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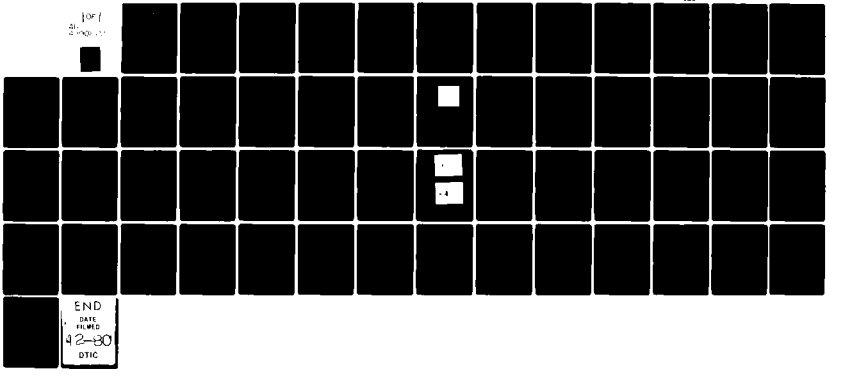

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# LOW LEVEL INFRARED IRRADIANCE

# OCULAR EFFECTS

FINAL REPORT

November 1978



By

William H. Bowie

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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Life Sciences Division
San Antonio, Texas 78216

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The ocular lenses of checker rabbits were exposed to infrared radiation at 1064 nm. Exposures were made with a cw laser with exposure times varying up to 3000 seconds. Exposures were repeated at weekly intervals with power levels chosen such that no retinal lesions were produced. Documentation of retinal and lenticular effects was provided by fundus and slit lamp photography. Opacities were observed on the posterior poles of the rabbit lenses. Following the exposure sequences, the lenses were removed and the capsule, cortex and nucleus separated. The components were homogenized and subjected to polyacrylimide gel electro-

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phoresis. In addition, measurements of intralenticular light scattering and posterior pole opacity size were made. However, the cortex of irradiated lenses contained more insoluble material than did the cortex of non-irradiated lenses. Opacity size and density may have been a function of both irradiation and anesthesia usage.

# FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee of the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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## SUMMARY

The ocular lenses of checker rabbits were exposed to infrared radiation of 1064 nm wavelength. Exposures were made with a cw mode laser for various periods of time to and including 3000 seconds. Exposures were made at power levels observed to produce no retinal lesions. Documentation of retinal and lenticular effects was provided by fundus and slit lamp photography. Opacities were observed on the posterior pole of the rabbit lenses. The lenses were removed and the capsule, cortex and nucleus separated. The components were homogenized and subjected to polyacrylimide gel electrophoresis. In addition, measurements of interlenticular light scatter and posterior pole opacity size were made. Little difference was observed between irradiated and non-irradiated nuclei. However, the cortex of irradiated lenses contained more insoluble material than did the cortex of non-irradiated lenses, but may have been a function of both irradiation and anesthesia drug dose.

## 1. INTRODUCTION

The following study was designed to investigate the interaction of near infrared radiation and the ocular lens. A Nd:YAG laser, operated in the continuous wave (cw) mode, was used to irradiate the lens of young, mature rabbits with radiation of 1064 nm.

Intentional long-term low-level exposure of the ocular lens to IR radiation has been studied very little, consequently, the effect such irradiation might have on the clear lens is virtually unknown. The largest data source available on the subject exists as a result of epidemiological studies of natural cataracts and typical glass blower's cataracts.

One of the first correlations of IR exposure and cataract formation was made by Vogt in 1919[1]. He exposed rabbits to radiation in the 800 nm to 1200 nm range and produced cataracts. His work was considered by Goldman[2] to indicate that the real source of such cataracts was a transfer of heat from the iris to the lens. In 1970, Clarke[3] examined the works mentioned above and concluded that a retinal burn would have occurred in all those experiments and that all cases reviewed represented acute phenomena.

Posterior pole opacities were produced by Bernat and Hryniewicki in 1959[4]. Their source of irradiation was a carbon arc lamp with "Appropriate filters allowing red and infrared rays of wavelengths greater than 680 nm to pass..." Their exposures must be considered acute due to the adverse reaction of surrounding ocular tissue to the irradiation. Also, the exposures by Wolbarsht, et al.[5], must be considered acute in that their longest exposure time was 180 sec, and all exposures caused some degree of cloudiness in the lenses. Anterior surface opacities were

reported for lenses exposed to 100 J or more. These results differ from the results of most other infrared studies in that posterior pole opacities have been more commonly reported.

Study of ocular opacities requires that an animal model be used and that the opacification process be accelerated. At least one investigator, other than Technology Incorporated, has endeavored to produce opacities as a result of chronic low-level exposure. Messmann[6] was able to produce posterior pole opacities several months after a 4-month exposure period during which "subliminal doses" were administered. The present work by Technology Incorporated produced tenuous opacities in the vicinity of the posterior suture line of rabbit lenses. Approximately one to two months of low-level IR exposure were required before any change in the naturally occurring posterior suture opacity was observed.

Epidemiological data are gathered from human exposure cases. A study was made in 1971[7] of lens opacities among steel workers in a British plant. A high degree of significance was found between heat exposure and total time on the job and anterior or posterior pole opacities. It was pointed out, however, that age also increased with time on the job and that such opacities were a function of age. Nevertheless, a difference in the prevalence of opacities between the exposed groups and the control group was found. There was also a correlation between the cataract type and heat exposure. A second example of an epidemiologic investigation is that by Hiller, Giacometti and Yuen[8]. They found that annual sunlight hours were related to the prevalence of cataract. They implied that this was due to the ultraviolet component of sunlight but gave no data on spectral distribution or on the type of cataract formed.

To date, there has been little or no attempt to differentiate between the iris heat transfer cataract and the direct effect of infrared radiation on a normal, healthy lens. All studies reviewed have irradiated the entire globe, including the iris, and have produced lenticular opacities. However, in a field combat situation, lasers may be used at night as well as during the day. During night operations, the pupil is dilated, exposing a very large area of lens and little iris. Also, various ocular filters are unlikely to be worn at night due to their attenuation of the available light. Thus, the potential for maximum lenticular exposure is established.

Many analytical studies attempting to relate lenticular proteins to cataracts have utilized the entire lens. On the other hand, most research into the etiology and biochemistry of cataract formation *per se* has studied the nucleus, cortex, and capsule as individual components. However, it is difficult to extrapolate biochemical results from natural cataracts to intentionally induced infrared cataracts. Naturally cataractous lenses are obtained as a result of eye enucleation or as a result of surgical correction of cataracts. Eye enucleations are performed in response to a serious pathological condition. Removal of cataractous lenses is performed, most often, late in life, and as a result of senile cataract formation. However, an old lens with senile, transient, or cortical cataracts bears little biochemical similarity to a young emmetropic lens. For this reason, careful biochemical analyses of IR exposed lenses are important.

## 2. EXPERIMENTAL METHODS

The experimental program was divided into two phases. Phase I was to define exposure conditions to be used during the bulk of the program. Phase II involved exposure of rabbits to the parameters defined in Phase I.

### 2.1 Phase I

Criteria published in American National Standard ANS Z136.1-1976[9] established maximum permissible exposure levels for human ocular exposure to a wide spectrum of laser radiations. Phase I exposures were made to determine the relationship between Technology Incorporated's data and the ANSI standards.

Early in the program, a beam scan was performed and revealed a very flat-topped broad beam of ∿3.8 mm diameter at the cornea. The beam scan and $1/e^2$ points are shown in Figure 1.

In both Phases I and II, the laser beam was aligned to coincide with a fundus camera reticle before each exposure session and between animals exposed on the same day. The beam was visualized on an IR phosphor excited by an ultraviolet lamp. Also, before each exposure session and again between animals, the beam power was adjusted and calibrated. Calibration was accomplished by placing a disk calorimeter in the position to be occupied by the rabbit. The calorimeter was connected to a Coherent Radiation power meter which had been calibrated against a Scientech 326 power meter.

Rabbit threshold data for the current experimental program were established using a beam diameter of 3.8 mm at the cornea. The energy delivered to the exposed eyes was calculated in joules, then converted to $J/cm^2$ for a 7-mm diameter pupil. ANSI data are also expressed as maximum

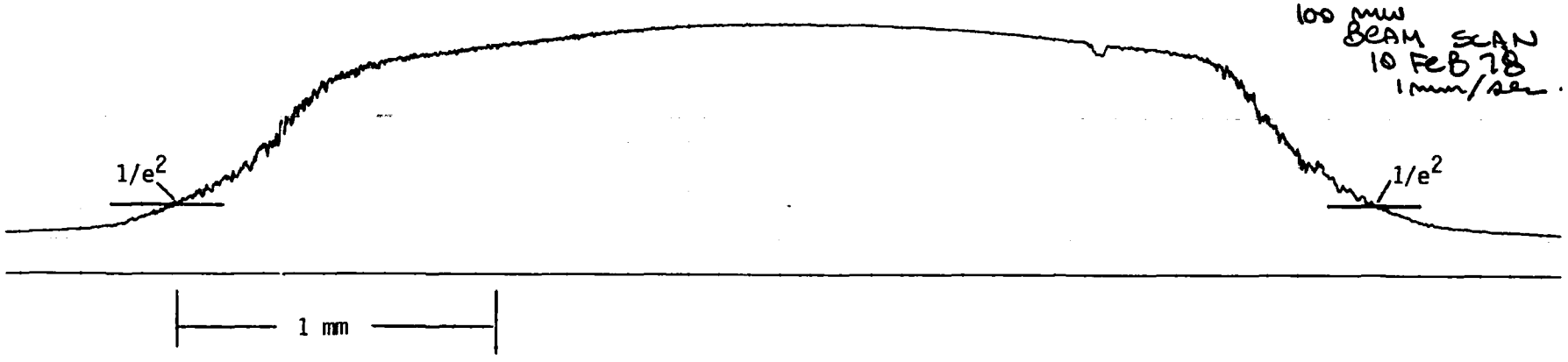


FIGURE 1. Transverse Scan of Nd:YAG cw Laser Beam

permissible exposure in $J/cm^2$ for a 7-mm pupil. Such MPE data generally incorporate a safety factor of approximately 10. Therefore, to convert our approximate lesion threshold values to MPE values, it was necessary to multiply our data by a factor of 10. Care was taken to ensure that the laser beam did not strike the optic disk or the highly mylenated area around the disk. Those areas are highly reflective and would have produced very high lesion thresholds if used for such studies.

Prior to exposure, a few drops of atropine sulfate (AS), or a small amount of 1% AS ointment was instilled into the conjunctival sack. When full mydrasis was attained, the animal was anesthetized with Nembutal Ketaset or Sernalyn and Xylazine (Rompun). After a deep plain of anesthesia was established, a 24-gage scalp vein infusion set was placed in the marginal ear vein. Nembutal was injected slowly and at regular intervals to maintain the desired anesthetic level. The animal's eyelid was taped open and a drip of normal saline positioned over the cornea to prevent drying of the tissue.

Power levels were limited in order to prevent production of retinal lesions. If occupational exposure to near infrared radiation occurred, and a retinal lesion were sustained, it would likely be noticed by the exposed individual or during a routine ophthalmological examination. However, if no immediate retinal damage was incurred, a subject might be exposed to the same source over long periods of time with the potential of lenticular opacities or other chronic ocular effects.

ANSI Z136.1-1976 indicates that direct ocular exposure to a given power level for durations greater than 1000 sec will not result in retinal damage if such damage has not been incurred by 1000 sec. However, we used

a maximum exposure time of 3600 sec. Periodic observations of the retina were made during exposure by means of a fundus camera. The exposure and examination apparatus is shown in Figure 2. If a lesion was detected, the power level was decreased, the beam moved to an unexposed area of the retina, and a new exposure begun. All animals were sacrificed at the end of Phase I, the eyes enucleated, and the lens removed and frozen for storage and electrophoresis.

