FOREWORD

This investigation was performed by the Department of Electrophysiology, The New York Eye and Ear Infirmary, under Contract No. AF 33(657)-7894 in support of Project No. 6301, "Aerospace Systems Personnel Protection", Task No. 630103, "Vision Enhancement and Protection in Aerospace Environment". Captain L. R. Loper of the 6570th Aerospace Medical Research Laboratories was contract monitor.

Primary responsibility was assumed by Jerry Hart Jacobson, M. D., Director of the Department of Research, Blossom Cooper, M. S., Physicist, acted as Assistant Administrator and was concerned with the physical equipment and problems of irradiance, dose and spectral characteristics. Nicholas Kremenic performed the physical calibrations. Dr. Kohtiao and Dr. Najac supervised and conducted the actual experimental exposures. Nan Pillsbury prepared the histopathology and histochemical slides. Dr. Morris H. Shamos, Chairman of the Department of Physics in Washington Square College of New York University, acted as Physics Consultant and rendered invaluable assistance.

ABSTRACT

The eyes of pigmented, grey chinchilla rabbits were exposed to thermal energy to determine its effectiveness in producing pathological changes in anterior ocular tissues. The study included the variation of the following parameters: tissue, rate of delivery of energy, and spectral characteristics. Preliminary findings relative to damage to the cornea and iris are presented.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

J. W. HEIM
Technical Director
Biophysics Laboratory

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INTRODUCTION

The objective of this project was to study the effect of visible and infrared energy, of wave length ranging between 350 and 2500 millimicrons, delivered to in vivo eyes, relative to the production of changes in the anterior ocular tissues.

This study, which was intended to be the first phase of a two-phase investigation, involved several experimental procedures:

1. Devising a suitable source of energy, including the calibration of output, control of exposure time and spectral characteristics.

2. Determining the threshold dose for the cornea and iris as a function of spectral characteristics.

3. Determining the effect of thermal energy of different spectral characteristics on the lens.

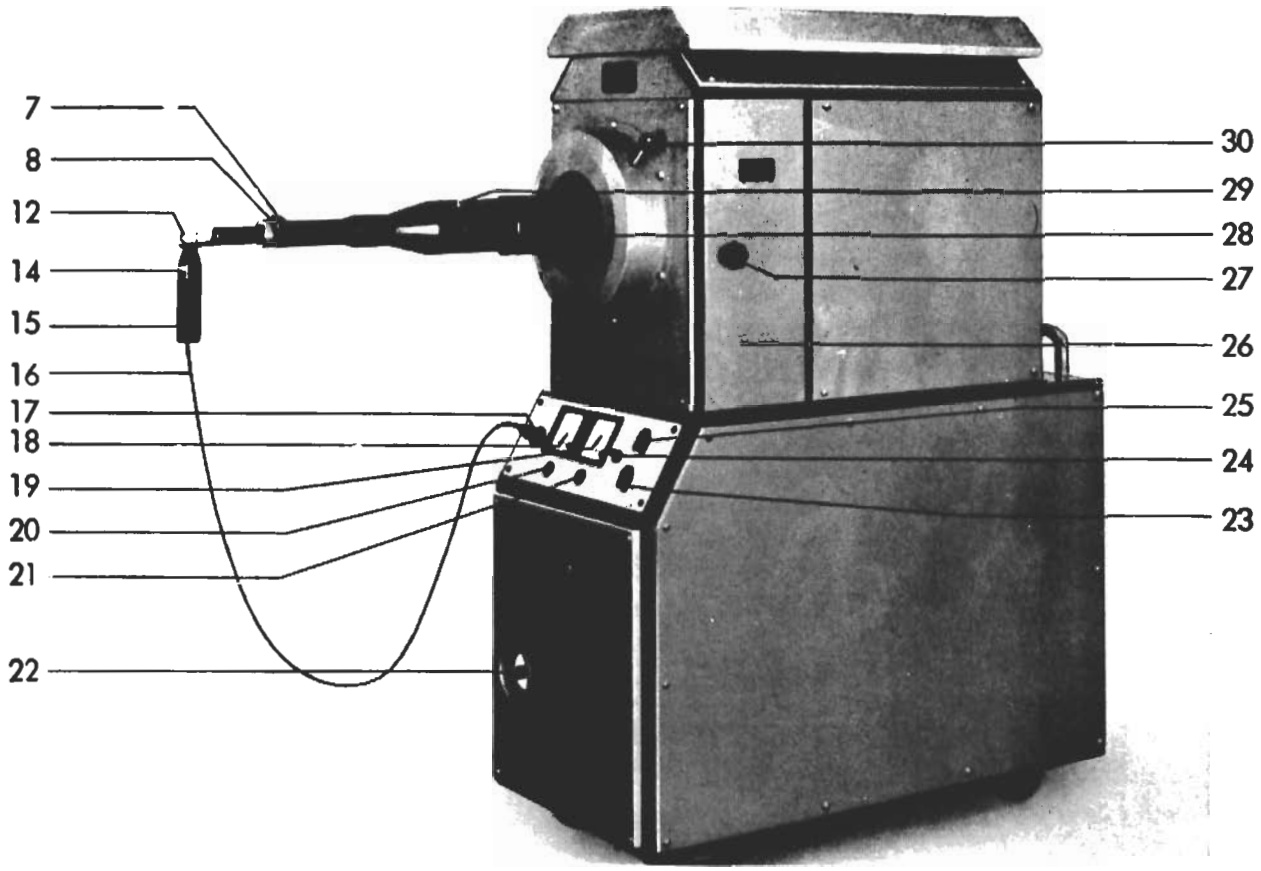
4. Developing techniques to analyze the physio-pathological response of the eye to thermal energy.

5. Perfecting a technique for implanting thermocouples within the eye to measure the temperature rise in the anterior chamber.

I. SOURCE OF ENERGY

A light source of the Meyer-Schwickerath type employing a high pressure Xenon lamp (Osram Model XBO 2001) originally altered to provide high retinal irradiance[1] was further altered to provide high corneal irradiance.

As originally designed, the Xenon lamp is in continuous operation at rated power and the short exposures are obtained with a shutter arrangement. We investigated the possibility of increasing the output of this lamp by pulsing it at high peak power for the short duration required. This was done by switching the voltage from storage batteries in parallel with the main circuit of the lamp for the required time.



7. Filter disc
8. Image field diaphragm
12. Mirror
14. Release knob
15. Handle
16. Cable
17. Cable connection
18. Ammeter
19. Voltmeter
20. Switch-OFF
21. Switch-ON
22. Protective switch
23. Multi-stage switch for normal load
24. Selector switch
25. Multi-stage switch for overload
26. Door
27. Door-handle
28. Optical beam director
29. Lever for iris diaphragm
30. Locking lever

