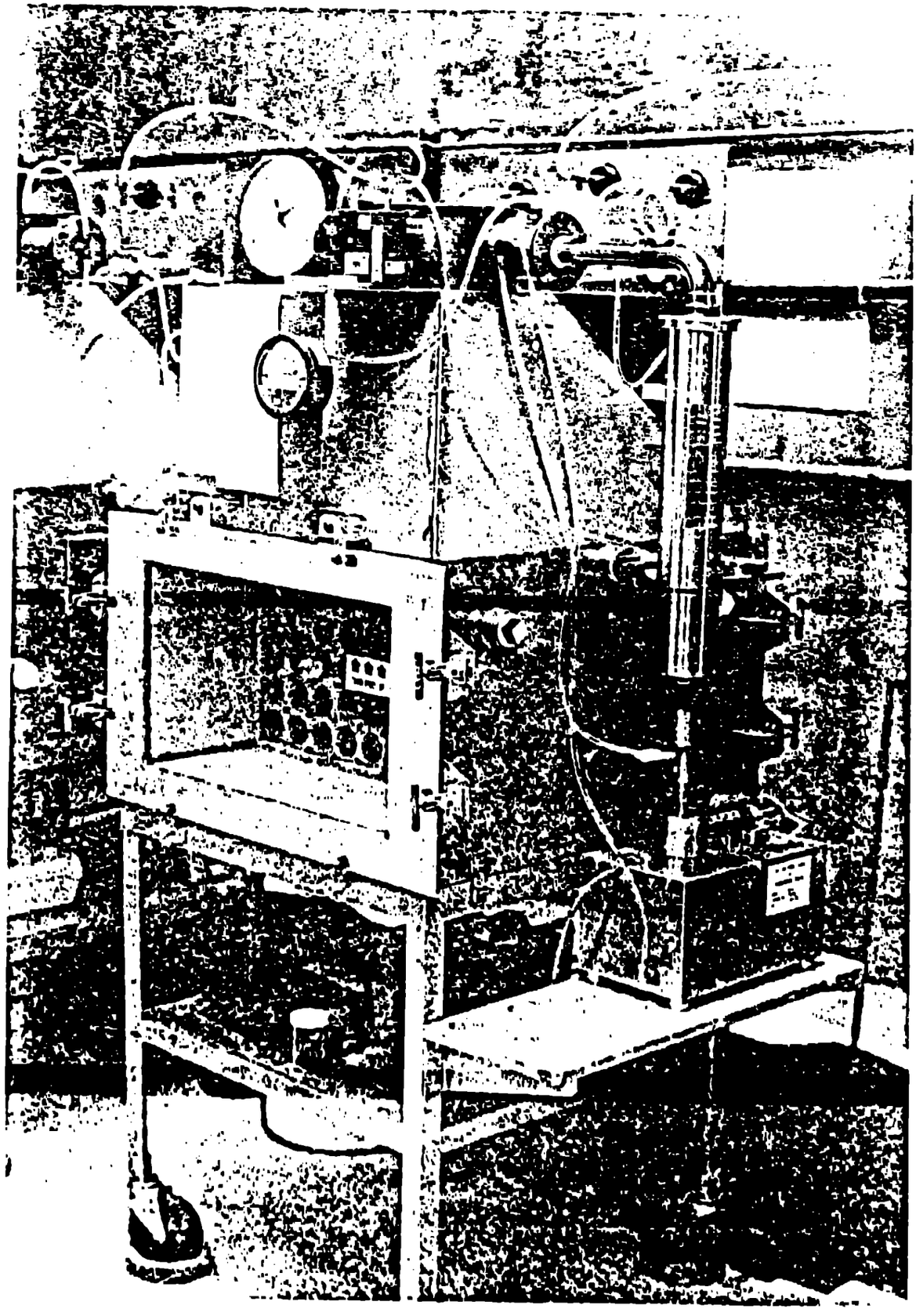


Figure 5