## 2.2 Phase II

### 2.2.1 Animals and Anesthetic

Nine English spot rabbits between 6 and 12 months of age were obtained. Upon receipt, the fundus of all animals was inspected and baseline slit lamp photographs obtained. Light anesthesia was used for all observations. That is, the animal retained a blink reflex, but was completely docile. The reflex was overcome with topical application of an ocular anesthetic (Ophthaine) to the eye.

During the early part of Phase II, long IR exposures were made with the animal under Nembutal anesthesia. However, it was soon discovered that the marginal ear vein was unable to tolerate repeated infusion of the drug. Over a period of several exposure sessions, the ear vein and immediate area became inflamed. The inflamation eventually was followed by necrosis and loss of that portion of the ear. The anesthetic was then changed to Ketaset and Rompun, alternately injected IM into the right and left flank for each exposure session.

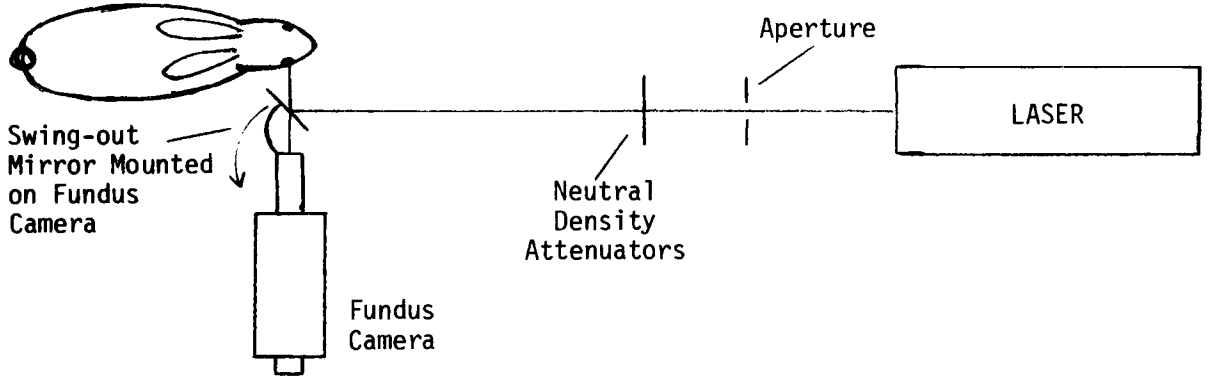


FIGURE 2. Exposure and Observation Apparatus

### 2.2.2 Exposure

The animals were exposed to total energy doses ranging from 100 J to over 20,000 J. Exposure levels did not exceed 200 mW and exposure times usually did not exceed 2 hours. The animals were divided into four sets of two each, according to exposure level, with one additional animal (control) receiving no IR exposure. One eye of each animal, excluding the control animal, was exposed, thus retaining one eye as an *in vivo* control. Two animals received 100 J, two animals received 1000 J, four animals received exposures from about 4,500 J to 10,000 J, and two animals received over 10,000 J. One additional animal was used to assess the effects of drugs on lenticular opacity.

In all cases, exposures were as close to the retinal damage ED50 threshold as possible, while avoiding an ophthalmoscopically visible lesion.

All laser exposures were made in the continuous wave (cw) mode. The apparatus was aligned and calibrated immediately before an exposure session. Occasionally, an exposure led to detectable retinal damage. When this was observed, the time of exposure to that point was noted, the beam power reduced, and the beam moved to an unexposed area on the retina. During movement, care was taken to insure that the beam stayed within the dilated pupil.

### 2.2.3 Scatter

Slit lamp photographs of the animals' eyes were obtained each month. A neutral density step wedge was simultaneously photographed on each frame. The device is shown diagrammatically in Figure 3. The wedge was illuminated by a constant amount of light reflected from a beam splitter

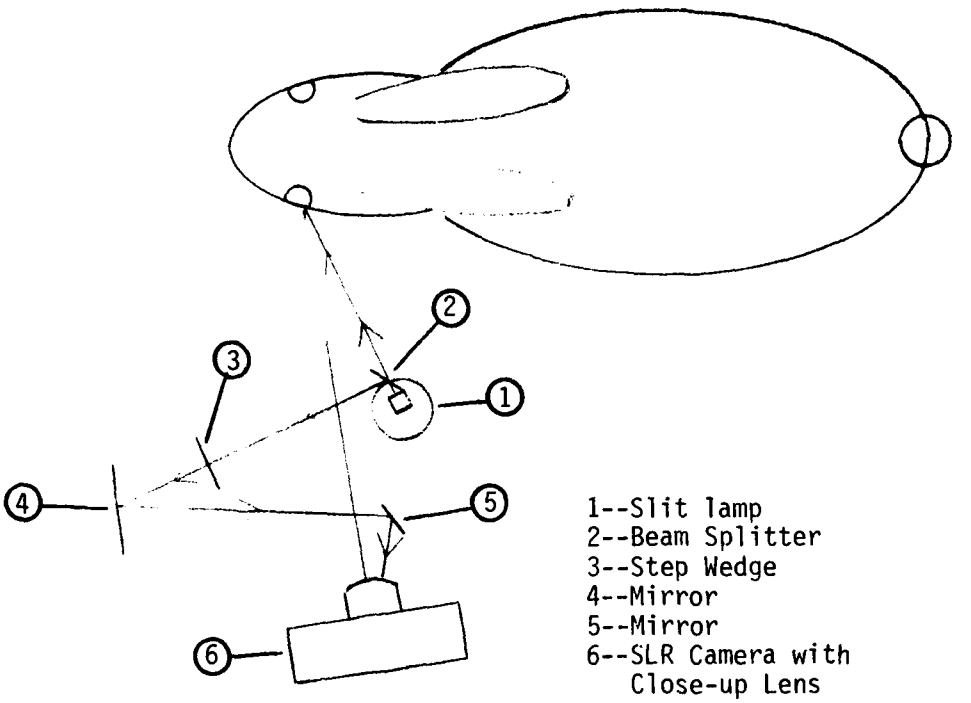


FIGURE 3. Schematic Diagram of Ocular Photography System. The optical path length from the camera to no. 3 is equal to the path length from the camera to the rabbit.

in front of the slit lamp exit slit. Thus, the illumination intensity could be monitored on each frame and the haze or scatter from the eye measured against the step wedge.

In practice, the step wedge image was scanned with a photodiode, thus producing a step-like analog trace on chart paper. The height of the steps were measured and a smooth curve drawn as a function of the step number. The ocular photograph was then scanned and the height of the nuclear scatter measured. A typical photograph and matching scan is shown in Figure 4. The measurement was compared to the step wedge curve and the approximate wedge density derived from the abscissa. Previously, a scan had been made of the step wedge alone and the percent transmission of each step calculated and plotted against the step number. A smooth curve was then drawn through the points (Figure 5). The step number inferred from the test photograph was projected to the calibration curve in Figure 5 and % transmission read from the ordinate.

### 2.2.4 Opacity Size

In addition to scatter, the development of posterior pole opacities was monitored from the slit lamp photographs. The opacities' edges often were indistinct, therefore, approximations were made in order to affect measurement.

Opacity size was derived from planimeter measurement of the posterior pole opacities. The sizes were expressed in arbitrary units and did not represent the actual opacity dimensions. Each opacity area was traced three times and the planimeter readings averaged. The step wedge was used as a size reference by measuring the center step dimension and arbitrarily