Figure 1. ZEISS LIGHT COAGULATOR AS DELIVERED

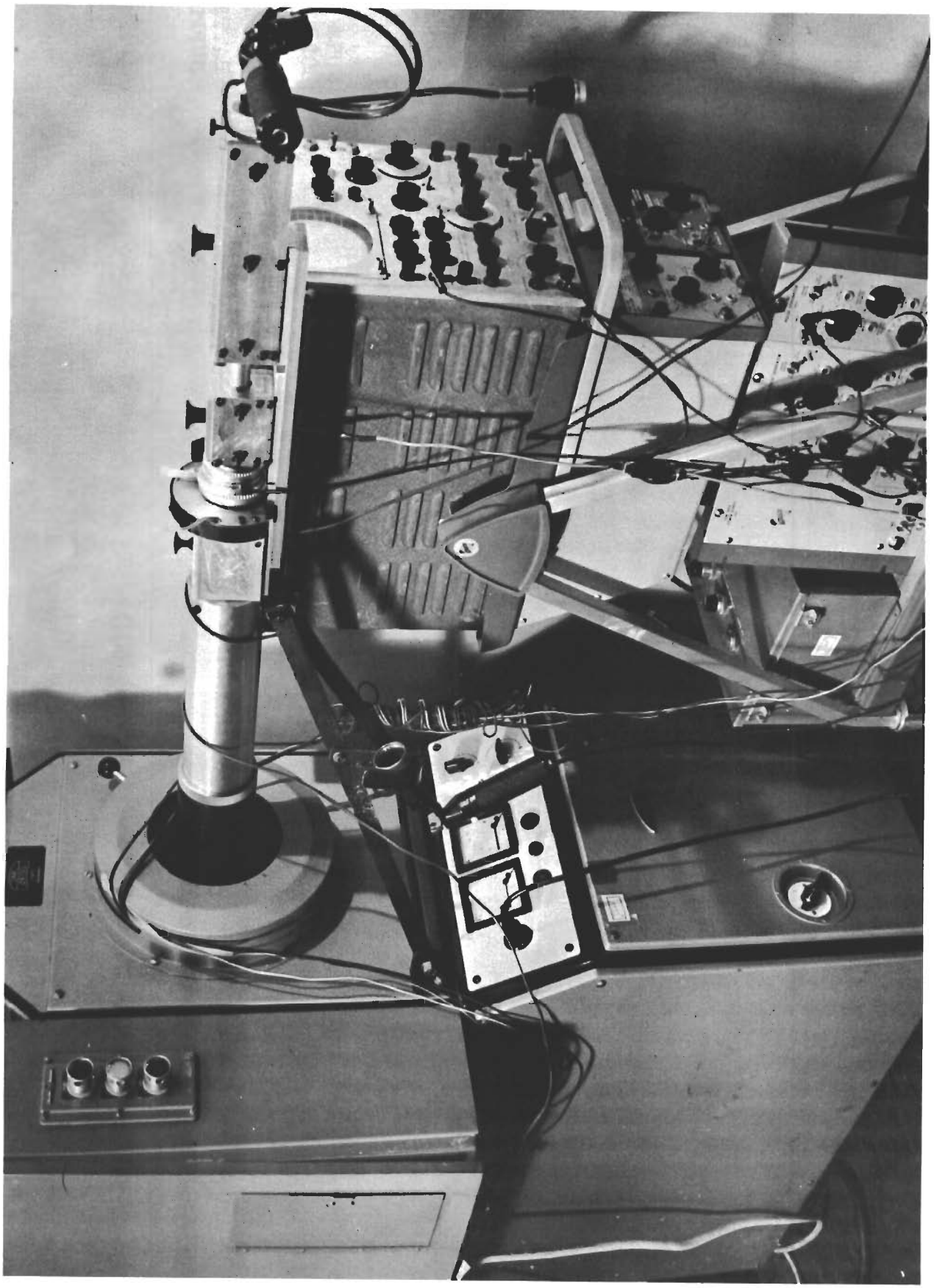


Figure 2. ZEISS LIGHT COAGULATOR MODIFIED FOR PULSING

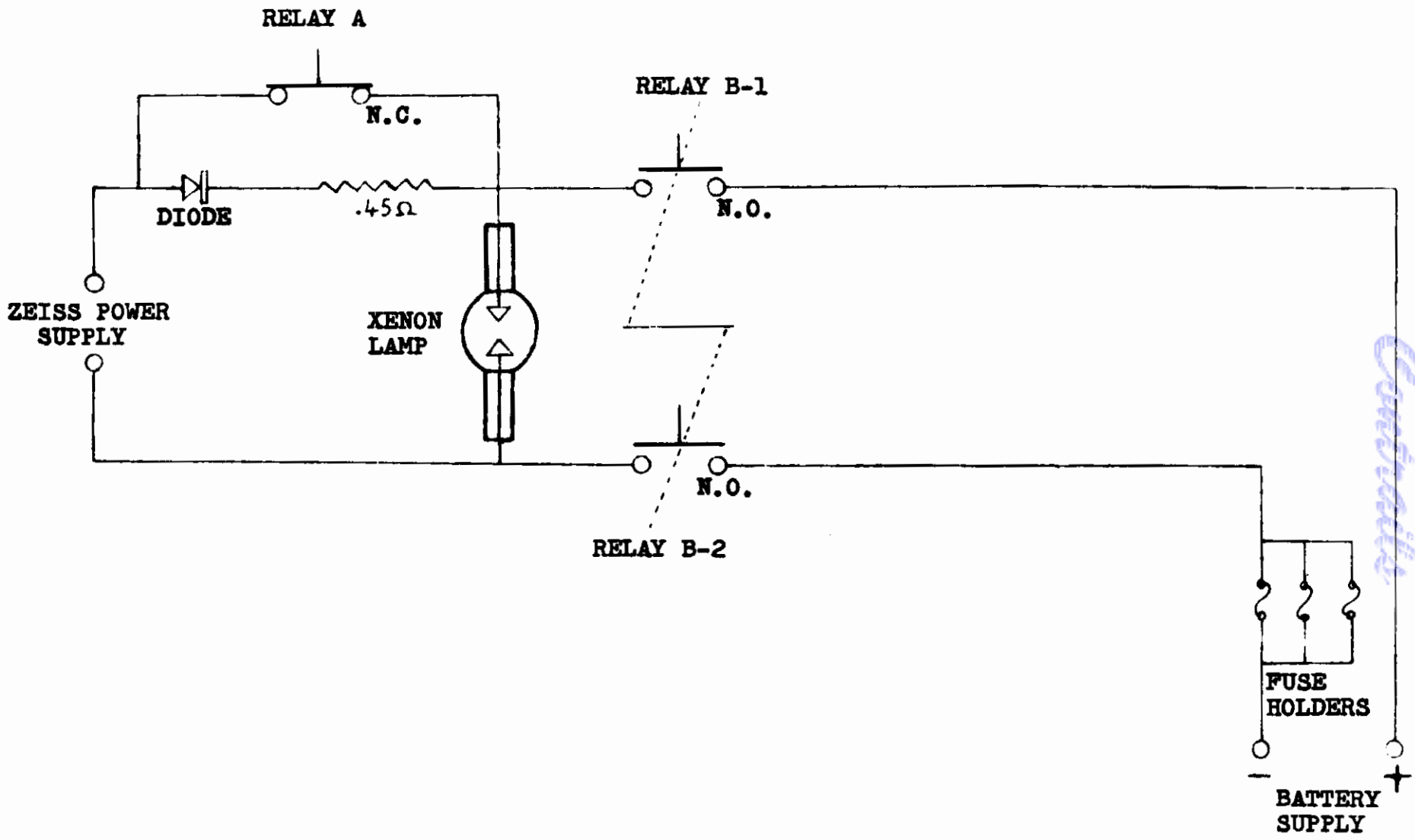


Figure 3. SCHEMATIC OF PULSING CIRCUIT

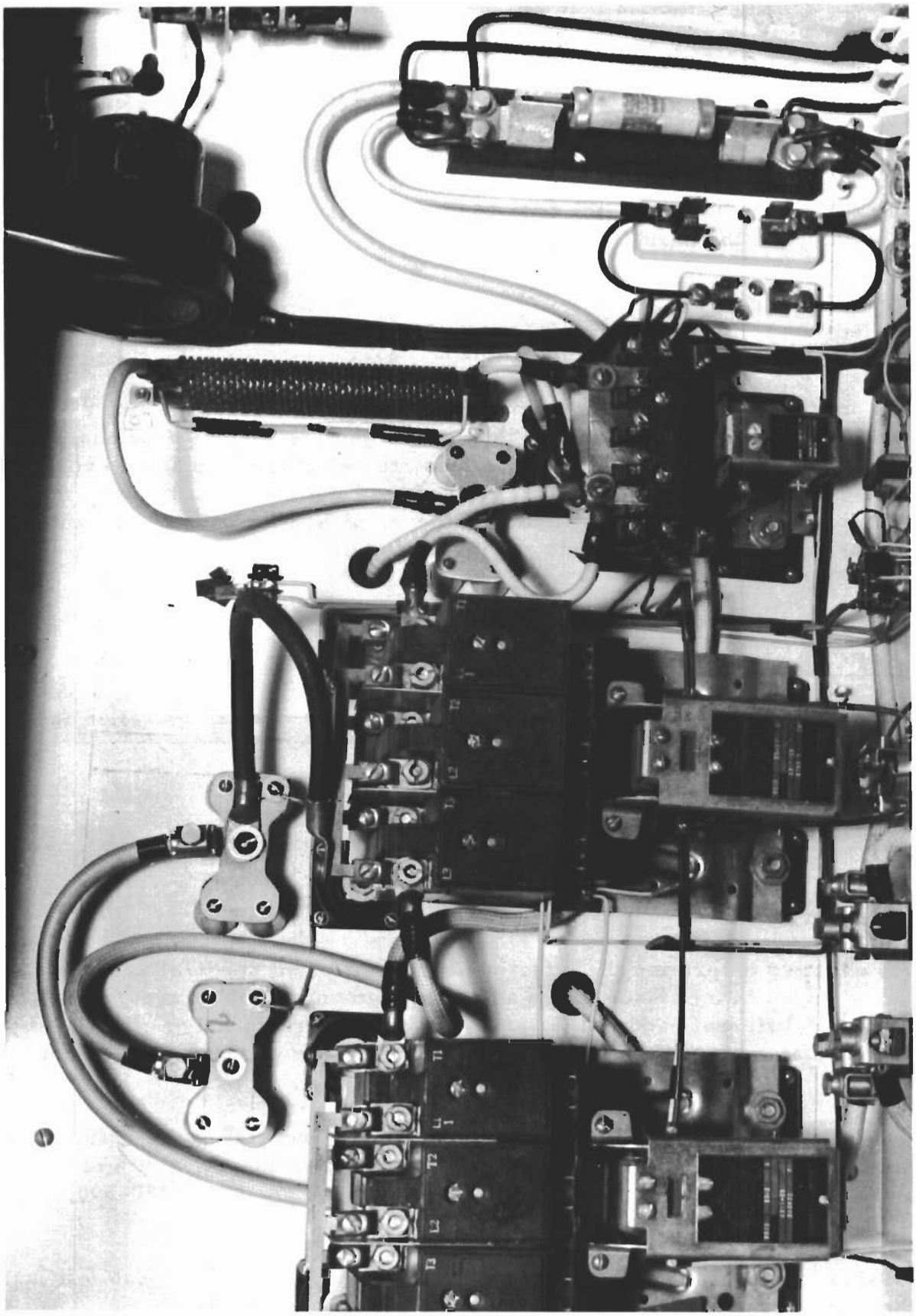


Figure 4. PHOTOGRAPH SHOWING PART OF PULSING CIRCUIT

II. EXPOSURE TIME

The exposure time was specified by the requesting laboratory to be 150 milliseconds. Control of the exposure time was formerly accomplished by the use of a silicon controlled rectifier and calibrated fuse in the pulsing circuit (placed in parallel with the original Zeiss circuit) [1]. The system has since been changed by eliminating the silicon controlled rectifier and adding a fast acting relay in the control circuit of the switching relays. A pulse of the required duration from a Tektronic Pulse Generator type 163 keeps this fast acting relay closed, which in turn keeps the parallel circuit closed.

III. OPTICAL SYSTEM AND IMAGE SIZE

To increase the corneal irradiance and provide a uniform beam, a lens of 30 mm. focal length was added to a two-lens objective system previously constructed for retinal irradiation. This lens, to be referred to as an eye simulator lens, provides a Maxwellian view of the light source with uniform energy falling on the anterior segment of the eye. A beam diameter of 2 mm. was used throughout the experiment. (Figs. 5 and 6).

IV. SPECTRAL DISTRIBUTION

The energy distribution of the Xenon lamp under normal operation is shown below:

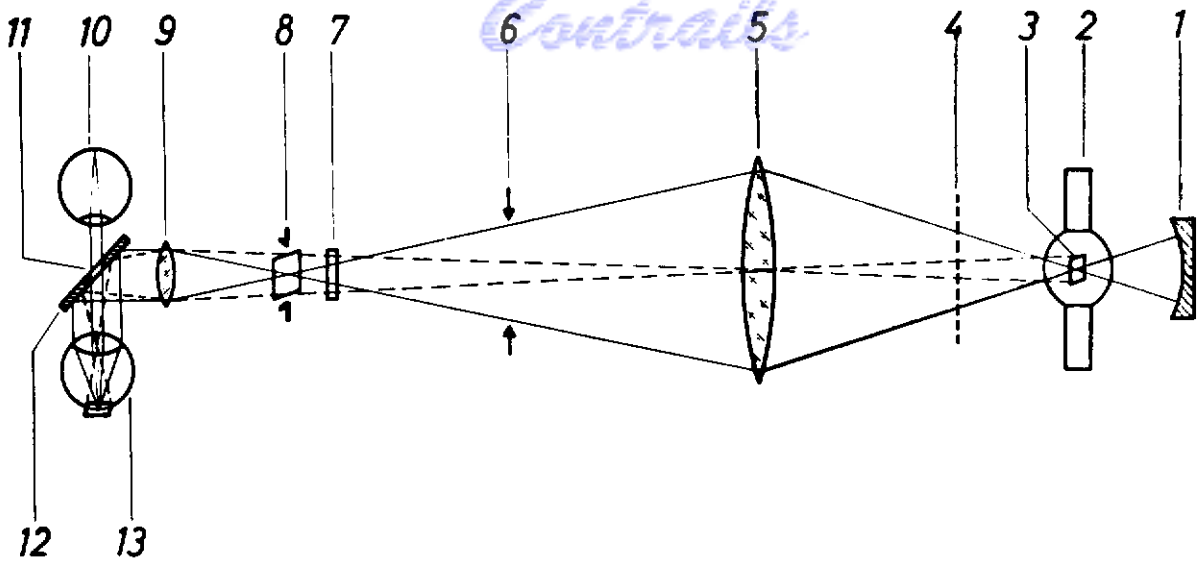
uv	-	390 mμ	14%
390	-	750 mμ	30%
750	-	infrared	56%

In order to determine the effectiveness of different spectral areas in producing changes in the tissue of the anterior segment of rabbit eyes, optical glass filters of different bandwidths, analyzed spectrographically at the Naval Material Laboratory, (NML)*, were used in appropriate combinations during irradiation.

