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RADIATION AND DEGRANULATION STUDIES ON UVEAL MAST CELLS

James Price
(Ph. D. Thesis)
DONNER LABORATORY

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INDEX

ABSTRACT

Origin and function of the tissue mast cell remain unsolved problems. In part this has been due to the problems found in attempting to establish adequate counting methodology. Tissue mast cells have usually been counted with the assumption that a Poisson distribution would fit their arrangement in tissue, and yet, an evident correlation exists with such tissues as blood vessels and nerves that do not have this distribution. Free peritoneal mast cells can be quantitated more accurately, but their existance outside tissue presents a classification problem.

Based on some prior evidence and for the above reasons, the choroid of the albino rabbit was studied to learn whether a mast cell density gradient could be established. Several unique anatomical features exist in the rabbit eye.

A density gradient that could be fitted to a simple exponential equation and that had several regions useful for statistical analysis, was demonstrated. Furthermore, it was reproducible and had a counting variance which was adequately constant for biological data.

From birth to maturity, a similar gradient was present. A weight range (age) from just before maturity was selected as a control group for the assay of several agents (radiation and adrenocorticosteroids) that are thought to affect mast cells.

Both radiation (gamma) and cortisone acetate were found to reduce the number of uveal mast cells by a statistically significant level, but they did not eliminate them even at unusually high dose levels.

I. Quantitation of Uveal Mast Cells and Their Relation to Growth

A. Introduction

Flat tissue preparations, standard histological sections, and peritoneal washings for free cells have been used by various investigators in an attempt to determine if the effect of various agents can alter this cell population. Though obscure in function and origin, they contain potent pharmacologic agents and it is not difficult to ascribe various roles to them (Michels, 1938; Selye, 1965; Riley, 1959; Asboe-Hansen, 1954).

Without an adequate method of separating this cell from circulating basophils, a stable compartment has been assumed for counting purposes. Anatomical variations are recognized, but no one has been able to define a population density for sampling. This paper, in part, will describe a unique density gradient that exists in the rabbit's choroid (uvea).

Mast cells are found in connective tissue concentrated along structures which form a tissue interface. These are principally blood vessels and nerve fibers (Riley, 1953, 1961). Therefore, their density in any sample is dependent on the local distribution of these structures. A smaller number of cells are found at a distance from them. Anatomic landmarks may be used in the hope that samples taken from approximate areas will contain the same number of cells (Selye, 1965).

Standard histological sections are subject to additional error introduced from volume changes such as fixational shrinkage or expansion, tissue edema, loss of other cellular components such as lymph cells from radiation or fat cells from starvation, and irregular cell diameters (Padawer, 1963; Liebow and co-workers, 1949). Some correction can be made using the formula (Erankö and Kohlberg, 1955):

$$N = \frac{an}{a + 2r - 2k}$$

where N = number/mm^3

a = section thickness

r = radius of particles

k = vertical height of the smallest fragment observed

n = counted number of particles/mm^3 tissue.

Considerable error is introduced when the cell radius is much greater than the section thickness or when the section thickness is irregular. Hellström and Holmgren (1950) achieved some correction by using thick sections and counting only those cells present in every fourth section. Variability in sectioning, secondary volume changes, and anatomic variation still were not corrected. In fact, their samples had a 400% numerical difference between the largest and smallest number. Tissue markers, when present, can be used for establishing a correction factor for volume changes but dimensional change does not always occur equally along three axes (Padawer, 1963; Valtonen, 1961). The use of free peritoneal mast cells for quantitation appears to be a more exact methodology (Padawer, 1963). The principal assumptions are that these cells are the same as those present in tissue and that extra-compartmental exchange is relatively slow. Questions concerning the above are presently unanswerable, but there is some evidence that exchange may be slow.

Absolute counts are necessary (Seeley and coworkers, 1937; Padawer, 1963) because only a small ascitic-fluid volume exists and any increase or decrease in this volume can cause significant errors if only counts per unit volume are determined. Some authors such as Ito (1957) have not been aware of this. A large volume of wash fluid in comparison to the peritoneal fluid will negate this error.

Flat preparations have several advantages. They are only subject to two dimensional axis changes and the cell population exists in the tissue. Usually, samplings are taken from subcutaneous connective tissue, hamster cheek pouch, and the peritoneum (Bates, 1935; Hellström and Holmgren, 1950; Smith and Lewis, 1955b; and others). Wide anatomical variation is present even when similar sites are sampled.

The eye has several tissues that can easily be processed for flat preparations. Only the uvea contains an adequate number of mast cells for study. Among the mammals, the rabbit has a unique vascular supply with the choroidal blood supply entering along a horizontal raphe and extending toward the periphery in a linear fashion (Prince, 1964; Prince and coworkers, 1960). Albino animals offer us the potential for observing these cells without their being obscured by pigment. Retinal vessels, extending only as two small horizontal twigs over the myelinated retinal fibers, are readily observed landmarks and aid the dissection.

Smelser and Silver (1963) reported the high choroidal concentration of these cells and also noted that their numbers were greater toward the posterior pole. Levene (1962) also noted an increased density posteriorly. On the basis of their work I examined this tissue to see if a reproducible density gradient for choroidal mast cells was present. Once established, this gradient could be used to assay the effects of such agents as ionizing radiation and corticosteroids on the mast cell. The albino rabbit, because of its distinct uveal blood supply, was selected, and rabbits in newborn and later stages of development were included to determine whether this cell was present at birth and whether changes occur with growth.

B. Experimentation

Commercial house-bred albino New Zealand male rabbits were divided into groups based on weight. In TABLE I these are listed as: group A, newborn 0.1 kg (mean weight); group B, unweaned 0.5 kg; group C, young adult 1.8 kg; and group D, 3.5 kg adult rabbits. The animals were killed by injecting 1 ml of solution containing 0.390 gm sodium pentobarbitol, 20% propyleneglycol, and 10% isopropyl alcohol (Euthenol-6) into a marginal ear vein, and the eyes were immediately enucleated.

Flat preparations were made according to the methods of Smelser and Silver (1963) with a slight modification. A vertical strip was cut through the choroid using the retinal vessels as landmarks (Fig. 1). The optic disc area was included in every section and the retina can easily be peeled away. Several cuts were made in the uvea to flatten the tissue and identify direction. A linearity of the choroidal vasculature, extending toward the periphery is apparent.

Fixation and staining were done according to the procedure described by Smith and Atkinson (1956). With this procedure mast cell granules are well preserved and the highly acidic toluidine blue will stain only these granules. After clearing, the mast cells stand out well and are easily counted.

Counts were obtained by counting all cells in continuous high power fields (magnification 440x) starting at the disc and proceeding superiorly and inferiorly.

C. Results

Figure 2 shows the density distribution for mast cells in each weight class (age). A definite increase in cell number occurs with development, and at birth a few but definite cells are present in the choroid. The mean weight

and variance for each group is given in TABLE I.

TABLE I

Rabbit Weight (in kg)

Group	Arithmetic Mean	Variance	Standard Deviation	Standard Error
A	0.10	0.0005	0.024	0.008
B	0.55	0.025	0.05	0.012
C	1.8	0.6	0.25	0.4
D	3.5	2.5	0.5	0.1

Several relationships might be useful for comparison purposes. Each of these density distributions was plotted on semi-log paper (Fig. 3); the data fit (a first order approximation) a series of positive and negative exponential functions of the form $Ao(e^{-k_1 r_1} - e^{-k_2 r_2})$. The values for the respective constants are listed in TABLE II.