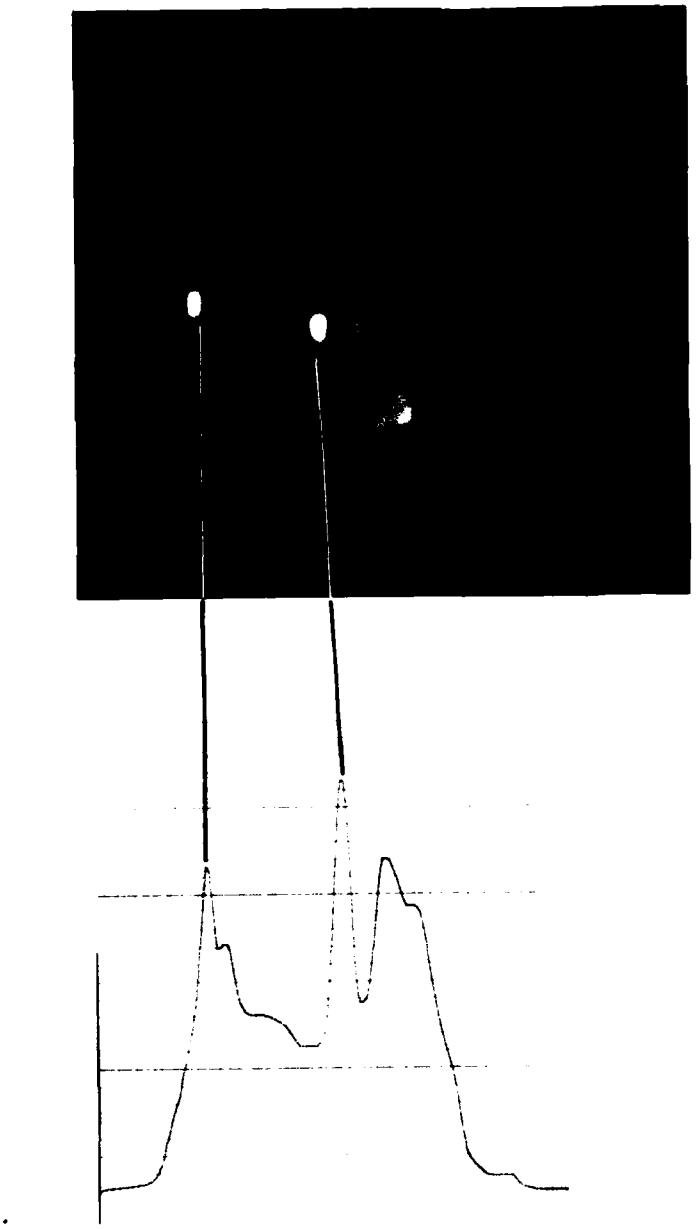

FIGURE 4. Typical Slit Lamp Photograph and Corresponding Densitometer Tracing

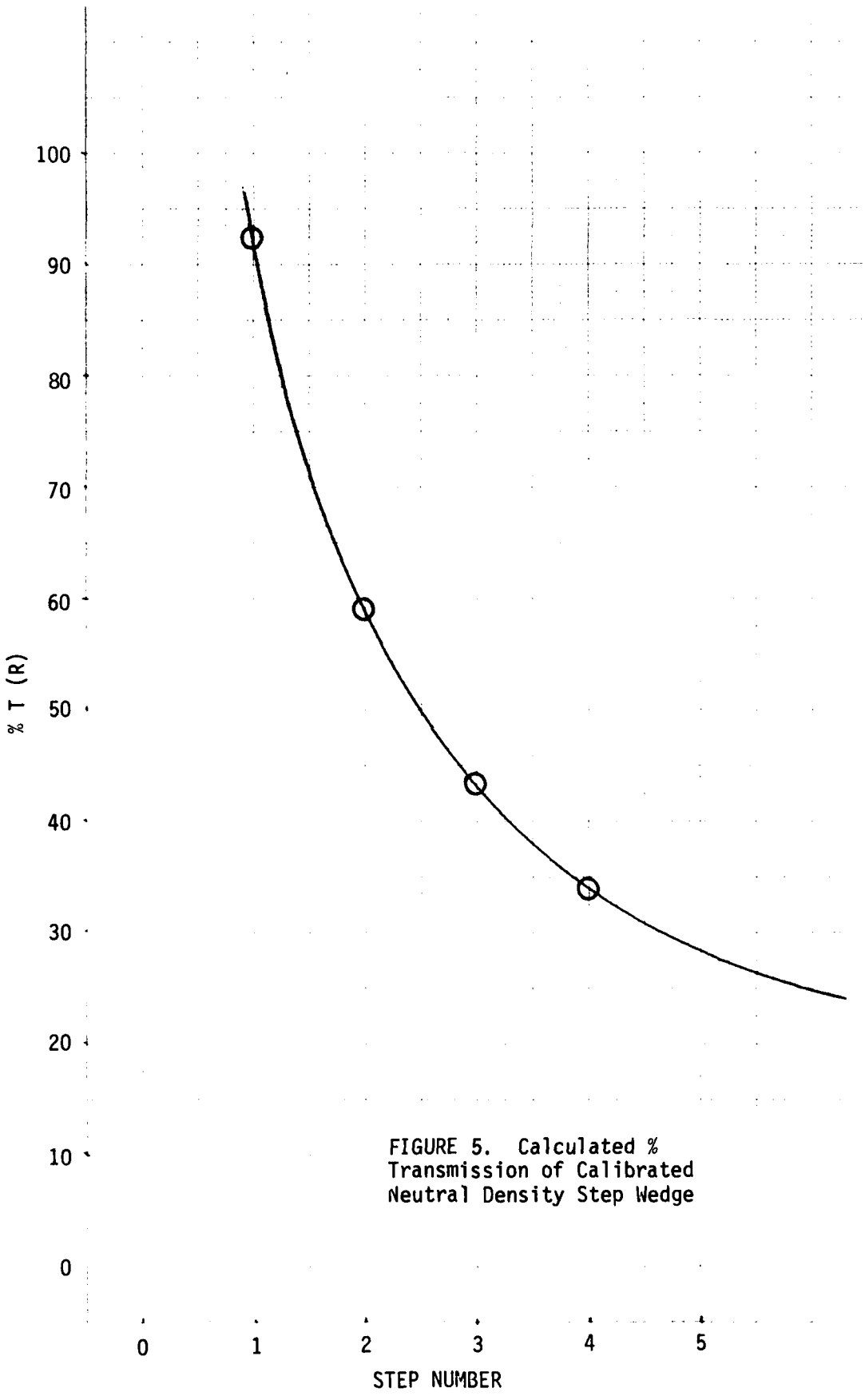


FIGURE 5. Calculated % Transmission of Calibrated Neutral Density Step Wedge

setting it equal to 1. The resulting ratio was used to scale the opacity size.

### 2.2.5 Electrophoresis

Polyacrylimide gel electrophoresis was conducted on the extreme exposure lenses (20 J vs. 2000 J) from Phase I and on the extreme exposure lenses (control and 20,000 J) from Phase II. The lenses were decapsulated and the capsule and lens placed in tris-glycine buffer on a rocking table and gently agitated for seven hours. After approximately five hours, the cortex was suspended in buffer and the remaining nucleus had ceased diminishing in size. The capsule, cortex, and nucleus were homoginized separately in tris-glycine buffer and centrifuged. The supernatant contained the water soluble protein fraction. The packed solids buttons were suspended and dissolved in tris-glycine buffer containing 8 M urea. Seven percent sucrose (wt/vol) was added to each sample to affect layering on the gel. Samples of 10 and 20 μl were placed on top of the gel columns with 5 μl of bromophenol blue added to one or two samples as tracking dye.

The tubes were run for 1/2 hour at 1 milliamp per tube, then the power increased to 2 milliamp per tube for 4.5 hours. At the end of 5 hours, the gels were removed and stained in 0.5% Amido black for 1 hour. Destaining required a minimum of 48 hours by the diffusion method. The destained gels were scanned with a densitometer and a permanent analog record obtained. No attempt was made to quantitate the data. However, each electrophoresis run compared one test lens and one control lens. This procedure allowed estimates of "more or less" protein content to be made between control

and test eyes. The estimates were based on band densities and were determined from height measurements of recorded peaks.

### 2.2.6 Raman Spectroscopy

Two Raman spectroscopy scans were made, utilizing an argon-ion laser.* The animal lenses were removed and placed in a balanced salt solution for scanning. One lens had received 1000 J, while the other had received no IR exposure.

* USAF School of Aerospace Medicine, Laser Effects Branch, Brooks AFB, Texas.

## 3. RESULTS

### 3.1 Phase I

The object of Phase I was to determine exposure technique and to verify exposure power levels for use in Phase II. The first step in determination of applicable power levels was an examination of ANSI threshold levels. A series of rabbit exposures was made above and below the stated ANSI threshold values. Exposures were from 1000 mW to 5 mW and exposure times were 5 sec to 3600 sec. Data from which our established lesion thresholds were derived are listed in Table 1. The relationship between threshold values, calculated ANSI values, and laser beam power is shown in Figure 6. The beam power curve superimposed over the threshold lines in Figure 6 is an approximation based on widely varying data. Nevertheless, the curve does appear to have an asymptotic approach to approximately 150 mW. The animals used for construction of the curves in Figure 6 received total energy doses from 21 to 2458 J (Table 2). Beam power refers to that beam radiometric value measured by a disk calorimeter and expressed in milliwatts.

The experimental paradigm used by Technology Incorporated called for the longest exposures possible. For this reason and based on the beam power curve in Figure 6, we elected to utilize a maximum power of 200 mW for Phase II.

TABLE 1.

DATA FOR ESTIMATED LESION THRESHOLD DETERMINATION

| Exposure Time (sec) | Corneal Power (mW) | Joules | Joules/cm$^2$ * | Lesion |
|---|---|---|---|---|
| 5 | 70 | 0.4 | 0.12 | No |
| 5 | 200 | 1.0 | 0.29 | No |
| 5 | 490 | 2.4 | 0.71 | No |
| 5 | 600 | 3.0 | 0.88 | No |
| 5 | 900 | 4.5 | 1.32 | No |
| 5 | 1000 | 5.0 | 1.47 | No |
| 5 | 1000 | 5.0 | 1.47 | No |
| 300 | 70 | 21 | 6.19 | No |
| 300 | 70 | 21 | 6.19 | No |
| 300 | 70 | 21 | 6.19 | No |
| 300 | 80 | 24 | 7.08 | No |
| 300 | 100 | 30 | 8.84 | No |
| 300 | 200 | 60 | 17.70 | Yes |
| 300 | 300 | 90 | 26.55 | Yes |
| 900 | 10 | 9 | 2.65 | No |
| 900 | 20 | 18 | 5.31 | No |
| 900 | 30 | 27 | 7.96 | No |
| 900 | 40 | 35 | 10.32 | No |
| 900 | 40 | 35 | 10.32 | No |
| 900 | 40 | 35 | 10.32 | No |
| 900 | 50 | 45 | 13.27 | No |
| 900 | 50 | 45 | 13.27 | No |
| 900 | 50 | 45 | 13.27 | No |
| 900 | 60 | 54 | 15.92 | No |
| 900 | 70 | 63 | 18.58 | No |
| 900 | 70 | 63 | 18.58 | No |
| 900 | 115 | 104 | 30.68 | Yes |
| 900 | 140 | 126 | 37.17 | No |
| 900 | 150 | 135 | 39.82 | No |
| 900 | 170 | 153 | 45.13 | No |
| 900 | 200 | 180 | 53.10 | Yes |
| 900 | 220 | 198 | 58.41 | Yes |
| 900 | 250 | 225 | 66.37 | Yes |
| 900 | 250 | 225 | 66.37 | No |
| 900 | 300 | 270 | 79.65 | Yes |
| 900 | 300 | 270 | 79.65 | Yes |

continued ...

TABLE 1
(continued)

| Exposure Time (sec) | Corneal Power (mW) | Joules | Joules/cm$^2$ * | Lesion |
|---|---|---|---|---|
| 3000 | 100 | 300 | 88.50 | No |
| 3000 | 100 | 300 | 88.50 | No |
| 3000 | 125 | 450 | 132.74 | No |
| 3000 | 150 | 540 | 159.29 | No |
| 3000 | 150 | 540 | 159.29 | No |
| 3000 | 175 | 630 | 185.84 | Yes |
| 3000 | 200 | 720 | 212.39 | Yes |
| 15 | 450 | 6.8 | 2.01 | No |
| 15 | 600 | 9.0 | 2.65 | No |
| 15 | 600 | 9.0 | 2.65 | No |
| 15 | 700 | 10.5 | 3.10 | Yes |
| 15 | 700 | 10.5 | 3.10 | Yes |
| 15 | 700 | 10.5 | 3.10 | No |
| 15 | 700 | 10.5 | 3.10 | No |
| 15 | 800 | 12.0 | 3.54 | Yes |
| 15 | 850 | 12.8 | 3.78 | No |
| 15 | 850 | 12.8 | 3.78 | No |
| 15 | 900 | 13.5 | 3.98 | Yes |
| 15 | 950 | 14.5 | 4.28 | Yes |
| 15 | 950 | 14.5 | 4.28 | No |
| 15 | 1000 | 15.0 | 4.42 | Yes |
| 15 | 1000 | 15.0 | 4.42 | Yes |
| 15 | 1000 | 15.0 | 4.42 | Yes |

* In terms of a 7-mm diameter pupil.

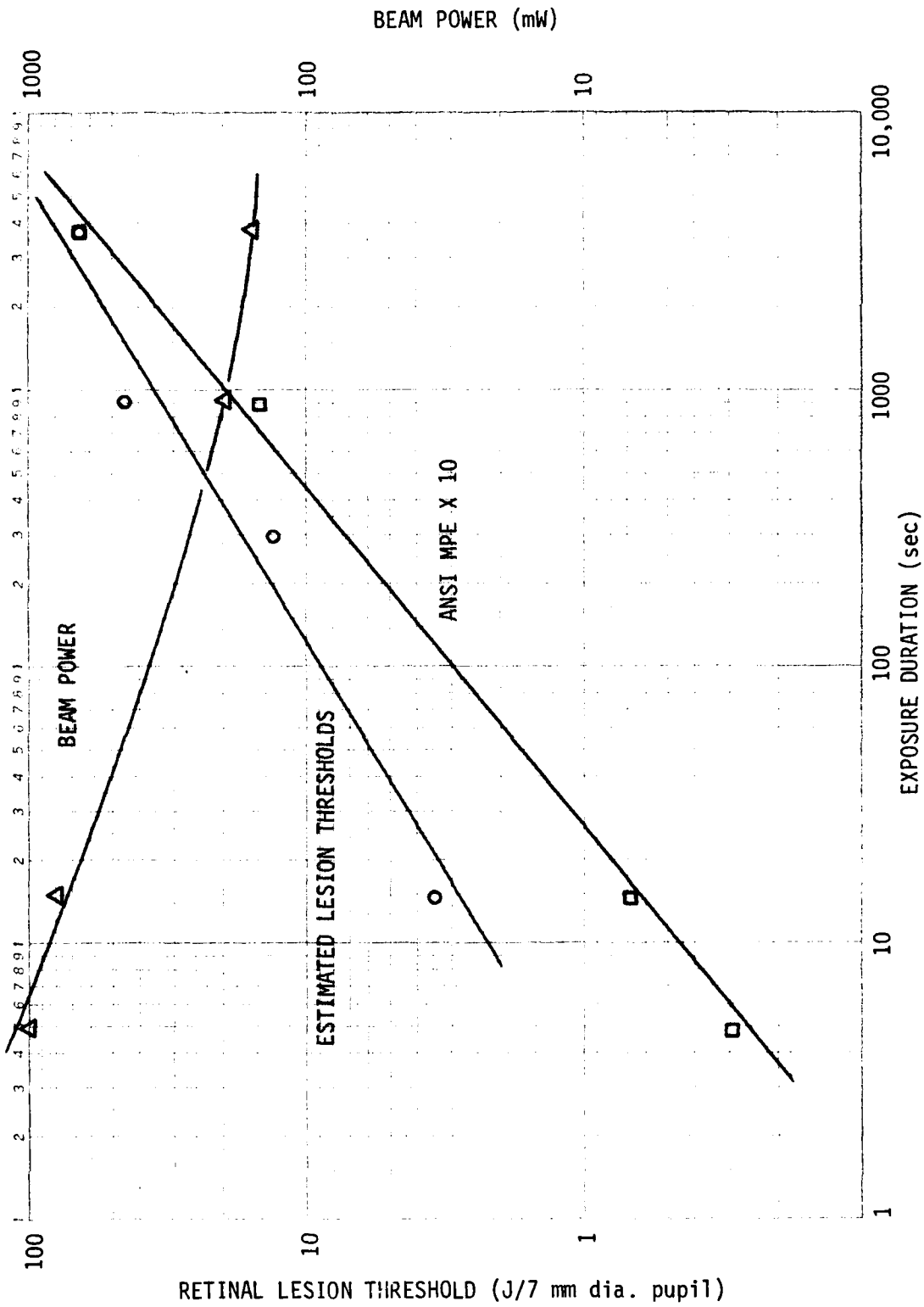


FIGURE 6. Retinal Lesion Thresholds for the 1064 nm Laser Line

TABLE 2.