The bandwidths of the filter combination and Xenon source is defined as the distance between the two wavelengths at which the energy is 50% of the peak value. The bandwidths used were as follows: 370-480, 480-590, 620-830, 870-1100, and 1200-1670 mμ.

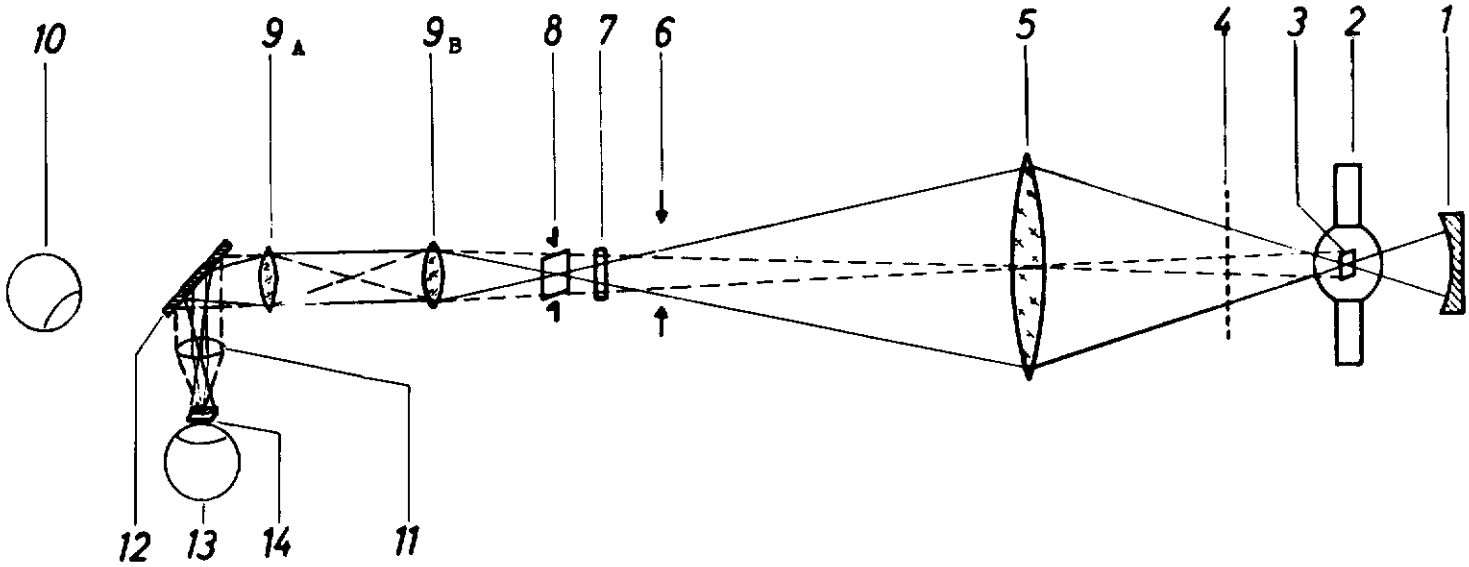
Table I presents the transmission of the individual filters. Table II gives the transmission of the specific filter combinations employed.

* (NML) - New York Naval Shipyard, Brooklyn, N. Y.



1. CONCAVE MIRROR
2. XENON LAMP
3. LUMINOUS PLASMA
4. PERFORATION DIAPHRAGM
5. CONDENSER
6. IRIS DIAPHRAGM
7. FILTER DISC
8. IMAGE FIELD DIAPHRAGM
9. OBJECTIVE
10. EYE OF PHYSICIAN
11. MIRROR APERTURES
12. EYE MIRROR
13. EYE OF PATIENT

Figure 5. SCHEMATIC OF ORIGINAL OPTICAL SYSTEM.



1. CONCAVE MIRROR
2. XENON LAMP
3. LUMINOUS PLASMA
4. PERFORATION DIAPHRAGM
5. CONDENSER
6. IRIS DIAPHRAGM
7. FILTERS
8. IMAGE FIELD
9. A & B; OBJECTIVE
10. EYE OF PHYSICIAN
11. SIMULATOR LENS
12. MIRROR
13. EYE OF RABBIT
14. CORNEA

Figure 6. SCHEMATIC OF NEW OPTICAL SYSTEM.

TABLE I

TRANSMISSION OF OPTICAL GLASS FILTERS

λ	GG13	KG3	H_2O	5-60B	Gr	2-60R	7-56IR
mμ	2 mm	1.5 mm	10 mm				
350	.30	.81	.87	.05	0	0	0
400	.71	.83	.89	.70	0	0	0
450	.87	.82	.90	.72	.02	0	0
500	.88	.84	.91	.18	.36	0	0
550	.89	.83	.91	0	.45	0	0
660	.89	.81	.91	0	.15	0	0
650	.89	.75	.91	0	.01	.85	0
700	.89	.69	.90	0	.01	.89	0
750	.89	.40	.87	0	.02	.89	0
800	.89	.24	.86	0	.02	.89	0
850	.89	.10	.85	0	.03	.89	.05
900	.89	.03	.82	0	.05	.89	.20
950	.89	0	.70	0	.08	.89	.37
1000	.89	0	(.82)	.01	.11	.85	.54
1050	.89	0	.70	.02	.15	.85	.68
1100	.89	0	(.72)	.03	.19	.85	.73
1150	.89	0	(.40)	.03	.24	.85	.78
1200	.89	0	(.26)	.04	.27	.85	.81
1250	.89	0	(.26)	.04	.34	.86	.83
1300	.89	0	.24	.06	.37	.86	.85
1350	.89	0	.05	.08	.43	.86	.86
1400	.89	0	0	.09	.46	.87	.87
1450	.89	0	0	.10	.51	.87	.88
1500	.89	0	0	.10	.54	.88	.89
1550	.89	0	0	.13	.59	.88	.89
1600	.89	0	0	.15	.61	.88	.89
1650	.89	0	0	.17	.64	.88	.89
1700	.89	0	0	.18	.66	.88	.89
1750	.89	0	0	.19	.68	.88	.89
1800	.89	0	0	.22	.70	.88	.89
1850	.89	0	0	.26	.72	.87	.89
1900	.88	0	0	.32	.74	.87	.90
1950	.88	0	0	.36	.75	.87	.89
2000	.88	0	0	.41	.76	.87	.89
2050	.88	0	0	.45	.77	.87	.89
2100	.86	0	0	.48	.78	.86	.89
2150	.87	0	0	.51	.78	.85	.89
2200	.86	0	0	.53	.78	.84	.88
2250	.86	0	0	.55	.79	.85	.88

TABLE I (continued)

λ	GG13	KG3	H_2O	5-60B	Gr	2-60R	7-56IR
mμ	2 mm	1.5 mm	10 mm				
2300	.86	0	0	.57	.80	.85	.88
2350	.85	0	0	.58	.80	.85	.88
2400	.85	0	0	.59	.80	.82	.88
2450	.85	0	0	.59	.80	.81	.87
2500	.84	0	0	.60	.80	.80	.87

GG13 - U. V. absorbing filter
KG3 - I. R. absorbing filter
H_2O - Water filter
5-60B - Blue filter
GR - Green filter
2-60R - Red filter
7-56IR - Infrared filter

( ) Average value for a 50 mμ bandwidth.

TABLE II

TRANSMISSION OF FILTER COMBINATIONS USED DURING IRRADIATION

1) 5-60 Blue + KG3

λ mμ	5-60B	KG3	Product
350	.05	.81	.041
400	.70	.83	.582
450	.72	.82	.591
500	.18	.84	.151
550	0	.83	0

2) GG + GR + KG3

mμ	GR	GG13 + KG3	Product
400	0	.59	0
450	.02	.71	.014
500	.36	.74	.266
550	.45	.74	.333
600	.15	.72	.108
650	.01	.67	.007
700	.01	.61	.006
750	.02	.36	.007
800	.02	.22	.004
850	.03	.09	.003
900	.05	.03	.002

3) GG + 2-60R + KG3

mμ	2-60R	GG13 + KG3	Product
600	0	.72	0
650	.85	.67	.569
700	.89	.61	.543
750	.89	.36	.320
800	.89	.22	.196
850	.89	.09	.080
900	.89	.03	.027

TABLE II (continued)

4) GG + 7 - 56IR + H_2O

λ mμ	7-56IR	GG13 + H_2O	Product
800	0	.77	0
850	.05	.76	.038
900	.25	.73	.182
950	.30	.62	.187
1000	.60	.73	.438
1050	.68	.62	.422
1100	.73	.64	.467
1150	.78	.36	.281
1200	.84	.23	.194
1250	.85	.23	.196
1300	.85	.21	.178
1350	.85	.04	.034
1400	.85	0	0

5) GG13 + GR + 7-56IR

λ mμ	GG13	GR	7-56IR	Product
800	.89	.02	0	0
850	.89	.02	.05	.001
900	.89	.04	.25	.009
950	.89	.06	.30	.016
1000	.89	.09	.60	.048
1050	.89	.12	.68	.073
1100	.89	.17	.73	.110
1150	.89	.22	.78	.153
1200	.89	.27	.84	.202
1250	.89	.32	.85	.242
1300	.89	.37	.86	.283
1350	.89	.42	.87	.325
1400	.89	.46	.88	.360
1450	.89	.50	.89	.395
1500	.89	.54	.89	.428
1550	.89	.57	.89	.451
1600	.89	.60	.90	.480
1650	.89	.63	.90	.505
1700	.89	.66	.90	.528
1750	.89	.68	.90	.545

TABLE II (continued)

λ mμ	GG13	GR	7-561R	Product
1800	.89	.70	.90	.560
1850	.89	.71	.90	.568
1900	.88	.73	.90	.585
1950	.88	.74	.89	.580
2000	.88	.75	.89	.587
2050	.88	.76	.89	.595
2100	.88	.78	.89	.611
2150	.87	.78	.89	.604
2200	.86	.78	.88	.590
2250	.86	.79	.88	.598
2300	.86	.80	.88	.605
2350	.85	.80	.88	.598
2400	.85	.80	.88	.598
2450	.85	.80	.87	.591
2500	.84	.80	.87	.585

V. DETERMINATION OF DOSE

Corneal irradiance was determined by means of a Naval Material Laboratory copper button calorimeter located at the corneal plane. However, to calculate the irradiance on the iris a correction factor due to absorption by the cornea and aqueous for each particular bandwidth had to be applied. Table III presents absorption values from a report by Prince. (2)

The NML calorimeter, which employs an iron-constantin thermocouple, was connected to a galvanometer recording system (Visicorder). This system provided the required sensitivity over a wide range of energies; it is essentially a black-body receiver with a response independent of wavelength. (Fig. 7).