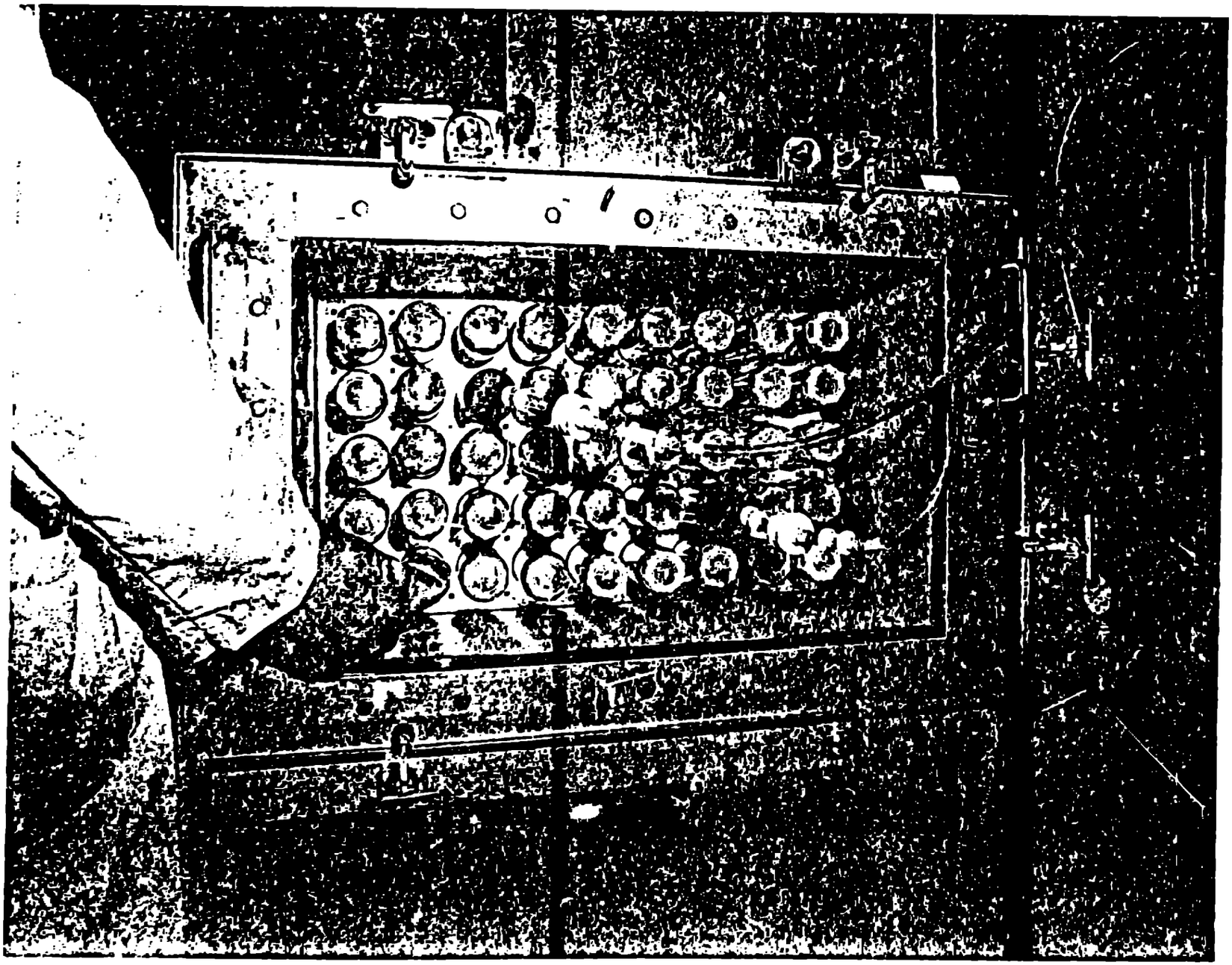
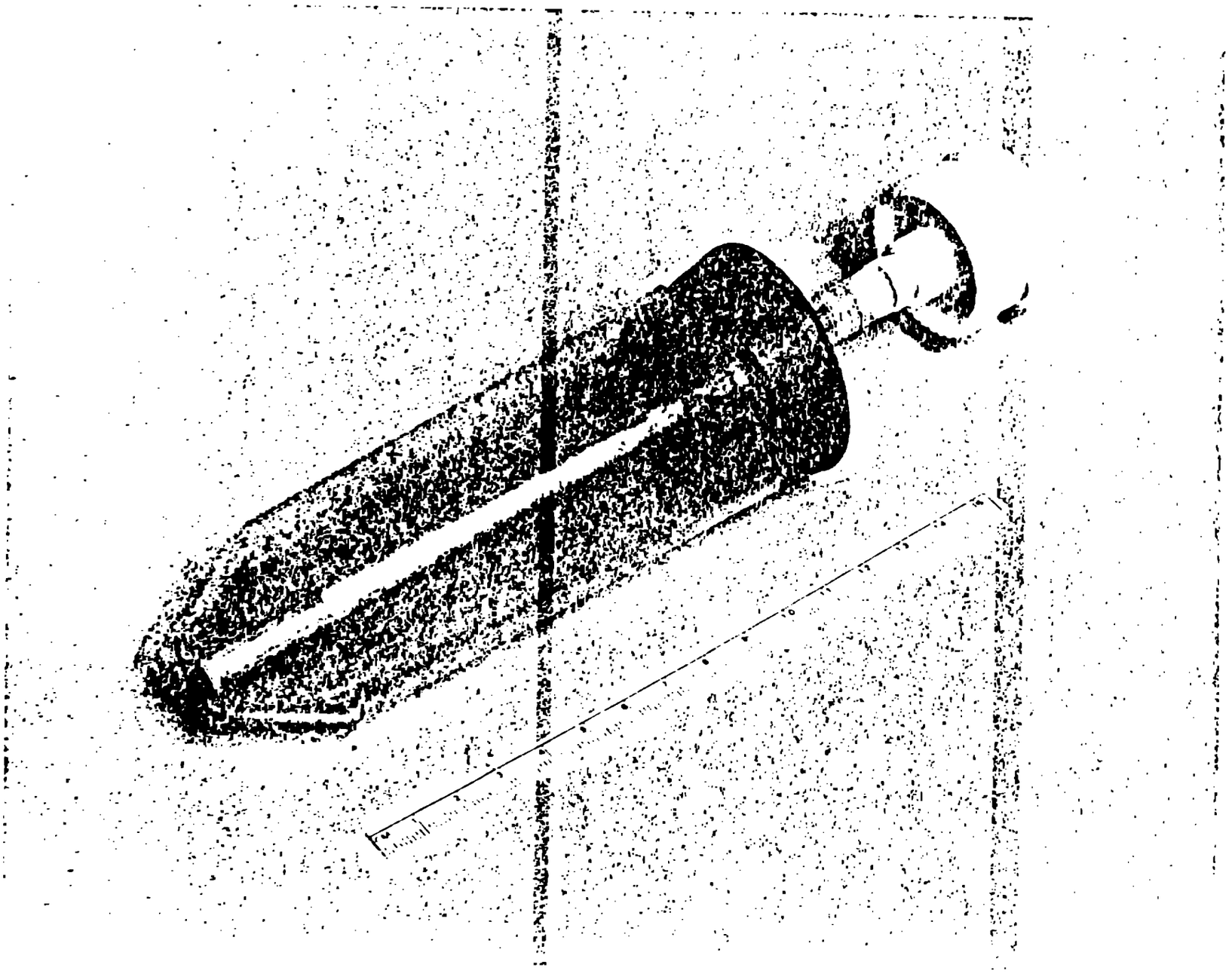