CUMULATIVE ENERGY DEPOSITION AT 1064 nm

| Animal No. | Eye | Energy Deposition (J) |
|---|---|---|
| 5 | OD | 21 |
| 5 | OS | 28 |
| 3 | OD | 188 |
| 6 | OD | 246 |
| 7 | OS | 270 |
| 3 | OS | 305 |
| 1 | OD | 493 |
| 2 | OD | 612 |
| 4 | OS | 734 |
| 4 | OS | 774 |
| 2 | OS | 774 |
| 8 | OS | 1020 |
| 8 | OD | 1119 |
| 7 | OD | 2458 |

## 3.2 Phase II

### 3.2.1 Exposure

Our original intent was to expose animals three times weekly. This was later changed to twice weekly, but even this schedule was difficult to comply with. The final frequency of exposure is shown in Figure 7. Table 3 lists the monthly exposure totals for all animals used in Phase II. However, due to the scarcity of data in some cases, not all animals listed in Table 3 will be discussed in later sections.

### 3.2.2 Scatter

During experimentation, backscatter increased, then decreased. This observation is illustrated in Figure 8, which shows the development of scatter from the central third of the lens. The significance of the peak value at the end of May or June is difficult to assess. It may be related to a natural aging process, the administration of drugs, reflection from the posterior pole opacity or some combination of these possibilities.

### 3.2.3 Opacities

All rabbits appear to have a horizontally oriented posterior pole opacity. It is not evident in very young animals, but it does increase in size and density as the animal matures. Several animals were examined at the supplier's, and in all cases, the opacity appears to have defined edges and to be a long, thin streak in the vicinity of the posterior suture line. The extent of the opacity is not evident in some

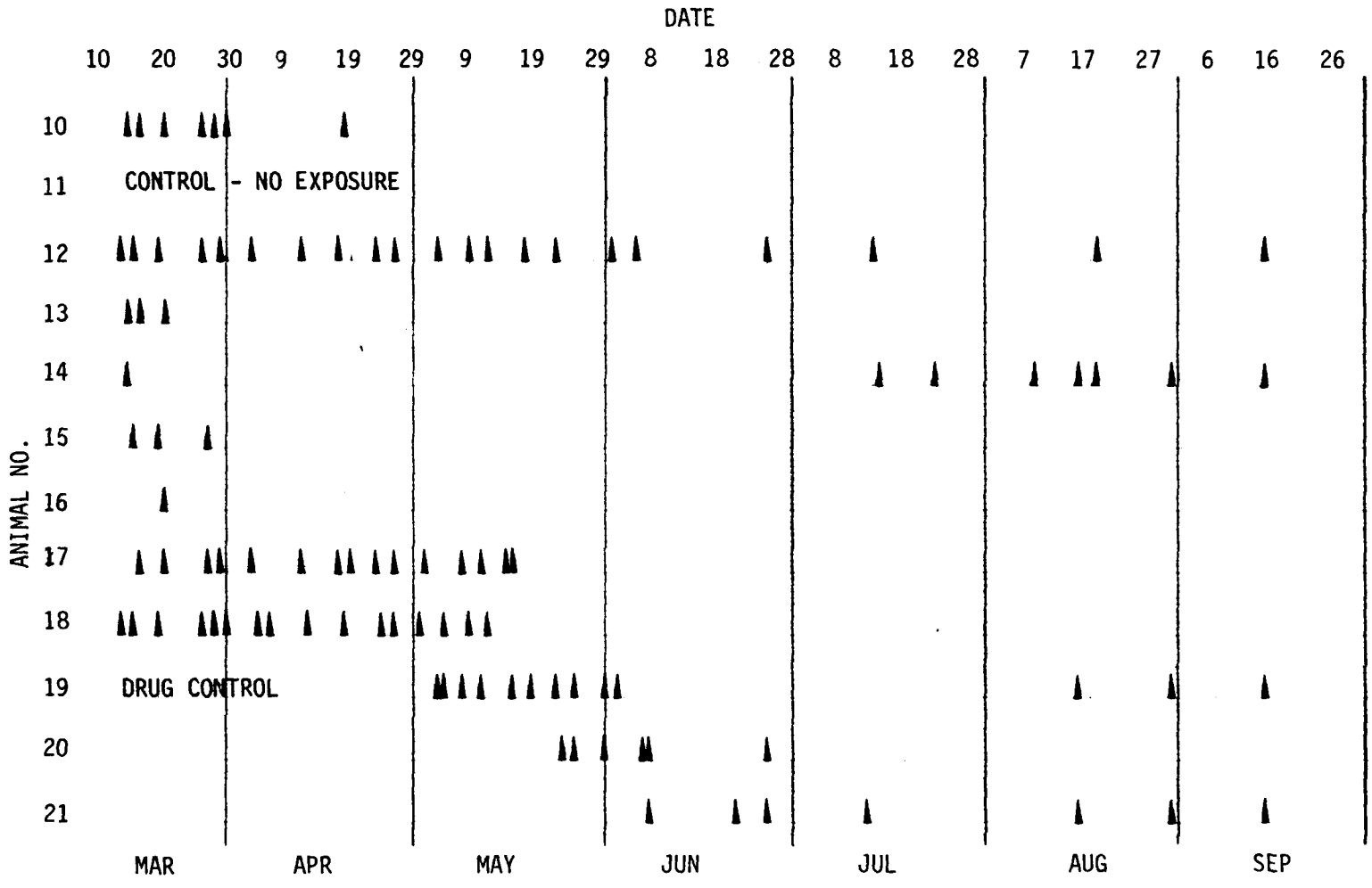


FIGURE 7. Exposure Dates of All Principal Animals

TABLE 3.

MONTHLY EXPOSURE TOTALS FOR EACH ANIMAL

| Animal No. | Month | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov |
| 14 OD | 100 | --- | --- | --- | --- | --- | --- | --- | --- |
| 14 OS | --- | --- | --- | --- | 540 | 3399 | 648 | --- | --- |
| 16 | 100 | --- | --- | --- | Died | | | | |
| 13 | 1000 | --- | --- | --- | --- | --- | Sacrificed | | |
| 15 | 1000 | --- | --- | --- | --- | --- | --- | --- | --- |
| 10 | 3687 | 814 | Died | | | | | | |
| 17 | 2330 | 4182 | 3525 | Euthanized | | | | | |
| 18 | 4185 | 6101 | 4672 | --- | Died | | | | |
| 12 | 4297 | 6086 | 5044 | 3432 | 648 | 702 | 486 | --- | Sacrificed |
| 20 | --- | --- | 2981 | 4311 | Died | | | | |
| 21 | --- | --- | --- | 6728 | 2520 | 4074 | 1148 | --- | --- |
| 11 | --- | --- | --- | --- | --- | --- | --- | --- | Sacrificed |
| 19 | --- | --- | --- | --- | --- | --- | --- | --- | --- |

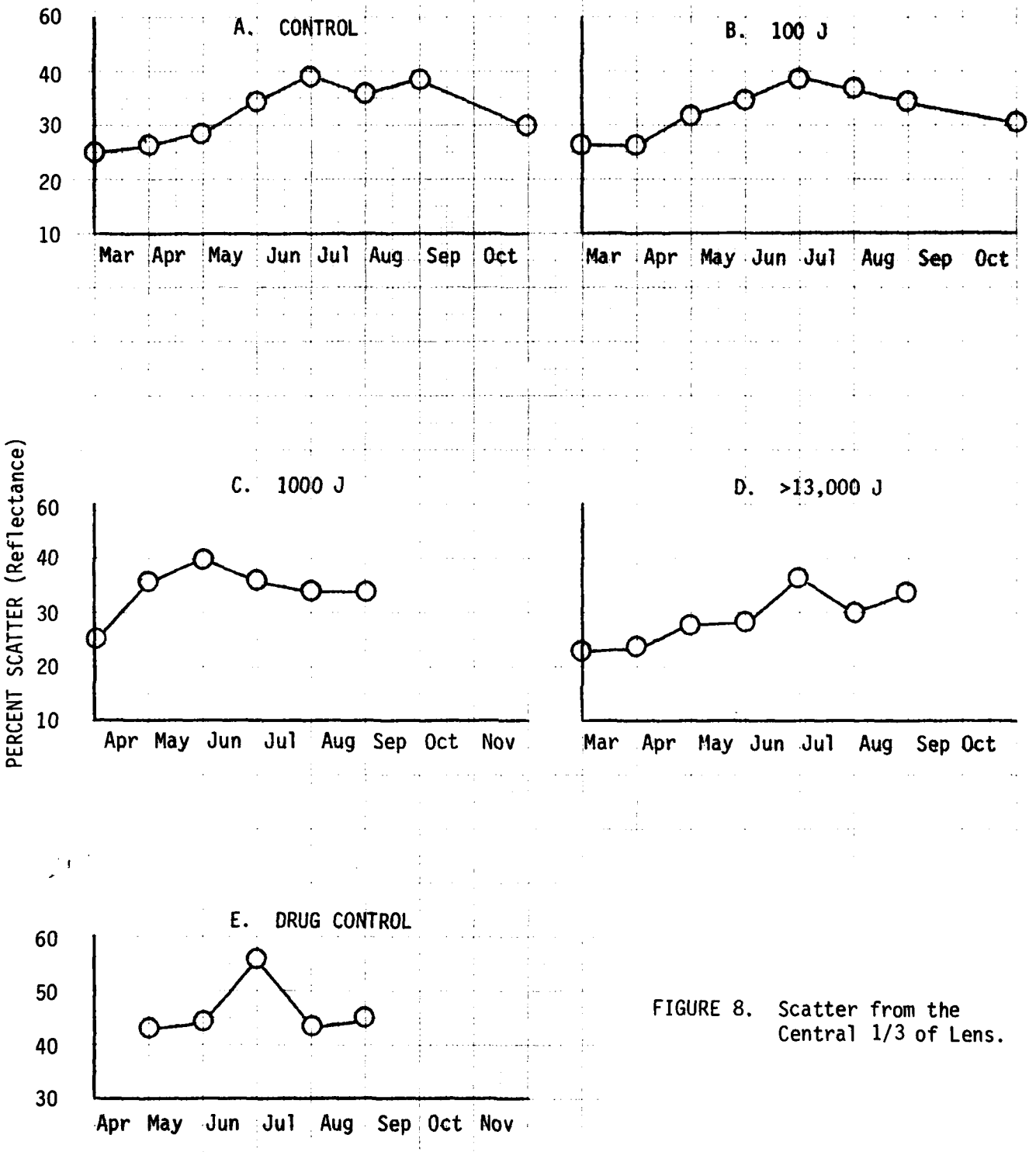


FIGURE 8. Scatter from the Central 1/3 of Lens.

of the earlier photographs (Figure 9a) due to the angle between the optic axis of the camera and rabbit eye. Further into the program, the angle was decreased to allow more complete visualization of the opacity (Figure 9b). Drawings of the opacities as of May and August are shown in Figures 10 and 11, respectively. It is important to remember that the edges of many of the opacities were indistinct, therefore, the drawings shown in Figures 10 and 11 must be considered approximations. The opacity size discontinuity between 1000 J and 10,000 J, as of May, was virtually nonexistent three months later.