VI. IRRADIATION OF RABBIT EYE

Chinchilla grey rabbits weighing 4-5 pounds, with brown pigmented irides, were used for all experiments. Animals were screened by slit lamp examination to eliminate undesirable specimens, since rabbit corneas frequently show punctate staining areas.

General anesthesia was obtained using pentobarbital sodium (25 mg/kg body weight), injected into the marginal ear vein. Pupils were maximally dilated with phenylephrine hydrochloride (10%) instilled in the conjunctival sac, for corneal burns. Pupils were constricted with pilocarpine (2%) for iris burns.

The anesthesized animal was positioned in front of the coagulator, lying on its side, at a distance dependent upon which tissue was to be irradiated. Excess fluid in the conjunctival sac resulting from initial examination was drained off by absorbent tissue. The corneal surface tear film was maintained by repeated closing of the eye lids. The pattern of the perforated diaphragm located in front of the lamp was focused at the level of the tissue to be burned.

A magnifying lens attached to the housing of the optical system was used to determine whether the beam was in focus on the tissue in question. (Fig. 8)

TABLE III

ABSORPTION AND TRANSMISSION OF RABBIT ANTERIOR OCULAR MEDIA*

λ mμ	Absorption Cornea	Transmission Cornea	Absorption Aqueous	Transmission Aqueous	Transmission Cornea x Aqueous
350	.38	.62	.42	.58	.36
400	.31	.69	.40	.60	.41
450	.27	.73	.37	.63	.46
500	.21	.79	.32	.68	.54
550	.27	.73	.34	.66	.48
600	.20	.80	.29	.71	.57
650	.19	.81	.27	.73	.59
700	.16	.84	.23	.77	.64
750	.16	.84	.22	.78	.65
800	.13	.87	.20	.80	.70
850	.10	.90	.19	.81	.73
900	.15	.85	.22	.78	.66
950	.14	.86	.22	.78	.67
1000	.14	.86	.22	.78	.67
1050	.12	.88	.19	.81	.71
1100	.13	.87	.19	.81	.70
1150	.10	.90	.17	.83	.75
1200	.09	.91	.15	.85	.77
1250	.10	.90	.16	.84	.76
1300	.10	.90	.17	.83	.75
1350	.12	.88	.20	.80	.70
1400	.16	.84	.27	.73	.61
1450	.33	.67	.44	.56	.38
1500	.50	.50	.58	.42	.21
1550	.53	.47	.69	.31	.15
1600	.46	.54	.68	.32	.17
1650	.31	.69	.50	.50	.35
1700	.19	.81	.32	.68	.55

*Prince, J. H., Spectral Absorption of the Retina and Choroid - 340-1700 mμ - Report RF Project 1069, Brooks Air Force Base, Texas. Mar. 1962