Figure 6



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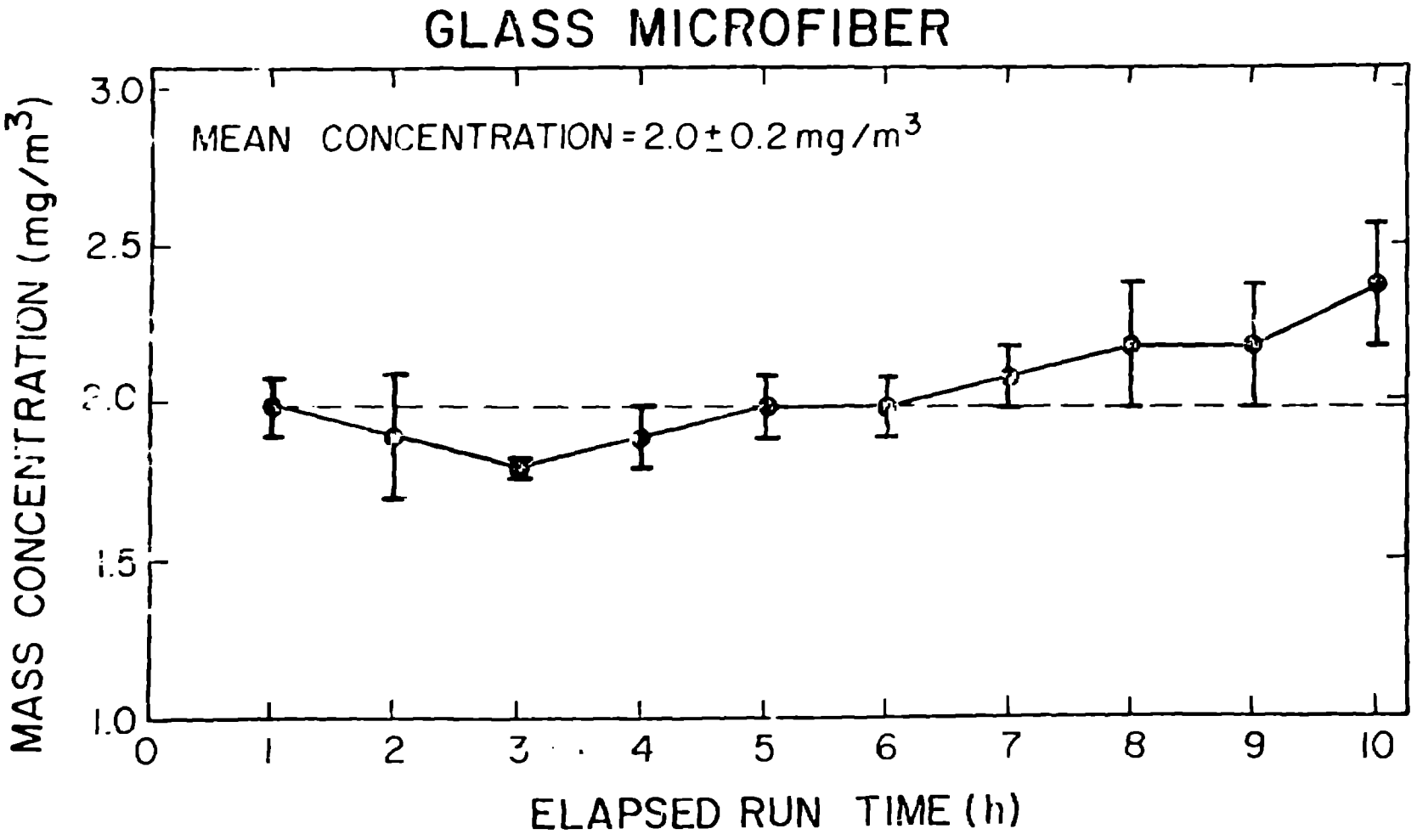

GLASS MICROFIBER
MEAN CONCENTRATION = 2.0 ± 0.2 mg/m3
MASS CONCENTRATION (mg/m3)
3.0
2.5
2.0
1.5
1.0
0
1
2
3
4
5
6
7
8
9
10
ELAPSED RUN TIME (h)