TABLE II

Constants for Exponential Functions

$Ao(-e^{-k_1 r_1} - e^{-k_2 r_2})$

Group	Ao	k_1	r_1	k_2	r_2
A	2	5.15	1.35	1.39	5.00
B	29	3.02	2.30	2.90	2.40
C	81	2.90	2.40	1.33	5.20
D	90	2.72	2.55	1.25	5.55

The peak of this density gradient approximates a gaussian curve and might be useful in quantitative studies. The counts made in the fields 4-11 were averaged for the 1.8 kg and 3.5 kg series (groups C and D respectively) and are given in TABLE III.

TABLE III

Fields 4 through 11 (Peak Region) for Groups C and D

Group	Arithmetic Mean	Number	Variance	Standard Deviation	Standard Error
C	19.29	308	113.01	10.63	0.61
D	23.32	267	184.28	13.58	0.83

(Means separate at the 1% level, t test.) Number of animals $\cong \frac{\text{number of fields}}{8}$. In a few instances <8 fields were counted because of tissue folds.

Furthermore, the segment of each curve including consecutive fields seven through 20 approximates a straight line; a best-fit least squares line was determined for each group (TABLE IV, Fig. 4), and in each case the fit was significant at the 1% level with a t test. These regions are outlined in Figure 5, and the hyperbolic confidence limit for group C can be seen in Fig. 4.

Figure 5 shows the standard error of the mean which exists for the counts per high power field at that distance from the optic disc. This particular group will be used as a control for the radiation and steroid studies.

In TABLE V the 95% confidence limit is given for the slope of the least squares line for each group. An increasingly negative slope occurs with growth, and by 0.5 kg the confidence limits (95%) no longer include the zero point.

TABLE V

Confidence Interval for Least Squares Line. Non-Irradiated Group

Group	95% Confidence Limits of Slope*
A	$-0.19 \leq B \leq +0.66$
B	$-0.98 \leq B \leq -0.56$
C	$-1.26 \leq B \leq -1.08$
D	$-1.64 \leq B \leq -1.43$

* Derived from

$$S_b = \frac{\frac{\theta^2_y}{\theta^2_x} - b^2}{N-2}$$

and $b - t_{0.05} S_b \leq B \leq b + t_{0.05} S_b$.

TABLE IV

Least Squares Line Data, Non-irradiated Group

Group	Area of Slope Selected	Number	$\bar{X}$	$\bar{Y}$	Slope	Y Intercept	θ^2_x	θ^2_y	Level of Significance of Fit
A	2 - 16	14	9.0	0.6	-0.063	1.13	18.66	0.15	1%
B	7 - 17	11	12.0	4.2	-0.755	13.26	10.00	6.66	1%
C	7 - 20	14	13.5	12.8	-1.169	28.60	16.25	22.60	1%
D	7 - 20	14	13.5	15.8	-1.535	37.17	16.25	43.08	1%

* Based on r_{xy} = correlation coefficient = $\frac{Cov_{xy}}{\theta_x \theta_y}$, where $Cov_{xy} = \frac{\Sigma(x)\Sigma(y)}{N^2}$, using the t test.

D. Discussion

A reproducible density distribution for choroidal mast cells which varies with weight (age) is present in the uvea of the rabbit (Fig. 2). It can be described with a family of exponential functions (Fig. 3), and several areas under the curve are useful for analysis; for example, the area under the peak, represented from 4 through 11 for group C in Fig. 5, was ranked according to counts per high power field. Figure 6 shows its histogram and cumulative frequency. An adequate approximation for a gaussian distribution exists in this segment of the distribution. A straight line, fitted by the method of least squares, was fitted to the segment from 7 through 20 with an agreement at the 1% level (t test). These lines for groups A through D are given in Fig. 4, with the hyperbolic confidence limits for group C represented. The slopes, intercepts, and other data for the least square lines are given in TABLE IV. Figure 7a shows representative photographs of the mast cells near the peak (maximum density) and Fig. 7b those toward the periphery. Granules stain metachromatically and the nucleus is seen as the central empty space. No other cells are stained.

This preparation demonstrates a reproducible density gradient with several regions which are useful for statistical analysis. Quantitative comparisons should be possible to demonstrate the effect of various agents on these cells.

Mast cells are closely related to blood vessels (Riley, 1953, 1961). Their distribution in this region closely follows the vascular distribution and is dependent in part on the functional and anatomical variations of the overlying retina. The choroid thickness and mast

cell density are maximum just under the rudimentary macular area of the rabbit retina. These cells, because of their association with vascular walls, may act as markers for the blood supply. The rabbit has a unique vascular distribution in the retina and choroid with the uveal vessels entering in the horizontal raphe posteriorly and extending toward the periphery (Prince, 1964). The exponential fall of the mast cell distribution may represent an exponential fall of vessels as they expand and supply an enlarging area toward the periphery. Many reports deal with the stage or time after conception but prior to birth when mast cells are first seen in mammals (Alfejew, 1924; Arvy, 1956; Fish, 1949; Ramsay, 1935; Ferrata and Michels, 1923; Gandolfo, 1924; Lombardo, 1906; Lehner, 1924; Knoll, 1936). Very few scientists have attempted to determine the relation of numerical changes to growth and development.

Webb (1936) reported an increase followed by a decrease in the rat subcutaneous mast cell during the gestational period, but others (Lindholm, 1959; Grahne, 1959; Zachariae, 1964), in samples of other tissue and in the human fetus showed a steady increase that continued toward a maximum after birth and was followed by a slow decline. Padawer and Gordon (1956b) demonstrated an increase with weight (age) after birth in rat peritoneal mast cells. After growth is completed, a slow decline in number may occur during the adult years (Constaninides and Rutherdale, 1957; Hellström and Holgren, 1947; Grahne, 1959; Lindholm, 1959). One author (Nozaka, 1962) felt that an increase in mast cells occurred with aging but that this may have been due to an increase secondary to chronic inflammation to which the region sampled (human cervix) is subject during adulthood.

In these rabbits, an excellent growth pattern can be seen (Fig. 2) with a family of density distributions observed increasing toward a maximum during development. Mast cells were found in the uvea of newborn rabbits and increased with growth (weight). The effects of senescence could not be evaluated because only a few rabbits among the 3.5 kg group were in this eye range (> 4 kg) and because of the difficulty in obtaining old house-bred animals.

This cell gradient should be useful to establish the effect of various noxious agents on mast cell number, to correlate tissue-histamine concentration with mast cells because a similar curve should be present, to study metabolic changes during and after the growth phase or before and after perturbing the system, and to correlate a similar concentration curve for the sulfated acid mucopolysaccharide associated with mast cells.

II. EFFECTS OF RADIATION

A. Introduction

Among papers concerned with the effect of radiation on tissue mast cells, no agreement can be found about the sensitivity of this cell to ionizing radiation. In part, this disagreement is attributable to the different types of radiation used, the lack of uniformity in the dose administered, the variation in sampling times, and the different laboratory animals and anatomical sites selected. No trend is evident among the various reports, -- perhaps because of the inherent sampling error in the usual assay for tissue mast cells.

When the most sensitive cells degenerate after irradiation, a net loss of volume occurs in lymph nodes, spleen, and thymus. More resistant cells have a relative increase in number because the tissue volume in which they exist is reduced. It is not unexpected therefore that studies of acute radiation effects on this form of tissue report that mast cells increase in number. Atomic bomb casualties (Liebow and coworkers, 1950), x-irradiated hamsters (Kelsall and Crabb, 1952; Crabb and Kelsall, 1956), and x-irradiated rodents (Arvy and coworkers, 1952; 1954a, 1954b), exhibit this relative increase in hematogenous tissue. Although an actual increase in mast cells might occur, more likely this finding represents a shrinkage of tissue with survival of a more resistant cell type (mast cells). The dose range, excluding those who succumbed to atomic bomb radiation, was from 1000 to 2000 rad.