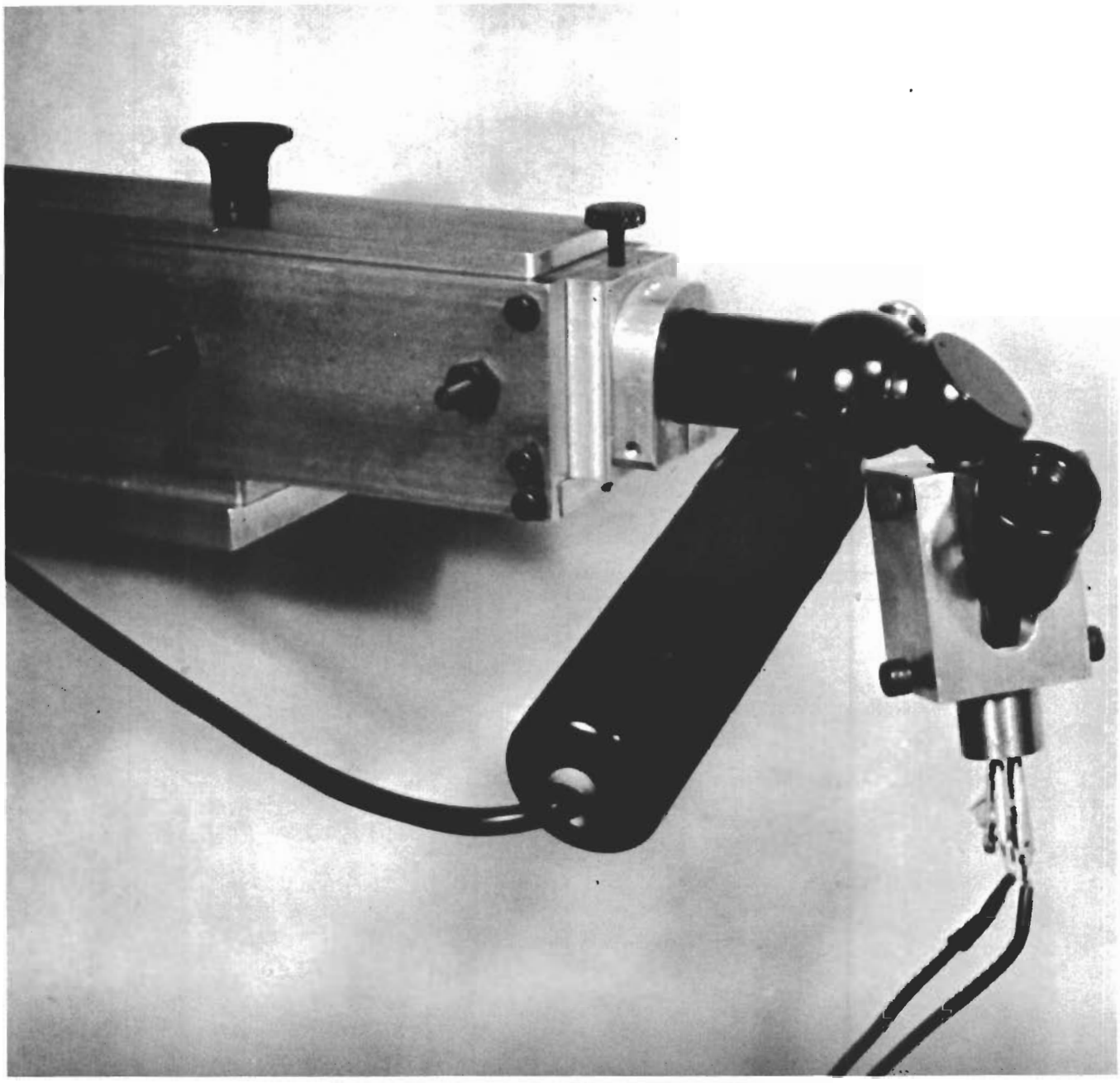


Figure 7. CALIBRATION WITH NML BUTTON CALORIMETER

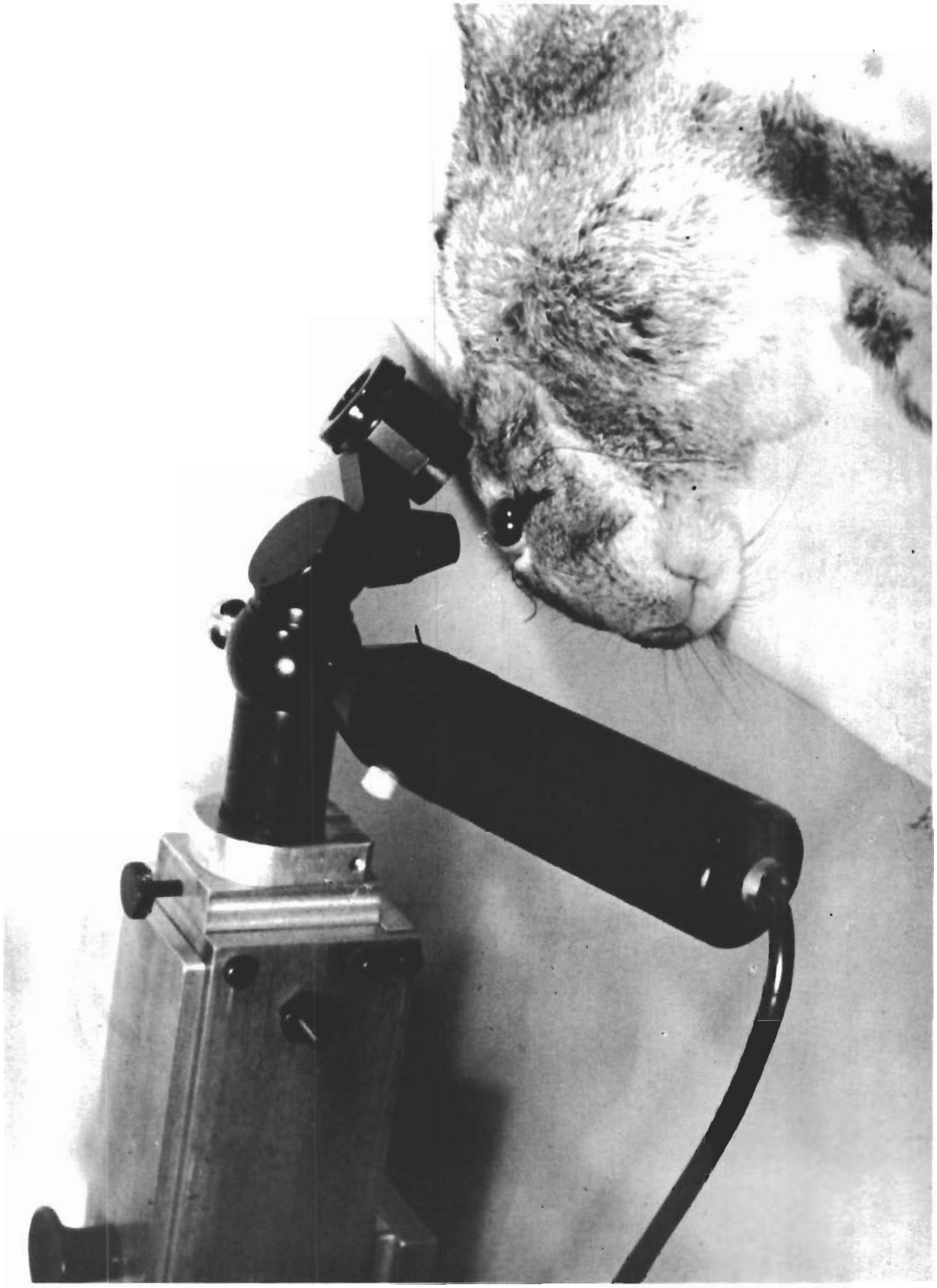


Figure 8. IRRADIATION OF RABBIT

VII. DEGREE OF DAMAGE

A. Cornea

1. Visual Evaluation of Corneal Lesions

Visible changes occurred in the cornea for all bandwidths previously mentioned. Gross changes in the cornea were determined by slit lamp examination after a few drops of fluorescein were placed in the eye. All burns were evaluated by the same observer to minimize individual variations. Table IV describes the various degrees of visible corneal damage.

TABLE IV

Criteria for Corneal Damage - Visual Evaluation

1.	S Burn:	Suspicious area
2.	-1 Burn:	Faint corneal haze (stain) noted as barely noticeable staining area on the superficial corneal epithelium. Fine granular appearance (similar to desiccation) is noted.
3.	+1 Burn:	Definite punctate or linear scratch-like staining areas on the corneal epithelium. This lesion usually disappears in 24 hours.
4.	+2 Burn:	Deep epithelial staining homogeneous, without punctate spots (deeper epithelium).
5.	+3 Burn:	Without aid of slit lamp, marked desiccation and charring of epithelium delineating area of the burn. Clinically, +2 and +3 burns are often accompanied by aqueous flare.

2. Histochemical and Histopathological Evaluation of Corneal Lesions

Histopathological and histochemical sections were prepared simultaneously, and correlation between morphologic and enzymatic evidence of damage were drawn. Lactic dehydrogenase determinations most readily indicated the presence of corneal damage. Succinic dehydrogenase studies were also performed on all eyes.

The eyes were enucleated one hour after irradiation. These were quickly frozen to -70 ^{o} C by soaking them in a beaker of iso-pentane solution immersed in a jar of acetone and dry ice. Cryostat sections of the lesion 5 microns in thickness were set aside for the various substrate use and H & E staining.

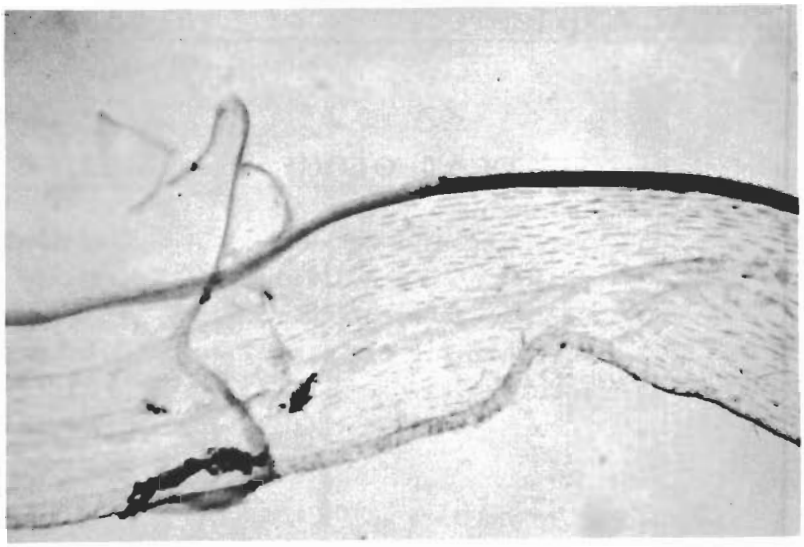
Tablve V lists the evaluation for the several lesions.

TABLE V

CRITERIA FOR CORNEAL DAMAGE -
HISTOPATHOLOGICAL AND HISTOCHEMICAL EVALUATION

VISUAL EVALUATION	HISTOPATHOLOGY	HISTOCHEMISTRY
-1 Burn	Swelling and vacuolization of the individual cells with reduction of the staining characteristics.	Lactic dehydrogenase activity diminished in all layers compared to surrounding normal areas. Some blue granular precipitation noticeable in the stromal cells.
+1 Burn	Epithelial layer shows marked disruption of architectural pattern. In some areas, individual cell differentiation is difficult to discern due to confluent necrosis. Bowman's membrane and stromal elements do not show any marked changes.	Aside from the architectural changes there is marked to complete absence of reductase activity of the epithelial and stromal layers.
+2 Burn	Denudation of entire epithelial layer.	Denudation of entire epithelial layer.

a)



b)

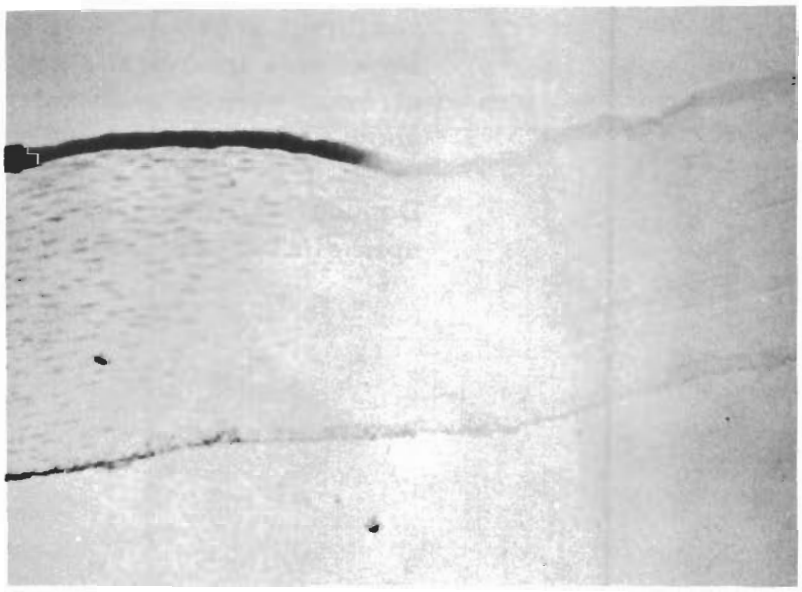


Figure 9. PHOTOGRAPHS OF HISTOCHEMICAL SLIDES OF CORNEAL LESIONS.
a) - 1 Burn b) +1 Burn

3. Visual Threshold Dose

Since the exposure time was kept constant at 150 msec. the lamp power was varied to determine the threshold in each case.

Immediate and 1 hour post-exposure examinations of 350 rabbit corneas exposed to threshold doses have revealed abrasions and/or desiccation of approximately 110 previously screened and normal corneas. However, a 24 hour examination failed to confirm these findings. A probable explanation for this phenomenon is that in seeking minimal corneal damage and thus delivering low doses, we damaged only the corneal epithelium which regenerates in a relatively short period of time. Thus, it should be noted that the criteria for a visual threshold dose are based on epithelial damage and anatomical changes observed within one hour.

Table VI presents the data obtained on corneal burns for the several bandwidths.

TABLE VI

CORNEAL DAMAGE AS A FUNCTION OF DOSE OF DIFFERENT SPECTRAL CHARACTERISTICS

Dose Range cal/cm²	Xenon+5-40BL+KG3 370-490 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²	Xenon+OG13+GR+KG3 490-590 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²	Xenon+OG13+2-60R+KG3 620-900 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²	Xenon+OG13+7-56IR+H₂O 870-1100 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²	Xenon+7-56IR 800-1120 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²	Xenon+OG13+GR+7-56IR 1200-1670 mμ* Lesion Visual 1 2	Lesion cal/cm²	No Lesion cal/cm²
0.30 - 0.39										-1 -1	.39	.39						
0.40 - 0.49				S S S S S S	.47 .49 .42	.48 .49 .45 .42 .44				-1 -1	.48	.44 .47 .49 .47 .48 .44 .41				-1 -1	.46	.41 .49
0.50 - 0.59			.56	-1 -1 -1 -1 -1 -1 S -1 -1 -1	.58 .59 .51 .52 .56	.53 .54 .57 .56 .53				S S S S S 9	.59 .59 .53	.58 .56				-1 -1 S S	.57 .56	.53 .52 .56 .56 .58 .51
0.60 - 0.69	S -1 1 S N -1 N S	.63 .68 .67 .60	.69 .66 .67 .68 .69 .69 .69 .69 .68 .60	-1 -1 -1 -1 -1 S	.62 .68 .67	.60 .62 .64 .62 .65 .61 .61 .68 .66 .69 .65 .62	-1 -1 S -1	.69 .67	.62 .65 .66 .60 .63 .63	-1 -1	.62	.61 .67 .65				-1 D -1 -1 S S S S -1 -1 S S	.61 .63 .62 .63 .60 .60	.62 .65 .63 .66 .63 .60 .60
0.70 - 0.79	S S S S -1 -1 S S S S -1 S S S -1 -1 S K	.77 .79 .75 .76 .74 .77 .78 .75 .75	.79 .75 .77 .78 .72	+1 +1 S S -1 S -1 S S S -1 S S S	.77 .73 .78 .74 .78 .77 .78	.72 .73 .74 .74 .76 .74 .70 .70	-1 -1 -1 -1 N -1 N S	.77 .78 .72 .71	.76 .78 .70	1 1 -1 -1	.71 .78	.70 .72 .73				-1 -1	.73	.73
0.80 - 0.89	-1 -1 -1 S +1 -1 -1 -1 -1 S	.87 .82 .89 .82 .81	.89 .86 .83	-1 -1 -1 -1 -1 S S S N 1	.82 .86 .87 .89 .89	.80 .89	-1 -1 -1 -1 -1 -1 N -1 -1 -1 -1 -1	.88 .89 .83 .86 .89 .82	.80 .86 .83 .85 .89 .83 .85 .82 .89 .83 .82 .86	-1 -1	.88	.80						
0.90 - 0.99	S S -1 -1 -1 S -1 -1 -1 -1 -1 -1 -1 S S S	.90 .90 .98 .96 .97 .95 .91 .93	.95 .94 .96				-1 -1 -1 -1 -1 -1	.96 .91 .94	.97 .99 .97 .92						.95 .90 .94			
1.9 - 1.09							-1 S -1 -1	1.09 1.05	1.05 1.0>1 1.02				-1-1	1.07				
1.10 - 1.19	-1 -1 -1 S	1.11 1.17	1.18				S S	1.13	1.10 1.16 1.12 1.15 1.14 1.15 1.12 1.19						1.12 1.16 1.10			
1.20 - 1.29	-1 -1 -1 K -1 K	1.23 1.29 1.29					-1 S -1 S S K -1 -1	1.28 1.24 1.29 1.26	1.20 1.21 1.27 1.22									
1.30 - 1.39							-1 -1 -1 -1 -1 K -1 -1 S S	1.30 1.35 1.39 1.34 1.39	1.30									
1.80 - 1.89													-1 -1 N -1	1.86 1.82	1.86 1.84 1.89			
1.90 - 1.99													-1 -1	1.90				
2.00 - 2.09													-1 S -1 -1 -1 S	2.07 2.05 2.05	2.0			
2.20 - 2.29													-1 -1	2.27				

