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H-DIVISION ANNUAL REPORT OF RESEARCH ACTIVITIES

(Group H-4, Dec. 1, 1947 to Dec. 1, 1948)

VOLUME II (Radiobiology)

Louis H. Hempelmann — Division Leader, Research Advisor
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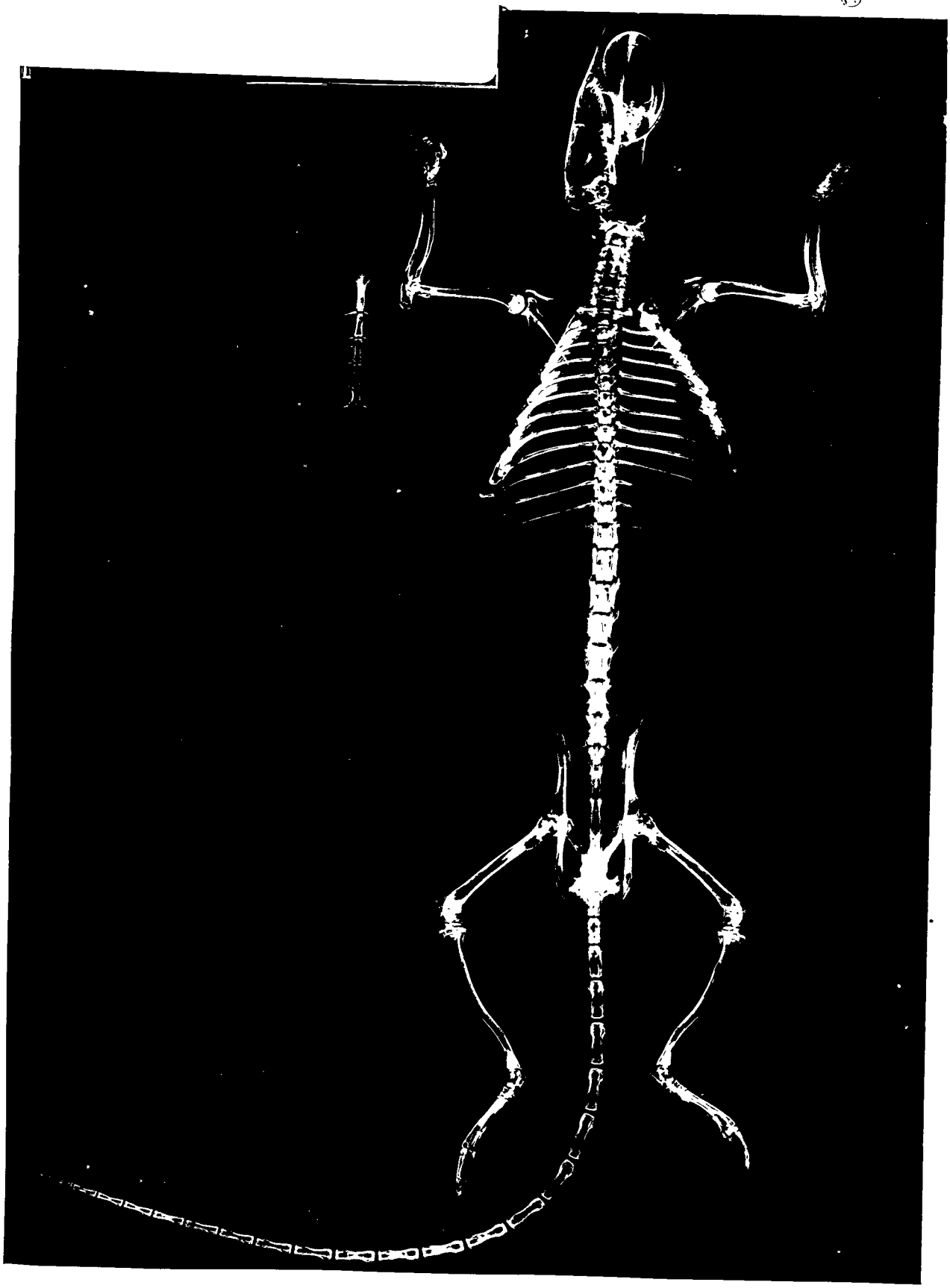


THE EFFECTS OF X-RAYS ON THE MITOTIC ACTIVITY OF MOUSE EPIDERMIS

Norman P. Knowlton, Jr., Louis H. Hempelmann, and Joseph G. Hoffman*

"This document is based on work performed under Contract No. 7405-eng-36 for the Atomic Energy Commission".

With the increased interest in various types of ionizing radiation as a result of the Atomic Energy Program, there is a great need for a practical method for the quantitative evaluation of the effects of sublethal doses of such radiation. Many investigators have shown that small doses of radiation result in a temporary but marked depression of the mitotic activity of lower animal, plant, embryonal, and tumor cells. This suggests that similar studies of mitosis in mammalian tissues might lead to a relatively simple and reasonably simple and reasonably specific method of expressing radiation damage. The usual technique of determining the mitotic index of a tissue involves the actual counting of many thousands of individual cells. Since this is extremely laborious, considerable effort has been devoted to developing simpler, more expeditious methods. This preliminary report describes a simplified technique of obtaining the mitotic index of mouse skin and indicates the surprising sensitivity of the mitotic activity of mouse epithelium to the effects of X-rays.

Groups of animals (CF_1 strain white mice, 6-8 weeks of age) were exposed to specific dosed of 250-KV peak voltage X-rays at the rate of 50 r/min and then autopsied at definite time intervals after exposure. Immediately after the animal had been

killed by crushing the cervical spine, the ears were removed and placed in 1% acetic acid. After 16 hrs at 5° C, a homogeneous layer of epidermis two cells thick was separated from the dermis according to the technique mentioned by Hoepke(3) and described in detail by Cowdry(2). The section of epidermis was then stained with Mayer's hematoxylin and mounted on slides for study. The cells in mitosis (arbitrarily defined as the period between the breakdown of the nuclear membrane in prophase and the complete separation of the cytoplasm in telophase) in a given number of microscopic fields outlined by a Whipple disc were then counted. The number of epidermal cells in the field delimited by each Whipple disc was carefully determined for animals of the strain and age used in this study so that the final value of the mitotic index can be expressed in terms of mitoses/100,000 cells. Variation in cell numbers from field to field is statistical in nature and introduces an error of 1-2% not encountered when individual cells are counted. The much larger number of cells which can be examined practically by the field method compensates for this error by reducing the over-all statistical error. It has been shown that X-ray dosage up to 325 r does not significantly alter the number of cells per field, so this method is valid for mouse skin after radiation exposure.

The change in mitotic index of mouse epithelium produced over a range of sublethal doses of X-rays from 5 to 325 r has been studied. The graphic response of the mitotic index in

animals receiving 35 and 325 r is shown in Fig. 1. Each point on the experimental curve represents the average mitotic index obtained by examining a total of approximately 200,000 epithelial cells in 4-5 experimental animals. The diurnal variation in mitotic activity has been taken into account in the exposure groups, since there is twice as much mitotic activity during the morning as there is in the evening. This has been previously reported[1] and confirmed in our laboratory by means of the control animals for the above experiments.

In both of the experimental groups the minimum point of mitotic activity is less than 1 mitosis/100,000 cells. This minimum was reached in less than 2 hrs after exposure. On the other hand, the time required for the mitotic index to return to normal varies from 5 hrs at 35 r to 6 days at 325 r. An "overcompensation" phenomenon is quite evident at the 325-r dosage level, with the mitotic activity more than double that of normal on the 8th day after irradiation. This phenomenon is being studied at the 35-r dosage level.

From the data presented above it is evident that the mitotic activity of mouse skin is extraordinarily sensitive to the effects of X-rays. Between the two dosages reported here it appears that the best index of damage is the time for the mitotic index to return to normal. Both the extent of the drop from normal and possibly the time in reaching the minimum point appear to be quite similar at these two extremes of dosage. However, the first point could have been reached earlier. By the use of this biological criterion of radiation effect our present program is to compare

the relative destructiveness of different types and different energy ionizing radiations.

It seems possible to postulate from the data at the dosage level of 35 r that the degree of depression of mitotic activity from normal may serve as an index of tissue damage at very low dosages. Experiments now in progress indicate that 5 r of 250-KV X-rays decreases mitotic activity to less than 25% of normal in 60-90 min.

The above work on the mitotic index in skin is being paralleled by similar studies in the jejunum, adrenals, and lymph nodes, but at the present time it appears that the skin is by far the most sensitive of the organs studied.

Experiments are in progress to determine the effect of rate of irradiation and of single or divided doses for various types of ionizing radiation on the mitotic index of mouse skin and other tissues. It is hoped that comparisons of the change in mitotic index and the shape of the recovery curve will be of value in evaluating these radiation effects.

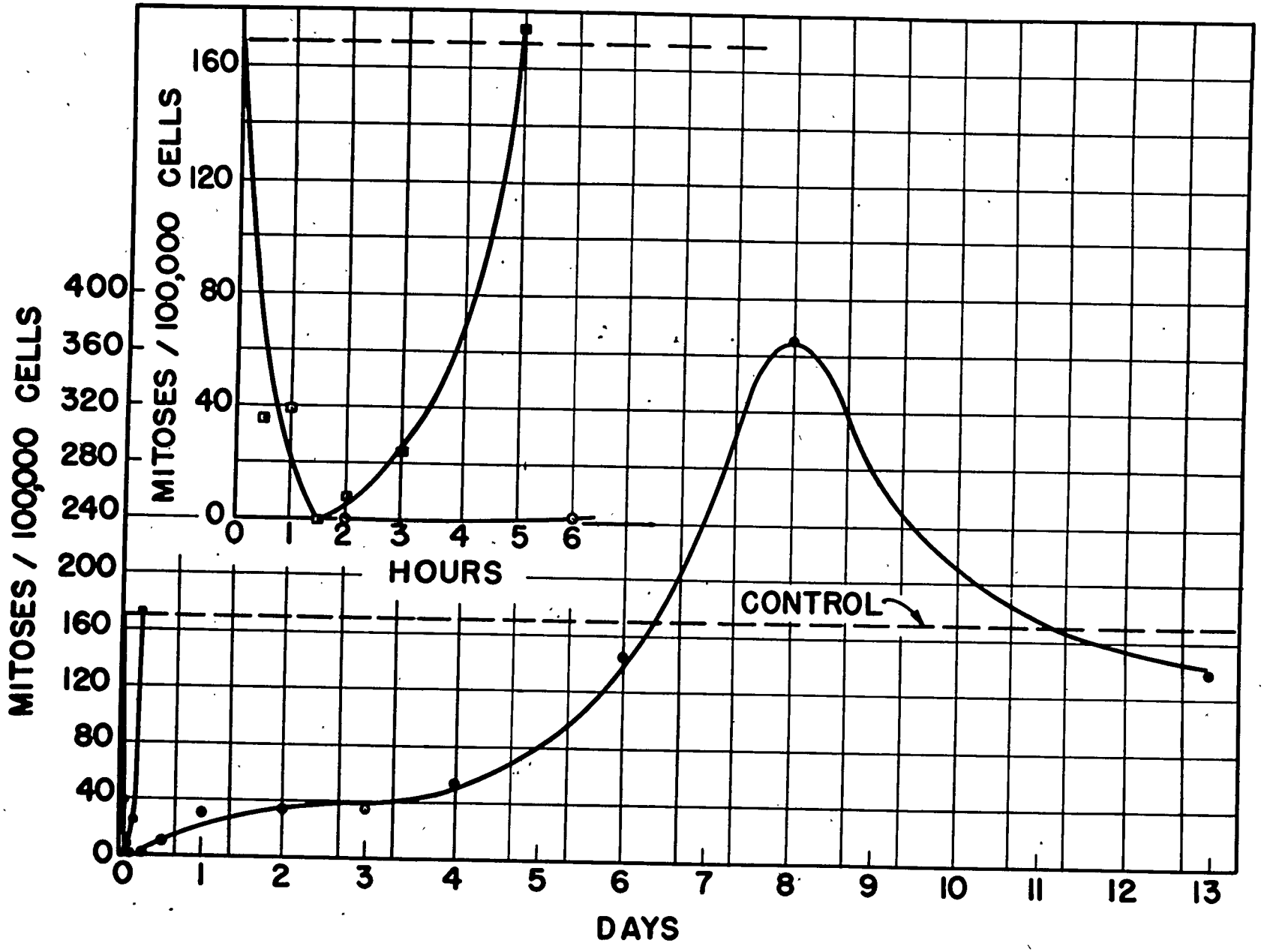
* With the technical assistance of Norma Lanter, Clare Morrison, Joan Thrap, and Julie Wellnitz. The writers gratefully acknowledge the personal help given them by Dean Robert A. Moore, Washington University, St. Louis, and Zola K. Cooper, University of Oklahoma School of Medicine, in organizing this experimental program.

REFERENCES

1. Cooper, Z. K., and Franklin, H. C. Anct. Rec., 78, 1-8 (1940)

2. Cowdry, E. V. A textbook of histology. Philadelphia; Lea & Febiger, 1938. Pp 530-531.

3. Hoepke, H. In Von Mollendorf's Handbuch der mikroskopischen Anatomie des Menschen, 1927, Bd. 3, Teil 1.


MITOSES / 100,000 CELLS
400
360
320
280
240
200
160
120
80
40
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DAYS
CONTROL
MITOSES / 100,000 CELLS
160
120
80
40
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0 1 2 3 4 5 6
HOURS

THE EFFECT OF X-RAYS ON THE MITOTIC ACTIVITY OF THE ADRENAL GLAND, JEJUNUM, LYMPH NODE AND EPIDERMIS OF THE MOUSE

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In the search for a suitable method of determining quantitatively the effects on mammalian tissue of sublethal doses of ionizing radiation, the following study of the mitotic indices of various mouse tissues following whole body irradiation was undertaken. It was hoped that by finding quantitative changes in the mitotic index of various tissues that it would be possible to use these changes as a measure of the relative effects of different types and different energies of ionizing radiation. The response of mitotic activity of four tissues was determined at intervals after exposures of groups of animals to doses ranging from 5 to 325 roentgens of 250 KV X-rays. The adrenal gland, lymph node, jejunum and epidermis were selected for this study because of their normally high mitotic activity and because of the simplicity of their structure which lends itself to the development of quantitative methods of counting mitoses. The effect on the epidermis at the 35 roentgen and 325 roentgen dosage level has been previously reported,(1) and is presented again to give continuity to the results reported in this article.

(1) Knowlton, N.P., Jr., Hempelmann, L.H. and Hoffman, J.G., The Effects of X-rays on the Mitotic Activity of Mouse Epidermis, SCIENCE 107, 625 (1948)

EXPERIMENTAL METHODS: All animals used in this experiment were CF_1 strain female

1. With the technical assistance of Norma Lanter, Clare Morrison, Joan Thrap and Julie Wellnitz.
2. This document is based on work performed at the Los Alamos Scientific Laboratory of the University of California under Government contract W-7405-eng-36, and the information contained herein will appear in Division V of the National Nuclear Energy Series (Manhattan Project Technical Section) as part of the contribution of the Los Alamos Laboratory.
3. The authors wish to acknowledge the assistance and advice of Gerold Tenney and his staff in the development of irradiation techniques and dosage measurements.

mice between 6-8 weeks of age from Gorworth Farms, Inc., New York. Only those mice were used which appeared entirely health after 10 to 14 days acclimation at this laboratory. Animals in the control groups were subjected to the same handling procedures as the irradiated mice.

The radiation was delivered by a 250 KV peak voltage X-ray machine at the rate of 50 roentgens per minute. No filters were used besides that inherent in the tube which was equivalent to approximately two millimeters of aluminum. The average energy of the X-rays delivered by this machine was 168 KV^{3}. The animals were contained in a flat lucite cage (1/32 inch wall thickness) during the exposure period. The lucite cage was placed on a thick lead plate to minimize backscatter of the incident radiation. The exact dosage delivered to each group of mice was determined by means of a 300 roentgen Victoreen ionization chamber placed at the center of the cage. It was shown that the variation in dosage delivered to the area occupied by the cage did not exceed eight percent. The results of this variation in dosage due to the inherent geometry of the tube and target was minimized for any group of mice by the free movement of the animals within this area.

Groups of irradiated and control animals were sacrificed at varying times after exposure. An attempt was made to choose those time intervals after exposure which would correspond to the minimum mitotic activity and to the period when the mitotic rate was returning to normal. The animals were killed by crushing the cervical vertebrae and tissues were removed in a definite order without delay. The ears were removed first, the inguinal lymph node was then dissected out, a segment of the jejunum distal to a point 2 centimeters below the pylorus was excised, and finally the right adrenal gland was removed. Approximately two minutes were required for the complete autopsy. Emphasis was placed on speed of removal of organs in order to avoid the modification of mitotic activity known to occur within a few minutes after death of an animal[2].

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(2) Thüringer, J.M. - Studies on Cell Division in the Human Epidermis. II. (a) Rate of Cell Division in the Prepuce; (b) Influence of Various Factors on Cell Divisions. Anat. Rec. 40, 1 (1928)

The adrenals, jejunum and lymph nodes were fixed in Bouin's fixative in all instances except in the first experiment in which the animals were exposed to 325 roentgens. At this dosage level the lymph nodes and jejunum were fixed in Zenker-acetic acid. All of the tissues were carried through routine histologic procedures and embedded in paraffin. All sections were cut 6 microns thick and were stained with Mayer's acid hematoxylin and an aqueous solution of eosin.

The epidermis was prepared by placing the ears in 1% acetic acid for 16 to 20 hours at 5° C. and then by blunt dissection separating the epidermis from the dermis in the manner described by several authors (3)(4)(5). The sheet of epithelium was stained with Mayer's hematoxylin and taken up through alcohol

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(3) Cowdry, E.V., A Textbook of Histology. Philadelphia: Lea & Febiger, 1938 pp. 530 - 531.

(4) Hoepke, H. - In Mollendorf's Handbuch der Mikroskopischen Anatomie des Menschen, 1927, Bd. 3, Teil 1.

(5) Baumberger, J.P., Suntzeff, V, and Cowdry, E.V. - Methods for the Separation of Epidermis from Dermis and Some Physiologic and Chemical Properties of Isolated Epidermis: Jour. Nat. Cancer Inst. 2, 413 (1942)

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solutions of increasing strength to absolute alcohol. The tissue was then cleared in methylsalicylate and xylol and mounted in Canada balsam.

In an effort to standardize the technique of counting mitotic figures a cell was considered to be in mitosis only between the time in prophase of elongation of the chromosomes before breakdown of the nuclear membrane and the time in telophase of complete separation of the cytoplasm of the daughter cells. This definition of mitosis was very satisfactory for all tissues except in the case of the epidermis in which the nuclear structures were distorted slightly by treatment with acid; therefore, the time of breakdown of the nuclear membrane was considered to be the beginning of mitosis.

The following techniques of measuring mitotic activity were devcloped to ascertain the number of cells in mitoses per unit area (or per crypt of Lieberkuhn) of the tissue section rather than per unit number of cells. This enables one to count in less time a greater number of mitoses and thereby reduce the overall statistical error. The statistical validity of the data is discussed under Experimental Results.

ADRENALS: The zonae glomerulosa and fasciculata of five serial sections of the adrenal gland cut from approximately the middle of the gland were searched thoroughly for cells in the process of division (6) (7) (8). Some of the mitotic figures were cut into two segments in the process of sectioning the tissue. Therefore, a record of halved mitoses were kept since the other portion of the particular figure could be seen in the corresponding area of the next section. When the total number of mitoses in the specified area had been determined, the microscopic image of the third of the five serial sections was projected at a standard magnification on a sheet of white paper and the outer boundary of the zona glomerulosa and the inner margin of the zona fasciculata were carefully outlines. The area of two zones at this magnification was determined by the use of a planimeter. In order to express the final results

(6) Nathanson, I.T. and Brues,A.M.- The Effect of Testosterone Propionate Upon the Mitotic Activity of the Adrenals in the Intact Immature Female Rat: Endocrinology, 29, 397 (1941).
(7) Blumenthal,H.T. - Aging Processes in the Endocrine Glands of the Guinea Pig: 1. Influence of Age, Sex and Pregnancy on Mitotic Activity and Structure of the Thyroid, Parathyroid and Adrenal Gland - Arch. Path. 40, 264 (1945).
(8) Blumenthal,H.T. - The influence of Time of Feeding on Periodicity in Activity in Thyroid and Adrenal Gland of Normal Male Guinea Pigs: Endocrinology 27, 481 (1940)

in mitoses per unit area the total number of mitotic figures observed on all five sections was divided by the total area of the projected image.

JEJUNUM: The sections of jejunum were cut perpendicular to the mucosa surface so that the epithelium of the villi and the crypts of Lieberkuhn was continuous. Since mitoses are almost entirely confined to the epithelium of the crypts, only this part of the intestine was examined. The mitotic figures in at least 200 crypts per animal were counted and the results are expressed in terms of mitoses per 100 crypts.

LYMPH NODES: The number of mitoses per standard area as outlined by a Whipple disc at approximately 900 diameters magnification was used to quantitate the mitotic activity of lymph nodes. At least 100 fields were counted by examining adjacent areas of a section of lymph note. No attempt has been made to translate mitoses per hundred fields into mitoses per 100,000 cells since there is a definite loss of cellularity, particularly after the higher doses of irradiation. The results expressed as mitoses per 100 fields can also be interpreted as mitoses per unit volume of lymph node.

EPITHELIUM: The technique used in counting mitoses in the epithelium of the ear has already been described[1] and the mitotic activity of the epidermis has been used by various authors in experimental work[9] [10] [11] [12]. In brief, it con-

(9) Cooper, Z.K. and Franklin, H.C.-Mitotic Rhythm in the Epidermis of the Mouse: Anat. Records 78, 1 (1940).

(10) Blumenfeld, C.M. -Periodic Mitotic Activity in the Epidermis of the Albine Rat: Science 90, 446 (1939).