Figure 8

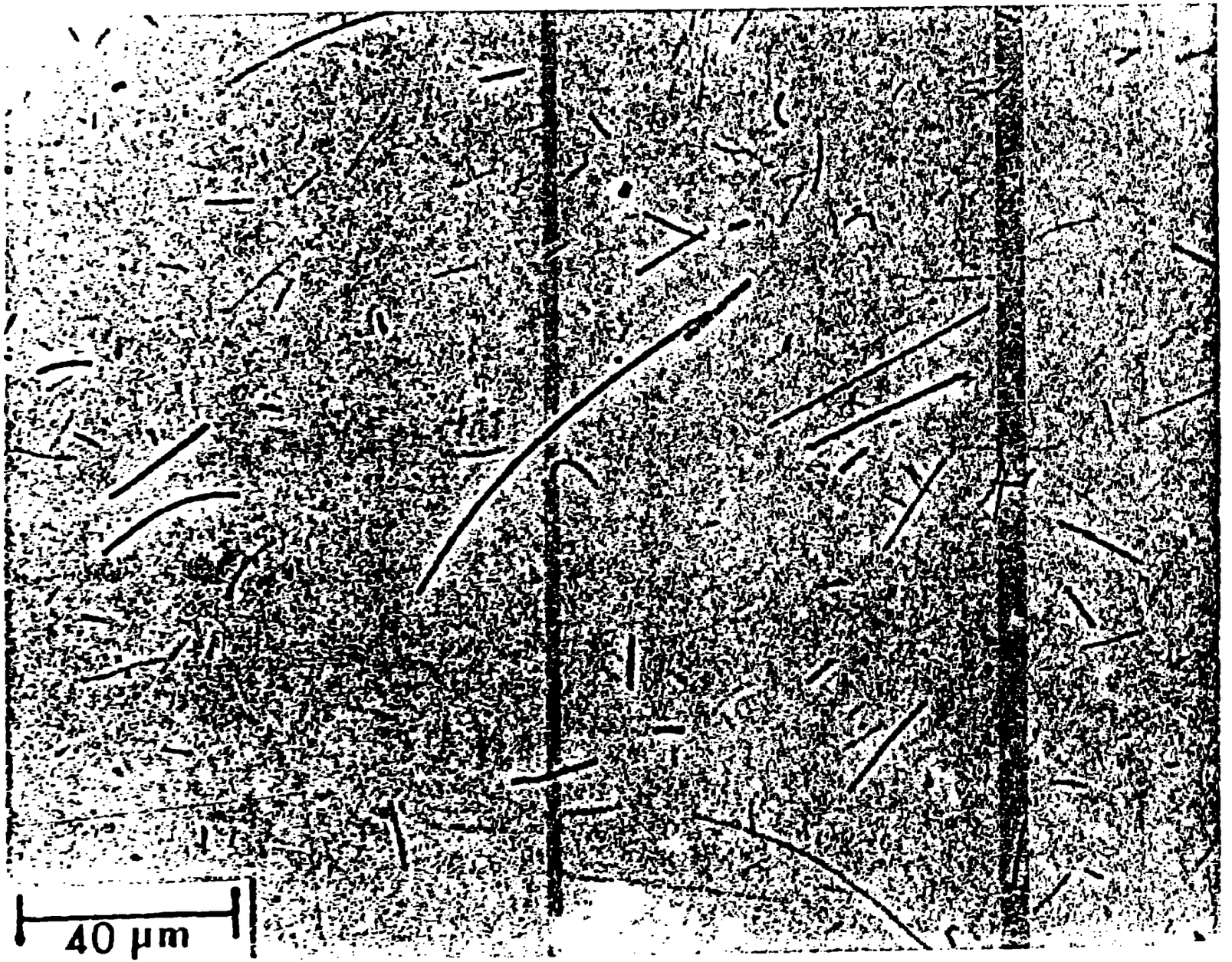


Figure 3