Opposing results are reported by Ito (1957). He found that in the lymph nodes of rats receiving 700 rad whole-body irradiation there was a decrease in mast cell

number. However, his tissue fixation was in 10% formalin, which is inadequate for proper fixation of these cells. Viklický and Trebichausky (1970) examined mouse spleens after 400 or 800 rad of gamma radiation and noted a decreased mast cell population. In mice, chronic, whole-body gamma irradiation will cause an increase in mast cells per unit volume in this type of tissue, but in those animals where leukemia develops, the count declines as the tissue volume expands or cell observation becomes more difficult (Spargo and coworkers, 1951).

Graham and Graham (1968) reported an increase in mast cells along radiation fistulas in biopsies obtained from patients. They felt that radiation was an actual stimulus to mast cell reproduction in exposed areas. Their assumptions ignore the increase in mast cells seen with chronic inflammation or the possibility that vascularization, with its closely associated mast cells, could account for an increased accumulation of these cells (Riley, 1953).

Flat preparations such as peritoneal spread or the hamster cheek pouch have been used for radiation studies by Choi and coworkers, 1966; Oh and coworkers, 1964; Michels, 1933; Conte and coworkers, 1956; Smith and Lewis, 1953, 1954a; Smith, 1958). Doses ranged from 25 to 1200 rad (measured as whole-body irradiation), and either the rat or hamster was used in their experiments. To determine mast cell counts, these investigators sacrificed the animals from minutes to several days after irradiation. Michels felt that the mast cells seen in peritoneal spreads were radioresistant at 400 rad and at 750 rad. Choi and coworkers (using phase contrast microscopy) did not see any morphological changes. Curiously, in another publication (Oh and coworkers, 1964), the same investigators reported a decrease in peritoneal mast cells after approximately

the same dose of radiation. Adrenalectomy prevented the loss of mast cells and, in the non-adrenalectomized animal, cell count recovery was complete by three days. Smith and Lewis (1954a) reported similar results (600 rad) in the hamster cheek pouch, and later Smith (1958) reported that antihistamines had a radioprotective effect. In three other reports by the same authors (1953, 1955a, 1958) they felt mast cell counts were inconclusive, because of tissue variation, and reported a radiation effect based on percent of abnormal cells noted. Conte and coworkers (1956) observed a transient increase after just 75 rad was received by the peritoneum. Higher doses (150 to 600 rad) did not produce any change in these cells. The studies of Dutta-Choudhuri and Ray (1959), and Detrick (1963), using similar doses (600 rad) were based on morphological changes.

Free peritoneal mast cells can be counted fairly accurately if absolute counts are used. Ito (1957), and Bercovici and Graham (1964), incorrectly used relative counts, and Ito used inappropriate fixation.

Morphological changes were seen in tissue mast cells after the internal administration of radioactive isotopes (Sanders and Adee, 1969; Guimaraes and Taylor, 1957; Fan and Sikov, 1958). The usual increase in number was observed in hematogenous tissue (Guimaraes and Taylor), and Fan and Sikov felt a decrease occurred in subcutaneous cells.

Pettersson (1954) attempted an extensive study in the subcutaneous tissue of the guinea-pig. He assumed a Poisson distribution of these cells but did observe a difference in concentration between skin regions. His comparisons were then made within regions, and he did not consider volume changes that could be caused by edema. He felt that a 50% reduction of mast cells occurs after

600 rad and that doubling the dose to 1300 rad does not increase their decline. His controls were the same animals biopsied prior to irradiation and a group of non-irradiated animals. Multiple biopsies might also alter the number of mast cells present, either from repeated trauma or from induced inflammation.

Because the above results are often contradictory between investigators, and at times among the same ones, further work seemed worthwhile on radiation effects on mast cells. The choroidal distribution of mast cells offered a method for analysis.

B. Experimentation

Albino male New Zealand rabbits in the weight range from 1.5 to 2.0 kg were randomly grouped in this part of the study. They were kept in a lighted room and allowed rabbit chow and water ad libitum.

Orbital irradiation was accomplished with a Co^{60} source of 1,500 Ci at a distance of 42 cm from the source, at a dose rate of 250 rad/min. The dose was measured with a Victoreen chamber (100 Roentgen) placed in a cadaver socket. A 3/8-inch sheet of plexiglas was used as an absorber for secondary radiation. Lead bricks (15 cm thick) were used for shielding, and only the head remained in front of the source. Though only the right eye was exposed, because of the size of the animal and the length of exposure, a considerable dose was received by the other orbit. For these reasons an unexposed group of equivalent weight was used as a control series.

Rabbits were exposed for the full dose at one time. The range of doses given and the number of animals in each group are indicated in TABLE VI. The rabbits were sacrificed at 3 or 8 days after exposure by injecting 1 cc of Euthanol-6 into a marginal ear vein. The right

TABLE VI

Sample Means, Area 4 Through 11, Irradiated Group vs Non-irradiated of the Same Weight

Dose in rad	Time after Radiation	Mean	Number	Variance	Standard Deviation	Standard Error	Separational Mean*
0	0	19.29	308	113.01	10.63	0.61	--
600	3	23.83	70	234.86	15.32	1.84	NS
600	8	19.10	88	87.74	9.37	1.00	NS
2,400	3	13.33	137	195.08	13.97	1.20	1%
2,400	8	19.62	151	204.42	14.30	1.17	NS
4,800	3	15.28	158	129.09	11.36	0.91	1%
4,800	8	28.87	89	315.34	17.76	1.89	2%
7,300	3	16.51	152	146.93	12.12	0.98	5%
7,300	8	22.77	64	247.24	15.72	1.98	NS
9,700	3	16.20	152	96.20	9.81	0.80	1%
9,700	8	14.10	207	118.73	10.89	0.76	1%
14,600	3	11.70	40	75.10	8.70	1.39	1%
14,600	8	19.50	48	117.60	10.80	1.58	NS
19,400	3	13.07	70	118.56	10.89	1.31	1%
19,400	8	19.55	55	197.76	14.06	1.91	NS

* Represents level of significance for separation of sample means from the comparable non-irradiated group (weight; group C). 5% level of t test considered significant.

eye was enucleated, fixed and stained by the methods identical to those described under Part I. Cell counts were made in the same way.

Two small series were given 850 r air-dose to their abdomens with similar shielding in reverse to their heads. This was to test whether an indirect mechanism might act to reduce mast cells.

C. Results

The counts per high power field for the area between the fourth and seventh high power fields of the mast cell distribution curve (Fig. 5), were averaged and the mean compared with group C (1.8 kg). Above 600 rad (orbital), a noticeable drop in mast cell number occurred on the third day after irradiation. The comparison of sample means between those sacrificed 3 days after irradiation and the non-irradiated groups was significant at the 1% level (t test) for all animals receiving more than 600 rad except those whose orbital dose was 7,300 rad (TABLE VI). The latter group had a separation of the sample means significant at the 5% level.

The same dose range was given to a series of rabbits sacrificed on the eighth day after irradiation. One group (9,700 rad) had a mean count for the same region which was less than the non-irradiated control group (1.8 kg), the difference being significant at the 1% level. The other groups were not significantly different from the non-irradiated controls, except for one that was higher; the 4,800 rad eight-day group had a mean count that was higher than that of group C, the difference being significant at the 2% level of significance (TABLE VI).

The data for the best fit least squares line determined from the linear portion of the uveal mast cell

distribution curve (Fig. 5, from the seventh through the twentieth consecutive high power fields) are given in TABLE VII. The lines for those groups receiving orbital irradiation are shown in Fig. 8a through 8g.