(11) Bullough, H.F.-Cyclical Changes in the Skin of the Mouse During the Oestrous Cycle: Endocrinology 3, 280 (1943).

(12) Blumenfeld, C.M. -Normal and Abnormal Mitotic Activity (1) Comparison of Periodic Mitotic Activity in Epidermis, Renal Cortex, and Submaxillary Gland of the Albino Rat: Arch. Path. 33, 770 (1942)

sists of counting the mitotic figures in at least 200 fields outlined by the Whipple disc in a homogeneous sheet of epithelium. It is possible to choose areas of skin where there are no hair follicles so that the variation in the number of cells from field to field is merely statistical in the area enclosed by a given Whipple disc for animals of a certain age and strain. It has been shown that there is no significant decrease or increase in cellularity of the epidermis at dosages as high as 325 roentgens. Thus, the mitotic index can be expressed in mitoses per 100,000 cells.

EXPERIMENTAL RESULTS: When this experimental program was begun, it was impossible to foresee which radiation levels and what time intervals after irradiation would give the most significant results. Initially it was decided to give one group of animals 325 roentgens, this being approximately two-thirds the median lethal dose of X-rays of this energy administered in the manner described above to mice of this age and strain. The delay in cell division at this dosage level was so extreme that a pilot experiment was undertaken using a smaller group of animals exposed to 75 roentgens. The results were still impressive and it was decided to go to lower dosage ranges without improving statistically the results of the above studies. The numbers of animals used and time intervals after exposure were modified as the study progressed. The experimental results of each exposure level are given below:

(a) 325 roentgens: 40 mice were exposed to 325 roentgens and twenty mice were used as controls in this experiment. Four irradiated and two control animals were sacrificed at two, six and twelve hours, and at one, two, three, four, six, eight and thirteen days after exposure. The change in mitotic activity of the tissues is shown in Graphs 1, 2a, 3 and 4. Statistical considerations of the data indicate that the changes are unquestionably real. Each point on the curves represent the examination of the following minimum number of mitoses and cells per points

	No. Mitoses Counted Per Point	No. Fields, Unit Areas or Crypts per Point	Total No. Cells Examined per Field Unit Area or Crypt	Total No. Cells Examined Per Point
Skin	3-600	800-1600 Fields	205	160,000
Lymph Node	2-275	400-800 Fields	310	100,000
Adrenal	0-194	1400 Unit Area	130	150,000
Jejunum	44-900	800 Crypts	100	80,000

In the case of the skin, there is no change in the number of cells per field at any time interval after irradiation.

In the case of the lymph node, however, there is a decrease in the number of cells following irradiation (Graph 5). If the results are expressed in terms of mitoses per cell rather than in terms of mitoses per unit area, there would not be as much depression below normal as there is shown in Graph 4. Hence, this method of expressing depression of mitotic activity of lymph nodes exaggerates this change, while in the skin there is no such false accentuation of the change of mitotic rate.

(b) 75 roentgens: A group of sixteen mice received 75 roentgens while ten were used as controls. Four irradiated animals were sacrificed at one and one-half, two, four, and seven hours. The mitotic activity of three tissues was determined. Four controls were used for determination of the mitotic index at one and one-half hours, but only two controls were used per point thereafter. The modification in mitotic indexis shown in Graphs 1, 2a and 3. Since the time interval between the first and last autopsies was only 5-1/2 hours, the diurnal variation was not taken into account.

The controls were lumped into one average normal for each tissue. It is evident that the curve of mitotic activity shows the drop produced by the X-rays but was not carried out long enough to show a possible over-shooting or normal mitotic index by the recovering tissue. It should be pointed out that later data, particularly in the 35 and 15 roentgen ranges, indicate that the one and a half hour point after exposure probably does not indicate the true minimum point of activity of these tissues.

The following are the data for the minimum number of mitoses and cells examined per point on the graphs:

	No. Mitoses Counted Per Point	Unit Areas or Crypts Per Point	Total No. Cells Examined Per Field Unit Area or Crypt	Total No. of Cells Examined per Point
Skin	4-176	400 fields	205	80,000
Adrenal	6-136	1000 unit areas	130	130,000
Jejunum	186-800	400 crypts	100	40,000

One should note that the fewer number of cells examined at this dosage level increases the chance of statistical variation. For the marked changes noted in these curves, however, it is evident that this is a real and not a statistical variation.

(c) 35 roentgens: Fifty mice were exposed to 35 roentgens and were sacrificed in groups of five at one-half, one, one and one-half, two, three, five, seven, nine, thirteen and sixteen hours. Tissues of five control animals were obtained at one-half, two, five, nine, thirteen and sixteen hour intervals. The data from this experiment are included in Graphs 1, 2b, 3 and 4. The data on the epidermis and the adrenal gland have been plotted only up to the 7 hour point since they show no significant deviation from the normal thereafter. The mitotic activity of the jejunum and lymph node has been graphed for only the first five hours for the same reason. It is notable that at this dosage level only the epidermis reaches zero mitotic activity, while the lymph node does not even drop to 50% of normal.

Each point on the curves at this dosage level represent the following minimum figures:

	No. Mitoses Counted Per Point	No. Fields, Unit Areas or Crypts Per Point	Total No. Cells Examined Per Field, Unit Area or Crypt	Total No. Cells Examined Per Point
Skin	0-289	500 fields	205	100,000
Lymph Node	150-262	1000 fields	310	300,000
Adrenal	37-222	1500 unit areas	130	200,000
Jejunum	223-623	300 crypts	100	30,000

(d) .5, 15 and 25 roentgens: Groups of thirty mice were exposed to 5, 15 and 25 roentgens. Ten irradiated animals from each exposure group and ten controls were sacrificed at one, one and one-half and two hours. The thirty control animals thus served as controls for all three exposure groups. In all three irradiated groups the epidermis was examined and the jejunum, lymph nodes and adrenal glands were studied only in the animals receiving 15 roentgens.

The response of mitotic activity of the epithelium at each dosage level expressed in terms of percent of normal activity is shown in Graph 6. (The data from the first 2 hours of 35 roentgen experiment are also included in this graph in terms of percent of normal).

The change in the mitotic activity of the adrenal gland is included in Graph 2b.

The mitotic index of the lymph nodes and the adrenal glands showed no significant change from normal with exposure to 15 roentgens and graphic presentation is not made.

The minimum number of mitoses and cells examined per point are show below:

	No. Mitoses Counted Per Point	No. Fields or Unit Areas Per Point	Total No. Cells Examined Per Field or Unit Area	Total No. Cells Examined Per Point
Skin	44-230	1400 fields	205	280,000
Adrenal	72-383	3000 unit areas	130	390,000

DISCUSSION:

It has been known for a long time that ionizing radiation causes delay in division of cells which have just entered mitosis[13][14]. It has been shown

(13) Henshaw, P.S. - Action of X-rays on the Gametes of Arbacia Puntulata: Amer. J. Roentg. 43, 917 (1940)

(14) Carlson, J.G. - X-ray Induced Single Breaks in Neuroblast Chromosomes of the Grasshopper: Proc. Nat. Acad. Sc. 27, 42 (1941)

by direct observation that irradiation of grasshopper neuroblast cells in early prophase produces changes in the chromosomes which appear to be in the backward direction toward the intermitotic stage rather than in forward direction of completion of mitosis[15]. Younger cells in the intermitotic stage which precedes

(15) Carlson, J.G. - Effects of X-radiation on Grasshopper Chromosomes: Cold Spring Harbor Symp. 9, 104 (1941)

the radiation-sensitive stage of prophase are able to enter mitosis at the proper time in their lives without a period of delay. Cells already in mitosis, past the radiation sensitive stage of early or mid-prophase, are able to complete mitosis although perhaps at a slower rate.[13] Such a delay in cell division is known to occur at low dosage levels (4 roentgens)[15]. This may be the reason for the fall in mitotic activity seen in the Graphs 1, 2, 3, etc. The overshooting of the normal mitotic activity noted particularly in the epidermis curve at 325 roentgens has been described before[16].

(16) Canti, R.G. and Spear, F.G. - Effectof Gamma Rays on Mitosis in Vitro: Proc. Roy. Soc. B, 105, 93 (1929)

and has been referred to as an "overcompensation" phenomenon. It has been explained as the result of a piling up of mitotic figures of two sources: (1) cells in which the division processes had been delayed by radiation until this time and (2) younger cells that were unaffected by radiation and which would normally have divided at this time without irradiation. Since cells damaged by ionizing radiation do not die until they attempt to divide (17)(18) (except in the higher dosage

(17) Lasnitski, I. - Effects of X-rays on Chick Tissue in Cultures: Brit. J. Radiol. 16, 61 (1943)

(18) Lasnitski, I., - Effects of X-rays on Chick Tissues in Culture: Brit. J. Radiol. 16, 137 (1943)

range), one can see that such an explanation demands that all cells must enter mitosis. This means that at the lower doses of radiation the amount of "overcompensation" should be equivalent to the depression of mitotic activity unless there is a marked change in time of mitosis or the doubling time of the cell.

The exact mechanism by which radiation affects mitosis is not known. It has been postulated that this effect is related to the interference with the transfer of nucleic acids from cytoplasm to nucleus (19).

(19) Mitchell, J.S. - Disturbance of Nucleic Acid Metabolism by X- and Gamma-Rays: Brit. J. Expt. Path. 23, 285 (1942)

Hance (20) has shown that when limited portions of an animal are exposed to

(20) Hance, R.T. - Mitosis in Hair Follicle Cells of Mice Following X-Radiation: J. Morph. 66, 409 (1940)

radiation, the mitotic activity in unexposed tissues is modified, although not to the same degree as the tissues of the same animal which had been irradiated directly. This would seem to show that there is an indirect, as well as a direct, effect of ionizing radiation on mitotic activity.

There are a number of features of the experimental results reported in this paper which are difficult to explain in terms of what is known about the action of ionizing radiation on cell division. Those features which deserve discussion are: (1) the apparent absence of the "overcompensation" phenomenon at lower dosage levels in epidermis, lymph node, and jejunum; (2) the abortive rise in the mitotic activity of the adrenals and jejunum of animals receiving 325 roentgens; and (3) the lack of correlation between the effect of radiation on the mitotic activity and the tissue damage. Each of these points will be discussed briefly.

It is impossible to make a definite statement about the "overcompensation" of mitotic activity seen in the tissues until the curves have been confirmed in greater detail using observations on more animals at more frequent time intervals after exposure and for more prolonged periods. As pointed out above, if there is a true overcompensation phenomenon, all cells should eventually go through mitosis at the lower dosages and the area above the normal curve should be equal to the area below this curve. Thos does not seem to be the case in epithelium and lymph nodes of animals exposed to 35 roentgens, both curves of which are based on enough experimental points to appear reasonably accurate in the desired ranges. The adrenals of the same animals do show changes in mitotic activity which could well be explained by the overcompensation phenomenon. Also, there is a definite overshooting of normal mitotic activity of the epithelium of animals exposed to 325 roentgens. Since this respohse does not appear for a matter od ays, it seems possible that other factors, such as skin erythema, with consequent increased metabolism of this tissue may play a role in the production of the marked increase in mitotic rate.

The abortive rise in the mitotic index of jejunum and adrenals of animals exposed to 325 roentgens id difficult to explain. Since it was not observed

at other dosages, it may represent a spurious observation which will not be confirmed. On the other hand, its observation in two tissues seems to attest to its real character. If this peak is real, its time of appearance during the depth of depression of mitotic activity would seem to suggest that more than one factor is responsible for the prolonged depression of mitotic activity. It is conceivable that the complementary action of different effects such as the direct and indirect effect previously reported by Hance (20) could be responsible for a curve of this nature if the time of the maximum action of each occurs at a different time.

Probably the most surprising feature of the data is the great effect of radiation on mitotic activity of the more radioresistant tissues. Thus, the lymphoid tissue and intestinal epithelium which are among the most radiosensitive tissues in the body show less interference of mitotic activity following exposure to X-rays than do the less radio-sensitive skin and adrenal gland. The term radiosensititity as used above is a concept based on the histological response of tissues to radiation. Tissues which are radiosensitive show more histological evidence of tissue damage than do radio-insensitive tissues. If our concept of how radiation affects mitotic processes is correct, one would expect them to show more interference with mitotic activity following radiation exposure than less rapidly growing tissues since more cells are in the mitotic stage (or more correctly, the radiation-sensitive pre-mitotic stage) at any given moment. Does the lack of correlation between tissue damage and interference with mitotic activity caused by radiation mean that these processes are not necessarily directly proportional?

There is a way of interpreting these data which seems fit in with the present concept of the mechanism of radiation action. One must remember two facts for which there is good experimental evidence: (a) all cells past late prophase

at the time of exposure complete cell division; and (b) cells in the radiation-sensitive pre-mitotic stage at the time of exposure to the lower dosages of ionizing radiation undergo mitosis after a delay. With these facts in mind, the curve of response of mitotic activity to radiation can be broken down into two compenents (Graph 7): Component 1 is made up of cells which were in mitosis at the time of exposure and are completing cell division. The time, T, at which this curve reaches zero is dependent upon the time of the mitotic period allowing, of course, for the fact that radiation may produce a lengthening of this period. Component 2 is composed of (1) cells, the mitotic process of which has been delayed by exposure to radiation and, (2) cells which were too young to be affected by the exposure. The time, R, will be a measure of the radiation-sensitive phase for the cells at this dosage. If recovery begins before Component 1 falls to zero, the dip in the resultant curve will not fall to zero. The socner the recovery begins (or the shorter the radiation sensitive phase), the less will be the dip in the curve of mitotic activity. It is reasonable to assume that a short-lived cell, such as a lymphocyte with a life span of hours, will compress its metabolic activities into a shorter time than will an epithelial cell which lives for several weeks. The radiation sensitive phase of a lymphocyte may be quite short in comparison to that of an epidermal cell. If this is true, than the mitotic activity of the lymphoid tissue and jejunal epithelium recovers sooner than that of the epidermis and adrenal gland and the dop in the curve of mitotic activity is not as marked. Such an explanation is admittedly hypothetical but, at least does not deny the relationship between mitotic activity and ultimate tissue damage.

CONCLUSIONS & SUMMARY:

1. The mitotic activity of the adrenal gland, lymph node, jejunum and epidermis of the mouse respond quantitatively to irradiation with 250 KV X-rays.

2. There is a lack of correlation between the sensitivity of the mitotic activity to ionizing radiation and the radiosensitivity of different tissues. It appears that the tissues which are most radiosensitive show less interference with mitotic activity than those tissues that show less histologic response to irradiation.

3. The "overcompensation" phenomenon seen in the response of the mitotic index to irradiation in several tissues has been discussed in relation to possible mechanism.

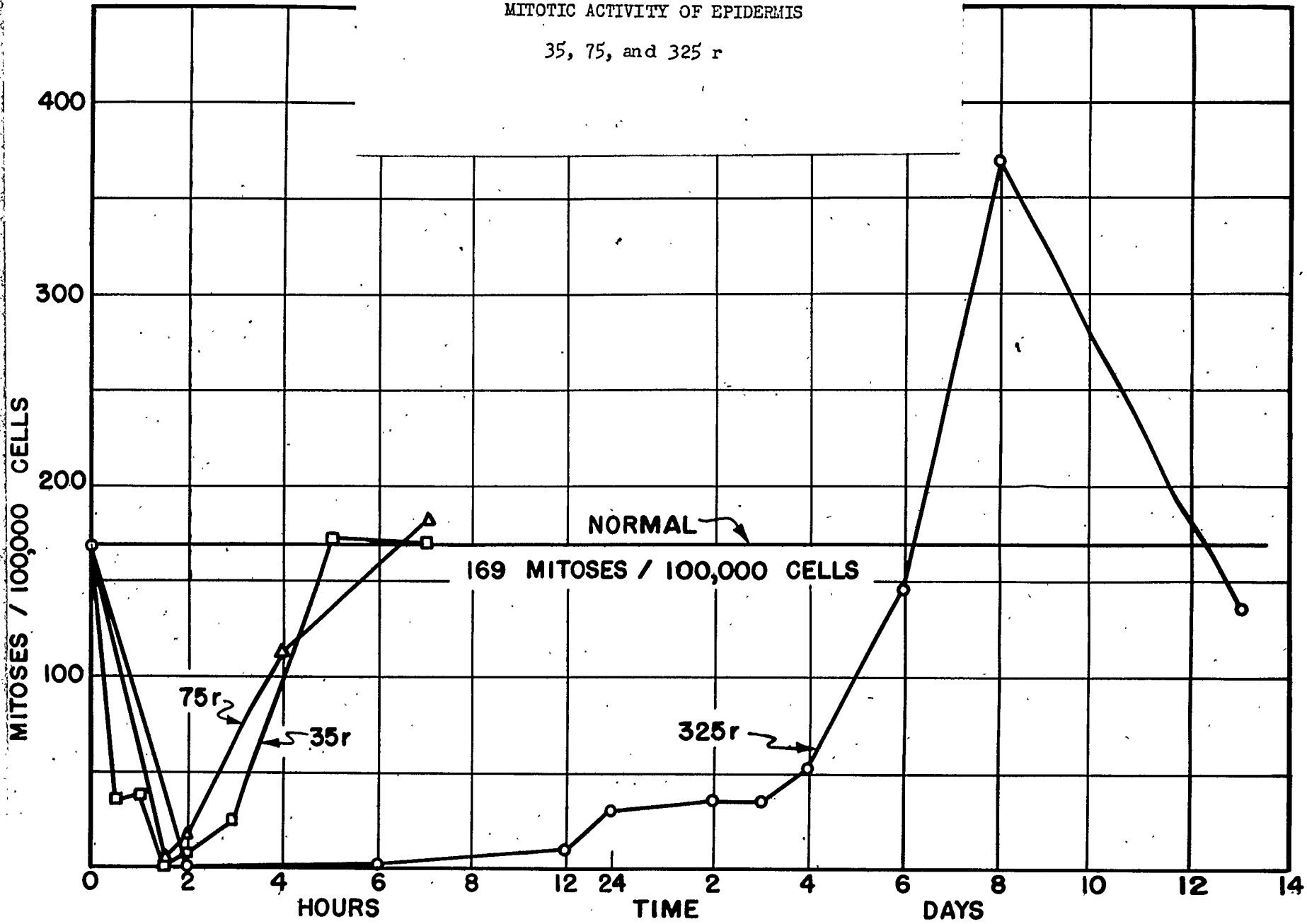
4. An attempt has been made to explain the abortive rise in the mitotic indices of the adrenal gland and jejunum by the use of the direct and indirect concept of radiation injury.

5. Although it is impossible to evaluate the fundamental significance of the change in mitotic activity after irradiation, it should be possible to use the depression as a measure of the biological effects of different types and different energies of ionizing radiations.