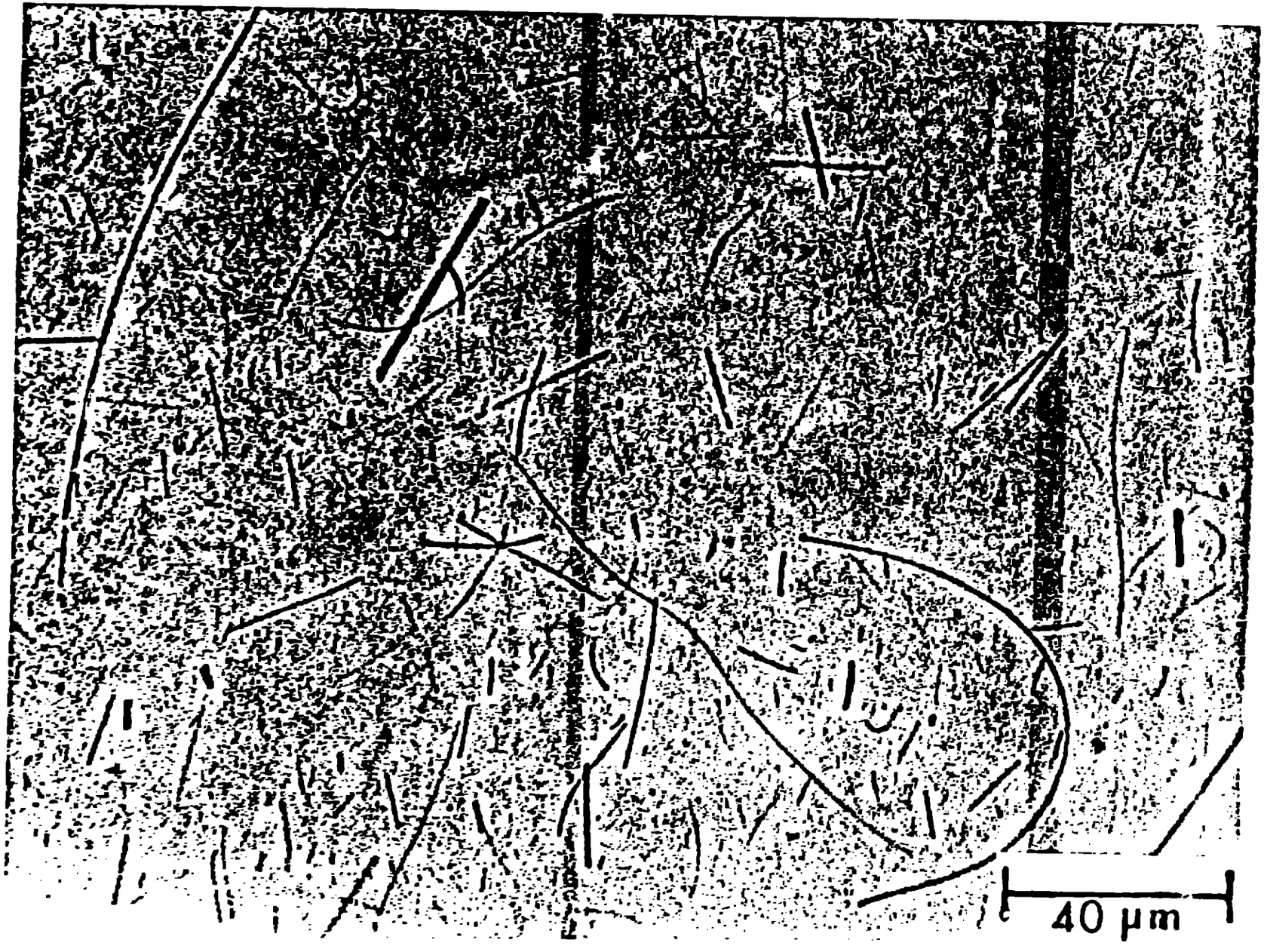


Figure 10