TABLE VII

Least Squares Line Data, Irradiated Group (Orbital)

Dose (rad)	Time after Irradiation (Days)	$\bar{Y}$	Slope	Y Intercept	θ^2_y	S_{yx}
0	0	12.8	-1.17	28.60	22.6	0.63
600	3	14.5	-1.39	33.26	35.9	2.11
600	8	13.4	-1.49	33.49	42.3	2.49
2,400	3	8.8	-0.64	17.49	7.8	1.04
2,400	8	12.0	-1.40	30.80	32.9	1.08
4,800	3	10.8	-0.95	23.50	15.4	0.93
4,800	8	18.4	-2.10	46.70	76.2	2.13
7,300	3	9.6	-1.35	27.83	31.5	1.12
7,300	8	13.8	-1.64	35.90	48.6	2.16
9,700	3	9.9	-1.21	26.24	25.1	1.10
9,700	8	8.5	-1.20	24.64	26.7	1.83
14,600	3	6.5	-0.89	18.50	13.6	0.93
14,600	8	14.5	-1.24	31.26	32.0	2.67
19,400	3	7.8	-0.87	19.44	13.5	1.13
19,400	8	12.0	-1.46	31.67	36.8	1.48

For all groups: $N = 14$, $\bar{x} = 13.5$, $\theta^2_x = 16.25$, and fit to straight line significance at the 1% level.

Above 600 rad all lines (for those sacrificed at the third day after irradiation) have a lower y intercept, have a lower mean $\bar{Y}$ value, and have a less negative slope than those sacrificed at the eighth day after irradiation. The one exception is the 9,700 rad series.

The 95% confidence interval for the slopes is given in the following table (TABLE VIII) along with the control group (1.8 kg).

For most doses above 600 rad, the slopes for those animals sacrificed on the third day after irradiation were flattened (less negative) below the slopes for those sacrificed at the eighth day and those of the control group. A drop in the "peak" region is more easily observed because of the number of cells present in this region, and this explains the change in the slope present at the third day after irradiation.

TABLE VIII

Dose in rad	Time after Irradiation	95% Confidence Interval for Slopes
0	0	-1.26 < B < -1.08
600	3	-1.72 < B < -1.06
600	8	-1.88 < B < -1.10
2,400	3	-0.80 < B < -0.48
2,400	8	-1.57 < B < -1.22
4,800	3	-1.07 < B < -0.82
4,800	8	-2.44 < B < -1.76
7,300	3	-1.57 < B < -1.14
7,300	8	-1.98 < B < -1.30
9,700	3	-1.38 < B < -1.04
9,700	8	-1.49 < B < -0.91
14,600	3	-1.03 < B < -0.74
14,600	8	-1.65 < B < -0.82
19,400	3	-1.05 < B < -0.68
19,400	8	-1.70 < B < -1.22

Since a number of studies (see above) have used abdominal irradiation to produce a radiation effect on mast cells, 850 rad were given to a group of rabbits whose orbits were shielded and kept out of the radiation cone. This was done to test whether an indirect effect might be the cause of a mast cell decrease. The dose selected was near the usual one in the above studies. Animals were sacrificed on the first and third day after irradiation, and no difference could be seen between their mean counts-(area 4 through 11) and those of the non-irradiated group in the comparable weight range (TABLE IX). The best fit least squares data are given in TABLE X and the 95% confidence intervals of the slopes in TABLE XI.

TABLE IX

Sample Means, Area 4 through 11, Abdominally Irradiated Group vs Non-irradiated Group of the Same Weight Range

Dose in rad	Time after Irradiation	Mean	Number	Variance	Standard Deviation	Standard Error	Separation of Mean*
0	0	12.29	308	113.01	10.63	0.61	--
850	1	19.04	79	154.28	12.42	1.41	NS
850	3	20.88	111	97.15	9.85	0.94	NS

* NS = not significant (5% level). The least square lines for this series are shown in Fig. 8H and the data for these lines in TABLES X and XI.

TABLE X

Dose in rad	Time after Irradiation (Days)	$\bar{Y}$	Slope	Y Intercept	θ^2_y	S_{yx}
850	1	11.40	-1.40	30.30	37.04	2.24
850	3	11.73	-1.70	34.68	51.34	2.00

For all lines: $N = 14$; $\bar{Y} = 13.5$; $0^2_y = 16.25$; straight line fit significant at the 1% level. r_{xy} and cov_{xy} calculated according to the formulas under TABLE IV.

TABLE XI

95% Confidence Interval for Slope of Least Squares Line Abdominal Irradiation (850 rad)

Time after Irradiation (Days)	95% Confidence Limits of Slope*
1	$-1.76 \leq B \leq -1.05$
3	$-2.02 \leq B \leq -1.38$

* Calculated from formulas given at the bottom of TABLE V.

D. Discussion

The contradictory results published on the effect of ionizing radiation on mast cells indicates at least that the cells are radioresistant. In none of these papers was a complete loss of tissue mast cells observed after irradiation. Morphological changes were frequently reported as a radiation effect, but among non-irradiated uveal preparations an enormous variation in morphology could be seen and changes of this form could not be quantitated. After receiving over 19,000 rad, countable mast cells still can easily be demonstrated in the choroid.

A fall in the cell count was observed in relation to the control group (1.8 kg weight) at three days after irradiation in each of the above groups that received 600 rad. The difference between the arithmetic means for the peak areas were significant at the 1% level for all but one, and that group had a significant decrease at the 5% level. By the eighth day, recovery to a normal cell count level occurred in all but one group (9700 rad). This indicates a rate of recovery faster than that of Fawcett (1955) and of Smith and Lewis (1954a). Abdominal irradiation at 850 rad with the head shielded, did not produce a drop in choroidal mast cells, thus indicating that the observed decrease may be a primary effect.

Recovery may occur by three mechanisms, either by cell division and repopulation, by migration into the tissue from the blood elements, or by a restoration of damaged granules. Radiation can depolymerize mucopolysaccharides and a loss might occur from the cytoplasmic granules (Jooyandeh and coworkers, 1971; Detrick, 1963). Granule repair might account for the recovery, and the increased count may be simply a return of metachromasia.

Though doubts exist about the mast cell's ability to undergo mitotic division, mitotic figures have been seen by a number of workers (Michels, 1938; Fawcett, 1953; Allen, 1961, 1962b). Padawer alone or with coworkers (1955, 1963, and 1966) watched thousands of free peritoneal cells treated with colchicine. Drastic alterations in cell structure were observed, but even with this mitostatic agent, he was never able to observe mitotic figures. Similarly, in cells observed under the phase-contrast microscope, Choi and coworkers (1966) never saw evidence of mitosis. Further confusion exists because fibroblasts can phagocytize mast cell granules and their appearance is identical to that of the original cells (Higginbothom and coworkers, 1956). It would be impossible to determine if the observed mitosis were in fact that of a fibroblast with phagocytized granules.

Tritiated thymidine labeling can be seen but the percentage labeled has been reported as 0.38% (Walker, 1961), 1.7% by Padawer (1961, 1963), and 5.7% by Allen in 1962a. The latter author (1962a) found that 75% of the thymidine labeled cells were also synthesizing DNA (fulgen-methyl green method).

Other evidence of mast cell division is indicated by the pathologic conditions of mastocytoma (Bloom and coworkers, 1960; Orkin, 1967) and of urticaria pigmentosa (Caplan, 1965; Klaus and coworkers, 1965). Also, tissue cultures have been achieved (Ginsburg and Lagunoff, 1967; Ginsburg, 1963).