### 3.2.4 Raman Spectroscopy

A cursory Raman spectroscopy scan was made on the lenses of one animal. The particular animal (no. 13) had received 1000 J in its right eye and no IR exposure in its left eye. The spectrum implied the presence of more water in the exposed eye than in the control eye. Also, there was little or no difference in quantities of detectable proteins between the two eyes. However, virtually the entire baseline of the control eye was elevated over that of the exposed eye (Figure 12).

### 3.2.5 Electrophoresis

The eyes of all animals from Phase I were saved and used as preliminary electrophoresis samples. All lenses, except those from animals no. 5 and 7 were used to determine the various parameters of gel concentration, buffer, power, run time, stain and destain. The lenses of animals no. 5 OD and 7 OD were saved for protein difference determinations.

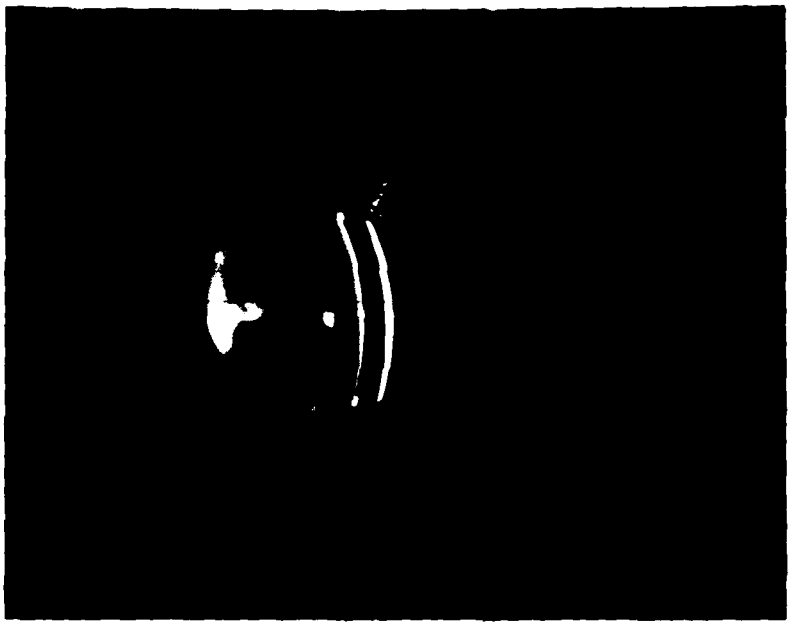

FIGURE 9a. Slit Lamp Photograph, Animal No. 120D, 8 March 1978.

FIGURE 9b. Slit Lamp Photograph, Animal No. 120D, 6 September 1978.

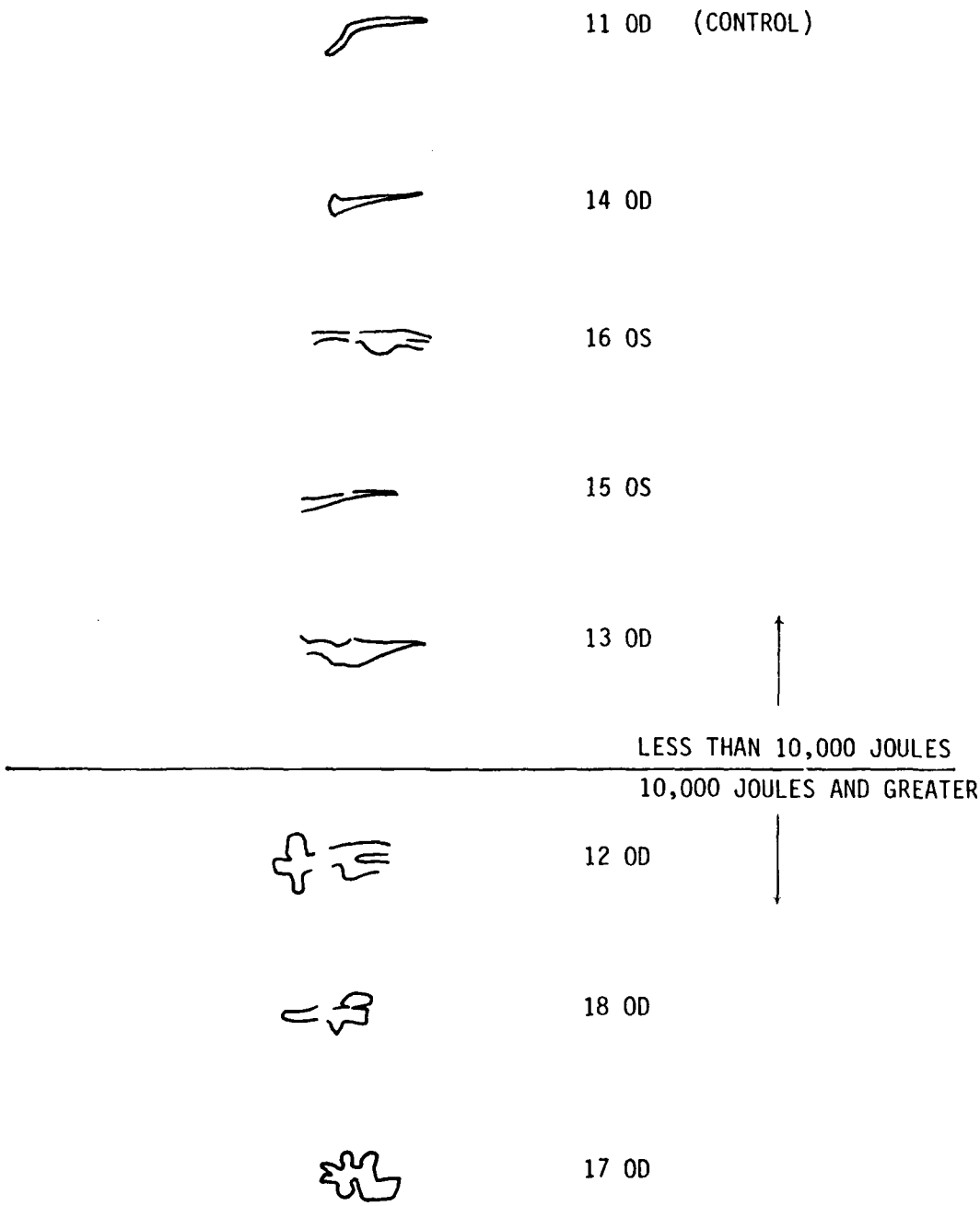


FIGURE 10. Approximate Relative Size and Shape of Posterior Pole Opacities as of 31 May 1978.

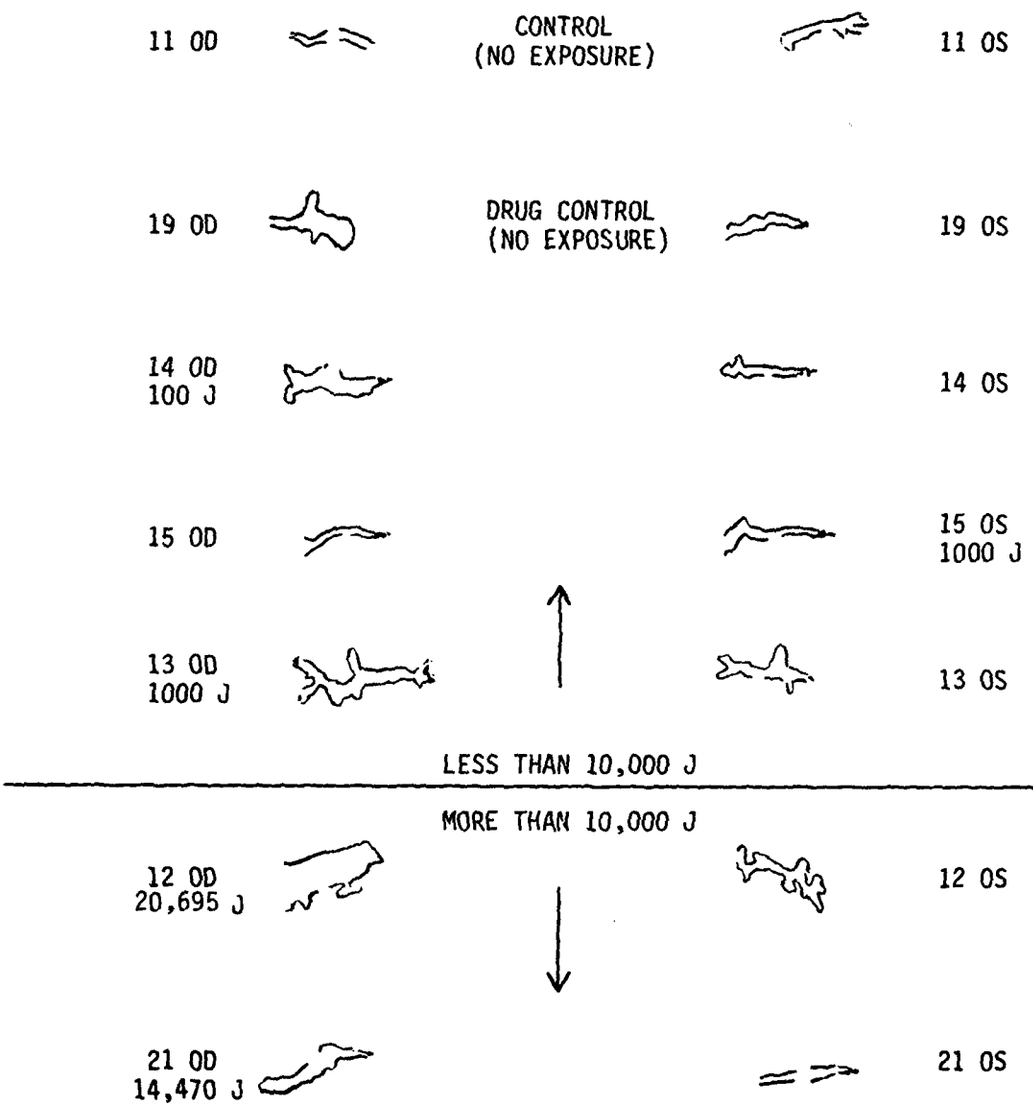


FIGURE 11. Approximate Relative Size and Shape of Posterior Pole Opacities as of 31 Aug 1978.