GRAPH I

MITOTIC ACTIVITY OF EPIDERMIS

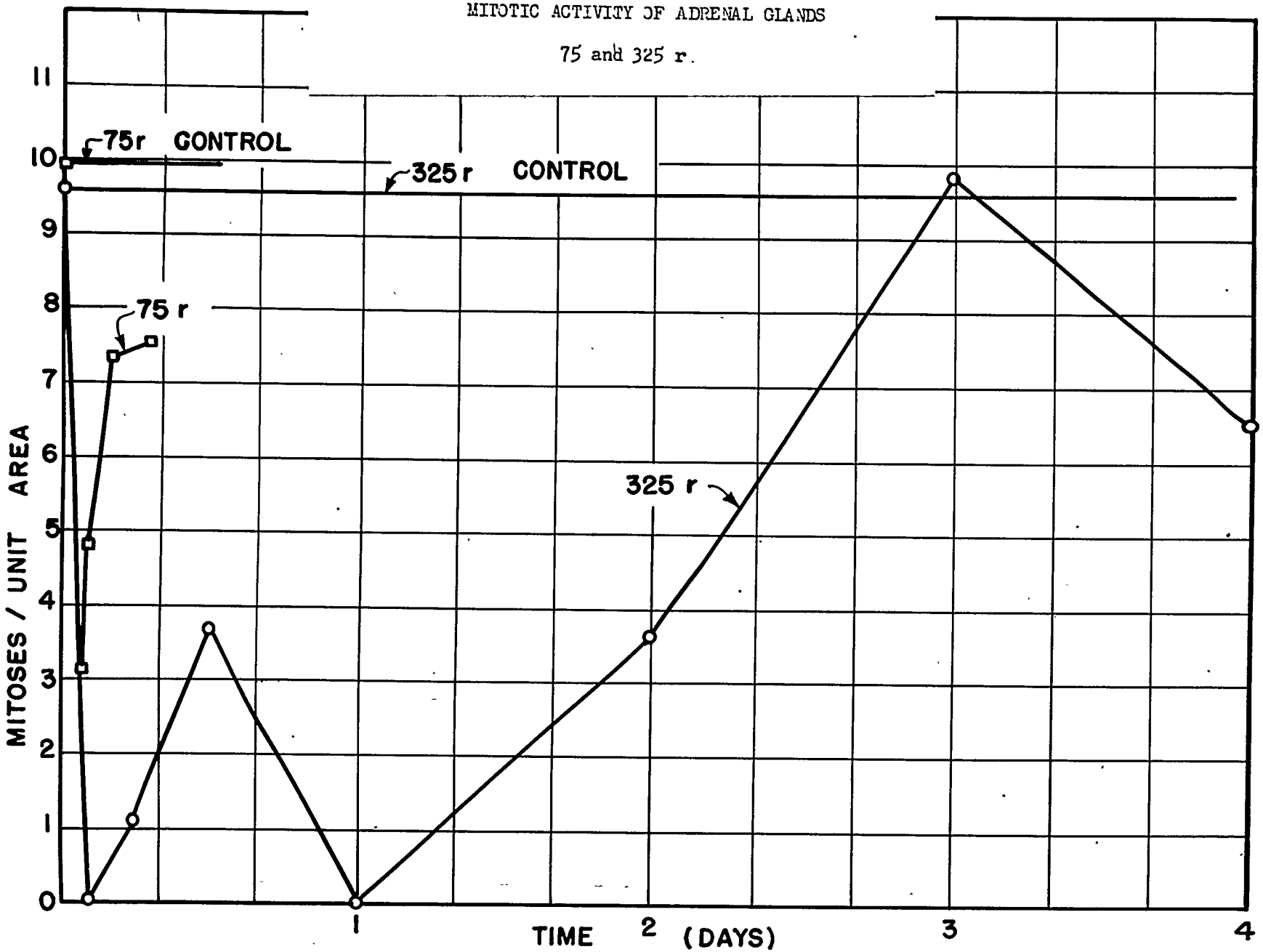
35, 75, and 325 r



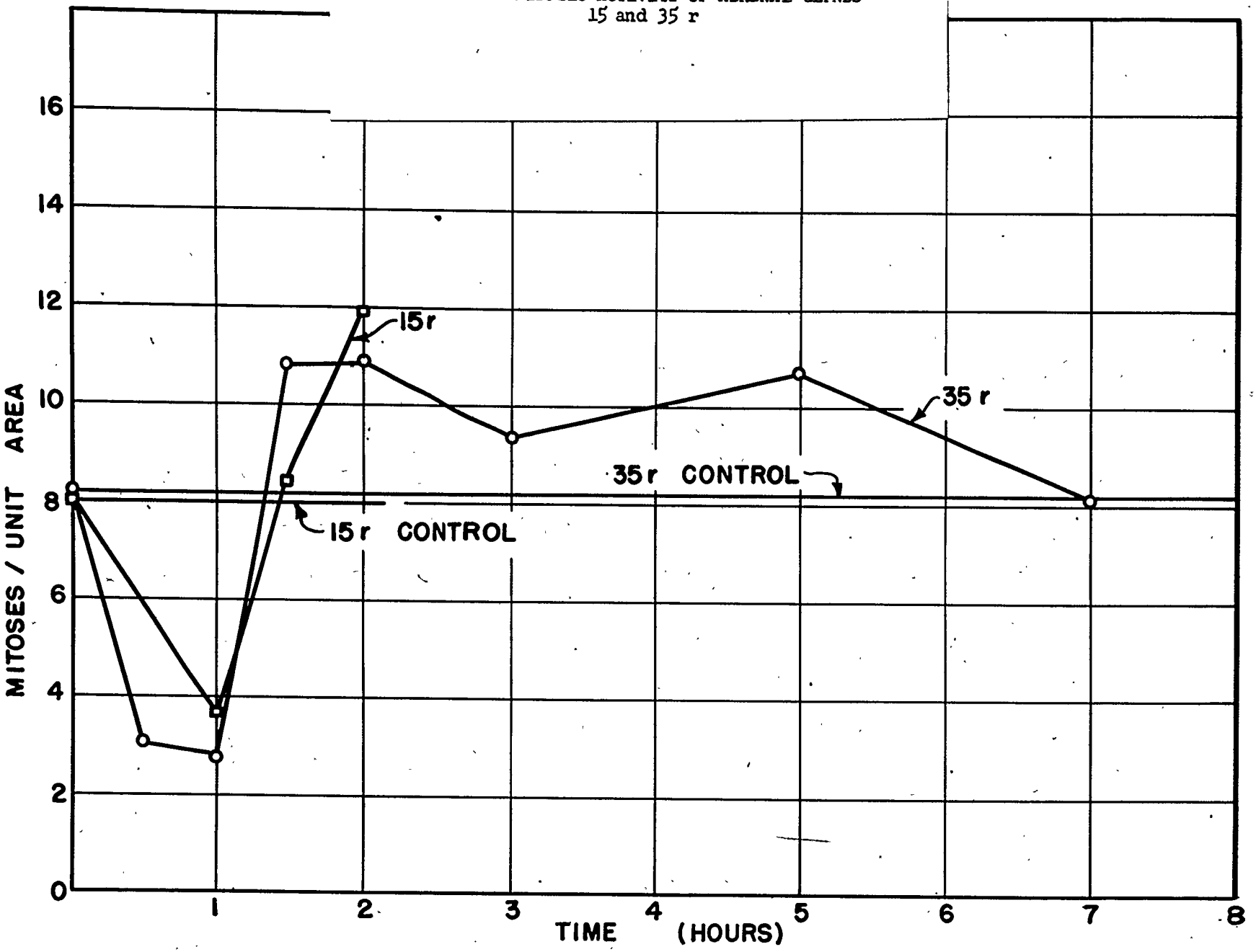
GRAPH 2a

MITOTIC ACTIVITY OF ADRENAL GLANDS

75 and 325 r.

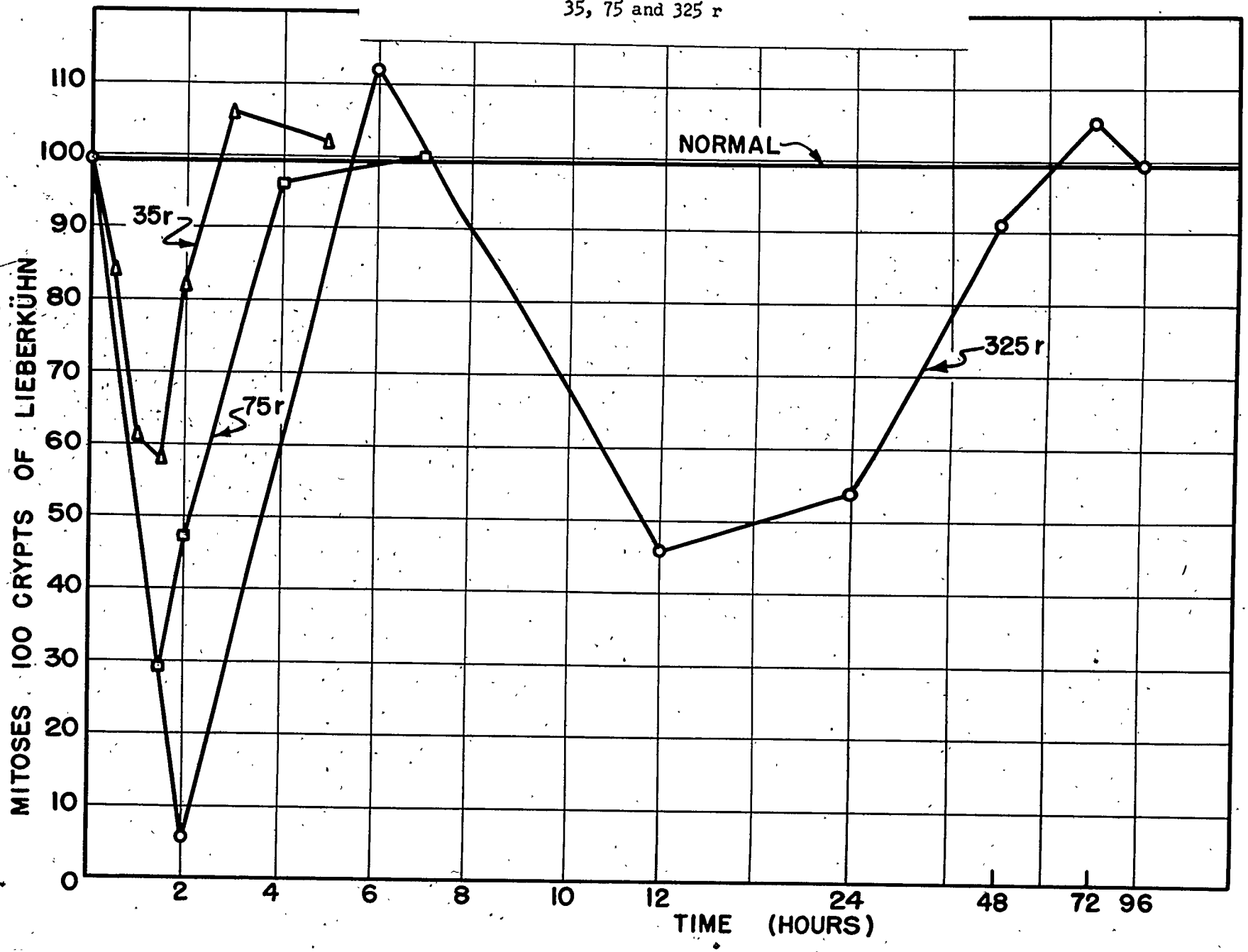


GRAPH 2b
MITOTIC ACTIVITY OF ADRENAL GLANDS
15 and 35 r



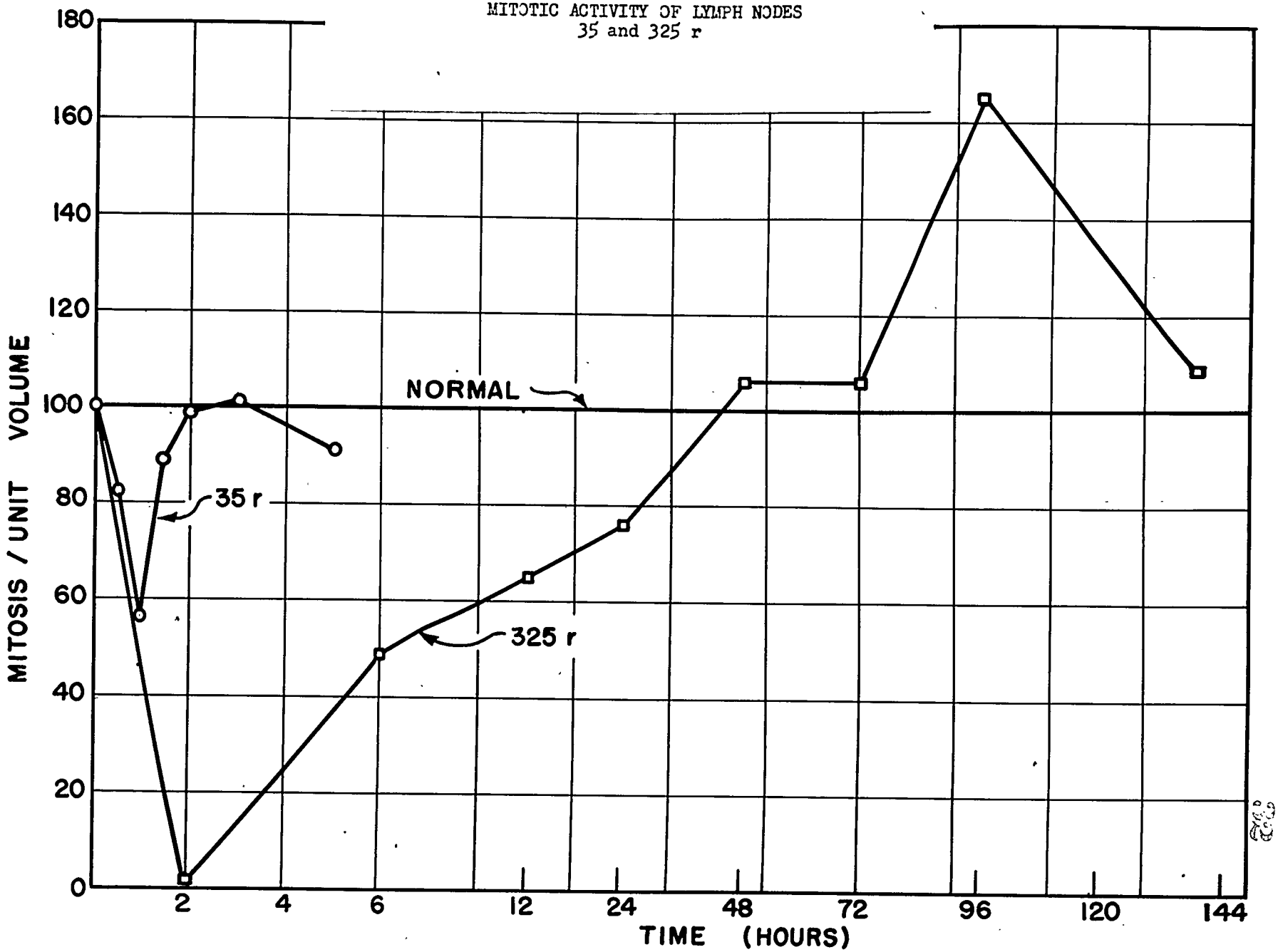
GRAPH 3

MITOTIC ACTIVITY OF THE JEJUNUM
35, 75 and 325 r



GRAPH 4

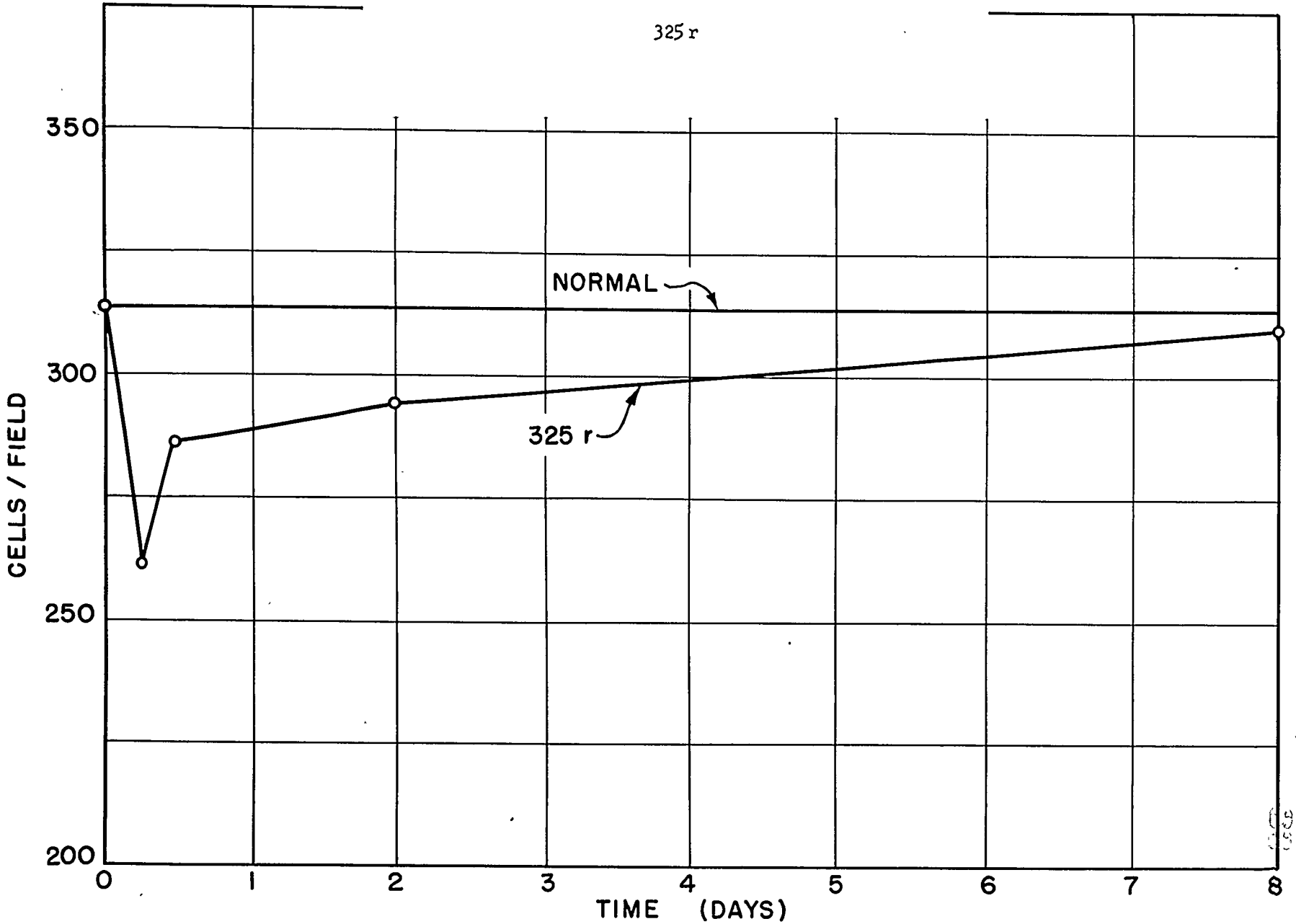
MITOTIC ACTIVITY OF LYMPH NODES
35 and 325 r



GRAPH 5

CHANGE IN CELLULARITY OF THE LYMPH NODES

325 r

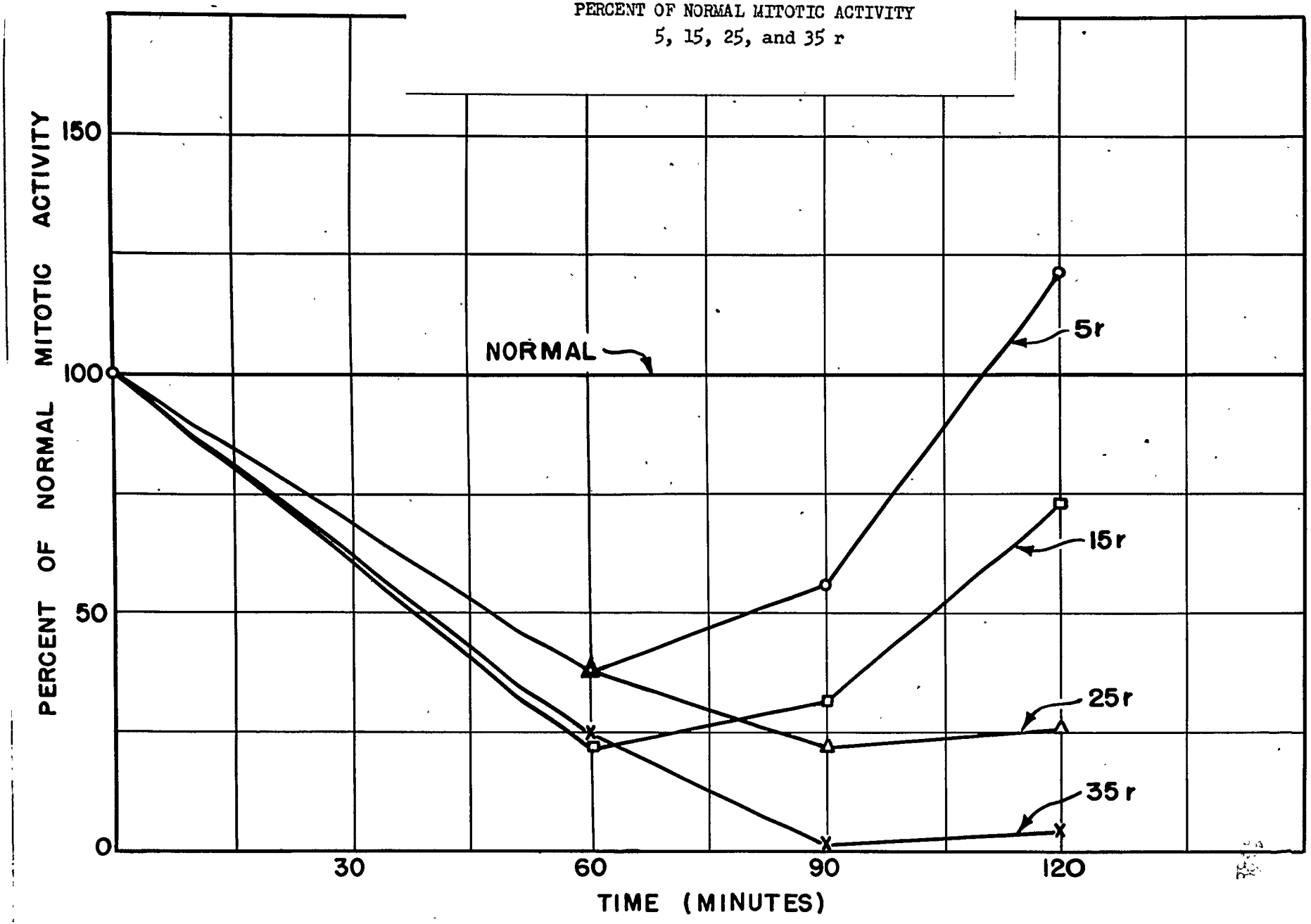


GRAPH 6

EPIDERMIS

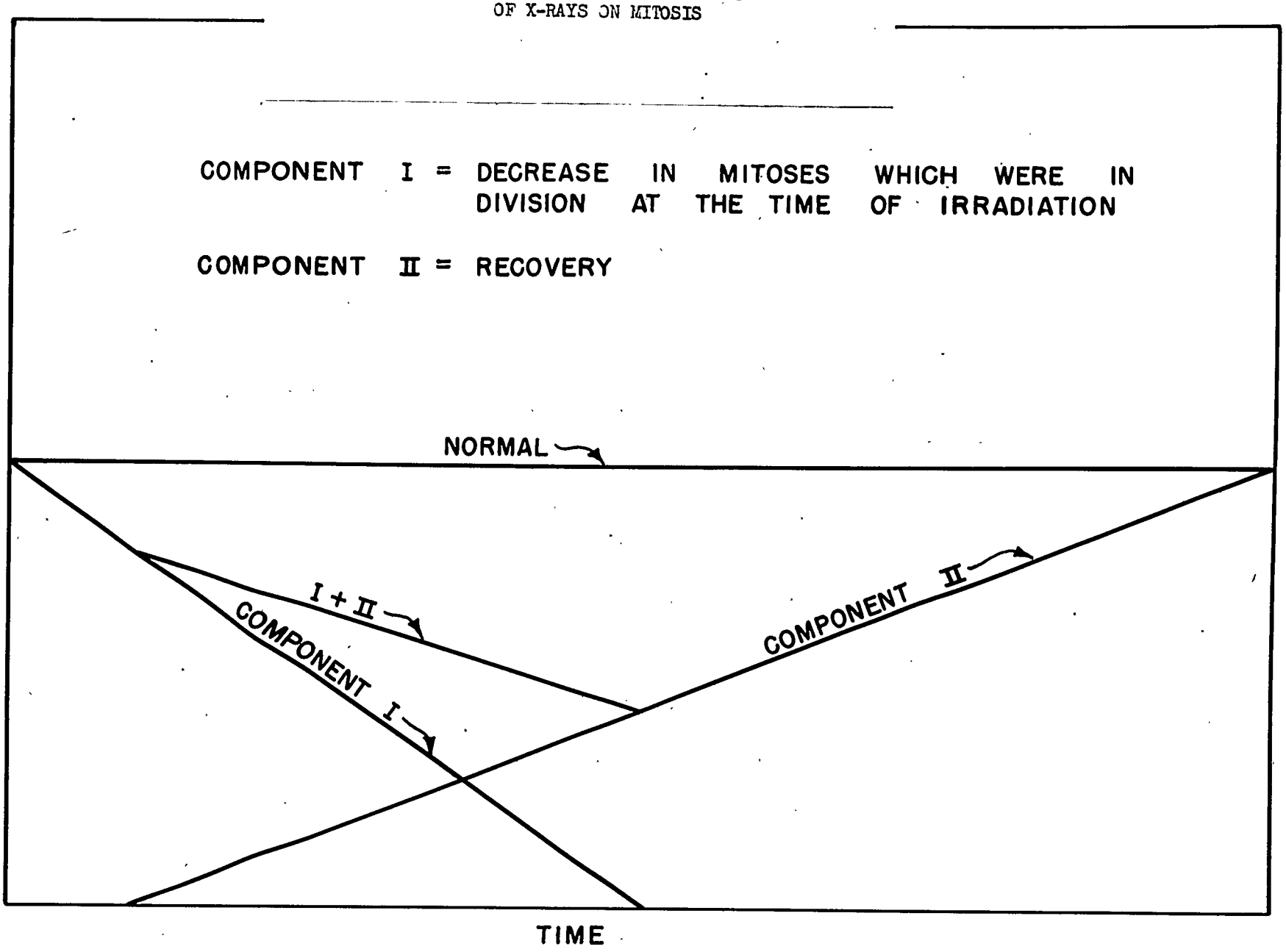
PERCENT OF NORMAL MITOTIC ACTIVITY

5, 15, 25, and 35 r



GRAPH 7

THEORETICAL CURVE OF EFFECT
OF X-RAYS ON MITOSIS



(Contribution from the Los Alamos Scientific Laboratory of
the University of California, Los Alamos, New Mexico)

THE EFFECT OF LETHAL DOSES OF X-RAYS ON CHILLED AND THYROIDECTOMIZED ANIMALS*

Louis H. Hempelmann, Teodore T. Trujillo and Norman P. Knowlton, Jr.