Even with the above evidence it seems likely that differentiated mast cells have little ability to undergo mitotic division. Questions concerning their origin have not been resolved (Selye, 1965). Replacement may come from the circulatory basophils or from undifferentiated precursor cells in connective tissue. Although the

consideration of tissue cells as separate entities from circulatory basophils is accepted, in practice it is difficult to find any distinctive feature between them. Quantitation studies are subject to the uncertainty that a continual entrance and exit may occur.

Further study of several factors should be interesting, in particular that of a radioactive sulfur uptake before and after irradiation and a tissue histamine determination. Histamine is bound in some fashion to mast cell granules (Riley, 1959) and is rapidly released by a number of agents (Selye, 1965). A gradient that should parallel the mast cell gradient in the choroid may be demonstrated, and irradiation may alter it. A histamine decrease in tissues exposed to irradiation has been reported (Eisen and Wilson, 1957; Eisen and coworkers, 1956; Feldberg and Loeser, 1954), and if a gradient could be established in the uvea it would be an ideal model for studying this effect. Studies which could be carried out with such a model include among others the rate of recovery of histamine after degranulation and autoradiography with tritiated thymidine during the growth phase as well as after irradiation.

III. EFFECTS OF CORTISONE

A. Introduction

Evidence exists that adrenal cortical steroids exert an effect on mast cells (Selye, 1965; Asboe-Hansen, 1954b, 1958; Boseila, 1963). Typically, morphologic changes occur after cortisone or hydrocortisone administration. These are described as: irregular size, shape and density of cytoplasmic granules, cytoplasmic vacuolation, degranulation, decreased cell diameter, and a change to orthochromasia in the granules (Wegelius and Asboe-Hansen, 1956; Smith and Lewis, 1954b, 1955b; Asboe-Hansen, 1952; Cavallero and Braccini, 1951; Stuart, 1951; Zachariae and Moltke, 1954; Boseila, 1963). In disagreement with the above are the following investigators: Baker (1953), Baker and coworkers (1951), and Devitt and coworkers (1953), who report that no alteration in mast cell morphology can be seen after cortisone or hydrocortisone administration.

In addition, Wenk and Speirs (1957) felt that in their experiments mast cells were neither reduced nor increased in number as a response to the steroids. Wenk and Speirs used peritoneal washings, one of the best methods for quantitating mast cells, and also followed blood basophil counts. Basophils were reduced in number after steroid administration.

Among those who noted alteration in mast cell morphology as a steroid effect, Smith and Lewis (1955b), and Zachariae and Moltke (1954), felt that neither could these cells be counted with any accuracy nor had any evident change occurred.

A reduction in mast cell count was thought to occur in the reports of Kelsall (1961), Asboe-Hansen (1952), Cavallero and Braccini (1951), Englebreth-Holm and Asboe-Hansen and Zachariae (1955). A relative increase in mast cells is seen in lymph nodes after adrenocorticotropin treatment in rats because of the loss of volume as lymphocytes regress (Baker and coworkers, 1951).

In further support of a steroid effect is the diurnal variation in circulating basophil counts, the reduction of the basophil count after steroid administration, perhaps the reduction of radioactive sulfur uptake in mast cells pre-treated with adrenocorticosteroids, the decrease in mast cell tumor (mastocytoma) size in response to steroids, perhaps the decreased production or binding of new histamine in mast cells after steroids have been given, and lastly, some indication of a diurnal change in mast cell activity (Niebroj, 1958; Code and Mitchell, 1957; Code and coworkers, 1954; Boseila, 1958a, 1959a, 1959b; Schayer and coworkers, 1954; Wenk and Speirs, 1957; Bloom, 1952; Osada, 1954, 1956; Asboe-Hansen, 1954a; James and coworkers, 1956).

Only a few of the above authors have attempted any quantitation. The choroidal density distribution of mast cells seemed a better system in which to observe and quantitate the effect of cortisone acetate on mast cell number. This steroid was given intramuscularly to a group of rabbits at several doses and for varying periods of times.

B. Experimentation

Albino, male New Zealand rabbits were randomly selected in the same weight range of 1.5 to 2.0 kg and kept in an illuminated room. Rabbit chow and water were available *ad libitum*.

Intramuscular injections of cortisone acetate were given in the thigh muscles for one or five days with either 50 mg per kg or 200 mg per kg, as the dosage. The

rabbits were killed by the injection of 1 ml of Euthanol-6 into the marginal ear vein the next day after their last steroid injection. Fixation, staining and counting were the same as described in preceding paragraphs.

C. Results

Cortisone acetate administered in one injection at a dose of either 50 mg/kg or of 200 mg/kg did not reduce the mast cell choroidal population among rabbits in this weight range. Administering a daily steroid injection for 5 days results in a decrease of the mast cell population, significant at the 1% level (TABLE XII).

TABLE XII

Dose mg/kg	Number of Injections	Arithmetic Mean	Number	Variance	Standard Deviation	Standard Error	Level of Significance*
50	1	21.81	174	164.54	12.82	0.97	NS
50	5	11.55	128	58.68	7.66	0.67	1%
200	1	21.30	76	122.37	11.06	1.27	NS
200	5	15.90	273	198.40	14.08	0.85	1%

* NS = not significant. Separation of mean from group C (1.8 kg).

The best fit least squares lines for the region including the 7th to the 20th consecutive high power fields are represented in Figure 9 and in TABLE XIII. In both instances, those animals that received a daily injection for five days had a lower Y intercept and mean $\bar{Y}$ value than those receiving only a single injection. In addition, they had values below those of the control series (group C). The animals receiving one injection had Y intercept and average $\bar{Y}$ values comparable to those of group C.

TABLE XIII

Cortisone Acetate Series
Regression Lines

Dose mg/kg	No. of Injections	$\bar{Y}$	Slope	$\bar{Y}$ Intercept	θ_y^2	Syx
50	1	12.1	-1.64	34.22	46.75	1.63
50	5	8.0	-0.85	19.47	12.85	1.00
200	1	12.6	-1.31	30.31	30.32	1.54
200	5	9.5	-1.27	26.60	26.89	0.92

For all groups: $\bar{x} = 13.5$; N = 14; significance of fit to a straight line = 1%; $\theta y^2 = 16.25$. (Calculations are the same as in prior footnotes to Tables.)

The 95% confidence limits are given in the Table below:

TABLE XIV

95% Confidence Limits for Slope of Least Squares Lines
Cortisone Acetate Group

Dose mg/kg	Number of Injections	95% Confidence Interval
50	1	$-1.91 \leq \beta \leq -1.37$
50	5	$-1.03 \leq \beta \leq -0.67$
200	1	$-1.55 \leq \beta \leq -1.07$
200	5	$-1.41 \leq \beta \leq -1.12$

D. Discussion

Even excluding those studies in which a numerical change in tissue mast cells occurs because of a large loss of other components such as lysis of lymphocytes in lymph nodes following steroid administration (Baker and coworkers, 1951; Kelsall and Crabb, 1952), or the loss of lipid cells with starvation (Baker, 1953), there remains a large indeterminate error in the counting and

preparation of mast cells to determine the effect of various agents on them. Merely a slight mechanical trauma, such as pinching or teasing tissue, can lead to degranulation (Higginbothem and Dougherty, 1956), and it is not unexpected that conflict exists concerning the tissue mast cell steroid effect.