* Effective Bandwidth

B. Iris

1. Visual Evaluation of Iris Lesions

With respect to the iris, it was possible, upon increasing the output from the Xenon lamp, to produce irreversible damage with radiation of the three visible regions and the total infrared extending from 825-2500 mu. Owing to corneal and aqueous absorption there was insufficient energy in the small infrared bandwidths to effect visible damage on the iris. Table VII describes the iris damage observed by gross visual examination.

TABLE VII

Criteria for Iris Damage - Visual Evaluation

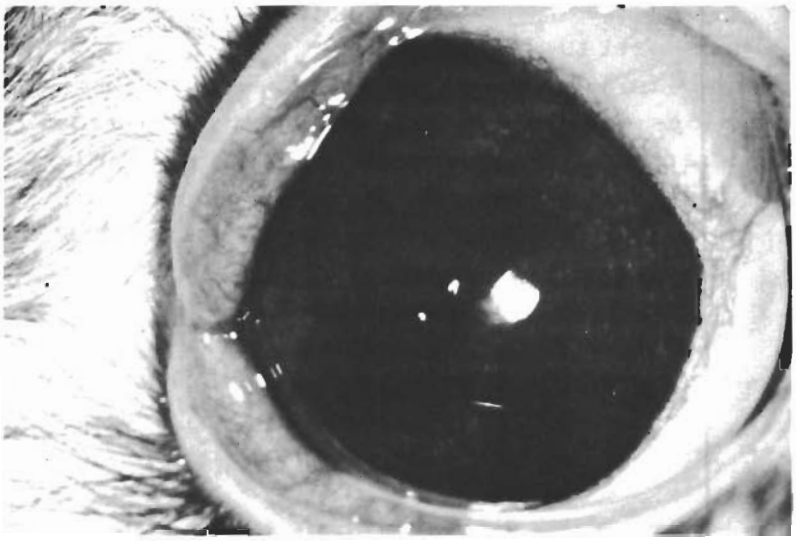
1.	S Burn:	Suspicious Area
2.	-1 Burn:	Barely visible, poorly delineated area of swelling and loss of luster of the iris surface. This is noted from 10 to 30 minutes after burning. Pupil usually becomes irregular and aqueous flare may appear.
3.	+1Burn:	Puckering of the edges of the burn with very minimal condensation of stroma, with pigmentary changes at the periphery. Aqueous flare and lens changes in area underlying burn.
4.	+2 Burn:	Well defined lesion with marked condensation of the stroma, leaving only pigment epithelium. Marked aqueous flare and lens changes underlying lesion.

2. Histochemical and Histopathological Evaluation of Iris Lesions

In conjunction with the visual evaluation of iris lesions, histochemical and histopathological studies were undertaken. Eyes were enucleated one hour after irradiation. In a manner similar to the corneal studies, these eyes were quickly frozen to -70 ^{o}C by soaking them in a beaker of isopentane solution immersed in a jar of acetone and dry ice. The enzyme succinic dehydrogenase was used for all iris lesions.

Table VIII describes our classification of iris lesions

a)



b)

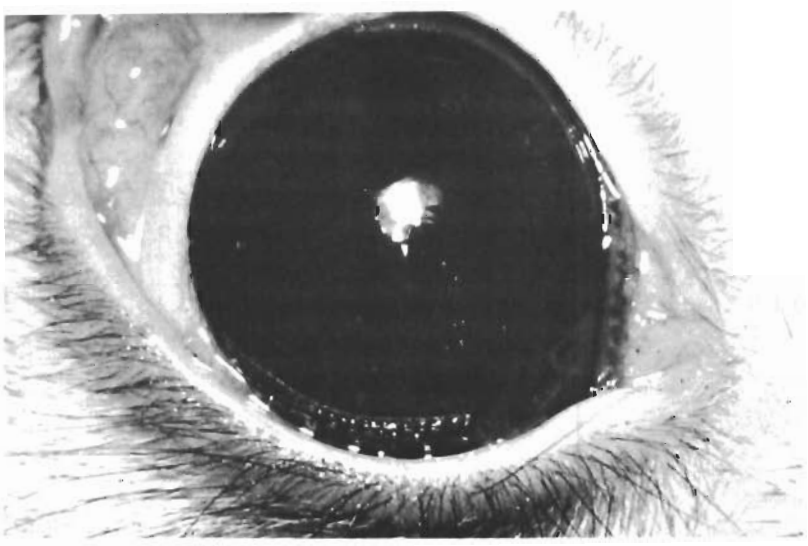


Figure 10. PHOTOGRAPHS OF IRIS LESIONS

TABLE VIII

CRITERIA FOR IRIS DAMAGE- HISTOPATHOLOGICAL AND HISTOCHEMICAL EVALUATION

VISUAL EVALUATION	HISTOPATHOLOGY	HISTOCHEMISTRY
S Burn	Congestion and engorgement of iris vessel. Slight stromal edema. Post pigment epithelium undisturbed.	Moderate diminution of formosan precipitate on the ciliary epithelium.
-1 Burn	Marked engorgement of stromal vessels with some extrovasation of red blood cells. Moderate stromal edema and some occasional round cells. Post pigment epithelium usually remains but occasionally some pigment migration anteriorly may be noted.	Diminished formosan precipitation noted on the ciliary epithelium of iris processes. Stromal elements not noticeably affected.
+1 Burn	Moderate disruption of the anterior border layer with moderate migration of pigment clumps anteriorly. There is marked stromal edema with vacuolization and some necrosis. Stromal vessels are engorged and show Peri vascular round cell infiltration. Post pigment epithelium shows moderate condensation and areas of necrosis.	Reductase activity completely inhibited in the stromal elements and ciliary epithelium.

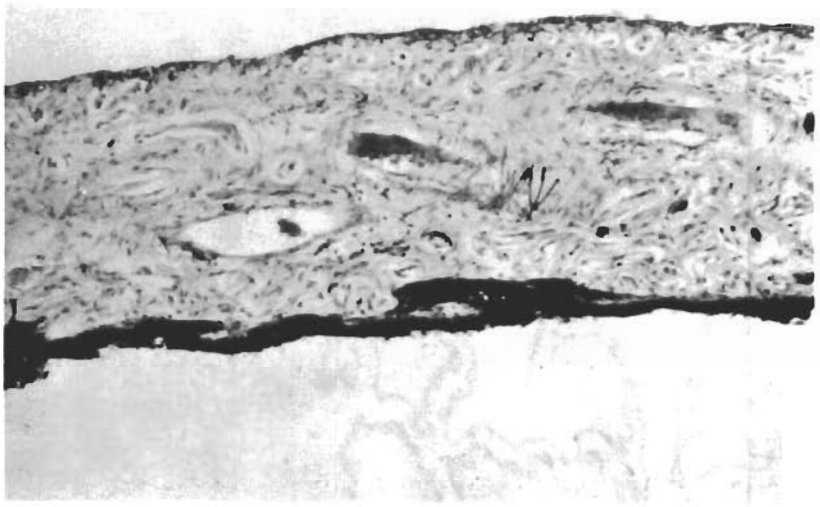
(continued)

TABLE VIII (continued)

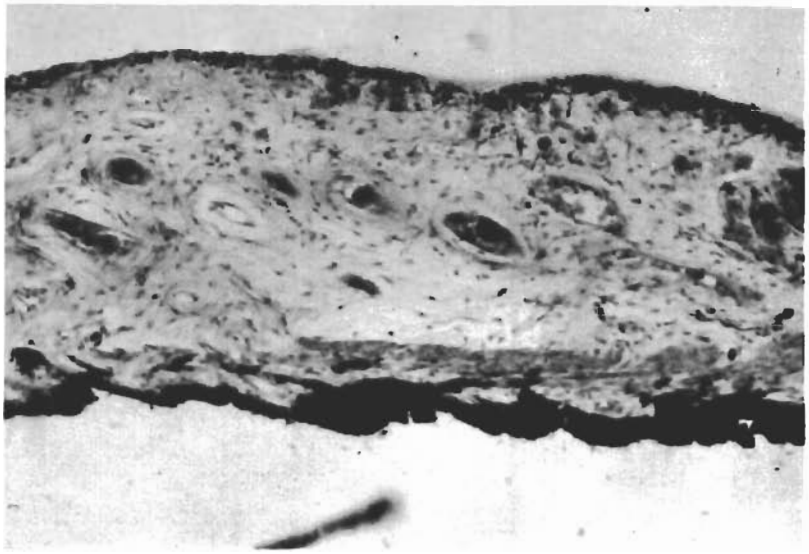
CRITERIA FOR IRIS DAMAGE -
HISTOPATHOLOGICAL AND HISTOCHEMICAL EVALUATION

VISUAL EVALUATION	HISTOPATHOLOGY	HISTOCHEMISTRY
+2 Burn	Marked condensation and shrinkage of anterior stromal layers with gradual sloping demarcation from surrounding areas. Marked distortion of architecture of the remaining posterior stroma and posterior pigment epithelium is evident. Posterior pigment epithelium usually shows necrotic changes.	Reductase activity completely inhibited in the stromal elements and ciliary epithelium.