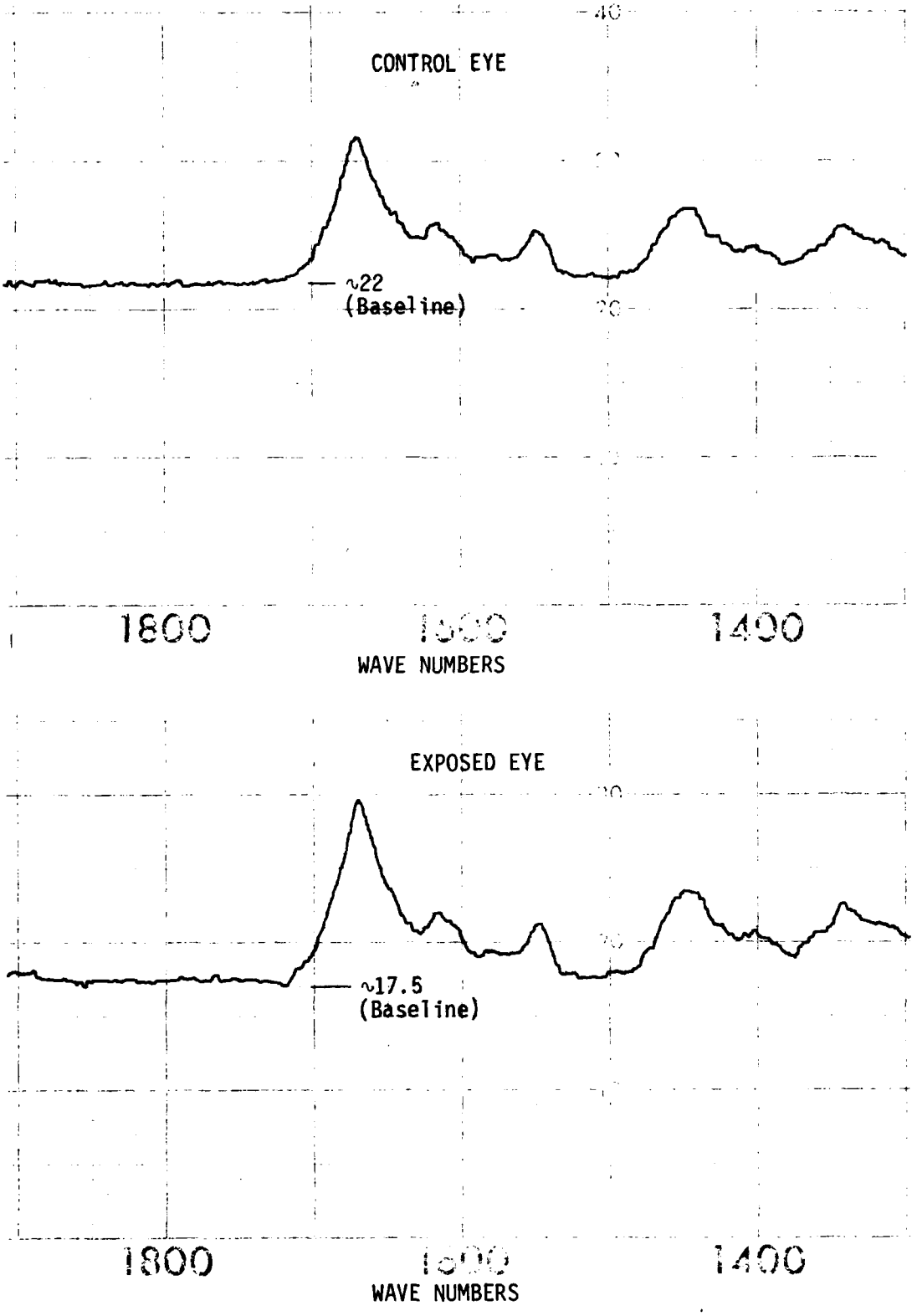


FIGURE 12. A Portion of the Raman Spectroscopy Scan of Animal No. 13. Note difference in baseline shift between the two eyes.

Electrophoresis results for this research study were not intended to be quantified. Instead, they were used to infer "more" or "less" differences between protein from highly exposed and lightly exposed eyes. Each electrophoresis run for data collection contained lenticular sample material from a highly exposed eye and a lightly exposed or unexposed eye. Such a procedure allowed direct comparison of proteins within each run. In all cases, only the maximum and minimum exposure levels were compared. Results from the preliminary sample material indicated that protein differences, if any, were masked by technique variability unless extreme exposures were compared.

Lenticular capsular material revealed no specific protein banding. However, occasionally, very faint cortex bands were detected in the material. On the other hand, clearly distinguishable differences were noted between cortical and nuclear material. Generally, the cortex of exposed lenses showed more urea soluble (insoluble) material and less water soluble material than unexposed lenses. There were slight differences between nuclei from the two lenticular treatments, but due to variability, these differences were thought to be insignificant.

# 4. DISCUSSION

## 4.1 Phase I

A series of eight animals was utilized to establish desired irradiance levels. The ANSI standard was used for a point of reference. Exposures were made around the reference point and the results compared with the ANSI data. Our collected data do not agree precisely with the established ANSI data (Figure 6). This may be due to an error in the safety factor estimation, insufficient number of data points, or interspecific tissue differences between test animals.

## 4.2 Phase II

### 4.2.1 Animals

A new series of nine rabbits was obtained after completion of Phase I. Based on data from Phase I, cumulative energy levels of 100, 1000, 10,000, and 50,000 J were selected as target totals.

With the exception of the death of animals no. 10 and no. 17, few problems were encountered until early June. Animal no. 10 died of a respiratory problem, while no. 17 was a self-mutilator and was subsequently euthanized. At this time, an additional animal, no. 19, was acquired as a drug control. In late June and during July, three animals were lost due to an undiagnosed respiratory problem. They exhibited symptoms of respiratory difficulty and did not respond to medication. Upon death, the animals were autopsied. The thoracic cavities of two animals were found to be filled with a clear, water-like fluid. Before death, one of the three

animals exhibited symptoms of lung embolism, with a large amount of bloody exudate from the nose and mouth. Upon autopsy, the animal was found to have severely hemorrhaged lungs, but very little chest fluid. We believe the lungs were weakened by disease and consequently ruptured during struggles, thus forcing fluid from the animal's chest.

A veterinary pathologist was consulted and performed one autopsy, after which he attempted to culture the chest fluid. One small colony of a human pneumococcus developed. According to the pathologist, such an organism is unknown in rabbits.

After the last autopsy, animal no. 14 developed symptoms characteristic of early stages of the illness seen in the diseased animals. On the assumption that the disease was indeed a human pathogen, the animal was isolated and treated with antibiotics. The treatment continued for five days, after which the distressed breathing had returned to normal. The animal was kept in isolation for another week and when no further symptoms were observed, was placed back into the experimental animal group. No exposures were made during the disease outbreak. It was felt best not to unduly stress the animals at this time, thus perhaps lowering the threshold of any natural defense mechanisms.

Following cessation of the respiratory outbreak, some animals continued to receive exposure, although not at the same frequency as before the disease onset. There was no evidence that the disease affected any ocular tissues. The corneas stayed clear and there were no conjunctival secretions or eruptions.

### 4.2.2 Experimental Results

Temperature increases in the human lens as a result of infrared exposure have been calculated to be from $9^{o}C$ to $1^{o}C$[5]. The $9^{o}C$ rise is an extreme condition and would not occur in an occupational setting. However, the $1^{o}C$ rise could result at chronic exposure levels, and it has been theorized that even a small increase in temperature would, over a long period of time, result in permanent lenticular pathology[5]. A temperature rise of $2.1^{o}F$ was measured in an *in vitro* system in our laboratory. A thermistor probe was placed in 1 $cm^3$ of water which was irradiated with a 200 mW laser beam of 1.06 μm. The beam was not allowed to strike the probe directly, therefore, any temperature rise was the result of water borne convection.

Dr. Wolbarsht contends that an IR induced cataract resembles a senile cataract[5]. Senile cataracts can be both cortical and nuclear[10]. The pathology observed to date in our work has been cortical. This type of opacicification is associated with a significant increase in the percentual water content of the lens[10]. Such an increase in water content might be considered a form of edema although certainly not a function of the lymphatic system. It is possible that the retention of water may be caused by an abnormally high salt concentration. Indeed, there is an increase in the calcium ion concentration in cataractogenesis and the aggregation of normal α protein and α protein from cataractous lens by calcium has been demonstrated[11]. An increase in the water content of cataractous lens is in agreement with Raman spectroscopy of a lens with a cortical opacity resulting from IR irradiation. The animal was exposed by Technology Incorporated during March, 1978, and had received a total of 1000 J. No

additional exposures were made from March to the data of the spectroscopy (10 October 1978).

The baseline displacement in Figure 12 apparently is not due to photoluminance[12]. If it were, the protein peaks would be partially obscured due to excitation and emittance of the sample background matter. More probably, the baseline shift is due to some type of particulate scatter. The proteins then are apparently a part of the scattering complex and as such, are excited normally over and above the background scatter.

The fact that there appears to be little difference in proteins between the control and exposed eyes is in agreement with Truscott and Augusteyn[13], who found nuclear and cortical proteins to be virtually identical in cortical cataractous lenses and normal lenses. Also, Francois, Rabaey and Boyen-Rikkers[14] have shown that many incipient cataractous lenses differ very little in their protein content from normal, healthy lenses.

A series of dialysis experiments was performed in which lenticular material homogenized in tris-glycine buffer was dialyzed against distilled water. After exhaustive dialysis for five days at $4^{o}C$, a very fine white precipitate was formed in the dialysate. The formation of this precipitate may be viewed as the result of salt removal and dilution of protein with water, or as a change in pH. This finding seems to correlate with the observation of increased water content in cataractous lens and the formation of opacities. Goldman, et al.[2] photographed the occurrence of clear "lakes" in swollen opaque corneas. These lakes are areas in which no

collagen is present and may represent areas of increased water content. Benedek[15], however, seems to imply that the cataractous lens does not form "lakes" *per se*, but aggregations of proteins which have indices of refraction different from that of the surrounding matrix. If so, such differences in refraction could cause turbidity and clouding in the lens.

The formation of protein aggregates is a common theme throughout cataract literature. But in nearly all cases reviewed, the assumption seems to be that they are merely centers of refraction. There seems to be little evidence that such aggregations could actually be opaque precipitates. The concensus is that expressed by Bruckner[16] when he stated, "If the aggregates become sufficiently large, light scattering becomes significant enough to make the lens turbid." Intralenticular aggregations of proteins have been detected by Tanaka and Benedek[17] in the intact cataractous lens. But they gave no indication whether the aggregations were soluble high molecular weight proteins (HMW) or "insoluble" proteins. The distinction between these two protein forms has been made by Zigman, et al.[18] and Francois, Rabaey and Stockmans[19]. Zigman, et al. separated the insoluble proteins from the HMW proteins by centrifugation. Our electrophoresis also distinguished between the two protein types. The fraction called "water soluble" by us actually contained the soluble HMW protein while that fraction termed "urea soluble" contained the insoluble proteins. The results shown by us agree with those of Zigman, et al., in that an elevation of insoluble protein was noted in exposed eyes when compared to the level of insolubles noted in control eyes. Zigman, et al., regards HMW as a precursor of the insoluble proteins. They also contend that interaction of the lens with light is more closely related to the

insoluble fraction than to the HMW fraction. Francois, et al.[19], observed an increase in insoluble albuminoids and a decrease in the relatively low molecular weight proteins in cataractous lens. These authors did not believe the HMW proteins were involved in a transition to the insoluble albuminoids. They did, however, specifically discuss the concept of precipitation as pertains to insoluble proteins.

Densitometric scans of slit lamp photographs from our laboratory revealed no nuclear cataract or opacity. The photographs and scans did, however, indicate a gradual increase, then a decrease in backscatter from the nucleus. In general, the scatter seemed slightly less for those highly exposed lenses as compared to the control and lenses which received up to 1000 J. It should also be noted that the posterior pole opacities were larger in the highly exposed lenses. No data have been found in which scatter *per se* was investigated. The fact that we generally observed an increase in scatter followed by a decrease could be the result of the radiation exposure, the use of drugs, or perhaps a combination of the two. Cumulative exposure and drug dosage was rapidly increasing to the end of the first week in June 1978. At that time, both exposure and consequently, administration of drugs essentially stopped. Scatter continued to increase for a short time, then either leveled or began to decrease. Opacity size also increase to the June peak, then began to decrease or to continue growth.