Adrenocorticosteroids or adrenocorticotropic hormone (ACTH) have a direct action on the mucopolysaccharides of connective tissue (Seifter and coworkers, 1953). A similar pharmacological action might be present on tissue mast cells or circulating basophils by an effect on the acid mucopolysaccharide of the granules. This cytoplasmic element, the granule, defines this cell; its loss could account for any morphological changes or diminished counts observed. Regeneration of the granules, not the cell, might be the mechanism for recovery. Smith and Lewis (1954b) feel that morphological changes do occur after ACTH administration, but uveal mast cells are too variable in form to use for quantitation purposes.

Cortisone acetate and related compounds reduce circulating basophils along with eosinophils (Code and coworkers, 1954), but a direct lytic action may not be the cause of their decline. Other factors, alone or in combination, such as a decreased production (or release) in hematopoietic tissue, margination and storage in blood capillaries or sinuses, increased phagocytosis of work out basophils, and movement of these cells into tissue spaces (Wenk and Speirs, 1957), could be the reason a decrease in circulating basophils occurs. The latter factor, an increased movement of cells into tissue spaces, might even lead to an increased mast cell count in tissue after adrenocorticosteroid administration or stimulation.

In this study, cortisone acetate administration for five consequent days at a dose of 50 mg/kg or 200 mg/kg led to a statistically significant fall in choroidal mast

cells of the rabbit. This action might be explained as a cell lysis, a loss of granules, a decreased production of these cells, and so forth. It seems that a drop in basophils resulting from administration of cortisone could not be explained either by an increased margination or by tissue entrance, as an increase in the tissue count might occur.

A decreased radioactive sulfur incorporation into the cytoplasmic granules was reported by Asboe-Hansen (1954a) after cortisone treatment and a reduced ability of mast cells to bind new histamine (after prior depletion) with steroid administration (Schayer and coworkers, 1954; Goth and coworkers, 1951) indicates an effect by steroids on mast cell granules. The granules contain a sulfated acid mucopolysaccharide (Schiller, 1963) that apparently binds histamine with a weak ionic linkage, and histamine is released by such agents as BW 48/80 (Uvnas, 1958; Riley and West, 1955). A depletion of this mucopolysaccharide could decrease histamine storage.

Further study would seem appropriate on the choroidal histamine level before and after steroid administration, and also on the radioactive sulfur uptake under the same circumstances. Because of the reproducible density gradient of mast cells in this tissue, an excellent opportunity exists to establish a similar gradient for the heparin-like acid mucopolysaccharides and histamine of the mast cell. Though excellent evidence exists for the occurrence of these two potent agents in mast cells, distribution studies of these cells and the extraction of them from tissue is not parallel in all instances (Jaques, 1961). This tissue seems to represent such an opportunity. The growth pattern (Section I) offers the additional chance to examine this distribution during growth.

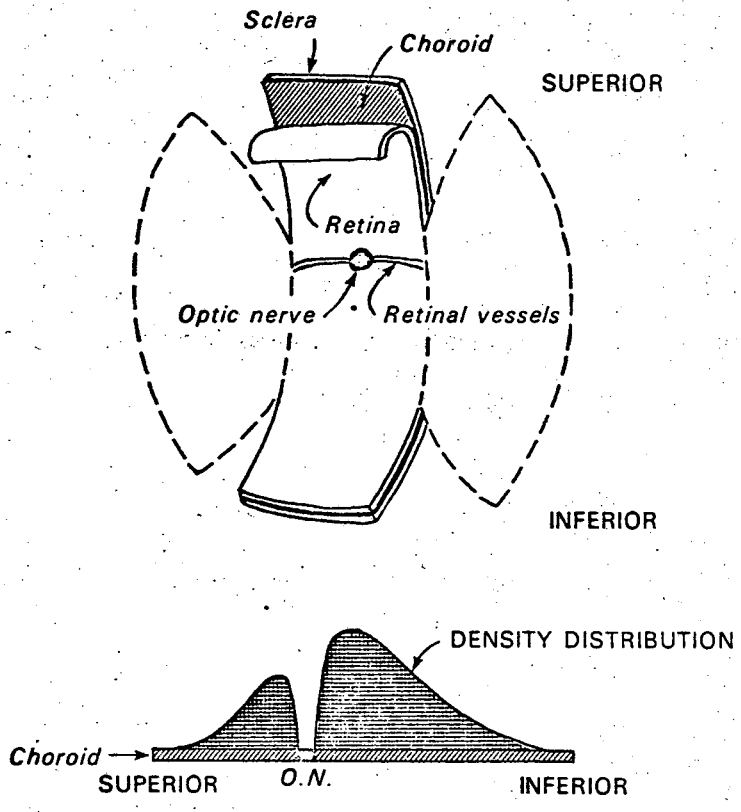

Figure 1. Choroidal mast cell preparation and density distribution.

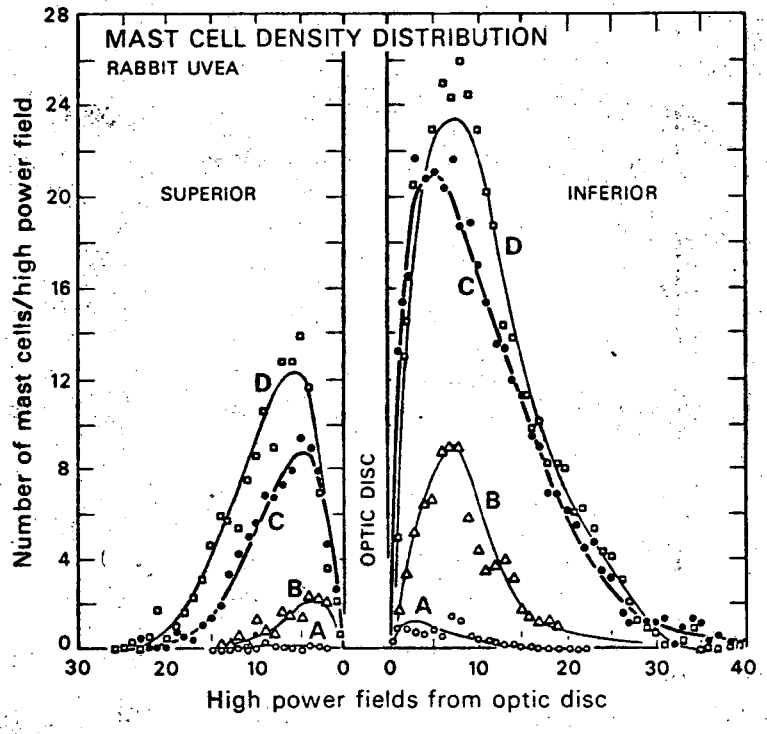

Figure 2. Density distribution of choroidal mast cells for the different weight (age) groups. Points represent average count at that distance from the optic disc.