During the past few years much information has been accumulated on the effects of ionizating radiation on living tissues. Despite this, however, there has been only a limited amount of progress in the treatment of radiation sickness. Rutin and antiheparin substances have been found to protect specific systems to some degree and chilling of animals has been shown to offer some protection from the lethal effects of radiation. In view of the latter results, an effort has been made in this laboratory to repeat and extend the study of the effects of chilling on the lethal effects of X-rays and to determine whether or not the lowering of the basal metabolic rate by thyroidectomy will give similar protection.

Five separate experiments were carried out using mice and rats as experimental animals:

1. Chilling of rats exposed to 1000 r. of X-ray.
2. Chilling of mice exposed to 525 and 625 r. of X-ray.
3. Chilling of new born rats exposed to 1000 r. of X-ray.
4. The effect of environmental temperature on the lethal effects of X-ray on mice.
5. The effect of lethal doses of X-ray on thyroidectomized rats.

CHILLING OF ADULT RATS:-- Wistar strain albino rats were exposed to 1000 roentgens of X-ray at a rate of 150 roentgens per minute. When chilling was done, nembutal or magnesium sulfate was used to facilitate the lowering of the body temperature. The magnesium sulfate was injected as a 5.0% solution using 100 mgm. of drug per 100

* This document is based on work performed under Contract No. 7405-eng-36 for the Atomic Energy Commission.

grams of body weight. When nembutal was used 4 mgm. of the drug per 100 grams of body weight were injected as a 0.6% solution. The drugs were injected intraperitoneally. There was some difference in the results obtained with the two drugs, but the variations were felt to fall within the error of the experiment. One-third of the animals received no drug or chilling; one-third was injected with magnesium sulfate or nembutal but were not chilled, and one-third received one of the drugs and was chilled. Of this latter group, half was chilled after exposure and half was chilled before and after exposure.

The body temperature of the animals was lowered by placing them in a special refrigerator at a temperature ranging from 5 to 8° C. Those animals which were chilled only after exposure had body temperatures of less than 30° C. for about three hours. The average minimum body temperature was 22° C. In the group of animals which were chilled before X-ray exposure, the body temperatures were lowered to 30° C. or less before being irradiated. After being exposed to X-rays the body temperatures were kept below 30° C. for at least two and a half hours with an average minimum temperature of 22° C. with a range of 18 to 26° C.

The animals which were not chilledwere placed in a Fisher oven at a temperature of 35 - 38° C. during the same period that the other groups were chilled. The rectal temperatures of each individual animal were followed during the process of chilling or while being kept in the Fisher oven. After the above procedures the animals were kept for 30 days and the mortality rate observed.

Table I shows the results obtained from the above experiment. In the group receiving magnesium sulfate there was a slightly greater survival of the chilled group but in the animals injected with nembutal this was reversed. The average percent 30 days survival for all of the animals receiving 1000 roentgens and no drug or chilling was 20%; for those receiving 1000 roentgens and one or the

other drugs 24.8%, and for all chilled animals 22.5%. Thus, there was no significant difference between the three major groups of animals.

CHILLING OF ADULT MICE:-- CF_1 strain albino mice 7 to 8 weeks of age were used in this experiment and exposed to either 525 or 625 roentgens. One half of the animals were chilled in the same manner as described in the previous experiment using adult rats and the other half served as controls receiving only X-ray. One-half of the chilled animals were chilled before and after X-ray exposure and the other half were chilled only after exposure. In this experiment there was no group of animals receiving drug and no chilling, as was done in the previous experiment. The dosages of magnesium sulfate and nembutal given intraperitoneally were as follows: magnesium sulfate, 14 mgm. in 0.7 cc. of distilled water per animal and nembutal, 1 mgm. in 1.0 cc. of distilled water per animal.

In the group of mice which was chilled before and after irradiation, body temperature was lowered to 30° C. or less before X-ray exposure. After X-radiation the body temperatures of all chilled animals were kept below 30° C. for approximately two hours. The average minimum temperature was about 21° C. with a range from 15° to 25° C. The unchilled group was kept at room temperature. Individual animal temperature changes were not studied but random rectal temperature measurements were made by means of a nichrome-advance thermocouple during the chilling period.

The results of this experiment are shown in Tables IIa and IIb. At the 525 roentgen dosage level there is a 3.4% increase in the 30 day survival with chilling and a 9.3% increase with the 625 roentgen exposure. These differences in the chilled and unchilled animals do not appear to be significant.

CHILLING OF NEW BORN RATS:-- Wistar strain albino rats 12 to 48 hours of age were used in this experiment. Each litter used in this experiment was divided into two groups. One group received 1000 roentgens of X-ray and the other group was chilled as well as irradiated. The chilling was done in three ways; immediately before X-ray exposure, immediately after X-ray exposure, or two hours after irradiation. The chilling was accomplished by placing the animals in the ice compartment of a refrigerator at a temperature slightly below 0° C. The body temperatures were lowered in 15 to 20 minutes to 2 to 5° C. as measured orally by means of a thermocouple. After chilling, all animals were labeled and returned to their respective litters.

The results of the experiment are shown in Tables IIIa, IIIb, and IIIc. All animals died inside of nine days except in the group chilled before exposure. In this group two animals survived the thirty day period. At the ninth day 33 of these 74 chilled animals were still alive and at the eleventh day 19 were still surviving.

THE EFFECT OF ENVIRONMENT TEMPERATURE ON THE LETHAL EFFECTS OF X-RAY ON MICE:-- 375 CF_1 strain mice 6 to 8 weeks of age were exposed to 675 roentgens of X-ray and divided into three groups of 125 animals each. The animals were placed in a special cabinet with compartments kept at 0-10° C., 18-24° C., and 36-37° C. The animals were fed and watered in their respective compartments for a period of thirty days.

The results of this experiment are shown in Table IV. None of the group kept at 0-10° C. survived the 30 day period, the last animal in this group having died at the 23rd day. At 18-24° C. 4.8% of the animals survived the 30 day period and 12.8% survived at 36-37° C.

THE EFFECT OF LETHAL DOSES OF X-RAY ON THYROIDECTOMIZED RATS:--Seventy-seven mature rats (37 males and 40 females) were thyroidectomized. After a post-operative recovery period of about one month 40 thyroidectomized (20 males and 20 females) and 40 normal rats of similar age were exposed to 1000 roentgens of X-ray. The animals were observed for 30 days and upon the death of a thyroidectomized animal a post-mortem examination was made to determine whether or not there had been thyroid regeneration. In the 28 thyroidectomized animals examined seven had a small portion of one thyroid lobe present.

The remaining 37 thyroidectomized rats (17 males and 20 females) and 37 controls of the same sex distribution were treated in the same manner as the above 80 animals except the dosage of X-ray was lowered to 800 roentgens. Post-mortem examination of the 24 thyroidectomized animals that died showed residual thyroid tissue on one side in 4 animals and a small amount of thyroid tissue on both sides of the neck in one animal.

The results of the above experiment are shown in Tables Va and Vb. There was 2.5% survival of both the thyroidectomized and the control groups at the 1000 roentgen dosage level. In the animals receiving 800 roentgens 40.% of the controls and 35.1% of the thyroidectomized animals survived 30 days.

RESULTS.

In adult rats receiving 1000 roentgens of X-ray there were no significant differences in percent 30 day survival between the animals receiving 1000 roentgens only (20%), 1000 roentgens and magnesium sulfate or nembutal (24.8%), and 1000 roentgens with drug and chilling (22.5%). Adult mice irradiated with 525 or 625 roentgens showed a slight but not significant increase in survival rate after chilling. When new born rats were chilled to 2-5° C. immediately before

being exposed to 1000 roentgens there appeared to be a significant increase in the average length of life of the animals in comparison with the unchilled rats. There was no significant difference in survival between the chilled and the control new born rats when the chilling was performed immediately after or two hours after X-ray exposure.

In adult mice exposed to 675 roentgens of X-ray and then kept at different environmental temperatures for 30 days there was an increased survival rate with an increase in temperature (0% survival at 0-10° C., 4.8% survival at 18-24° C., and 12.8% survival at 36-37° C.) There was no significant difference in the percent thirty day survival between normal and thyroidectomized rats exposed to 800 or 1000 roentgens of X-ray.

SUMMARY:

New born rats chilled to 2 - 5° C. before exposure to lethal amounts of X-ray irradiation showed an increased survival rate as compared to unchilled control rats. The lowering of the environmental temperature of adult mice exposed to 675 roentgens of X-ray decreased the thirty day survival rate. Adult rats and mice showed no significant increase in the thirty day survival rate when chilled after or before and after irradiation. Adult rats were not protected from the lethal effects of X-radiation by thyroidectomy.

TABLE I - CHILLING OF ADULT RATS

RATS - MAG. SULFATE

Group	Total No. Rats	R	% 30 Day Survival	Mean Life	Remarks
1. Controls (Rad.)	60	1000	21.6%	12.4 days	
2. Rad. + Mag. Sulfate	60	1000	26.6%	13.9 days	
3. Rad. + M.S. + Chilling	36	1000	30.5%	13.6 days	Chilled after exposure
4. Rad. + M.S. + Chilling	29	1000	27.6%	16.1 days	Chilled before & after exp.
3 & 4	65		29.0%	14.7 days	All chilled animals

RATS - NEMBUTAL

Group	Total No. Rats	R	% 30 Day Survival	Mean Life	Remarks
1. Rad. Controls	60	1000	18.3%	11.7 days	
2. Rad. + Nembutal	53	1000	22.6%	11.9 days	
3. Rad. + Nem. + Chilling	29	1000	6.9%	8.5 days	Chilled after exposure
4. Rad. + Nem. + Chilling	26	1000	23.1%	13.1 days	Chilled before & after exp.
3 & 4	55		15.0%	10.7 days	All chilled animals

TABLE IIa - CHILLING OF ADULT MICE

MAGNESIUM SULFATE:

Chilled Animals	Total No. Mice	R	% Survival 30 Days		Total No. Mice	R	% Survival 30 Days
After Exp.	90	525	55.5%	Controls	98	525	45.9%
Before & After Exposure	86	525	36.4%	Controls	92	525	42.4%

NEMBUTAL:

After Exp.	84	525	62.0%	Controls	89	525	66.1%
Before & After Exposure	74	525	86.5%	Controls	86	525	69.7%

MAGNESIUM SULFATE:

After Exp.	100	625	64.0%	Controls	99	625	54.5%
Before & After Exposure	92	625	59.8%	Controls	99	625	45.4%

NEMBUTAL:

After Exp.	97	625	60.8%	Controls	100	625	55.0%
Before & After Exposure	99	625	25.2%	Controls	97	625	16.5%

TABLE IIb - 30 DAY % SURVIVAL OF CHILLED MICE

GROUP	525 R	625 R
CHILLED AFTER	58.6%	62.4%
CHILLED BEFORE AND AFTER	59.3%	41.8%
ALL CHILLED	59.0%	52.3%
CONTROLS	55.6%	43.0%

TABLE IIIa - NEW BORN RATS CHILLED IMMEDIATELY BEFORE EXPOSURE

	CONTROLS - 78 RATS			CHILLED - 74 RATS		
	No. Surviving After Exposure			No. Surviving After Exposure		
DAY	I	II	III	I	II	III
0	29	20	29	23	22	29
1	26	18		23	20	
2	26	14		23	16	
3	26	10	27	23	12	25
4	26			22		
5						
6		4			5	
7	26		13	22		20
8		4			5	
9	0	0	0	15	0	18
10				13		16
11				8		11
12				2		
13						
14				0		2
30						2

TABLE IIIb - NEW BORN RATS CHILLED IMMEDIATE AFTER EXPOSURE

	CONTROLS - 39 RATS			CHILLED - 43 RATS		
	No. Surviving After Exposure			No. Surviving After Exposure		
DAY	I	II	III	I	II	III
0	11	9	19	12	9	22
1	11	9	14	12	9	22
2	9		9	6		
3				5		8
4	6	3		5	4	
5			7			3
6	6	0		4	0	0
7			0			
8	0			1		
9	0	0	0	0	0	0

TABLE IIIc - NEW BORN RATS CHILLED 2 HOURS AFTER EXPOSURE

	CONTROLS - 23 RATS	CHILLED - 49 RATS		
DAY	NO. SURVIVING AFTER EXP.	NO. SURVIVING AFTER EXP.		SUM OF CHILLED
0	23	24	25	49
1	22	24	25	49
2	21	21	24	45
3			23	
4	17	17	23	40
5				
6	10	13	18	31
7				
8	1	2	2	4
9	0	0	0	0

TABLE IV - ENVIRONMENTAL TEMPERATURE RESULTS

GROUP	NO. OF MICE	TEMPERATURE	NO. SURVIVING 30 DAYS	% SURVIVAL
I	125	0 - 10° C	0	0 %
II	125	18 - 24° C	6	4.8%
III	125	36 - 37° C	16	12.8%

TABLE Va - THYROIDECTOMIZED RATS - 1000 r.

DAYS AFTER EXPOSURE	NO. OF ANIMALS SURVIVING			
	CONTROLS		THYROIDECTOMIZED	
	Female	Male	Female	Male
0	20	20	20	20
3	20	20	19	18
4	17	13	13	7
5	17	12	13	4
6	15	10	9	4
7	13	9	7	4
8	10	9	7	2
9	7	6	5	2
10	4	5	5	2
11	4	4	4	1
12	4	2	2	1
13	3	2	2	1
15	2	2	2	1
16	1	2	1	1
18	1	0	1	1
20	1	0	0	1
30	1	0	0	1

TABLE Vb - THYROIDECTOMIZED RATS - 800 r.

DAYS AFTER EXPOSURE	NO. OF ANIMALS SURVIVING			
	CONTROLS		THYROIDECTOMIZED	
	Female	Male	Female	Male
0	20	17	20	17
1	20	17	19	17
3	20	17	18	17
7	18	17	15	17
8	17	17	12	16
9	14	15	9	15
10	11	14	9	13
11	10	13	9	13
12	8	13	9	11
13	8	12	7	11
14	8	11	6	11
15	8	11	6	10
16	7	11	5	10
18	5	11	4	10
20	4	11	3	10
30	4	11	3	10

(Contribution from the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico)

DETAIL AND SURVEY RADIOAUTOGRAPHS*

Wm. Ward Wainwright**,***

The use of "quasi-microscopic" technics in radioautography has left much to be desired in the study of the cellular distribution of radioactive elements and labeled compounds (20). The introduction of nuclear track emulsions in radioautographic technics (9) has added the possibility of studying the localization of radioactive substances at oil immersion magnification. Alpha tracks have been studied at high magnification (9,1) in conjunction with soft tissue sections mounted on the emulsion (10,9). The localization of beta emitters has been studied at high magnification in tissue sections and blood smears (6, 5). The principles of radioautographic technic have been reviewed recently (1,4,11,12). Technics for special purposes have been described (14,15,5,18,3).

The much used survey or contact type of radioautograph (2,13) illustrated in Figure 1 is indispensible for a study of the gross distribution of radioactive materials. This figure shows the distribution of plutonium in the tibia of a young rat. The section was cut unsoftened (16) at 10 u . The survey examination, however, gives only the gross picture and does not reveal directly the detailed localization of this heavy metal.

A detail radioautograph is equally indispensible. Its application to bone is seen in Figure 2 which was prepared by mounting a second thin section of the tibia on nuclear track emulsion. The detail radioautograph makes possible the determination of the localization of plutonium with respect to cells.

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** Grateful acknowledgement is made to Mrs. Julie Wellnitz and Mrs. Norma Lanter for technical assistance.

*** On leave from Washington University School of Dentistry.

The interpretation of a detail radioautograph depends upon the determination of the point of origin of the emission. Although this is far more easily accomplished for alpha particle emitters, as in Figure 2, much information may be obtained from the examination at high magnification of a beta particle sensitive nuclear track emulsion (5,7,8). A description of the types of nuclear tracks is given by Powell and Acchialini (19).

Nuclear particles leave their points of origin in the tissue and travel in random directions. About half of the particles enter the emulsion. The silver grains which lie in their path are struck and rendered developable. The series of silver grains in a single path, when developed, become a nuclear track (A, Figure 2). The tracks end somewhere within the emulsion with a mean free path length determined by the type and energy of the emission. Thus, the track can be retraced towards the locus in the tissue from which it originated by focussing up and down with the fine adjustment of the microscope at high magnification. For purposes of orientation the points of origin may be marked on an enlarged photomicrograph with map tacks. As marker after marker is placed at the beginning of the tracks the pattern of localization of the radioactive substance can be visualized. The orderly localization of plutonium in the region of the zone of eroded cartilage cells of the tibial epiphysis is shown in Figure 3.

Outlines of survey and detail radioautographic technics are given in Table 1. The necessity for the use of pressure to insure intimate contact between tissue and emulsion for survey radioautographs has been demonstrated by Sherwood's studies (21) of microradiographic technic. Nitrocellulose sections are mounted on nuclear track plates by carrying a flat section wet with approximately 75% alcohol on a section lifter to the emulsion. The water and alcohol render the gelatin surface sticky enough to hold the section securely throughout processing. The section is quickly

covered with filter paper (Whatman's No. 1) and the excess alcohol taken up by moving a tissue roller back and forth over the plate. The nitrocellulose is immediately removed by immersion in ether-alcohol.

Selection of the emulsion is greatly simplified by a realization of the functions of the survey and detail radioautographs. For survey radioautographs large grain size is not objectionable. Even the shortest tracks, such as those from an alpha particle, may radiate a distance of 20 u. Thus, the total width of the radioautographic image of a point source of alpha particles may be as great as 40 u (4). For beta emitters the images will be much wider (4,17). Therefore, since the fastest emulsions have a grain size sufficiently small to show all the detail possible by the contact radioautographic technic, the selection of an emulsion resolves itself to a matter of the emulsion speed desired. Fast X-ray films offer the advantage of the shortest possible exposure time for survey radioautographs. Processing of the emulsions must be carried out according to the manufacturers' recommendations.

SUMMARY

Radioautographic examination of thin tissue sections is accomplished by two complementary procedures. Survey radioautographs may be obtained by the well-known method of pressing the tissue, mounted on a glass microscope slide, in contact with a fast X-ray emulsion. Detail radioautographs for examination at high magnification under high dry and oil immersion objectives may be obtained by recently announced methods in which the section is mounted directly on a nuclear track emulsion.