Animal no. 11 (Figure 13) received a control treatment which consisted of no exposure or drugs except those small amounts required for photography

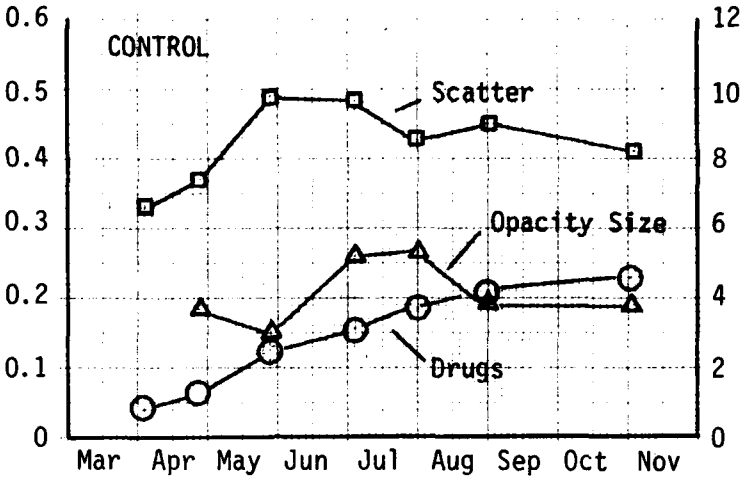


FIGURE 13. Average Composite Data, Animal No. 11

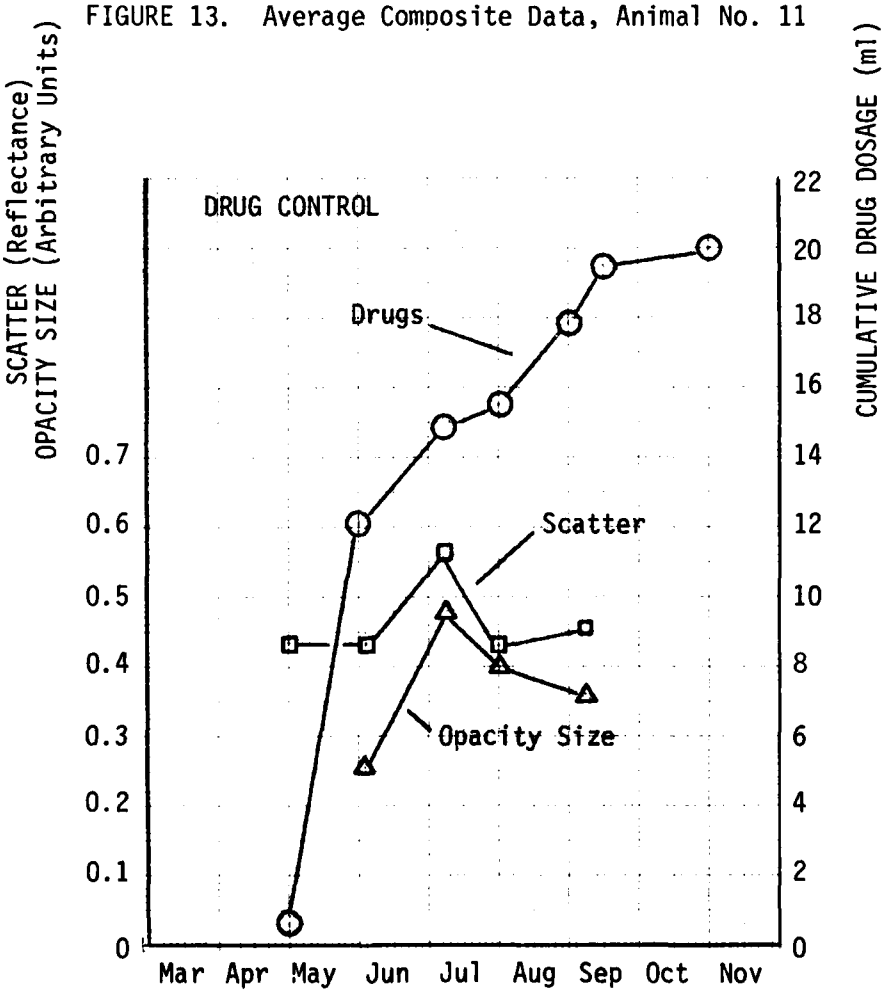


FIGURE 14. Average Composite Data, Animal No. 19

and observation. The curves shown in Figure 13 indicate no significant increase in opacity size. However, there appears to be an initial rapid increase in scatter then a slow decrease. This generalized scatter profile was seen in all other animals, except no. 13 (Figure 18a).

There were indications across nearly all animals that as nuclear scatter decreased, posterior pole opacity size increased and vice versa, particularly in the latter months of the test period. An exception, although not serious, was animal no. 14 (Figure 15). Scatter was low and the opacity large, but their progressions were roughly parallel. No particular opacity-scatter relationship was noted for the two control animals (Figures 13 and 14). The averaged data in Figure 8 revealed, for the control eyes, virtually the same scatter as did the eyes receiving 100 J exposure. The eyes receiving 1000 J seemed to reach a peak scatter value sooner than the other eyes, but that value was not any higher than the others. The eyes receiving over 13,000 J had the least scatter. There is no evidence from our data that IR exposure caused lenticular scatter through alteration of the nucleus.

Animal no. 12 received 20,000 J in its right eye (Figure 16). However, the drugs administered affected both eyes. If Figures 16 and 17 are compared, a striking similarity between opacity size and scatter will be noted. The obvious implication here is that the observed scatter and opacities are drug related. On the other hand, the differences observed in animal no. 13 (Figures 18a and 18b) seem to be exposure related. Study of these two figures will show an obvious difference between the exposed eye and the control eye. Animal no. 19 was used as a drug control and received no exposure. No obvious drug related effect was noted (Figure 14).