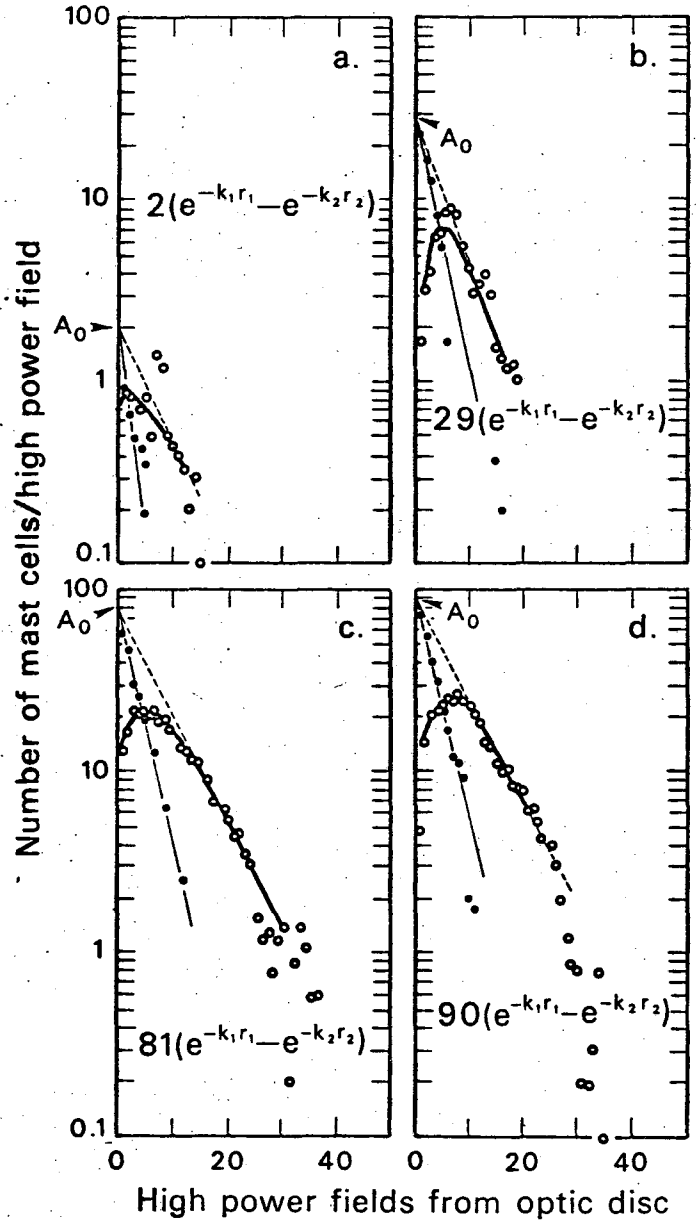

Figure 3. Density distributions (mast cells) plotted on semi-log paper.

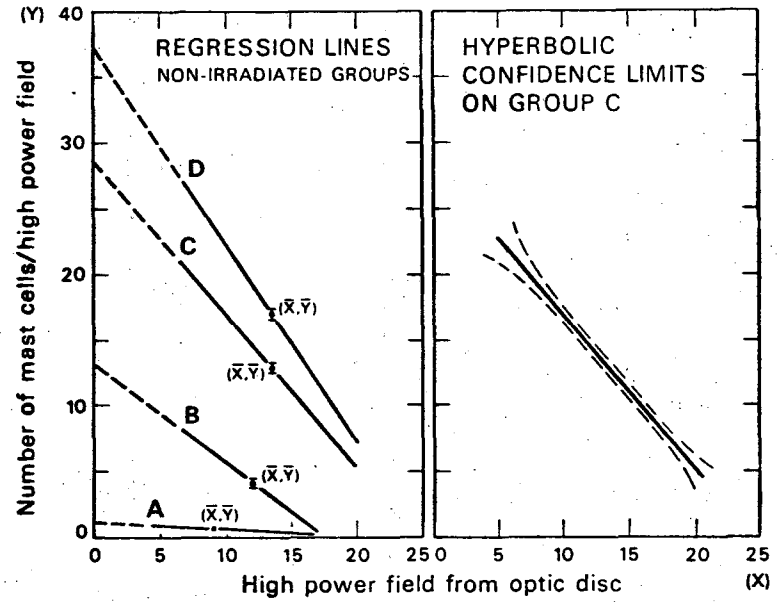

Figure 4. Regression lines (best fit least squares lines) for different weight (age) groups and the hyperbolic confidence limits for group C (1.8 kg).

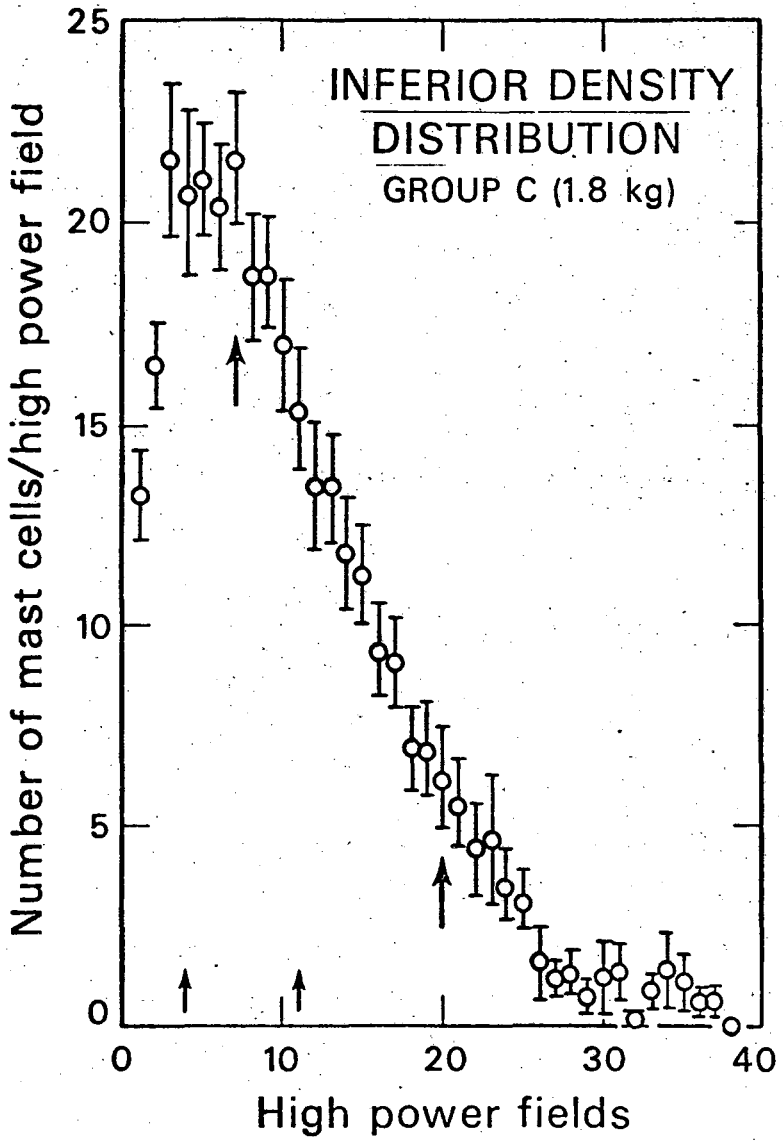

Figure 5. Mast cell density distribution for group C (1.8 kg). Error bars are the standard error of the mean. The arrows represent segments of the curve selected for analysis (see text).