a)



b)



c)

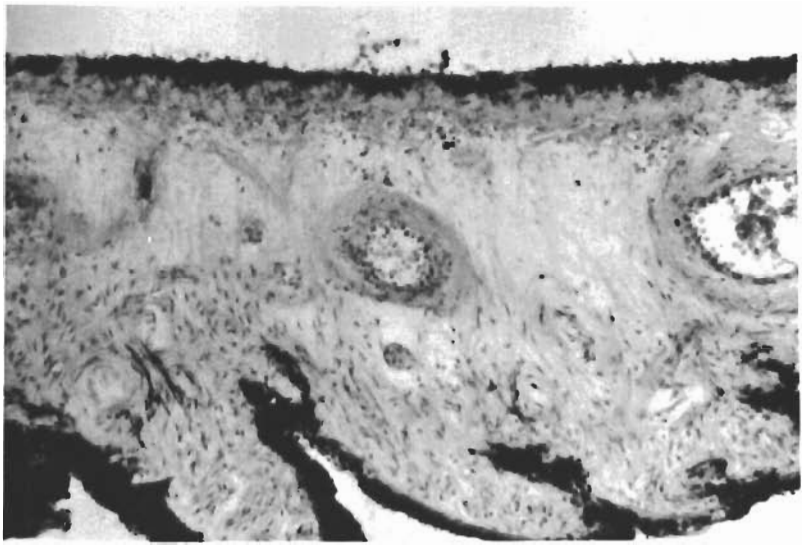
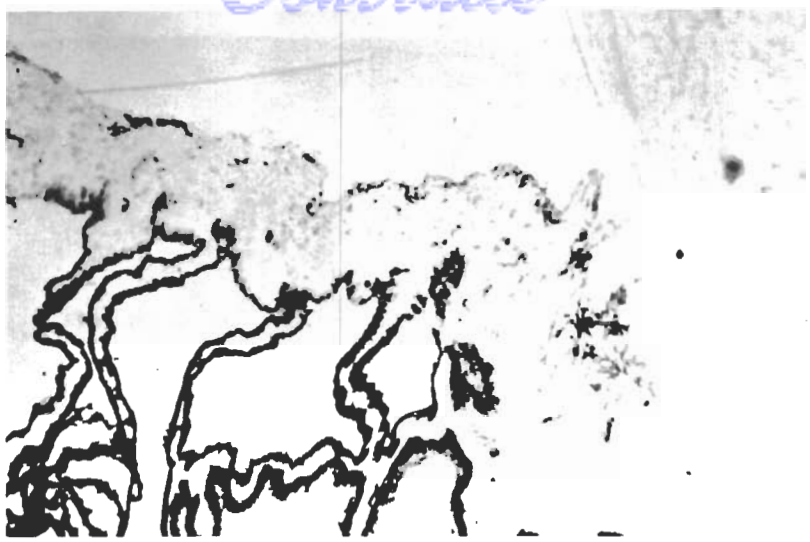
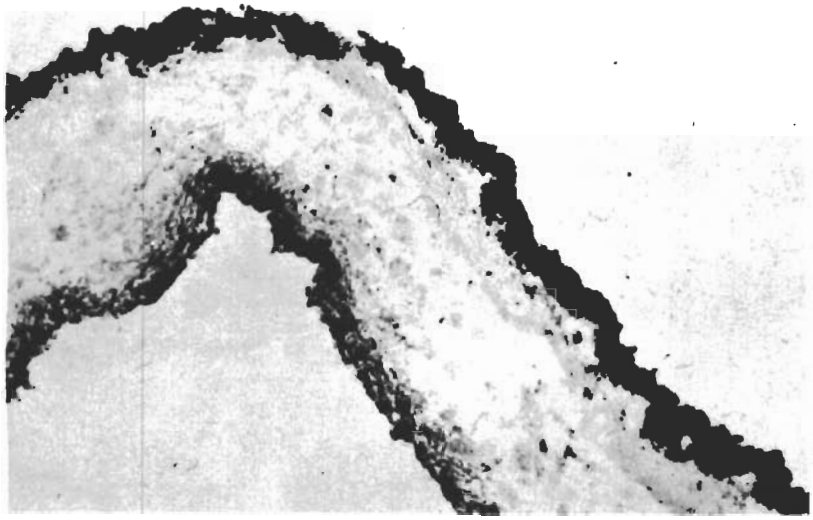


Figure 11. PHOTOGRAPHS OF HISTOPATHOLOGICAL SLIDES OF IRIS LESIONS.
a) S Burn b) -1 Burn c) + 1 Burn

a)



b)



c)

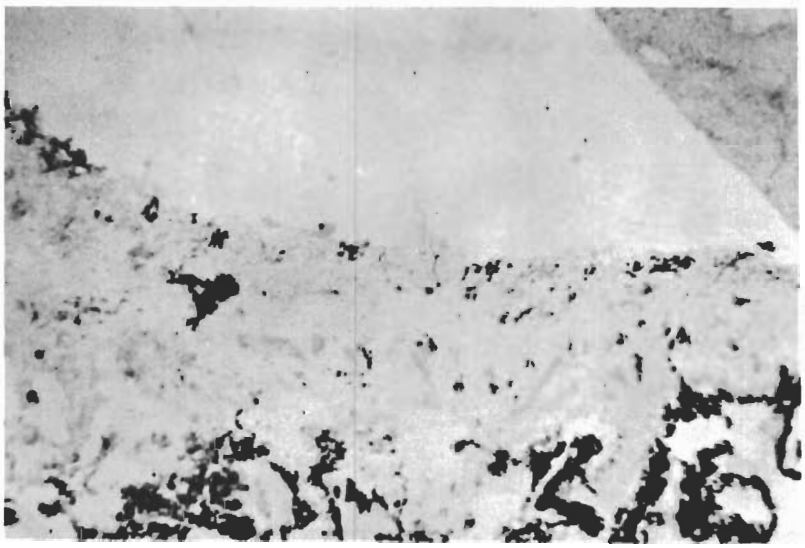


Figure 12. PHOTOGRAPHS OF HISTOCHEMICAL SLIDES OF IRIS LESIONS.
a) S Burn b) -1 Burn c) + 1 Burn

3. Visual Threshold Dose

The data obtained from the investigation of the threshold dose as determined by slit lamp examination are presented in Table IX.

As stated previously, the corneal irradiance was determined by means of an NML button calorimeter located at the corneal plane. However, to calculate the irradiance on the iris a factor [2] due to the absorption by the cornea and aqueous for a particular bandwidth had to be applied. [2]

Table IX presents the data obtained for iris burns with the several bandwidths. Visual evaluation was immediate, 5 minutes and 1 hour post irradiation.

TABLE IX

IRIS DAMAGE AS A FUNCTION OF DOSE OF DIFFERENT SPECTRAL CHARACTERISTICS

Uncorrected Dose Range cal/cm²	Xenon+5-60B+KG3 370-480 mμ			Xenon+GG13+CR-KG1 440-590 mμ			Xenon+GG13+CR-60B+KG1 620-810 mμ			Xenon+7-54IR 880-1120 mμ		
	Lesion Visual 1 2 3	Lesion cal/cm²	No Lesion cal/cm²	Lesion Visual 1 2 3	Lesion cal/cm²	No Lesion cal/cm²	Lesion Visual 1 2 3	Lesion cal/cm²	No Lesion cal/cm²	Lesion Visual 1 2 3	Lesion cal/cm²	No Lesion cal/cm²
0.10 - 0.19	N+1+1	.15	.15 .15 .16 .19 .17 .16 .16 .14 .13 .14 .11						.19 .19			
0.20 - 0.29			.22 .27 .27 .29						.20 .29 .23 .28			
0.30 - 0.39	S S S S -1 S	.39 .35 .34 .33	.33 .31 .38 .39 .35 .33 .36			.36 .39 .32 .32 .37 .38 .32 .37 .38			.30 .31 .32			
0.40 - 0.49			43			.47 .48 .47 .45						
0.50 - 0.59			.51 .52 .50 .54 .51 .51 .50 .51 .52 .54 .54 .58 .56 .56 .52 .53	S N S	.54	.58 .56 .51 .55 .55 .55 .52 .57 .59	S	.56				
0.60 - 0.69	S S	.67	.60 .65 .65 .66 .66 .62 .67	-1-1-1 -1	.69 .65	.60 .67 .62 .63 .68 .62 .61 .6 .69 .67 .68	N-1 S -1-1-1 -1-1-1 -1	.65 .65 .61 .68	.60 .60 .68 .63 .68 .67 .62 .61 .62 .68 .69 .64			
0.70 - 0.79	-1 -1-1 S -1	.70 .76 .75	.70 .73 .76 .75 .71 .79	S N S -1 -1	.70 .77 .78	.79 .74 .73 .71 .74 .79 .71 .73 .74 .74 .79 .76 .74	N S S -1-1-1 S S S S S-1 -1-1 S	.77 .73 .76 .79 .73	.75 .76 .76 .78 .72 .76 .78 .76 .73 .73			
0.80 - 0.89	N-1-1 -1-1-1 S S-1 S N S -1	.81 .84 .81 .84 .84	.84 .88 .85 .83 .86 .82 .87 .82 .86 .89	-1-1-1 -1 -1 -1 S S S S S S -1-1-1 S-1-1 -1 -1 S	.80 .80 .80 .80 .87 .84 .87 .81 .87 .88 .85	.87 .83 .87 .82 .83 .85 .82	-1-1-1 S S-1 -1-1-1 +1+1+1 S S-1 S-1 S -1 S S N S-1	.80 .80 .80 .84 .82 .86 .84 .83	.80 .80 .87 .83 .83 .85 .87 .86			
0.90 - 0.99	N N S S-1 S S S -1	.99 .96 .92	.99 .96 .93	-1 -1 -1 -1-1-1 -1-1-1 -1-1 S -1 +1	.95 .91 .96 .98 .91 .94 .94 .97	.90 .90 .98 .98 .93 .95 .92 .99 .92	-1-1-1 -1-1-1 S N-1 -1	.94 .91 .97 .96	.94 .93			

TABLE IX (continued)