REFERENCES

1. Axelrod, D.J., and Hamilton, J.G.- Preparation and Measurement of Isotopes and Some of Their Medical Aspects.- U.S. Naval Medical Bulletin, March-April Supplement. Pp. 122-141 (1948)

2. Arnon, D.I., Stout, P.R., and Sipos, F. - Am. J. Botany 27:791 (1940)

3. Bloom, W., Curtis, H.J., and McLean, F.C. - Science 105, 45 (1947)

4. Boyd, G.A., J. Biol. Phot. Assoc. 16:65 (1947)

5. Boyd, G.A., and Williams, A., Atomic Energy Commission Declassified Document U R 29 (1948)

6. Boyd, G.A., Atomic Energy Commission Declassified Document U R 33 (1948)

7. Boyd, G.A., Casarett, G.W., Altman, K.I., Noonan, T.I., Salomon, K., Science 108:529-533 (1948)

8. Boyd, G.A., Williams A., and Casarett, G.W. - To Be Published

9. Endicott, K.M., and Yagoda, H.,- Proc. Soc. Exp. Biol. Med. 64:170-172 (1947)

10. Evans, T.C.,- Proc. Soc. Exp. Biol. Med., 64:313-315 (1947)

11. Evans, T.C., - Nucleonics 2: 52-58 (1948)

12. Gorbman, A., - Nucleonics 2: 30-43 (1948)

13. Hamilton, J.G., Soley, M.H., and Eichorn, K.B. - Univ. Calif. Publ. in Pharmac. 1:339 (1940)

14. Leblond, C.P., Stevens, C.E., and Bogoroch, R.,- Science 108: 531-533 (1948)

15. MacDonald, A.M., Cobb, J., and Solomon, A.K.,-Science 107: 550-552 (1948)

16. McLean, F.C., and Bloom W.,- Anatomical Record 78: 333-359 (1940)

17. McLean, F.C., in Maximow and Bloom. Textbook of Histology. Philadelphia: W. B. Saunders, 5th Ed., p. 128 (1948)

18. Pelc, S.R.,- Nature 160: 749 (1947)

19. Powell, C.F., and Occhialini, G.P.S., - Nuclear Physics in Photographs. Oxford at the Clarendon Press (1947)

20. Radin, N.S.,- Nucleonics 1: 24-33 (1947)

21. Sherwood, H.F.,- Rev. Scientific Instruments 18: 80-83 (1947)

LEGENDS

FIGURES 1, 2 & 3: Tibia of a 9 day old Wistar rat, 16 gm., injected intraperitoneally at 7 days of age, approx. 10 gm., with 5.9 ug. plutonium as citrate. Ten u sections of unsoftened bone. (Rat 13; Spec. 133; Embedded in nitrocellulose, R.S. 1/2 Sec.)

FIGURE 1 Survey Radioautograph, by contact with Eastman Ultra-speed Dental X-ray film, exposed 31 days.
A. Indicates area of the detail radioautograph.
(Pl. 001494)

FIGURE 2 Detail Radioautograph, tissue section mounted on Eastman NTA plate, exposed 7 days.
A. Alpha track (tissue end in focus).
B. Zone of vesicular cartilage cells (tissue plane out of focus).
C. Zone of eroded cartilage cells.
D. Primary spongiosa.
(Pl. 063; stained with hematoxylin eosin; Bausch and Lomb Ampliplan 6, Zeiss oil immersion apochromatic 60; extension of camera 50 cm.)

FIGURE 3 Localization of plutonium, in the detail radioautograph of Fig. 2, marked by placing map tacks at the tissue end of each nuclear track. Note the orderly distribution of plutonium as against the apparent haphazard arrangement of tracks seen in Fig. 2.

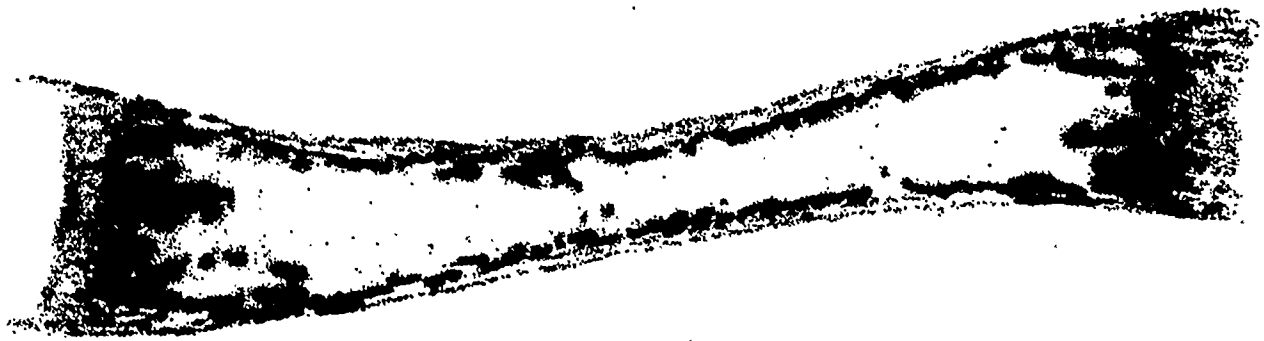

TABLE I.

OUTLINE OF RADIOAUTOGRAPHIC TECHNIC

	Survey Radioautograph	Detail Radioautograph
Mount	Contact Mount tissue on microscope slide (stain after radioautographic exposure). Secure film to slide with tab of scotch tape. Wrap appropriately to prevent scatter to next film. Clamp between glass slides with 2 (7 lb.) paper clamps. Expose in box, painted black inside and out.	Mount tissue on emulsion.
Emulsion	Fast X-ray Film	Alpha graph: Alpha or beta sensitive nuclear track emulsion. Betagraph: Beta sensitive nuclear Track emulsion.
Exposure	Estimate exposure time from experience with comparable material.	Approximately 1/3 exposure time of survey radioautograph.

THE NATURE OF THE REFRACTIVE GRANULES IN HUMAN LYMPHOCYTES

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The existance of refractive bodies in the cytoplasm of human lymphocytes has been known since Galls original description in 1936. Recently it has been observed in this laboratory that the number of these refractive bodies per lymphocyte increases in persons chronically exposed to low level doses of ionizing radiation. This observation led to an interest in the nature of these bodies in the hope that such information would illucidate the mechanism of tissue damage caused by low level ionizing irradiation. The observations of the optical properties, the histochemistry, and the method of formation of these bodies are reported in this article.

PROPERTIES OF THE REFRACTIVE BODY

A. General Descriptions

The refractive gramules in human lymphocytes vary in size from about one micron in diameter down to the limit of definition of an ordinary microscope (approximately 0.3 microns). They are usually round, but sometimes slightly oval in shape. The refractivity is comparable to that of the granules of the eosinophil, but they have none of the disc-like appearance of those specific gramules. The gramules are usually observed only in the lymphocyte but rarely one or two similar gramules may be seen in the monocyte. The number in monocytes has not been quantitated. In wet, unstained preparations of normal human blood the number of granules in each lymphocyte varies from none up to ten or twelve. About 50% of lymphocytes have no gran-

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ules, 30% have one granule, 10% have two granules, and the remaining 10% is made up of lymphocytes with three or more granules. When one or two in number, the granules are usually located near the hof of the nucleus, but with higher numbers, are generally scattered at random throughout the entire cytoplasm. In fresh wet preparations the granules appear to have two types of motion - one, a quick vibratory activity consistent with Brounian movement and, secondly, a directional motion which is apparently the result of the streaming of cytoplasm. In some cases the granules in fresh preparations of lymphocytes show free movement throughout the entire cytoplasm. The movement of this body is markedly greater than that of the mitochondria which appear to be more rigidly fixed in the cytoplasm. In older preparations, as the lymphocyte loses its motility, the granules usually settle close to the nucleus in the region of the hof and lose their directional motion but continue to show a normal amount of Brounian movement.

B. Optical Properties:

The optical properties of the refractive body are summarized in Table I. The value of 1.45 - 1.48 for the refractive index was determined by observing the body in various media of known refractive index, a method commonly used in clinical microscopy. The body appears very refractive in water (refractive index 1.333). It cannot be seen in pure glycerin (refractive index 1.47) but is again easily visualized in cedarwood oil (refractive index 1.515). The optical phenomenon related to the Becke line of microscopic crystallography are reversed depending upon whether the refractive index of the medium is higher or lower than that of the body. Thus focussing upward from the body suspended in water causes a change in appearance from black to brilliant white, while the same maneuver of the body is mounted in cedarwood oil causes it to change from whiteto black. By use of glycerin and water mixture in wet preparation of blood and various oils on dry unstained blood smears,

it was possible to limit the value of the refractive index of the body to the figures mentioned in Table I.

Observation of the particle under the phase microscope shows the granule to have a high phase contrast. This is to be expected because of the high refractive index. Phase microscopy at high magnification gives no better optical definition than does ordinary microscopy. When observed in a polarizing microscope, the granule shows no double refraction phenomenon and, therefore, must be isotopic. No inherent color was noted in the granule in any of the different types of optical observations.

6. Solubility Properties:

The solubility of the refractive body in organic solvents and other clinical agents is shown in Table II. One of two techniques was used to determine solubility depending upon whether or not the solvents were muscible with water. In the case of water-muscible solvents, it was possible to run solutions into wet preparations of blood and to observe the effect on individual bodies. Spedial plastic slides with parallel grooves 5 mm. apart were made and used instead of ordinary glass microscopic slides. A small drop of blood was placed between the grooves and covered with No. 0 coverslip. By placing drops of the solvent to be tested at one end of the grooves and applying a piece of filter paper to the other end, it was possible to flush the system with the solution. If the blood preparations were allowed to dry slightly before employing this technique, the lymphocytes anchored themselves to the glass slide and their positions were not influenced by the streaming fluids. Thus the granules in the same lymphocyte could be examined before, during and after treatment. In the case of water-immersible chemicals, unfixed dried blood smears were immersed in the solvent for varying periods of

time. The smear was then washed with water, covered with a drop of methylene blue in isotonic saline under a coverslip. At least twenty lymphocytes were examined for the presence or absence of granules.

It must be emphasized that the solubility data given in Table II is not absolute. The volume of the particles is so small (approx. 10^{-9} to 10^{-10} cm.) that ordinary concepts of micro-chemical solubility do not apply. One must be satisfied with relative solubilities in various solvents.

D. Staining Properties:

Table III lists the staining properties of the refractive bodies as determined by various histo-chemical procedures. The peculiar refractivity and solubility of the bodies made it necessary to modify many of the standard techniques. Interpretation of the results was often difficult, if not impossible. Thus, it was found that the refractiveness of the body was so marked that it was impossible to determine whether small amounts of a water soluble dye had been taken up by the body. In certain instances it was possible to circumvent this difficulty by changing to to a medium of a higher refractive index (e.g. osmic acid). It was also obvious that no conventional stain which employed ethyl alcohol solvent could be used without modification. Since most fat stains, including the Sudan stains, are strong tinctures, they easily dissolve the refractive bodies before they can be stained. Watering the stains to the point that they do not attack the granules renders them less efficient in staining lipids and makes difficult the interpretation of the results.

Results:

1. Lipid Stains:

As pointed out above, the Sudan stains are used in organic solvents which by

themselves dissolve the lymphocytic granule. Sudan III was used in 40% ethyl alcohol, scarlet red in a mixture of equal parts 70% ethanol and acetone, and Sudan Black B was used in 70% alcohol. When the solvents were diluted with water in order to avoid dissolving the granule, the Sudan stains became increasingly insoluble in the solution and no staining could be demonstrated. Thus, the results with these stains are actually inconclusive.

The Nile blue sulfate staining was carried out by allowing an isotonic solution of the stain to run into a wet preparation of blood. The staining of the granule was a slight but definite blue.

If equal parts of a 2% aqueous solution of osmic acid and whole blood are mixed on a slide, covered with a coverslip, and sealed with petroleum jelly, there is an apparent staining of the granules in the first 10 to 20 minutes. In order to show that the blackening is due to the reduction of osmic acid, glycerin can be added to the medium to increase the refractive index to 1.44 with no change in the black color of the granule.

2. Polysaccharide Techniques:

The Feulgen-Bauer stain for glycogen and Gomori's procedure for glycogen and miscin were carried out according to the prescribed technique except that the alcohol fixation was eliminated because of the solubility of the granule on this solvent. The periodic acid procedure for polysaccharides as outlined by Hotchkiss was done using the alternate procedure of aqueous solutions.

3. Protein Stains:

Methlene blue, toluidine-blue and pyronin Y flooded into a wet preparation all appear to stain the residue left after treatment with 70% ethanol. If one attempts to stain the granules with these dyes before treating them with organic solvents, only a few of the larger ones are definitely stained and the smaller ones, because of the high refractivity of the body and their minuteness, are not definitely

stained. This also holds true if one fixes the blood beforehand with 20% formalin, Na_2CO_3 or lanthanum acetate. In the Feulgen reaction the preparations were not placed in 95% alcohol to extract the substance in plasma which is Feulgen positive but otherwise it was carried out routinely.

Incubation with ribomuclease caused no changes in the staining properties of the residue at a concentration of 0.5 mg. per ml. but the pH of this medium was not accurately controlled. Stowell (1947) has shown that there is no significant differences in the enzymatic activity between pH 5 to 8 when using a concentration of crystalline enzymes of 0.1 mg./ml. and incubating for 3 hours. The incubation in our experiment was carried out for as long as 24 hours with no demonstrable change in staining properties.

4. Supravital Stains:

Neutral red stains the granules a reddish-orange color. The color can be seen only slightly in wet preparations due to the high refractive index of the granule, but if neutral red is added to heparinized blood and frozen-dried smears are made, the red refractive granules can be demonstrated. The frozen-dried films are made by freezing the blood film before it dries by plunging it into liquid nitrogen and then dehydrating it at 0.2 microns of Hg. This preserves the normal cell contour and gives less distortion of the cell constituents than ordinary drying. The reddish-orange color of the granule would indicate that the pH of the granule was about 7.0 since neutral red is a scarlet red at a pH of 6.8 and yellow at a pH of 8.0.

5. Enzymes:

The peroxidase test of Sato and Sekiyz unmodified gave negative results.

MECHANISM OF FORMATION OF THE REFRACTIVE BODIES:

It is known that toxic agents in the human body cause an increase in the number of refractive granules in the cytoplasm of the lymphocyte. In order to see if this is a direct action, several methods of injuring the leukocytes *in vitro* have been studied.

A. The Influence of Neutral Red on the Development of the Refractive Granules:

It was noted in studying unstained wet preparations of blood that there were fewer refractive granules in these lymphocytes than in blood films stained supravitally with neutral red and Janus green. It has long been known that neutral red and other basic dyes increase the number of cytoplasmic vacuoles in certain cells. In order to show statistically that there was an increase in the number of granules in stained preparation as compared to wet unstained preparations, a group of thirteen individuals were studied. The number of granules per lymphocyte were determined using both wet unstained blood and blood stained with neutral red and Janus green. The stain itself, which is much stronger than that usually used in supravital studies, was prepared by adding 0.6 ml. of a saturated absolute ethyl alcohol solution of neutral red and 0.2 ml. of Janus green in a similar solution to 2.25 ml. of absolute ethyl alcohol freshly prepared by the sodium phthalate method. This solution is then allowed to flow on to clean microscopic slides and the excess immediately wiped off. The results of counting the granules in these preparations are tabulated in Table IV. It can be seen that there are approximately twice as many granules per lymphocyte in the preparations containing neutral red and Janus green. Also, the number of lymphocytes with six or more granules, show a striking difference in the two preparations with 28 such lymphocytes in the stained preparations and only one in the unstained blood.

On the basis of the above, the next step was to determine which of the two supravital stains were responsible for the increase in granules. Thus, the blood of nine individuals was examined using four different preparations on each person. The slides were made without stain, with neutral red and Janus green, with neutral red alone and with Janus green alone. The results are tabulated in Table V. It can be seen from this table that neutral red is the stain responsible for the increase in granules as the blood stained with Janus green alone shows no increase in granules in comparison to the unstained blood. The discrepancy between the granules pe

lymphocyte in the similar preparations used in Tables IV and V can be explained on the basis of the fact that the individuals included in Table IV were, in general, from a group who were receiving more exposure to ionizing radiation than the ones used in Table V.

Thus, it can be concluded that neutral red in the concentration used for routine examination of blood in our laboratory has an effect on the lymphocytes manifested by the increase in refractive granules in the cell. It is not felt that this is due to the smaller granules becoming more evident to the observer since the criterion used for distinguishing these specific granules from the neutral red vacules is not the taking up of neutral red, but the refractivity which is not changed by the adding of this stain.

B. X-Ray Irradiation In Vitro:

Since it has been shown that chronic and acute exposure of humans to penetrating ionizing radiation causes an increase in the number of refractive bodies in the cytoplasm of the lymphocytes, in vitro studies were carried out to determine whether or not this was a direct effect upon the cell. Twenty milliliters of heparinized whole blood was divided into four equal portions. Two of the portions were exposed to 1000 roentgens of 250 KV X-rays and two served as controls. After irradiation one of the exposed specimens was kept at room temperature and one at 5° C. The two controls were similarly kept at these different temperatures. A determination of the granules per lymphocyte was carried out on each of the four blood samples using wet films stained supravitally with neutral red and Janus green at time intervals of one, six and twenty-four hours.

At least 100 lymphocytes were studied from each specimen at these specified time intervals. The results showed that there was no difference in the number of granules per lymphocytes between the four groups at any of the three time periods. It was also noted that the number of granules per lymphocyte did not change during this 24 hour period in any single blood specimen.

It would thus appear that in vitro irradiation with 1000 roentgens of X-rays does not increase the number of granules per lymphocyte and that in twenty-four hours there is no in vitro increase in number of granules in the control blood at 5° and at room temperature.

It should be pointed out that during this twenty-four hour period there were many lymphocytes in all four specimens which apparently died and also there was a development of large, slightly refractive vacuoles in the dying cells. A few of the lymphocytes in the control blood kept at room temperature showed definite life manifested by motility after three days, but careful observations of the differences in motility between the four groups were not made.

DISCUSSION:

In general, it may be concluded from the properties of the refractive granule that it consists of two types of compounds. The major portion is soluble in 70% alcohol and other organic solvents, while there is a residue which remains after treatment with these solvents.

The major portion of the granule is soluble in organic solvents considered fat solvents. The reduction of osmic acid is a non-specific reaction which is an indication that the compound is unsaturated. Neutral red is also a non-specific stain, but is known to stain lipids. The results with Nile blue sulfate cannot be too highly regarded since the size of the granule is such that it is very difficult to make out whether the inside of the granule is stained or if the color is due to some material which could be absorbed on the surface.

If one assumes that this portion of the granule which is soluble in organic solvents is a lipid and attempts to classify it on the basis of its solubility characteristics, the following deductions can be made. The material is probably not a lecithin, cephalin, or sphingomyelin since these types of lipids are not soluble in acetone. Cerebrosides (phrenosin and kerasin) and sphingomyelin are

insoluble in ether. The cold alcohol solubilities tend to rule out sphingomyelin, phrenosin, kerasin, cephalin and neutral fats in general. It should be noted that the granule is isotopic and that frequently lipids are anisotropic. It should be pointed out also that the refractive index of human fat is about 1.46 and this is consistent with that found for the lymphocytic granule. Thus, it appears that the lipid component of the granule does not fit with those usually considered in biochemistry.

The residue left after treatment of the granule with organic solvents may represent the absorption of cytoplasmic protein on the surface of the granule before or during treatment with solvents. This residue stains with ordinary basic dyes and these same dyes also appear to stain the intact granules although it is difficult to be sure in all cases because of the high refractiveness of the body. When the residue is treated with ribonuclease there is no change in its staining properties. This is an indication although not absolute proof that it is not ribonucleic acid in composition.

It can be concluded that this body appears to contain a lipid-like compound and a protein - perhaps is a lypoprotein complex. The chemical properties of the refractive body and the increase after treatment with neutral red suggest that this body may be part of the Golgi apparatus. Dustin, in a discussion of the conclusions of many authors attempts to simplify the concept of the Golgi apparatus by describing it as a dynamic region of the cell where vacuoles staining vitally with basic dyes are associated with lipoprotein cytoplasmic membrances or structures. He states that in the simplest form they appear as lipoid granules vitally stained with neutral red. Bourne (1942), after stating that the Golgi bodies are soluble in fat solvents and therefore contain a high percentage of fats and mentioning thir reduction of osmic acid concludes that they are probably fat and protein, with the latter on the outside, so that they cannot be stained with fat dyes. He also points out that the injection of neutral red into animals produced droplets in the area

of the secretory granules, but distinct from the Golgi canal. Many observers have noted the indrease of vacuoles near the Golgi body in the cytoplasm of cells exposed to very small concentrations of neutral red. They call this a "toxic reaction" or "protective secretory activity" on the part of the cell.

Dustin points out that in many cases the basophilic material of the Golgi apparatus is of the ribonucleoprotein type as indicated by ribonuclease studies. Hibbard and Lavin, on the other hand, using ultraviolet microscopy on the Golgi apparatus in chicken gizzard epithelium, conclude that the region occupied by the Golgi apparatus does not contain appreciable amounts of nucleoproteins, nucleic acids, or proteins containing tryptophane and tryosine.

In general, the literature on the Golgi apparatus is quite confusing, namely because of the large varieties of cell types studied. There appears to be, however, some parallelism between the present concept of the Golgi apparatus and the refractive granules in the human lymphocyte. It can be stated that the development of the granules in the lymphocyte is a non-specific response to some alteration in cell metabolism brought about in specific cases by neutral red, ionizing radiation, and possibly certain heavy metals.

SUMMARY:

1. On the basis of optical properties, solubility studies and staining reactions, it is concluded that the refractive granule in human lymphocytes is probably composed mainly of a lipid and that it is either surrounded by or contains a small amount of protein which is probably not ribonucleoprotein.
2. Neutral red in the concentrations used at this laboratory for routine supravital preparations of blood increases the number of these refractive granules in lymphocytes over the number observed in unstained lymphocytes.
3. An effort is made to correlate the development of this refractive granule with the present concept of the Golgi apparatus.

TABLE I.

OPTICAL PROPERTIES

1.	General	Quite refractive in wet preparations
2.	Refractive Index	1.45 to 1.48
3.	Double refraction	Absent
4.	Phase contrast	High
5.	Inherent color	None

TABLE II.

SOLUBILITY PROPERTIES

SOLVENT	RESULTS
1. Absolute methyl alcohol (Refractive Index 1.32)	Moderate decrease in refractivity and size
2. Ethyl alcohol (R.I 1.38)	Little if any change from normal
35%	Slight loss of refractivity and size
50%	The granule appears to be dissolved with only a small dark, non-refractive residue remaining.
70%	Similar to 70%, except there is more shrinkage of the entire lymphocyte.
75-100%	
3. Ether (using dry film)	No evidence of granule after treatment.
4. Acetone (R.I. 1.358)	There is at first a swelling of the cytoplasm and then the granule disappears.
5. Chloroform (R.I. 1.44)	As the chloroform layer approaches the granule, it loses its refractivity and disappears.
6. Ether-Ethylalcohol-Water Mixture (1:1:1) (R.I. 1.35)	The granule loses a slight amount of refractivity and decreases slightly in size.
7. Water (R.I. 1.333)	The cytoplasm of the lymphocyte becomes greatly swollen, but the granule remains normal in appearance.
8. Dilute acid (1% acetic acid)	No effect
9. Strong acid (conc. HNO_3)	The granule disappears.
10. Dilute alkali (3% Na_2CO_3)	No effect
11. Strong alkali (conc. KOH)	The granule disappears
12. Sodium choleate and soap	In both cases the cytoplasmic membrane is broken with release of the granule, but with no change in the granules appearance.