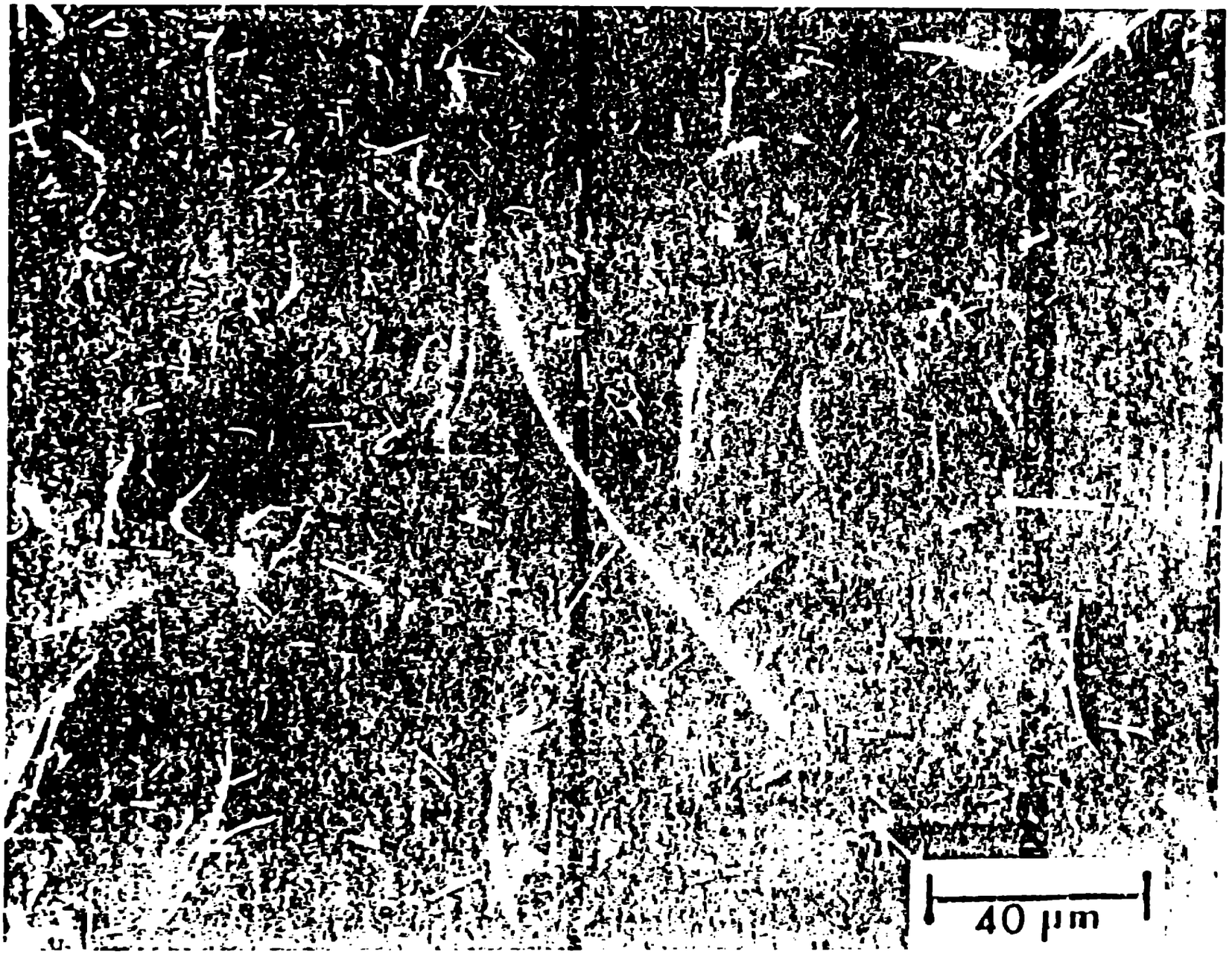
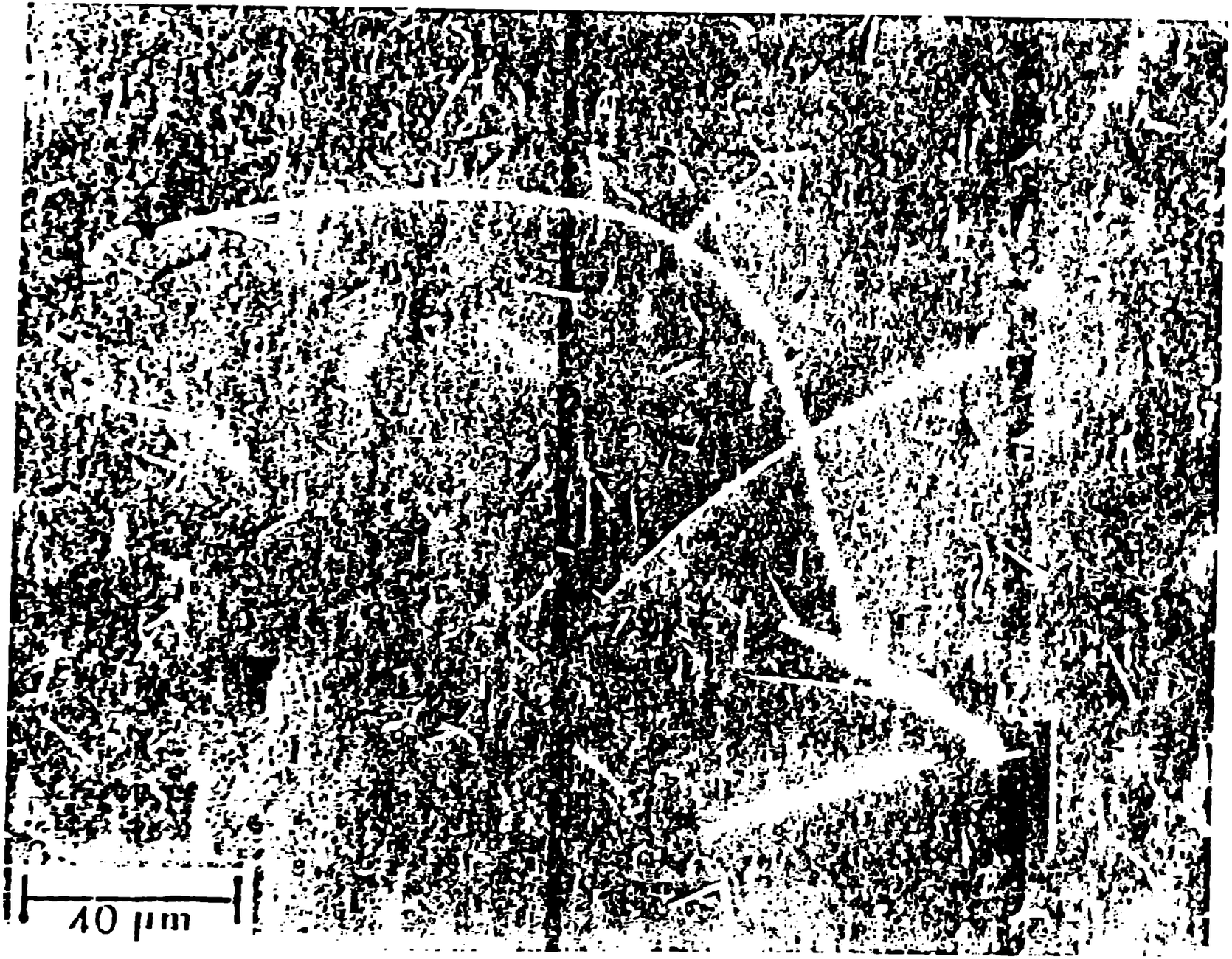