IRIS DAMAGE AS A FUNCTION OF DOSE OF DIFFERENT SPECTRAL CHARACTERISTICS

Uncorrected Dose Range cal/cm^2	Lesion Visual 1 2 3	Lesion cal/cm^2	No Lesion cal/cm^2	Lesion Visual 1 2 3	Lesion cal/cm^2	No Lesion cal/cm^2	Lesion Visual 1 2 3	Lesion cal/cm^2	No Lesion cal/cm^2	Lesion Visual 1 2 3	Lesion cal/cm^2	No Lesion cal/cm^2
1.00 - 1.09	S S-1 S S-1	1.08 1.09		-1-1-1 -1 S S -1 -1 -1-1-1 -1-1-1 S	1.00 1.00 1.01 1.05 1.05 1.04 1.06 1.02	1.06 1.02 1.05	S S S N N S	1.07 1.09	1.03 1.01 1.03			
1.10 - 1.19	-1-1-1 -1-1	1.10 1.18	1.19	S S -1-1-1	1.16 1.12	1.19 1.19	-1-1 -1-1-1 -1	1.16 1.10 1.10	1.10 1.16			
1.20 - 1.29	-1-1-1 -1-1-1 +1+1+1 -1-1-1	1.22 1.21 1.29 1.22		-1		1.22						
1.50 - 1.59												1.50 1.59
1.60 - 1.69												1.63 1.67 1.62 1.63
1.70 - 1.79												1.78
1.80 - 1.89												1.87 1.83 1.86 1.88 1.81 1.82
1.90 - 1.99												1.95 1.92 1.95 1.92 1.95
2.00 - 2.09										S S	2.08	2.00 2.00 2.08 2.05 2.02 2.05 2.09
2.10 - 2.19										-1	2.10	2.14 2.19 2.18 2.10 2.10
2.20 - 2.29										-1	2.21	2.20 2.20 2.27 2.23 2.26
2.30 - 2.39												2.34 2.32 2.32 2.32 2.37
2.40 - 2.49										-1-1 S S	2.44 2.46	2.48 2.44 2.42 2.47 2.46
2.50 - 2.59										S S	2.59	2.52 2.59 2.52 2.52 2.56 2.57 2.50
2.60 - 2.69												2.64
2.70 - 2.79										N S-1	2.74	
2.80 - 2.89										-1	2.84	2.88 2.88
2.90 - 2.99										N-1-1	2.92	2.94

C. Lens

Producing lens damage has proved to be more difficult and lengthy than corneal and iris damage. Of prime importance in the production of lens damage is the location of the exposure relative to the iris. We have observed immediate lens damage behind the iris. However, as previously reported, the lenses of rabbits have been irradiated in the pupillary area with no immediately visible damage. To determine whether damage occurs at some later date, we undertook the following study:

a) irradiation with both visible and infrared energy - 60 cal/cm^2 /sec.

b) irradiation with visible energy - 30 cal/cm^2 /sec.

c) irradiation with visible energy - 15 cal/cm^2 /sec.

d) irradiation with infrared energy - 15 cal/cm^2 /sec.

For each of the four conditions, six rabbits were irradiated and examined after 30, 60, and 90 days.

Table X presents data on lens damage. The -1 lens lesion represents a faint posterior subcapsular opacification which appeared within 30 days and which disappeared in 60 - 90 days.

TABLE X

LENS DAMAGE

	Uncorrected Dose -cal/cm ^{2}	Initial Evaluation	2nd Period 30 days	3rd Period 60 Days	4th Period 90 Days
1. No Filtration					
	9.21	N	-1	-1	N
	8.65	N	-1	-1	N
	9.33	N	-1	-1	N
	9.08	N	-1	-1	N
	10.05	N	-1	-1	K
2. 7-56 (IR) (850 mu - IR)					
	1.88	N	N	N	N
	1.77	N	N	N	N
	1.82	N	-1	-1	-1
	1.84	N	-1	-1	N
3. GG+KG (Visible) (370-900 mu)					
	2.40	N	-1	-1	N
	2.48	N	-1	-1	N
	2.48	N	-1	-1	N
	4.59	N	-1	-1	N
	2.48	N	-1	-1	-1
	4.26	N	-1	-1	N
	4.21	N	-1	-1	N
	2.14	N	-1	-1	N
	4.17	N	-1	-1	K

N - No visible lesion

K - Animal sacrificed

D. Aqueous

There remains investigation of the aqueous flare - a condition probably caused by some insult to the iris. Although we have been able to produce the condition upon irradiating the iris, there is as yet no correlation between dose and degree of flare.

VII. THERMODYNAMIC CONSIDERATIONS

To determine the characteristics of the heat distribution, thermocouples, 38 gauge, were placed at various depths in the anterior ocular tissues. Histological examination and histochemical analysis of the ocular tissues irradiated were performed to determine changes.

A. Surgical Technique

Chinchilla grey rabbits weighing 4-5 pounds were used for all experiments. 150-200 mg. pentobarbital was administered parenterally for effecting anesthesia. Homatropine (2% solution) was instilled in the eyes one hour prior to the operation. A retrobulbar injection of 1-2 ml. of 2% procaine solution was given for immobilization of the eye. A complete peritomy was performed. Occasionally, tenotomy of a muscle was performed to facilitate exposure of the eye.

The base intra-ocular pressure was obtained with the aid of a Schiotz Tonometer. Guide needles, 31 gauge, were inserted at the depths of interest of the anterior ocular tissues. One ml. of heparin was injected slowly into an ear vein.

Thermocouples were threaded through the guide needles with care, and the needles withdrawn. Any extensive manipulation of the anterior chamber, whether intra or extra-ocularly, initiated a transformation of the normal aqueous to that of a plasmoid type, such that fibrin deposition or even complete coagulation of the aqueous occurred.

Since the pre-inserted needles are of a larger diameter than the thermocouple wires, the tracts created by the former are too big for the smaller diameter thermocouple wire to plug, thereby permitting escape of aqueous. In earlier experiments, it was found that the change of the aqueous to a plasmoid type helped the plugging of the tracts left by the needles. However, the deposition of fibrin on the thermocouple and on the anterior of chamber interfered with the proper transmission of energy to the thermocouples and surrounding tissue, as plasmoid aqueous is of denser consistency than normal aqueous.

Heparin (1 cc) injected intravenously immediately after inserting the guide needles dissolved the precipitated fibrin one hour after administration. We also found the intra-ocular pressure returned to the original base pressure within this time.

Once the thermocouples were in place, they were made taut in the eye, thereby approximating the actual position of the guide needles. The outside extensions of the thermocouples were fixed and properly labelled. The rabbit was then immobilized in a carriage and further anesthesized if necessary.

B. Technique for Implantation of Thermocouples

1. Triangulation Method

An instrument was devised, whereby the approximate depth of the pre-inserted guide needles in relation to the anterior surface of the cornea can be measured. This consists of two calibrated copper rods attached to a 26-gauge syringe needle, forming an isosocles triangle. The protruding end of the preplaced guide needle was threaded through the syringe needle and a perpendicular (copper rod) was dropped down to the anterior surface of the cornea. Direct measurements were read from the calibrated rods. Knowing the exact angles formed by the triangle, the anterior chamber depth was calculated by using the formulae:

$$a)\ \sin\theta = \frac{\text{opposite side}}{\text{hypotenuse}}$$

or

$$b)\ \cos\theta = \frac{\text{adjacent side}}{\text{hypotenuse}}$$

2. X-Ray Method

With the thermocouples in place, an x-ray of the anterior ocular tissues was taken at a distance of six feet. This distance was necessary in reducing to a negligible amount the usual enlargement of the image print on the film, when taken at closer distances. Direct measurement of the thermocouple depth from the anterior surface of the cornea could be accomplished.

C. Thermal Burns

Thermocouple leads were connected to separate galvanometers on the Honeywell recorder. Readings and interpretation of the deflections were recorded as a function of spectral quality, irradiance, exposure time and beam size. These parameters were similar to conditions for determining pathological effects due to the thermal energy.

Rabbits were studied by this technique but insufficient experimental time did not permit acquisition of statistically valid data.

VIII. DISCUSSION OF RESULTS

This report covers the first 12 months of a projected 24-month project to study the effects of thermal energy delivered to *in vivo* eyes upon anterior ocular tissue.

It must be emphasized that all data must be interpreted with the fact in mind that this is an interim report, based upon a minimal number of animals.

Although more data are essential, evidence as to the dose required to produce minimal corneal changes as a function of spectral bandwidth have been obtained and the data are presented in Table VI and summarized in Table XI. Results indicate that the region from 620 - 1100 mu is less effective than the regions from 370 - 590 and 1200 - 1670 mu in producing corneal lesions. Data on corneal absorption taken from other studies [2] indicate that results are in good agreement with the concept of damages vs. absorbed dose. It is to be noted that this visual threshold dose is based on epithelial damage and anatomical changes observed within one hour and regenerating within 24 to 48 hours.

Insufficient time precludes further irradiation of animals with sufficient energy to effect changes in the deeper layers of the cornea (stroma, Descemet's membrane and endothelium).

Studies so far have indicated that the corrected iris dose (corneal dose x transmission factor of cornea and aqueous) necessary to produce minimal iris lesions increases with wavelength. This phenomenon is in keeping with the decrease in absorption of energy by the iris. The LD50 iris dose of approximately .3- .5 cal/cm^2 of visible energy has produced a minimal lesion which is irreversible. The data are presented in Tables IX and XII.

Table X presents information acquired in connection with irradiation of lenses. No conclusions can be drawn from these data other than that the damage was reversible and further studies are necessary.

TABLE XI

LD50 cal/cm^2 - VISUAL THRESHOLD CORNEAL DOSE

Filters	Transmission of Filters mμ	Effective Wavelength mμ	Dose cal/cm^2
5-60B + KG3	350-530	370-480	.70
GG13 + GR + KG3	450-650	480-590	.75
GG13 + 2 - 60R + KG3	620-900	620-830	1.20
GG13 + 7 - 56IR + H_2O	850-1350	870-1100	
7-56IR	850-IR	880-1120	≈1.9
GG13 + GR + 7 - 56IR	850-IR	1200-1670	.70

TABLE XII

LD 50 cal/cm^2 - VISUAL THRESHOLD IRIS DOSE

Filter	Effective Wavelength mu	Corneal Dose cal/cm^2	Transmission* Cornea and Aqueous	Corrected Iris Dose cal/cm^2
5-60B + KG3	370-480	.95	.41	.39
GG13 + GR + KG3	480-590	.78	.53	.41
GG13 + 2-60R + KG3	620-830	.80	.66	.51
7 - 56IR	880-1120	≈2.7	.47	1.17

* Data taken from curves in report
Institute for Research in Vision, Ohio State University
Jack H. Prince - Spectral Absorption of the Retina and Choroid (340-1700 mu)
Report RF Project 1069 - Contract AF 41 (657) - 306 - Brooks Air Force Base, Texas, Mar. 1962

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