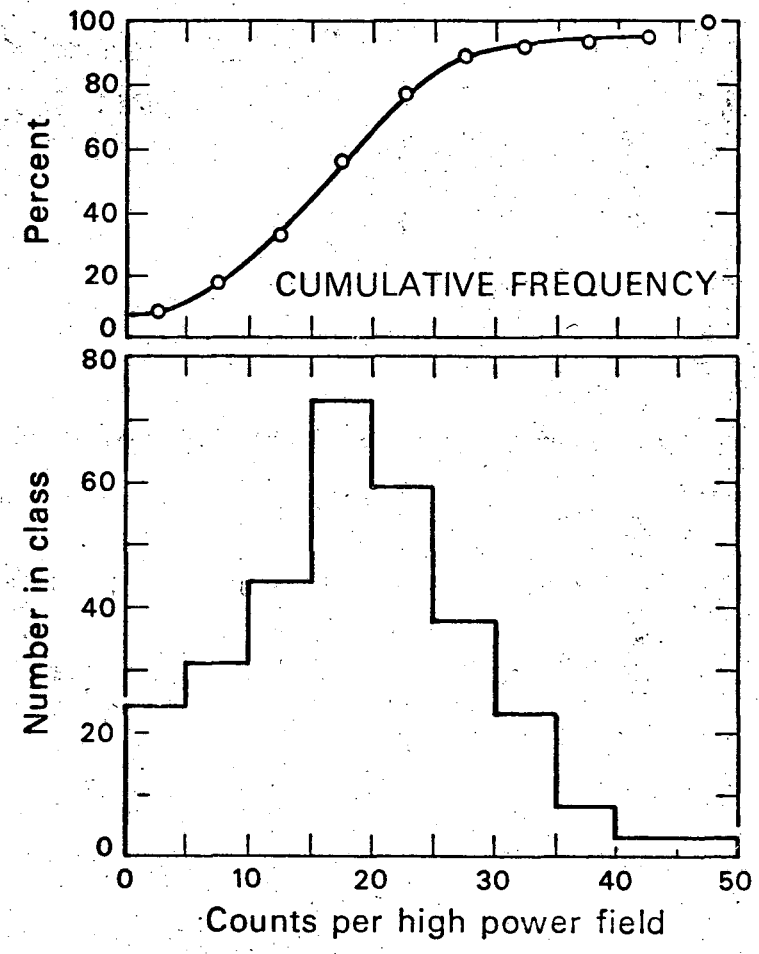

Figure 6. Histogram and cumulative frequency for counts per high power field (ranked) in group C (1.8 kg).

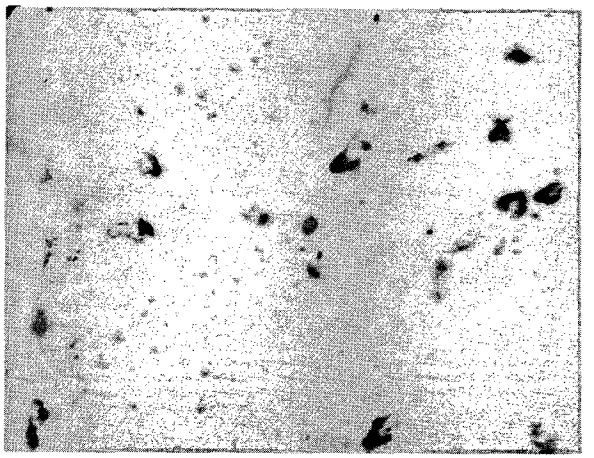

Figure 7A. Photomicrograph (x400) of mast cells near the region of maximum density. The central unstained region of the cell is the nucleus. Cells outside of the plane of focus are seen as indistinct forms.

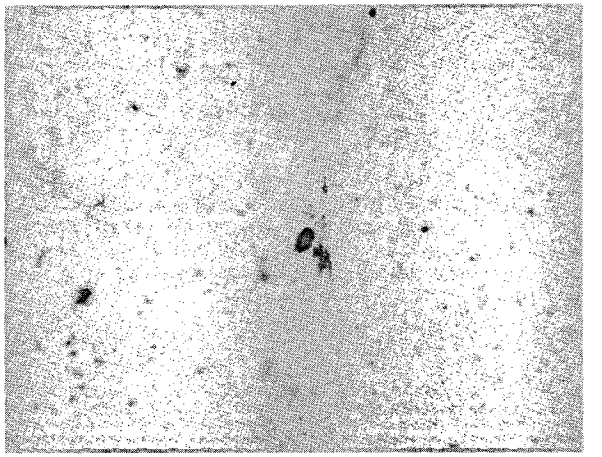

Figure 7B. Photomicrograph (x400) of the peripheral choroid. Flat mast cell preparation.

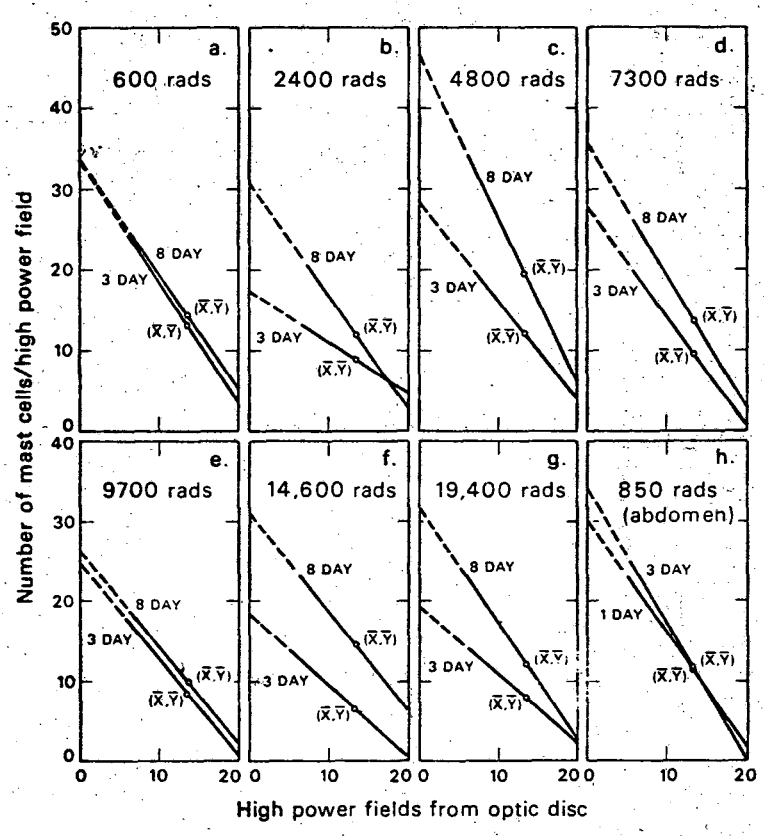

Figure 8. Best fit least squares regression lines for irradiated groups (7th through 20th high power field segment of density curve).

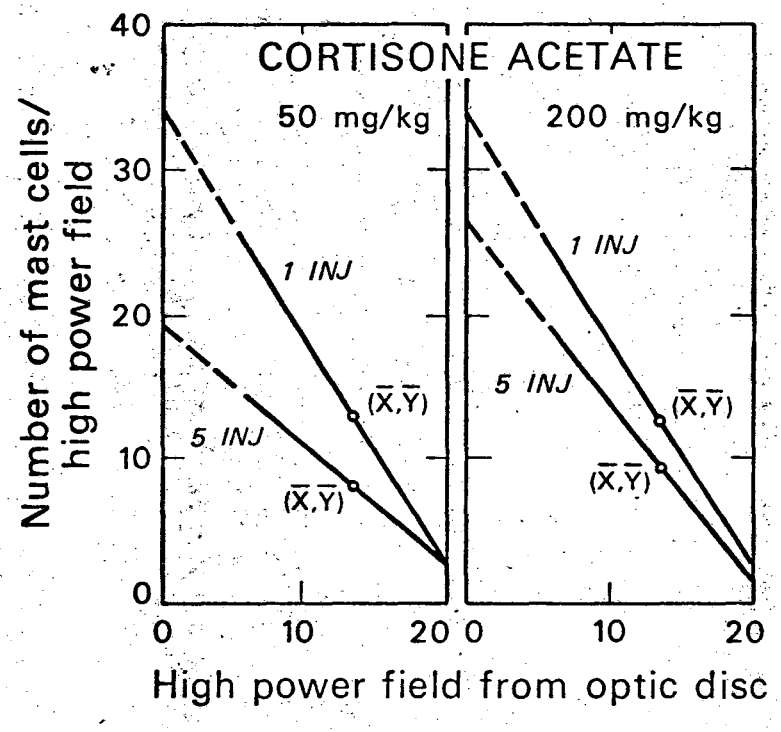

Figure 9. Best fit least squares regression lines for groups receiving cortisone acetate injections.

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TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720