Figure 11



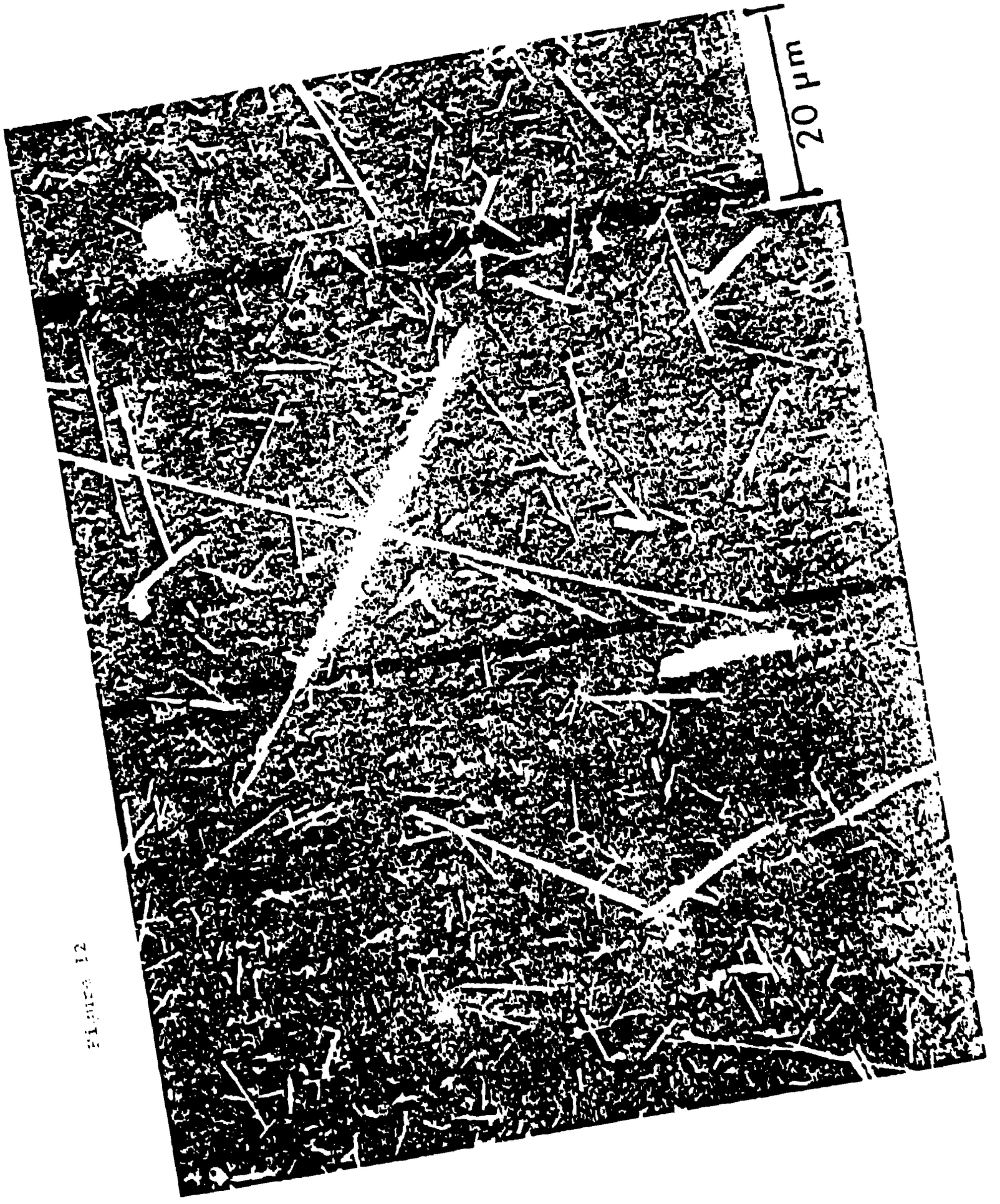



Figure 12

Figure 13

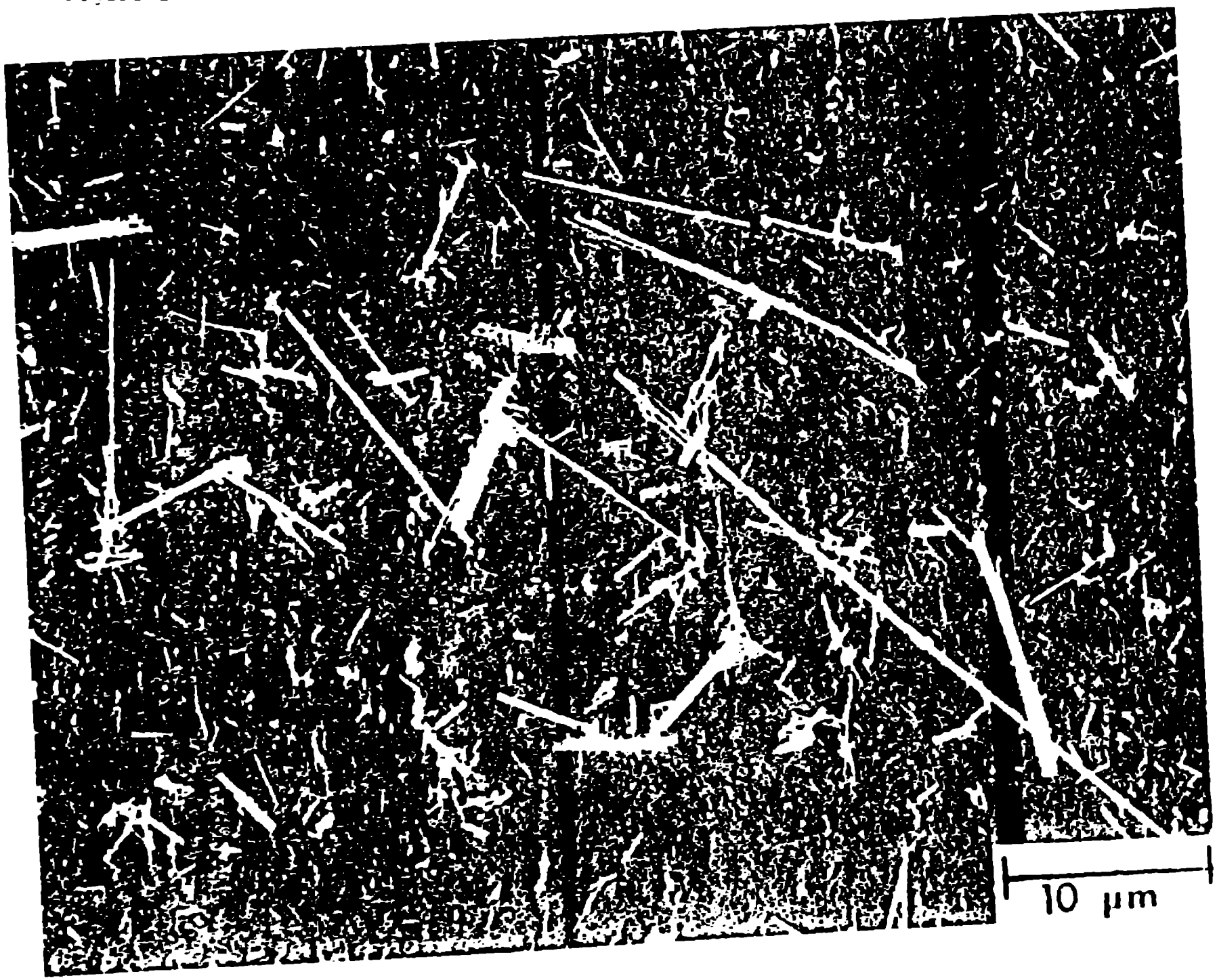
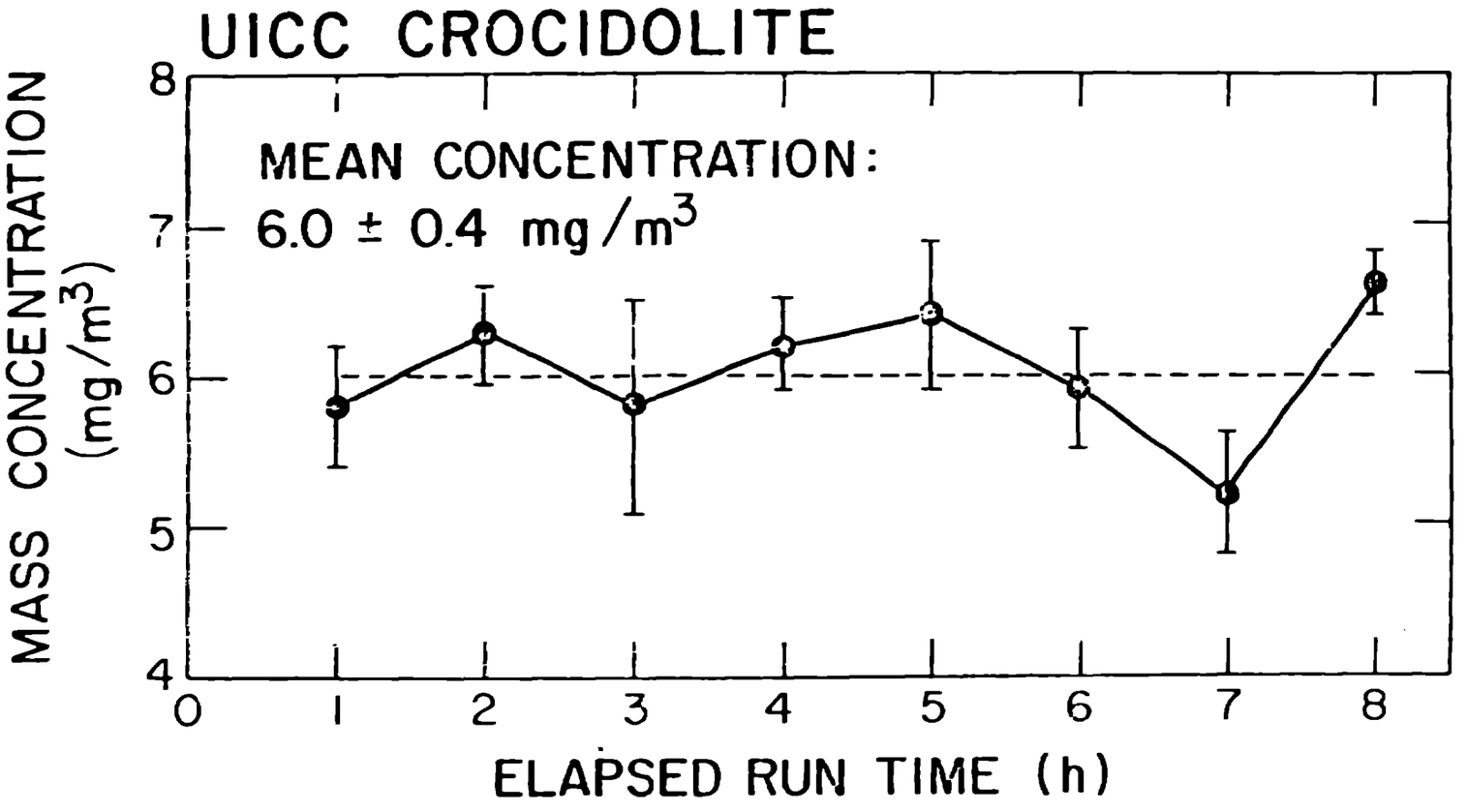


Figure 11


UICC CROCIDOLITE
MEAN CONCENTRATION:
6.0 ± 0.4 mg/m3
MASS CONCENTRATION (mg/m3)
8
7
6
5
4
0
1
2
3
4
5
6
7
8
ELAPSED RUN TIME (h)