TABLE III.

STAINING PROPERTIES

STAIN	RESULTS
A. Lipid Stains	
1. Sudan stains (Sudan III, Scarlet red, Sudan black B)	Inconclusive
2. Nile blue sulfate	There is a slight bluish tinge to the granule.
3. Osmic acid	The granule stains black.
B. Polysaccharide Techniques	
1. Feulgen-Bauer Reaction	
2. Gomori's Glycogen-Mucin Technique	Negative results
3. Hotchkiss Periodic Acid Procedure	
C. Protein Stains	
1. Methylene blue	There is a definite staining of some of the granules, but one cannot be sure that all granules stain presumably because of their refractiveness, however, the residue left after treatment with 70% ethyl alcohol stains in every case.
2. Toluidine blue	
3. Pyronin Y	
4. Feulgen Reaction	Negative results
5. Ribonuclease for Ribonucleic Acid	Negative results
6. Gram's Stain	Negative results
D. Supravital Stains	
1. Neutral red	The granule stains a reddish-orange color.
2. Janus green	Negative results
3. Trypan blue	
E. Enzymes	
1. Peroxidase (Method of Sato & Sekiya)	Negative result

TABLE IV.

COMPARISON OF UNSTAINED AND NEUTRAL RED-JANUS GREEN STAINED LYMPHOCYTES

Type of Preparation	Lymphocytes Counted	Number of Granules in Lymphocytes											Total Granules	Granules per Lymphocytes	Lymphocytes With 6 or More Granules
		0	1	2	3	4	5	6	7	8	9	10 or more			
Unstained	360	181	108	42	17	8	3	0	0	0	1	0	299	0.83	1
Neutral red-Janus Green	360	165	66	48	25	14	14	12	3	3	4	6	605	1.67	28

TABLE V.

COMPARISON OF UNSTAINED, NEUTRAL RED-JANUS GREEN, NEUTRAL RED, AND JANUS GREEN STAINED LYMPHOCYTES.

Type of Preparation	Lymphocytes Counted	Number of Granules in Lymphocyte											Total Granules	Granules per Lymphocyte	Lymphocytes With 6 Or More Granules
		0	1	2	3	4	5	6	7	8	9	10 or more			
Unstained	225	133	71	18	2	1	0	0	0	0	0	0	117	0.52	0
Janus Green	225	157	51	10	2	2	0	1	1	0	1	0	107	0.48	3
Neutral Red	225	89	74	27	18	9	4	3	0	1	0	0	264	1.17	4
Neutral Red-Janus Green	225	101	56	26	16	10	7	5	2	0	0	2	300	1.33	9

THE CHANGES IN THE BLOOD OF HUMANS CHRONICALLY EXPOSED TO LOW LEVEL GAMMA RADIATION*

Norman P. Knowlton, Jr.

(Contribution from the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico)

At the Los Alamos Scientific Laboratory a number of individuals are exposed continually to small amounts of ionizing radiation. Although it is well known that small doses of radiation (0.1 to 10 r.) in single acute exposures cause no change in the peripheral blood picture, it is possible that if a large enough group of individuals with such exposures are studied over a long period of time one might obtain changes in the average blood counts which are statistically significant.

In view of this possibility, ten individuals who had daily film badge exposure records and weekly blood counts over a seventy-seven week period were selected for study. The following is a statistical study of the changes in the total white blood count and the absolute neutrophil and lymphocyte counts for these ten individuals.

A group of 24 individuals have been used as controls in this study and, although they do not have blood studies as frequently as the exposed group, the large number of individuals tends to correct this deficiency.

A. INDIVIDUALS STUDIED:

Exposed Group: These ten individuals (all males) received an average of 16.21 roentgens of gamma rays per man over a 77 week period (0.211 roentgens per week). The extremes of dosage varied from 13.06 to 24.20 roentgens. Table II shows the exact dosage received by each man. The number of blood counts done on each individual during this 77 week period varied from 37 to

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74 with an average of 64 blood counts per man. The number of counts obtained 77 on each man are tabulated in Table IV. During this study period all 10 men carried out a similar experiment involving materials which emit gamma rays. In general, the radiation was received uniformly over the 77 week period with perhaps double the average amount during the periods between 11 and 21, and weeks 25 and 32. The radiation delivered during a week period was received in the five day work week and usually they received approximately one-half of their weekly dosage during one of the five days. One of the individuals had been working on this particular experiment for 14 months before this blood study was begun (Case 4); one for 7 months (Case 6) and one individual (Case 7), who is included in the study, began work seven weeks after the study began. The other seven individuals began working on this operation 0 to 20 weeks before the study period. None of the ten individuals had significant exposure to ionizing radiation before starting on this experiment.

Control Group: This group consists of 24 individuals (23 males and 1 female), who were picked for study specifically because their jobs involved no appreciable exposure to neutrons, beta, gamma or X-radiation. They had slight exposure to alpha radiation, but this is not considered an external hazard because of its low penetrating power in tissues (approximately 50 microns) and the alpha exposure was such that inhalation or ingestion was unlikely. These 24 individuals have had no known exposure to other toxic materials, such as dusts, organic solvent vapors, etc. The blood counts of these persons were studied over approximately the same time period as those of the exposed group. The number of blood counts done on each individual during this period varied from three to nineteen, with an average of ten blood counts per individual.

B. METHODS OF STATISTICAL STUDY:

Exposed Group: This group was studied during a period of 77 weeks from December 1946 to June 1948. This period was divided into two time periods of

of 38 and 39 weeks. In the second, 39 week period, there was one week during 78 which no blood examinations were made (the December holiday season 1947) and so the two periods are considered as equal in length. The analysis of the hematological data consists of an attempt to demonstrate a difference in the blood picture, of the individuals between the two 38 week periods. This was done by two methods:

(1) To compare the weekly average of the blood counts of the ten men during the first and second thirty-eight week periods.

(2) To study the difference between the blood counts of each individual during the 1st and 2nd 38 week periods.

Control Group: Since there were fewer counts per individual in this group the hematological data was considered as an average of all individuals and individual changes were studied only in the case of the total white blood count. The data includes all blood studies from May 1946 to July 1948 on these individuals. This period was divided into two approximately equal periods - May 1946 to June 1947 and July 1947 to July 1948. It should be mentioned that no blood counts were excluded from the data on any of the 24 control cases or the 10 exposed individuals over the time periods studied.

C. RESULTS:

Exposed Group: Table I shows the difference in the total white blood count between the first and second 38 week time periods. In this data the average counts of all ten men for each week are considered, thereby reducing the variation of the counts from the mean in comparison to the variation present if each individual is considered separately. The mean WBC in the first 38 weeks is 7892 and for the second period 6945 - a drop of 12.0%.

Table II shows the change of the total white blood count of each of the ten

individuals for two 26 week periods from December 1946 to May 1947 and from December 1947 to May 1947 to rule out possible seasonal effects on the blood count. The drop in total white blood count is from 7770 to 6720 cells per cubic millimeter = a drop of 13.5 per cent. The individual drops vary from 5.6 to 17.1 per cent with an average of 12.4 per cent. Table II also gives the exposure record of the ten individuals.

Table III shows the difference in the absolute neutrophil counts in the first and second time periods. The average weekly counts of all ten men were used for the statistical test. There is a drop from 4881 to 4366 neutrophils per cubic millimeter = a decrease of 10.5 per cent.

Table IV shows the changes in the absolute neutrophil counts of each individual. Nine out of ten of the men show a drop and one shows a rise of 2.5 per cent. The changes vary from minus 13.4 to plus 2.5 per cent with an average decrease of 10.4 per cent.

Table V shows the difference in the absolute lymphocyte counts in the two 38 week time periods. These figures are obtained from the average absolute lymphocyte counts of all ten individuals for each week. There is a drop from 1920 to 1611 lymphocytes per cubic millimeter of blood = a decrease of 16.1 per cent.

Table VI demonstrates the changes in the absolute lymphocyte counts of each of the ten individuals. All ten show a decrease with a range from minus 1.7 to minus 26.6 per cent. The average drop is 16.6 per cent.

Figure I shows the average weekly blood counts of the exposed group and a graphic representation of their exposure record.

Control Groups The analysis of the total white blood counts of the control group of 24 individuals is shown in Table VII. There are 11 decreases, 12 rises, and one count that is constant during the period studied. The change

in the WBC varies from plus 20 to minus 28 per cent with an average of minus 1.0 per cent. The means of the average WBC for each individual show a change of minus 1.3 per cent, while the average of the sum of all counts for each time period shows a change of plus 0.4 per cent. 80

The comparison of the neutrophils and lymphocytes in the two time periods has been done on the basis of the average percent of these blood cells in the differential count since the total white blood count has remained approximately constant. Table VIII shows the average percent neutrophils and lymphocytes in the first and second part of the study period. It is evident that there is no drop in the percent neutrophils and a small (3.1%) drop in the per cent lymphocytes.

D. DISCUSSION:

The statistical analyses for changes in the total white blood count and the absolute neutrophil and lymphocyte counts in ten individuals exposed to approximately 0.2 roentgens of gamm rays per week over a 77 week period show highly significant changes in the blood of twenty-four individuals who received no known exposure to beta, gamma, X-rays or neutrons. The exposed group showed a 12-13 per cent decrease in the total white blood count, a 10-11 per cent fall in the absolute neutrophil count, and a 16-17 per cent drop in the absolute lymphocyte count. The control group showed a drop of 0-1 per cent in the total white blood count, no change in the per cent neutrophils, and a decrease of 3 per cent in the per cent lymphocytes.

It would appear that this group of 10 individuals exposed to doses of gamma radiation which is considered below tolerance have had significant changes in their peripheral blood. These changes which are relatively small are only recognized when blood examinations are made frequently over a long period of time. It must be emphasized that, although no other agents which might cause these changes have been uncovered, it is quite possible that they existed. Such factors as

the altitude at Los Alamos (7,400 feet above sea level), dietary changes, previous exposure to ionizing radiation, illnesses, unmeasured gamma radiation, and the remote possibility of inhalation or ingestion of radioactive materials have been investigated and, although they appear to be negligible, it is always a possibility that one or more of them might have been casual agents.

It is improbable, but not beyond the realm of possibility, that such dosage levels would give changes in the blood picture. With dosages as low as 20 to 30 roentgens in a single acute exposure, it is possible to show a significant decrease in the lymphocytes of the peripheral blood of a single animal. At a dosage of 30 roentgens this decrease may be twice the drop seen in this study. Although it is known that a certain amount of ionizing radiation given in an acute exposure causes a greater effect than if the same amount is given over a long period of time, there must be a definite relationship between the two. It is evident in animal experiments that this factor is not a one to one ratio, but that it probably takes at least ten times the amount of total radiation in chronic exposures to obtain the drops seen in single acute exposures. This is quite a reasonable phenomenon for there is constant repair during chronic irradiation, but with an acute dose the entire damage is done during a brief period before effective repair has been instigated. The ratio between dosages giving a similar depression in acute and chronic exposures would be closer to one to one if one considers what dosage in an acute exposure will give a residual depression of bone marrow or lymphoid activity months after exposure. In such a case the time for repair could be made equal to that present in a chronic exposure study.

In view of these findings, it is important that hematological and exposure data at various other laboratories be analyzed to determine whether or not these are real changes. If confirmatory results are subsequently obtained, the present concept of tolerance doses should be carefully reviewed.

E. SUMMARY: 82

A group of ten individuals exposed to 0.2 roentgens of gamma radiation per week showed a significant fall in the total white blood count and the absolute neutrophil and lymphocyte counts during a 77 week period. The degree of fall would not have been predicted from experimental irradiation in animals and other unknown factors might have also been casual agents. It is suggested that hematological and exposure data in other laboratories be analyzed statistically to confirm or disprove the effect of such low dosages of ionizing radiation on humans.

TABLE I

EXPOSED GROUP

STATISTICAL ANALYSIS OF CHANGE IN WBC:

$\bar{x}$ = Average of first 38 weeks

$\bar{y}$ = Average of second 38 weeks

$\bar{x}$ =	7892	$\bar{y}$ =	6945
n_x =	38	n_y =	38
σ_x =	543	σ_y =	565

$\bar{x} - \bar{y}$ = 947 = 12.0 per cent drop

Student T Test

$t = 7.5$

PROBABILITY = approx. 10^{-8}

TABLE II

EXPOSED GROUP

Individual Changes in WBC

CASE NO.	WBC WEEKS 1-26	WBC WEEKS 53-77	% DROP	ROENTGENS WEEKS 1-26	ROENTGENS WEEKS 27-52	ROENTGENS WEEKS 53-77	TOTAL
1	8250	7455	10.5	6.30	6.15	1.39	13.84
2	7712	6510	15.6	7.23	9.74	7.23	24.20
3	6312	5527	12.4	5.77	5.30	2.76	13.83
4	6657	5767	13.4	7.21	4.17	2.25	13.63
5	6445	5412	16.0	8.34	8.04	3.23	19.61
6	7925	6750	14.8	9.61	4.53	4.02	18.16
7	7457	6582	11.7	4.66	7.46	3.80	15.92
8	9292	7705	17.1	5.39	4.80	2.87	13.06
9	10,702	8932	16.5	4.78	6.12	2.78	13.68
10	6945	6555	5.6	6.25	6.62	3.31	16.18
AVERAGE	7770	6720	12.4				16.21

Difference between the two means = 1050 = 13.5% drop

TABLE III

EXPOSED GROUP

STATISTICAL ANALYSIS OF CHANGE IN NEUTROPHIL COUNT

$\bar{x} = 4881$ $\quad \bar{y} = 4366$

$n_x = 38$ $\quad n_y = 38$

$\sigma_x = 393$ $\quad \sigma_y = 499$

$\bar{x} - \bar{y} = 515 = 10.5$ per cent drop

$t = 4.93$

PROBABILITY = approx. 10^{-6}

TABLE IV

EXPOSED GROUP

INDIVIDUAL CHANGES IN NEUTROPHIL COUNTS

CASE NO.	NEUTROPHILS PER MM^3, AVERAGE # Counts	Wks. 1-38	#Counts	Wks. 39-77	% CHANGE
1	34	5367	28	4668	- 13.0
2	36	5131	36	4553	- 11.3
3	38	4022	26	3781	- 6.0
4	24	4212	13	3662	- 13.1
5	38	3776	36	3270	- 13.4
6	33	4276	28	3711	- 13.2
7	29	3953	36	3471	- 12.2
8	38	4844	31	4263	- 12.0
9	38	7908	34	6953	- 12.1
10	33	4668	31	4785	+ 2.5
AVERAGE		4816		4312	- 10.4

Difference between the two means = 504 = 10.5% drop

TABLE V

EXPOSED GROUP

STATISTICAL ANALYSIS OF CHANGE IN LYMPHOCYTE COUNT

$\bar{x} = 1920$ $\quad$ $\bar{y} = 1611$

$n_x = 38$ $\quad$ $n_y = 38$

$\sigma_x = 260$ $\quad$ $\sigma_y = 220$

$\bar{x} - \bar{y} = 309 = 16.1\%$ drop

$t = 4.93$

PROBABILITY = approx. 10^{-7}

TABLE VI

EXPOSED GROUP

INDIVIDUAL CHANGES IN LYMPHOCYTE COUNTS

CASE NO.	LYMPHOCYTES PER MM3, AVERAGE Weeks 1-38	Weeks 39-77	% CHANGE
1	1749	1720	- 1.7
2	1863	1410	- 24.3
3	1664	1308	- 21.4
4	1385	1121	- 19.1
5	1727	1455	- 15.8
6	2331	1835	- 21.3
7	2019	1948	- 3.5
8	2786	2066	- 25.8
9	1706	1589	- 6.9
10	1603	1177	- 26.6
AVERAGE	1883	1563	- 16.6

Difference between the two means - 320 = 17.0% drop

TABLE VII
Control Group

TOTAL WHITE BLOOD COUNT

Case No.	FIRST TIME PERIOD No. Counts	Sum of WBC's	Average WBC	SECOND TIME PERIOD No. Counts	Sum of WBC's	Average WBC	% Change
11	7	46375	6625	4	27400	6850	+ 3
12	3	26550	8850	4	28000	7000	- 21
13	6	42150	7025	4	23700	5925	- 16
14	9	79425	8825	10	83500	8350	- 5
15	8	54000	6750	11	79750	7250	+ 7
16	9	74250	8250	9	73125	8125	- 2
17	6	53100	8850	6	63600	10600	+ 20
18	7	57225	8175	7	58450	8350	+ 2
19	6	43500	7250	6	36750	6125	- 16
20	5	35250	7050	4	20200	5050	- 28
21	7	47075	6725	5	37250	7450	+ 11
22	6	37950	6325	4	30400	7600	+ 20
23	3	25050	8350	4	34200	8550	+ 2
24	6	47850	7975	5	41250	8250	+ 3
25	7	42875	6125	5	27000	5400	- 12
26	1	6950	6950	2	15400	7700	+ 11
27	1	8450	8450	3	25425	8475	0
28	5	37625	7525	2	15950	7975	+ 6
29	4	35600	8900	3	24675	8225	- 8
30	4	19400	4850	3	15075	5025	+ 8
31	4	40400	10100	3	29400	9800	- 3
32	3	37125	12375	4	48100	12025	- 3
33	3	28200	9400	3	30675	10225	+ 9
34	5	51000	10200	2	17950	8975	- 12
TOTAL	125	977375	191900	113	887225	189300	- 24
		÷ 125	÷ 24		÷ 113	÷ 24	÷ 24
AVERAGE		7820	7995		7852	7887	- 1.0%

Average of All WBCs 7852 - 7820 = + 32 = 0.4%

Average of Case Averages 7887 - 7995 = -108 = 1.3%

TABLE VIII

CONTROL GROUP

PER CENT NEUTROPHILS AND LYMPHOCYTES

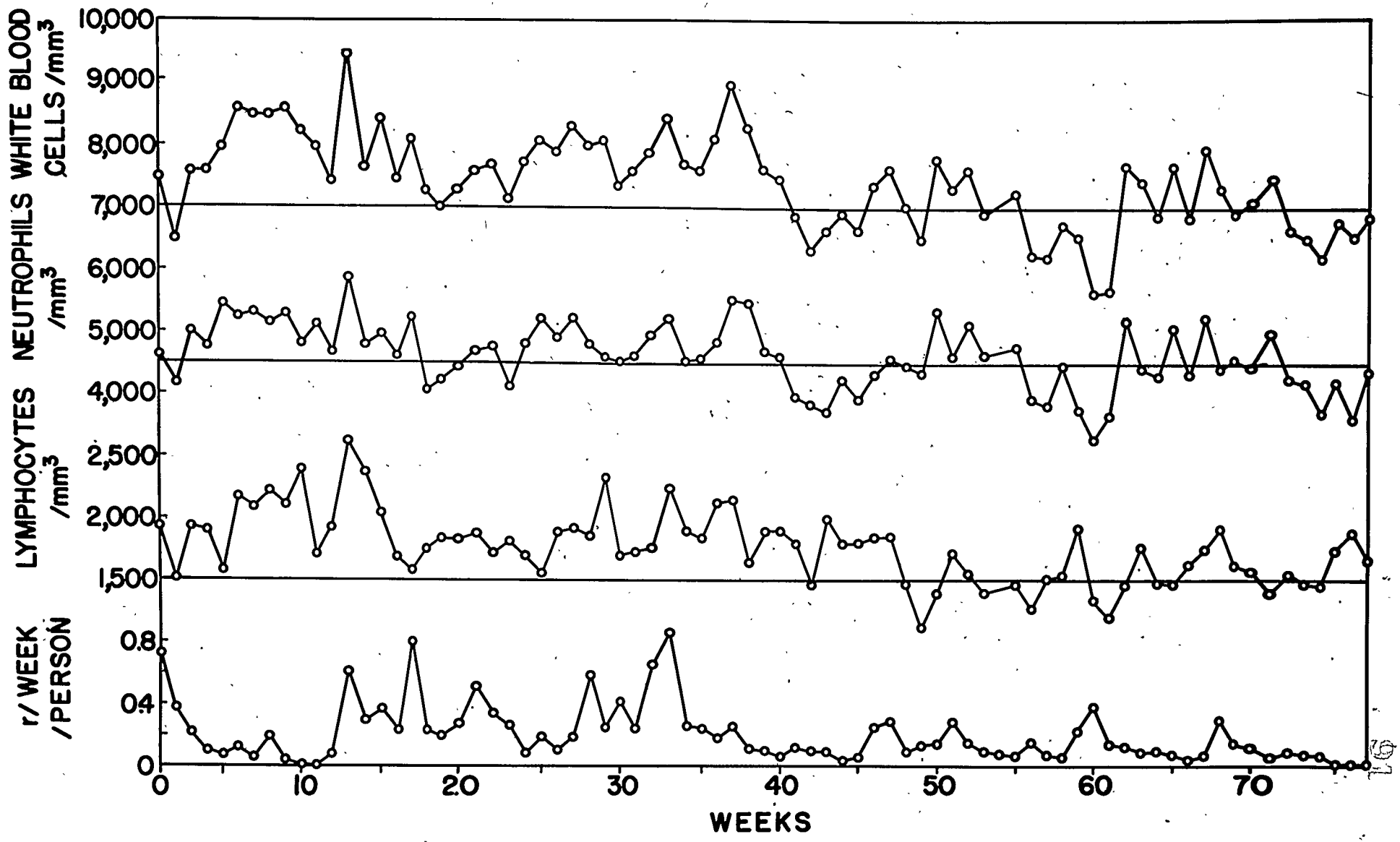
NEUTROPHILS:

	No. Counts	Average %	% Change
1st Year	125	58.39	0.0%
2nd Year	113	58.43	

LYMPHOCYTES:

	No. Counts	Average %	% Change
1st Year	125	29.3	- 3.1%
2nd Year	113	28.4	

BLOOD CHANGES FOLLOWING CHRONIC EXPOSURE TO GAMMA RAYS



(Contribution of the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico)

BETA RAY BURNS OF HUMAN SKIN: A REPORT OF FOUR CASES*

Norman P. Knowlton, Jr., Louis H. Hempelmann, Edgar Leifer & John R. Hogness

The magnitude of the Atomic Energy Program and the widespread use of radioactive isotopes in research laboratories since World War II has resulted in a large increase in the number of persons exposed to radiological hazards. With this increase in use of such isotopes, experimenters must be acquainted with the hazards of these laboratory tools. Four cases of beta ray burns of the hands are reported with the purpose of supplementing the present information in the literature and emphasizing the early clinical signs and symptoms, as well as the laboratory findings present in such cases. It is hoped that persons exposed to dangerous amounts of beta rays may realize the warning signs and symptoms and discontinue any hazardous operations before more damage is incurred.