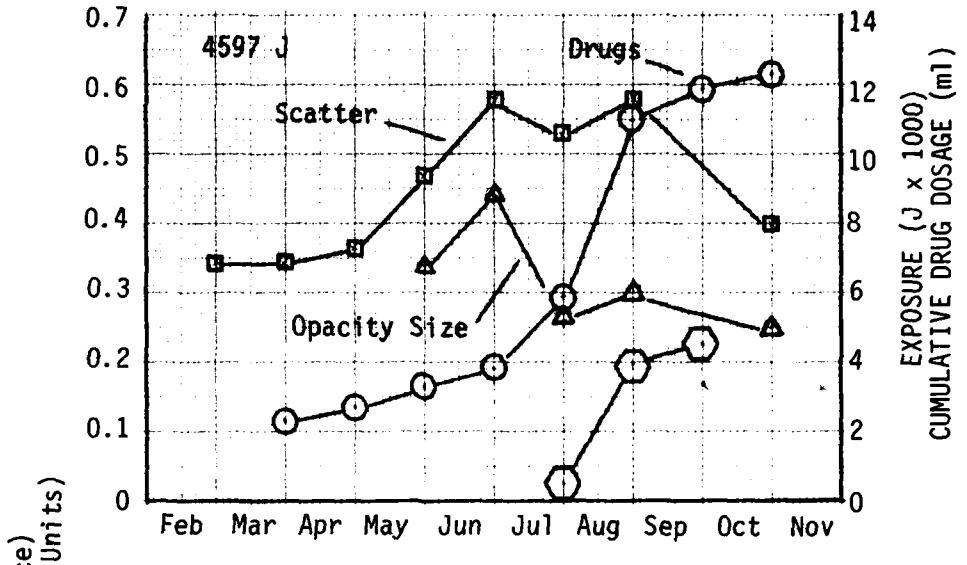


FIGURE 15a. Composite Data, Animal No. 14 OS
Exposure - ⬡

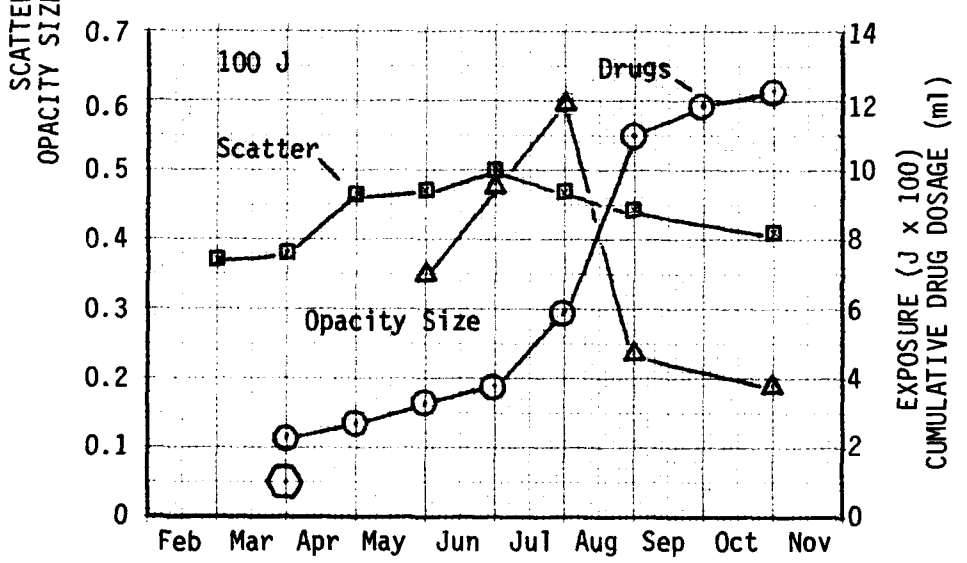


FIGURE 15b. Composite Data, Animal No. 14 OD
Exposure - ⬡

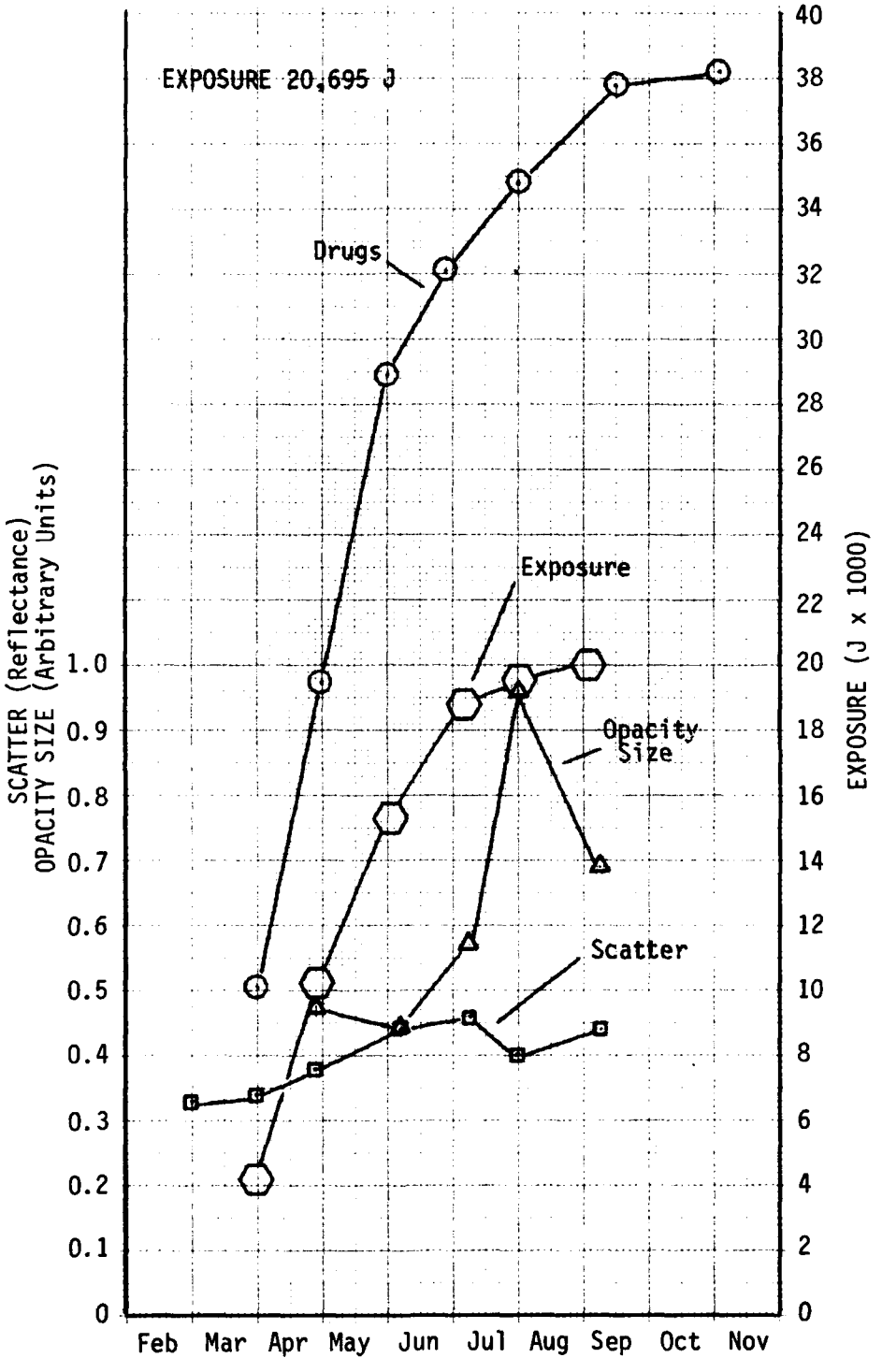


FIGURE 16. Composite Data, Animal No. 12 OD

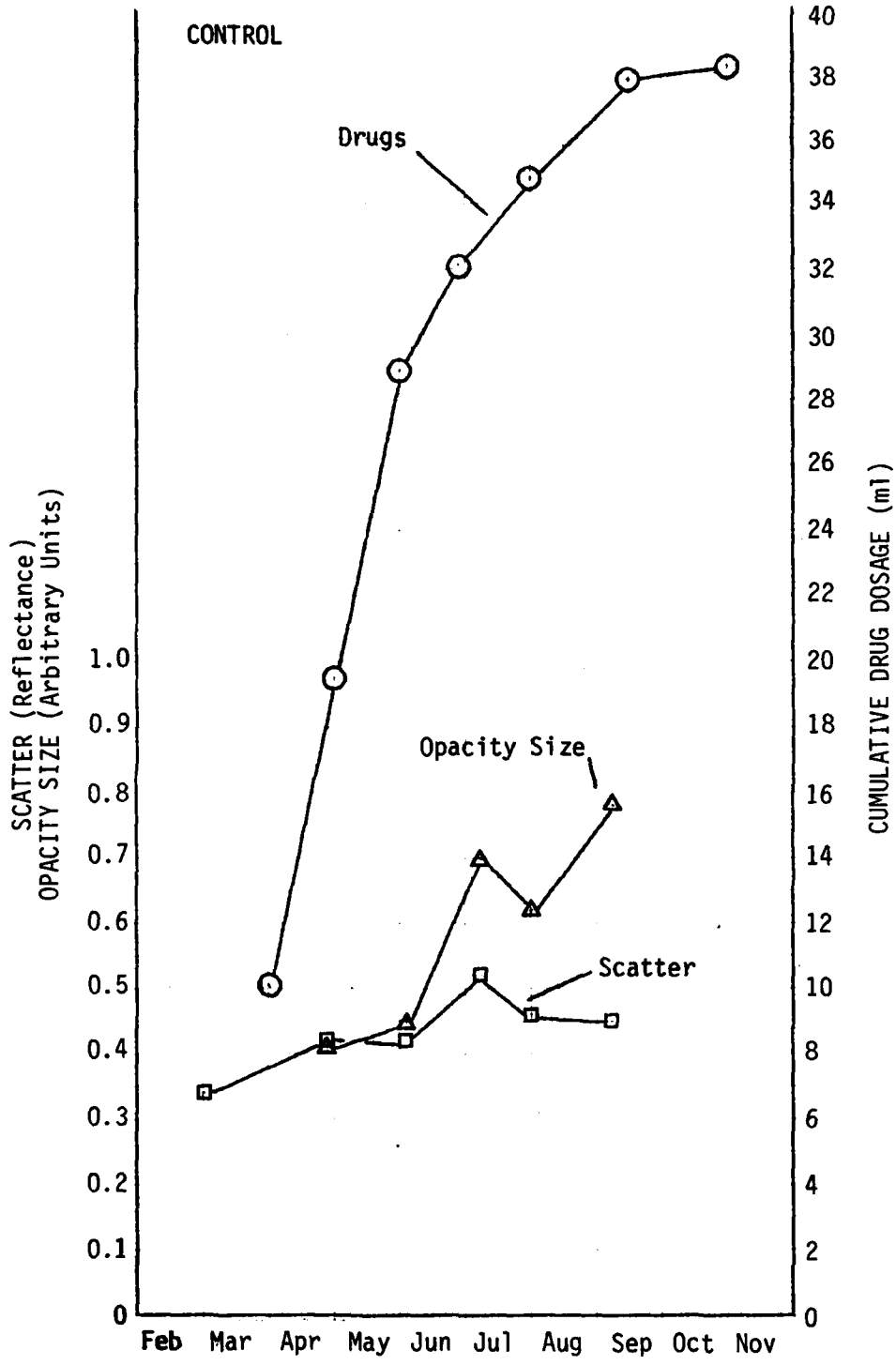


FIGURE 17. Composite Data, Animal No. 12 OS

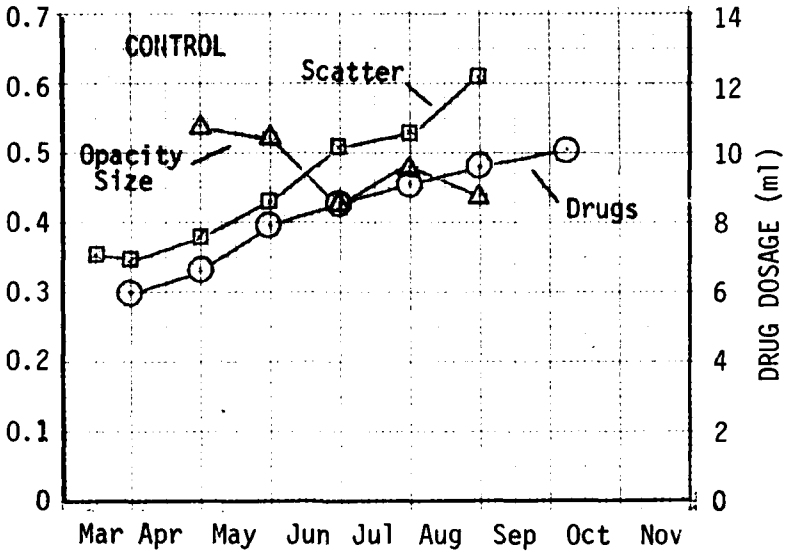


FIGURE 18a. Composite Data, Animal No. 13 OS

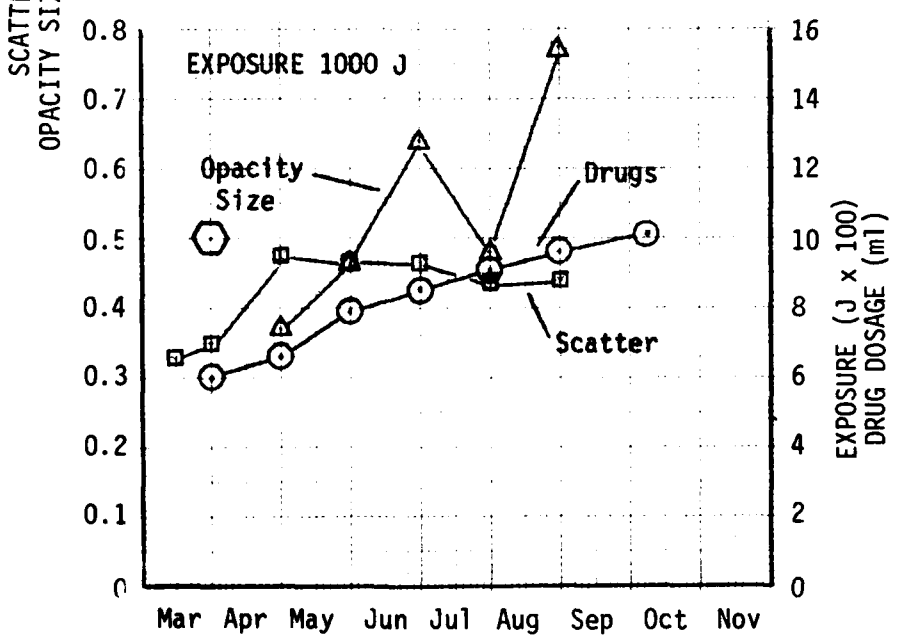


FIGURE 18b. Composite Data, Animal No. 13 OD
Exposure - ⬡

The same negative observation can be seen in animal no. 11 (Figure 13) which received no drugs or exposure.

The posterior pole opacities observed in our work began in or at the posterior suture line and resembled ionizing radiation cataracts. The very tenuous cob-web appearance of these opacities made their quantification difficult, so any size determinations were made cautiously. With this in mind, it seems safe to say the higher exposed animals had the largest opacities. However, at this point in time, it is not safe to assume the opacity sizes were related to exposure alone. There is a possibility that drug dosage may have been a contributing factor.

We believe the lenticular effect of chronic IR exposure is cumulative and does not obviously alter light scatter or transmission of the lens nucleus within the parameters of our exposure paradigm. Data from our laboratory indicated little difference between nuclei of exposed and unexposed eyes. However, the cortex of unexposed lenses contained more soluble material than did the exposed lenses. Also, the cortex of exposed lenses contained more urea soluble material than did unexposed lenses. Further, differences between the urea soluble fractions could be noted in the more numerous bands present in the exposed eye.

Our electrophoresis data essentially substantiate our scatter observations, in that there is little difference between the nuclei of exposed and unexposed lenses. On the other hand, there is a substantial difference between the cortex for the two cases.

## 5. CONCLUSIONS

When a lens is chronically irradiated with cw IR irradiation of 1064 nm, little or no immediate effect is noted. The lens is a slow growth organ and has a relatively low metabolic rate, therefore, any alterations resulting from chronic irradiation will require some time before becoming visible. Such alterations may increase the natural aging process which usually results in cataractous lenses. The mechanisms involved result in an increase in calcium content, an increase in water content, an increase in insoluble proteins and a decrease in soluble proteins.

Our work tentatively relates opacity size to IR exposure and drug dose. Chronic IR irradiation appears to affect an inverse relationship between nuclear scatter and posterior pole opacity. However, neither IR irradiation or drug dosage appears to effect nuclear scatter directly. Very high cumulative irradiation dosage results in high cumulative drug dosage. The resulting opacity may be related to either or both of these factors. However, the opacity size-scatter relationship mentioned above still holds. The effect of lower irradiation doses (1000 J or less) seems to be related more to energy input than to drug dose, but this relationship is not clear.

Evidence for both an infrared related lenticular effect and a drug related lenticular effect has been presented, but a complete disassociation of the two effects is not possible in the present study.

If there is concern about cw IR effects, more work must be done. Many more animals must be exposed for longer periods of time in order to produce statistically valid data. Only through such a larger effort can the effect of chronic cw IR irradiation of the ocular lens be defined.

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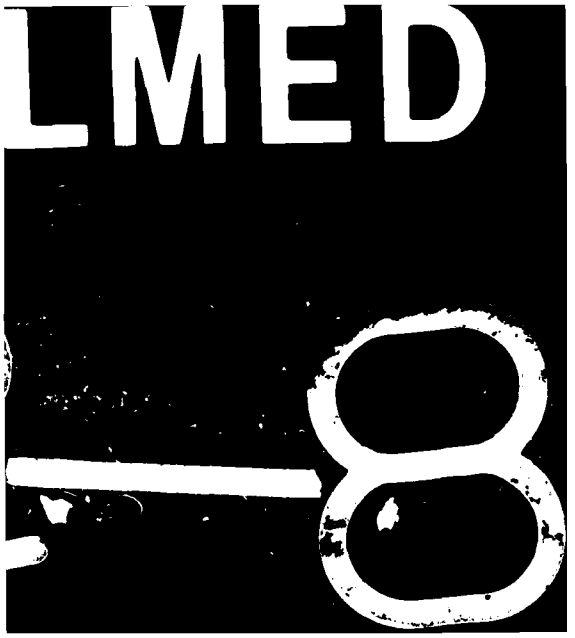
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