All four patients were injured while undertaking procedures of a similar type at the recent Atomic Tests at Eniwetok. This involved the handling of radioactive materials which emitted large amounts of beta and gamma radiation. The major portion of the radiation consisted of beta rays with an average energy of about 1 Mev. The ratio of beta counts to gamma counts in a Geiger-Mueller type tube was about 6 to 1. A [redacted] deviation in procedure resulted in the handling of these radioactive substances directly with gloved and sometimes with bare hands. No measurement of the dosage of beta rays received by the patients was obtained at the time of exposure, but film badge and pocket ionization chambers worn by the individuals showed readings for gamma rays varying from 1.5 to about 17 roentgens delivered to the thoracic region. In view of the fact that the hands were much closer to the active material, it must be presumed tha

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the amount of gamma radiation received by the hands was somewhat larger, probably by a factor of ten.

The physical properties of 1 Mev. beta rays are such that approximately 90 per cent of the incident rays are absorbed by the first 2.5 mm. of tissue and that 99 per cent are absorbed in 5 mm. of tissue. Calculations of dosage show that these four individuals received in the neighborhood of 5000 rep. (roentgen equivalent physical) to the outer surface of the skin. The dosage at 2.5 mm. below the skin surface would be about 500 rep. and 50 rep. at a 5.0 mm. depth.

In view of the fact that there was definite evidence that the whole body exposure did not exceed 17 roentgens of gamma rays in any case, it was felt that there was minimal, if any, danger of important whole body effect and the individuals were treated primarily for local beta ray burns of the hands. This assumption was borne out in the course of the disease in that at no time was there any significant evidence of whole body damage.

Since the amounts of radiation received and the clinical courses of the patients differed to some extent, each case is presented separately. The following are brief synopses of the clinical courses of each patient.

CASE I:

This 26 year old white male received 10 roentgens of gamma rays to his whole body and probably 5000 to 10,000 rep. of beta rays to his hands in about a 60 minute period. At the time of exposure he noticed a tingling and itching of his palms which he assumed to be due to excessive perspiration. Approximately 2 hours later he noticed a stiffness of his fingers and a slight swelling of his hands. He was seen by a physician 34 hours after exposure. At this time the second and third fingers of the right hand showed slight swelling, erythema and tenderness. On the left hand the volar aspect of all digits and the distal portion of the palm showed mild erythema and swelling. There was a blanched area over the proximal phalanx of second finger of the left hand.

In the next 24 hours there was a definite decrease in the erythema and swelling of both hands while the blanched area of the index finger of the left hand started to become vesicular in character and by three days after exposure there was a definite vesicle formed with a surrounding area of erythema.

On the 4th day the center of this vesicle took on a blackish-blue color and there was erythema of the volar surface of the second, third and fourth fingers of the left hand and slight selling with no erythema of the index finger and thumb of the right hand.

On the 7th day the right hand showed blanched and tender areas along the thenar aspect of the index finger and on the pad of the distal phalanx of the thumb. By the 8th day these areas became vesicular. From the 8th to the 25th day there was a continuous extension of the erythema of both hands with blisters forming two to three days after the onset of the reddening. (Figures 1 and 2) By the 25th day the erythema had disappeared and there was a maximum of bleb formation. At this time there was one large confluent blister covering all four fingers and the distal portion of the palm of the left hand, and a comparable bleb covering the right hand with extension into the palm.

From the 25th day after exposure the clinical course has been one of constant slow improvement. By the 28th day dehydration of the blebs of both hands was quite noticeable and on the 32nd day most of the blistered areas were quite dry.

On the 39th day the hands were surgically debrided. At this time the skin of both hands showed epithelization except in the area over the proximal phalanx of the second finger of the left hand which was white and necrotic and lesser areas of non-healing over the second, third and fourth fingers of both hands.

A second debridement was done on the 53rd day and at this time the only unepithelized areas were over the second and third fingers of the left hand and a small area over the proximal phalanx of the second finger of the right hand.

Surgical debridements were carried out on the 67th days and 82nd day (Figure 3)

and there were still persistent unhealed areas on the second and third fingers of the left hand and on the proximal phalanx of the second finger of the right hand.

Five months after exposure these last mentioned lesions were still not healed (Figure 4). The dimensions of all three lesions were about one by two centimeters and there were white necrotic areas in the center of each of the three lesions with easy bleeding on trauma. The areas were surrounded by invaginating folds of thin, pink epithelium. There was minimal healing during the third, fourth and fifth months of the disease. Skin grafting of these lesions is to be performed in the near future.

There is much ankylosis of the phalangeal joints of the three involved fingers with soft tissue atrophy and deep scar tissue formation. It is felt that surgical repair will result in only poor final function.

CASE II.

This 27 year old white male received 17 roentgens of gamma rays to his thoracic region and 8,000 to 16,000 rep. of beta rays to his hands over about a 60 minute period. During this exposure he felt an itchy sensation of both hands. Four hours after exposure he noticed that his left hand felt swollen. He was seen by physicians 34 hours after exposure and at this time all the digits of the left hand were swollen and erythematous with the second and third fingers more severely involved. There were no blanched areas on the left hand and the right hand appeared perfectly normal.

During the 3rd and 4th days there was a definite decrease in erythema of the left hand and no symptoms referable to the right. From the 7th to the 11th day the left hand developed erythema and tenderness over all four fingers and the distal portion of the palm and on the 11th day tenderness and erythema was noted on the right index finger.

On the 12th day blisters began to develop in the reddened areas and up to the 28th day there was spreading of the erythema with blebs forming in all erythematous areas. Figure 5 shows the extent of the blebs on the 21st day. By the 28th day the blisters covered the volar surfaces of all the fingers and the distal portions of the palms of both hands.

At about the 28th day the erythema subsided and progressive healing started. The blebs were (~e dehydrated by the 35th day (Figure 6) and part of the dead epidermis pealed off spontaneously. Surgical debridement was done, however, on the 39th day and there was a thin epithelization over most of the burned areas (Figure 7). When a second debridement was done on the 53rd day there was a small unhealed area on the second finger of the right hand and on the second and third fingers of the left hand. On the 67th day a third debridement was performed and at this time both hands were entirely epithelialized. The new epithelium was quite thin and had a tight, shiny appearance as can be seen from Figure 8.

Five months after exposure there was a secondary breakdown of the epidermis over the most heavily exposed areas with cracking and serous drainage. Skin grafting of the palmar surfaces of the more seriously involved fingers has been advised. At this time there is no loss of mobility of the digits of either hand.

CASE III.

This 26 year old white male received 4.5 roentgens of gamma rays to his whole body and probably 5,000 to 10,000 rep. of beta rays to left hand in about a 40 minute period. He first noticed symptoms of swelling and stiffening of his left hand about 6 hours after exposure. He was seen by the authors 40 hours after exposure and at this time there was mild erythema and edema of the left hand. The right hand was free of signs and symptoms (this was true during the entire course of the disease). The generalized erythema and edema of the left hand decreased up to about the 3rd day at which time a secondary erythema appeared over the volar-thenar aspect of the index finger.

On the fourth day there were circumscribed dark red areas over each phalanx of the index finger and the middle and distal phalanges of the third finger - all on the volar-thenar aspect. From the 7th to 14th day (Figure 9) these five areas became a dark bluish color and vesicular in character. By the 15th day these blisters began to

coalesce and a new bleb appeared on the proximal phalanx of the third finger. At this time the 4th and 5th fingers had become erythematous. On the 17th day erythema was noted over the distal heads of the second and third metacarpals.

By the 28th day there was a confluent blister including all four fingers and the heads of the 2nd and third metacarpals. The entire reaction reached a maximum extent on the 32nd day at which time all erythematous areas, including one on the lateral margin of the distal phalanx of the thumb, had become blistered. On the 35th day the bullae had begun to dehydrate and desgumate.

Surgical debridement was performed on the 39th, 53rd and 67th days (Figure 10) and at each operation further healing was observed. On the 67th day there were whitish necrotic areas over the second and third fingers corresponding to the location of the initial bluish colored blebs seen on the 4th day.

116 days after exposure tendons were visable in both of the ulcerated areas (Figure 11). The tendon visable in the lesion on the second finger appeared to be partially necrotic. There was little, if any, healing of the lesions during the 3rd and 4th months of the disease. During the 5th month of the disease there was definite improvement of the lesions (Figure 12), but from a functional standpoint the second and third fingers of the left hand showed only slight joint mobility. Skin grafting of these two fingers is to be started in the near future.

CASE IV.

This 27 year old white male received 1.5 roentgens of gamma rays to his whole body and probably 3,000 to 4,000 rep. of beta rays to his left hand in about a 60 minute period. He had no symptoms until 11 days after exposure at which time the patient noticed redness and swelling of the skin over the volar aspect of the proximal phalanges of the second, third and fourth fingers of the left hand. (At no time during the course of the disease were there any signs or symptoms referrable to the right hand). The patient was seen by a physician on the 13th day at which time there was no vesicle

formation but a bluish discoloration beneath the skin in the above areas. By the 15th day blisters had developed on all of the erythematous areas. The middle phalanges of the second and third fingers showed erythema and there was a bluish discoloration over the head of the second metacarpal.

On the 19th day vesicles had formed over the middle phalanges of the second and third fingers.

The middle phalanx of the 4th finger and distal phalanx of the second finger showed an early erythema. By the 25th day the process had ceased to progress and there were blebs present over the volar surfaces of all the fingers and over the head of the second metacarpal. From this time on dehydration progressed rapidly and by the 32nd day the blisters were completely dehydrated. There was desquamation of the burned surfaces by the 47th day with epithelization over all of these areas. This patient has had no signs of residual damage or tissue breakdown over a period of five months and appears to have complete healing of the entire hand.

DISCUSSION:

A. CLINICAL FINDINGS:

The clinical course of these four patients can be divided into three distinct phases. Phase I consisted of the initial erythema and edema with blanching of areas which received the greatest amounts of exposure. This phase reached a peak in about 48 hours and then rapidly subsided. There was then a period of relative remission of signs and symptoms for 3 to 5 days at the end of which Phase II of the disease began. Phase II was first manifested by the development of a secondary erythema which in some cases was complicated by an extravasation of blood into the erythemic areas. These reddened areas became vesicular 8 to 12 days after exposure. There was then a period about two weeks long during which the erythema spread and the blisters formed. At the end of this time (24-32 days after exposure) the active disease process ceased to progress and the blisters began to dehydrate and desquamate. During the last part of Phase II

epithelization occurred over all areas below which the vascular supply had not been seriously damaged. Phase III was primarily the chronic state of the disease. Those areas of skin which had had serious interference with the vasqular supply remained unhealed and skin grafting will be indicated at a later date. There is an atrophic epidermis probably permanent in the less involved areas with a loss of secondary epithelial structures (hair, sabaceous glands, etc.). The secondary ulcerations with the concurrent hyperkeratoses, talangictases and carcinomas which occur many years after exposure must be included in this phase.

It is interesting to note that the two individuals who probably received the largest amounts of radiation apparently noted a sensation of tingling and itching of the hands at the time of exposure. It is important to realize that irradiation of this magnitude produces a sensory perception.

The initial erythema and edema seen in these patients in the first 48 hours hours may be due to a local release of histamine in the skin with a resulting dilation of the superficial capillaries. In areas which were more heavily exposed, there was a definite blanching which may have been due to direct damage to the capillaries resulting in a vasoconstriction phenomenon or to thromboses with occlusion of the vessels. The secondary erythema which occurred after 5-7 days was probably due to a vascular reaction to dead epithelial cells which were continuing to die over a long period of time.

It has long been recognized that many cells which have been injured by radiation can survive for a considerable period of time, but when they attempt to divide at a later date are unable to do so and die. There is good statistical evidence that the intermitotic time for human epidermis is between 17 and 35 days and this would suggest that cell death as a result of the acute exposure will occur over a 3 to 4 week period.

The bluish discoloration of some of the erythematous areas was a result of ex-

travasation of blood from the injured dermal plexus of capillaries. These hematomatous areas in all cases showed poor healing and in some cases non-healing. This non-healing is due to the severe damage to the capillaries which must, in the process of repair, nourish the healing epidermis.

All of the areas of the skin which were involved in the secondary erythematous process showed blister formation. The blisters were usually discrete initially and subsequently coalesced with the formation of large blebs which, in some cases were continuous over all five digits and most of the palm. These blebs were tense and frequently caused sufficient pain despite generous sedation to justify repeated drainage. It is important that, in spite of spontaneous and surgical drainage of these blebs aerobic and anaerobic cultures of the blister fluid was negative except on one occasion in one case in which staphalococcus albus was found. Thus, the course was not complicated by secondary infection. The development of a cellulitis resistant to chemotherapy would have been very detrimental to the course of the disease. The blisters began to dehydrate at various time intervals after exposure and were either spontaneously desquamated or removed by surgical debridement.

The new epithelium which was formed over the involved areas was, in general, quite thin and delicate except in Case IV, in which there appears to be complete healing. A blotchiness of the underlying vascular tissue, which probably represents minor vascular damage, was noted in this case.

The effects on certain secondary skin structures in these patients have been quite definite. Nail growth was retarded in all cases to about one-half the normal rate (several of the authors serving as controls) and there was a slight depression in the nail which was being formed at the time of exposure. There was a definite loss of hair from the backs of the fingers in Case II, but this was not noted in the other three individuals. The dorsal surfaces of the fingers showed some increase in pigmentation in Cases I, II and III.

There were definite changes in the capillary loops of the nail beds. Inside of the first 5 days both an abnormal amount of dilation and constriction of the loops were apparent with an increased tortuosity and in some capillaries actual thrombosis with interruption of blood flow was observed. By 10-15 days there was a definite loss in the number of capillary loops and during the third and fourth weeks some of the remaining loops had formed collateral loops into the area of destroyed capillaries. At the end of 4 months there was still a decreased number of capillaries and the collateral loops were apparent and functioning.

It should be mentioned that all of the erythema and vascular lesions were confined to the volar surface of the hands. The dorsal surfaces showed minor desquamation but, in general, there was a sharp line of delineation between the volar and dorsal surfaces. Another important feature of the cases was the lack of damage to the right hands of Cases III and IV and the lesser reaction seen in the right hands of Cases I and II. This can be explained by the nature of the operation which involved more contact of the radioactive material with the left hand.

Case II, who apparently received the largest exposure had complete epithelization of the burned areas. This individual had unusually thick epidermis on the palmar surfaces of his hands and this was no doubt extremely important in reducing the dosage received by the more vital underlying vascular tissue. One extra millimeter of tissue overlying this vascular bed would decrease the amount of radiation to these capillarie by a factor of about 50%.

The TPR charts are not shown but, in general, there were slight elevations of temperature and pulse corresponding to the development of the blisters. The respiratory rates and blood pressures remained in normal range throughout the illness. The salient clinical features have been placed on Graphs Ia, IIa, IIIa and IVa, the numerals corresponding to the case numbers.

B. LABORATORY FINDINGS:

The laboratory examinations in these cases showed significant changes in the total white blood counts, the absolute neutrophil counts, the refractile granules in the cytoplasm of the lymphocytes, and the sedimentation rate. The total white blood count is the average of counts obtained from both sides of a hemacytometer. The differential count, used for the calculation of the absolute neutrophil and lymphocyte figures, was made in wet films stained suprovitally with neutral red and Janus green. A total of 200 cells were examined for the differential count, 100 by each of two technicians. To determine any quantitative change in the number of refractile granules in the lymphocytes, one hundred lymphocytes were examined, 50 by each of two technicians, and the average values used. The same two technicians did all of the differential counts and lymphocyte studies and, although there was constantly a slightly larger number of granules counted by one of the technicians, this possible source of error is constant throughout the data. The hemoglobin values were obtained from a standard Evelyn photometric hemoglobinometer. The per cent reticulocytes and platelet counts were determined in a wet preparation stained with brilliant cresyl blue and at least 1000 erythrocytes were counted. The sedimentation rates were obtained in a standard Wintrobe tube.

The total white blood count, absolute neutrophil and lymphocyte counts, sedimentation rate, and lymphocyte granules are presented for each case with the salient clinical features in the accompanying graphs. The pre-exposure blood data as plotted is taken from approximately one year of routine blood studies, and is plotted with no reference to the time intervals between each one. The refractile granules in the lymphocytes are plotted by two methods. The per cent of lymphocytes with six or more granules is used because there were control counts obtained by this method previous to exposure and also the cases reported in the literature previously have been studied in this fashion. The granules per lymphocyte is a less arbitrary method of reporting

the findings and is now used routinely by this laboratory. In the pre-exposure portion of the curves showing the percent of lymphocytes with six or more granules all of the values have been averaged since fewer lymphocytes were examined per count for these routine pre-exposure observations.

The response of the total white blood count, the neutrophil count and the sedimentation rate appeared to run parallel with the development of the blisters on the hands and the fever response which was seen. Apparently there was enough tissue destruction occurring during this time to give these hematological changes. Case I, who showed the most severe formation of blebs showed the greatest changes in these blood tests.

The absolute lymphocyte counts did not show any significant changes at any time after exposure. Despite this fact, however, there was a definite morphological change in these cells manifested by an increased number of refractile granules in the supravital preparations. This change was much earlier than the increase in the neutrophils or the sedimentation rate. It should be noted that in Case II the exposure record showed that he received more gamma irradiation than any of the other individuals yet the increase in refractile granules was minimal and the healing of the burned areas was complete before the two patients (Cases I and III), who had a greater rise in these lymphocytic bodies. This suggests that this response is related to the local process in the hands and may be an early indicator of the seriousness of the burn.

There was a 1 to 2% rise in reticulocytes in all cases which is very difficult to explain. These individuals were at sea level for about 10 days and returned to Los Alamos (elevation 7400 feet). It appears unlikely that this increase in altitude caused a reticulocyte response since all four individuals had lived at Los Alamos for at least one year before their short sojourn at sea level. The reticulocytosis is more likely due to an erythroid response in the bone marrow to the tissue destruction.

The hemoglobins, erythrocyte counts, and platelets at no time showed variations

from normal which could not be explained by inherent experimental or statistical errors.

The prothrombin times, bleeding times, and clotting times were done frequently during the course of the illnesses and at no time showed abnormalties. The absolute monocyte, eosinophil and basophil counts showed no significant variation from the normal.

Urine analyses at all times were considered normal and tests for radioactivity in the urine, which were done 72 hours after exposure, were negative.

Blood chemistry, including serum proteins with albumin-globulin ratios, serum bilirubin, alkaline phosphatase, and blood urea nitrogen with daily nitrogen balance studies were all normal during the illnesses.

C. TREATMENT:

The initial treatment consisted of immobilization, elevation and chilling with ice packs of the involved hands. The individuals were placed on a high protein diet with supplementary multiple vitamins, and given rutin 20 mg. t.i.d.

Duracillin therapy, 300,000 units each day, was started in Case I after drainage of the blebs and with the development of low grade fever and increase in sedimentation rate and after the first debridement in Cases II and III. This was considered to be prophylactic therapy in view of the possible disasterous effects of concurrent infection.

The surgical debridement done in Cases I, II and II on the 39th, 53rd and 67th days were done under general anesthesia due to the extreme tenderness of the tissues. An attempt was made to clean off all dead skin and areas of soft necrosis without damaging the forming epithelium. Pressure dressings were applied with vaseline gauze proximal to the injured areas. These dressings were kept on for 14 day periods.

It should be mentioned at this point that the Phase III of the clinical course

has actually just been entered and that these individuals will have to be followed closely for many years in order to see the complete course of their disease. It is very probable that skin grafts will be necessary in Cases I, II and III, and the outlook for "takes" of these grafts is entirely dependent upon the state of the vascular bed beneath the present ulcerated areas.

D. CONCLUSIONS:

In these four cases of beta ray burns to the hands, it has been seen that, although in general beta rays are not considered a serious external radiologic hazard, this form of ionizing radiation is a definite external hazard in large dosages. The clinical response can be divided into three phases:

I. A sensation of tingling and itching at the time of exposure and a short time later a slight erythema and edema (sometimes associated with blanching of the skin) which lasts for 2-3 days.

II. A secondary erythema which soon produces vesicles and blebs which dehydrate and desquamate after about 2 weeks, leaving a new layer of thin epithelium.

III. A chronic phase with atrophic epithelium, loss of secondary epidermal structure, telangiectases, hyperkaratoses and carcinomas.

The only significant hematological findings were a neutrophilia, an increased sedimentation rate, an increase in the number of refractile granules in the cytoplasm of the lymphocytes, and a low grade reticulocytosis. Blood chemistry and urine findings were consistently normal. Local treatment consisted of routine burn therapy with accessory chilling and later surgical debridement. General treatment involved the use of rutin, high protein diet, multiple vitamins, and parenteral penicillin. Prognosis is dependent upon the status of the vascular supply to the burned areas in view of probable skin grafting and the careful late

follow-up necessary to recognize early malignant changes.

E. SUMMARY:

Four cases of beta ray burns to the hands have been presented with careful consideration of the clinical course, laboratory findings, treatment and long range prognosis.

FIGURE 1

CASE I - 11 DAYS AFTER EXPOSURE

THIS DEMONSTRATES THE EARLY VESICLES ON THE SECOND FINGERS OF BOTH HANDS AND THE SHARP MARGINATION OF THE ERYTHEMA ON THE FOURTH FINGER OF THE LEFT HAND.

FIGURE 2

CASE I - 18 DAYS AFTER EXPOSURE

THIS DEMONSTRATES THE LARGE, TENSE, CONFLUENT BIEBS WHICH FOLLOWED THE SECONDARY ERYTHEMA.

FIGURE 3

CASE I - 82 DAYS AFTER EXPOSURE

THERE IS AN ULCER OVER THE FIRST PHALANX OF THE SECOND FINGER WITH A SLIGHTLY VASCULAR, FIBROTIC GRANULATION TISSUE COVERING IT. THE SUPERFICIAL DESQUAMATION OVER THE OTHER FINGERS AND PALM IS ALSO QUITE EVIDENT.

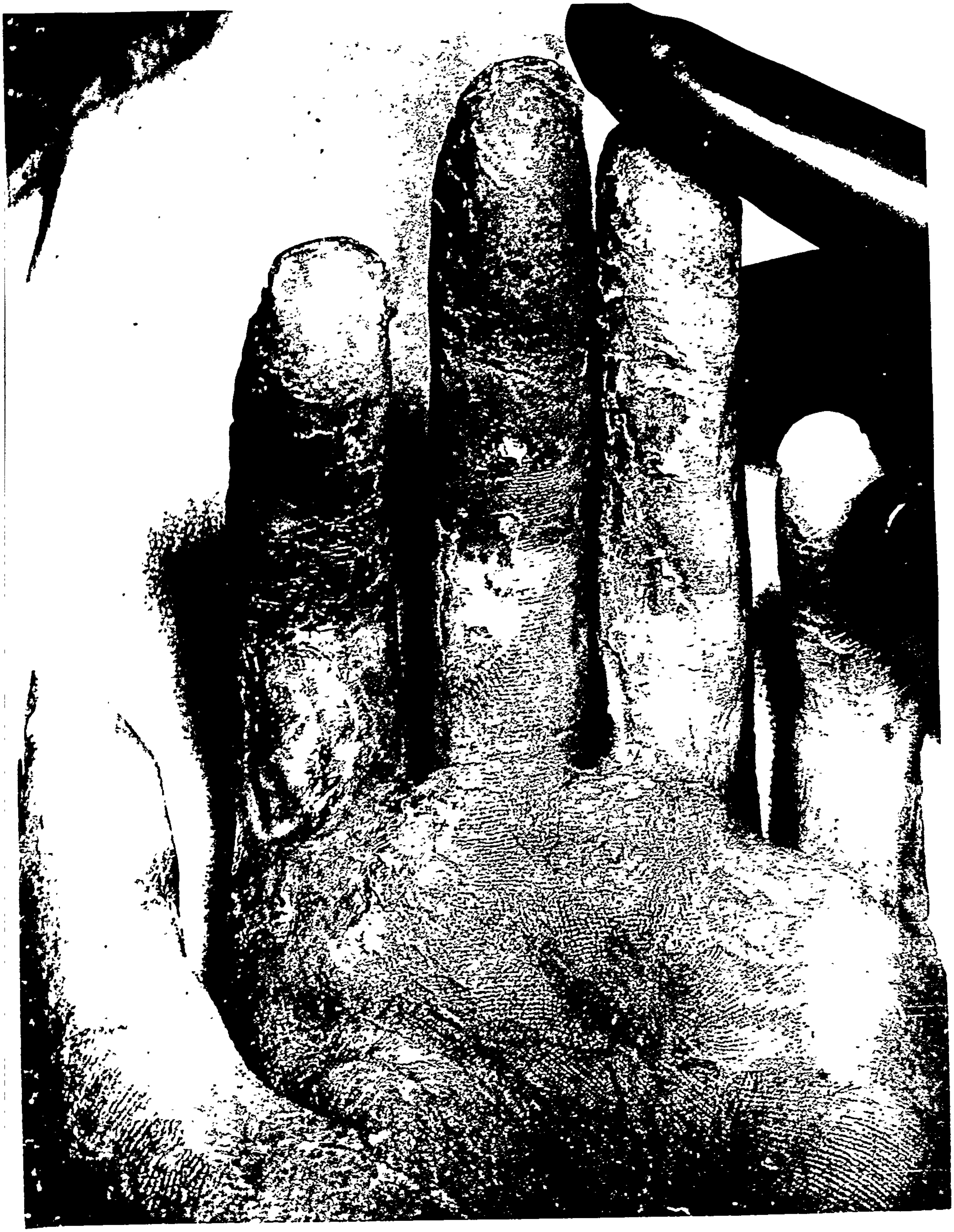


FIGURE 4

CASE I - 158 DAYS AFTER EXPOSURE

THERE IS STILL AN UNHEALED ULCERATED AREA OVER THE FIRST PHALANX OF THE SECOND FINGER WITH AN ATROPHY AND FLEXOR CONTRACTION OF THE DIGIT.

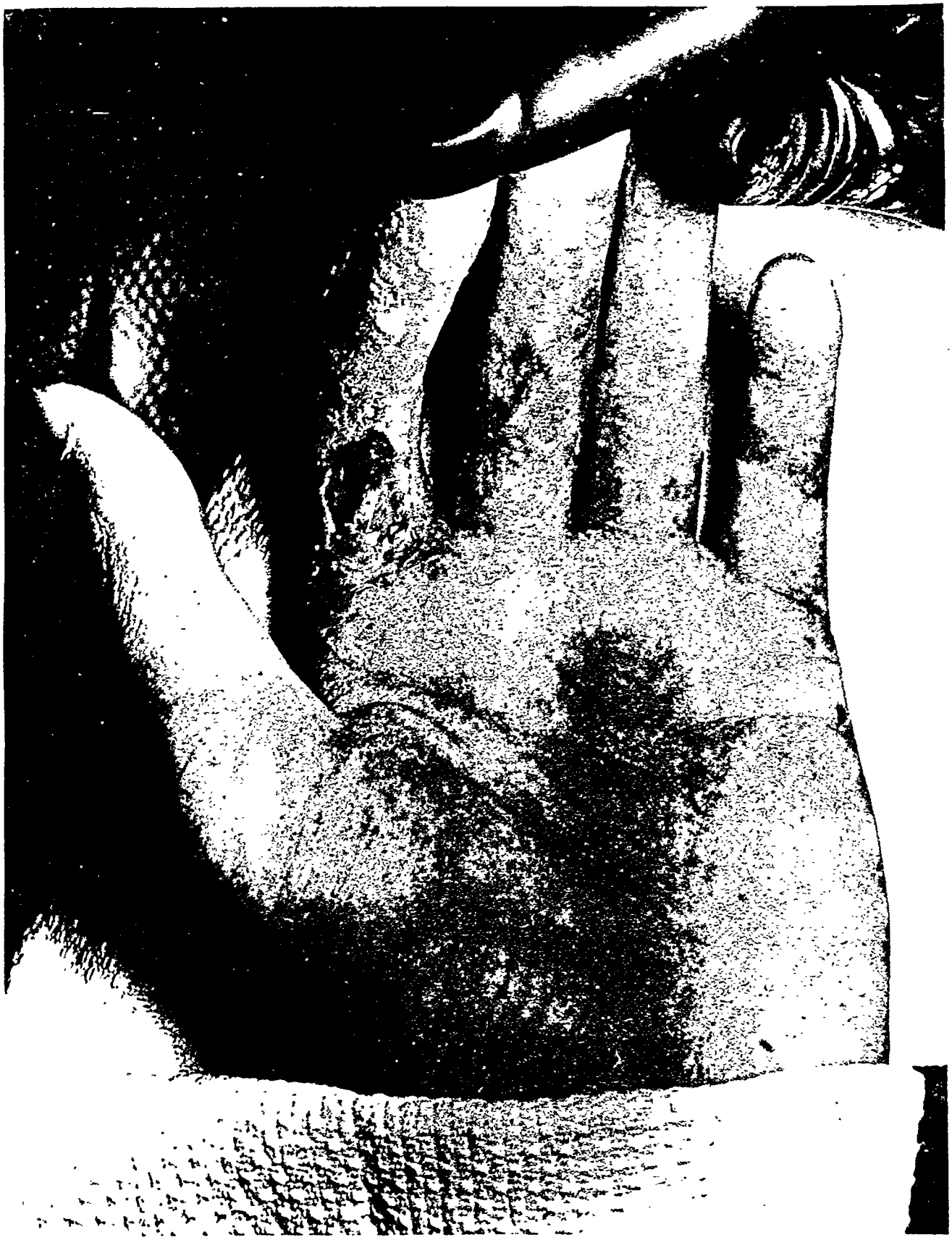


FIGURE 5

CASE II - 21 DAYS AFTER EXPOSURE

THIS DEMONSTRATES THE TENSE DIGITAL BLEBS WHICH COVER THE ENTIRE PALMAR SURFACES AND EXTEND AROUND TO THE LATERAL ASPECTS OF MANY FINGERS. THE BLISTER ON THE THENAR ASPECT OF THE LEFT PALM IS AT THE SITE OF A FAIRLY RECENTLY HEALED PRESSURE-FRICTION BLISTER INCURRED PRIOR TO THE IRRADIATION.

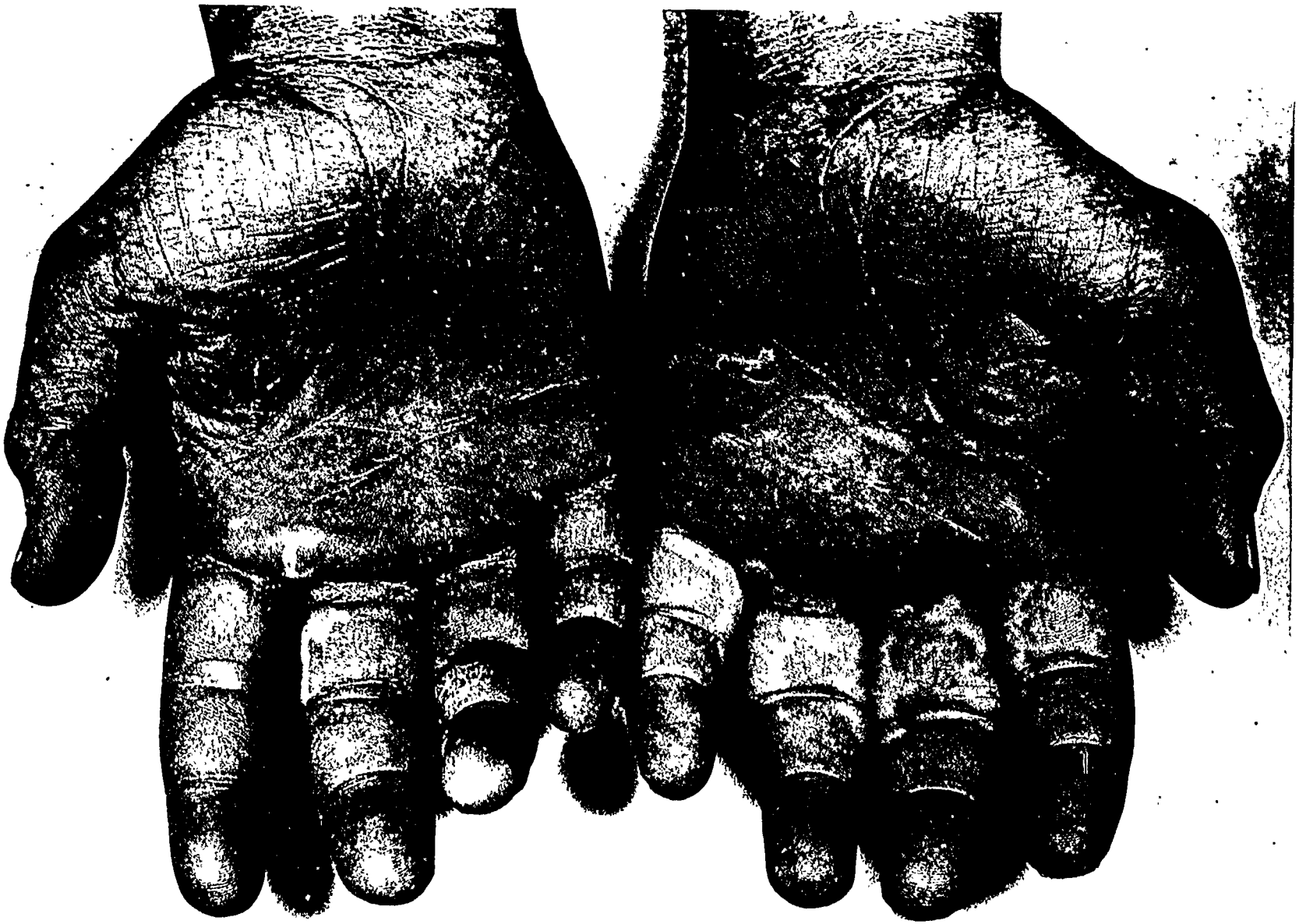


FIGURE 6

CASE II - 35 DAYS AFTER EXPOSURE

THERE IS MUCH SPONTANEOUS DESQUAMATION OVER THE BURNED AREAS WITH A TANNED APPEARANCE OF THE PROXIMAL PORTIONS OF THE PALMS.

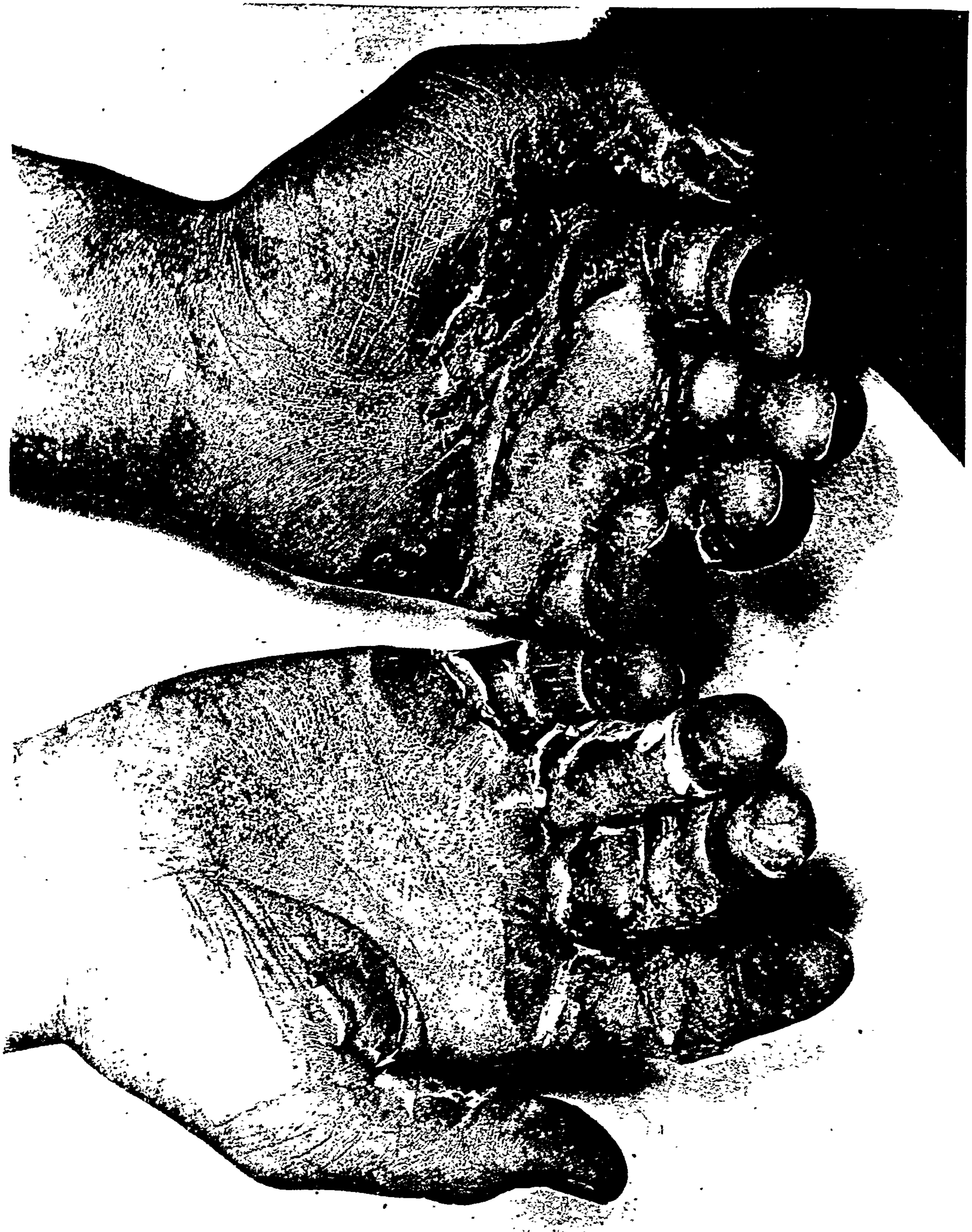


FIGURE 7

CASE II - 39 DAYS AFTER EXPOSURE

THIS SHOWS THE VERY THIN EPITHELIUM FOUND BENEATH THE THICK, DEAD SKIN WHICH HAD JUST BEEN REMOVED SURGICALLY. THESE AREAS WERE VERY PAIN SENSITIVE.

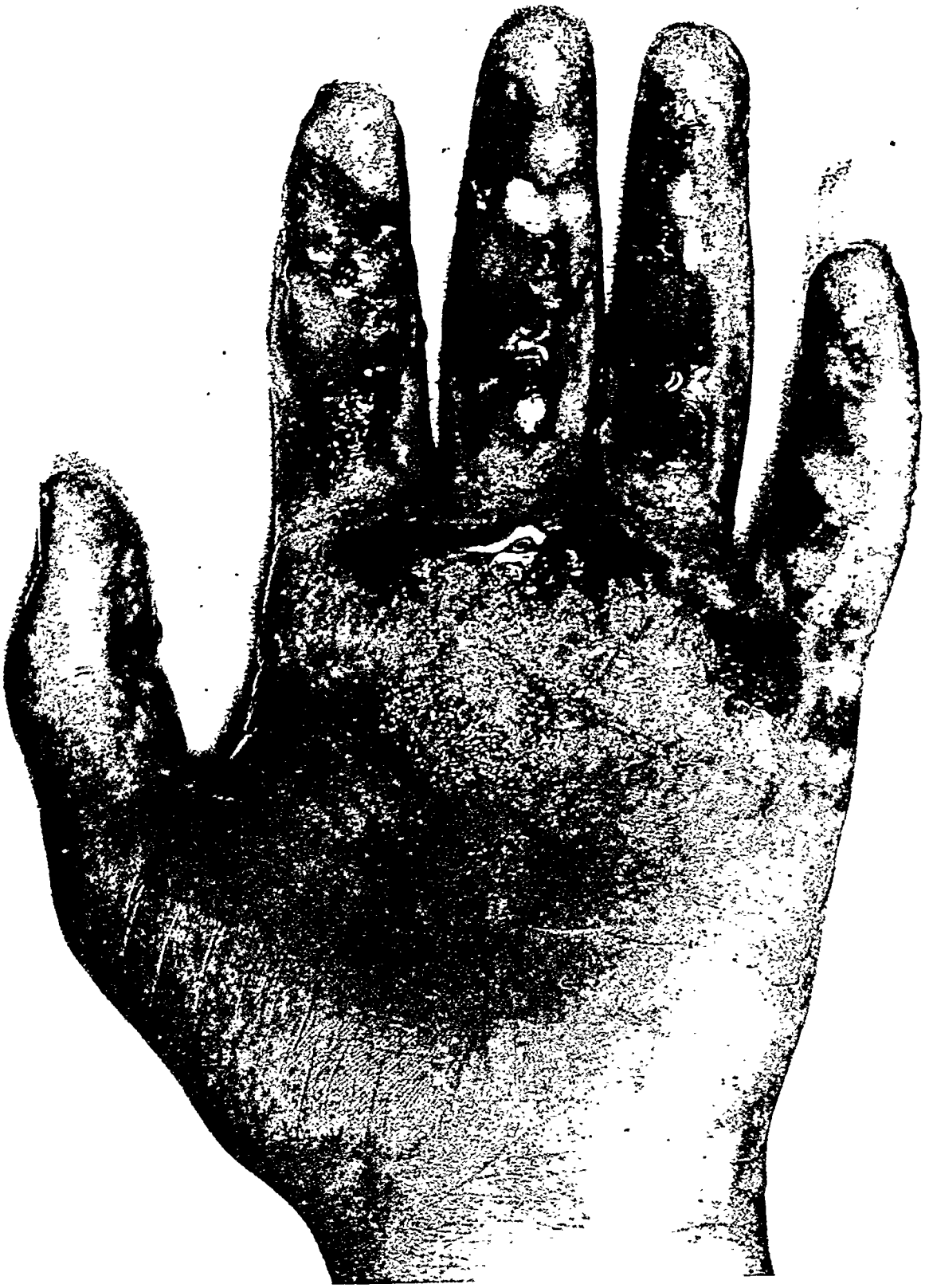


FIGURE 8

CASE II - 82 DAYS AFTER EXPOSURE

THERE HAS BEEN COMPLETE EPITHELIZATION OF BOTH HANDS, BUT THE SHINY, ATROPHIC EPIDERMIS IS EVIDENT OVER BOTH INDEX FINGERS. THE SECONDARY BREAKDOWN OCCURRED IN THESE AREAS AT ABOUT THE 160TH DAY.

FIGURE 9

CASE III - 14 DAYS AFTER EXPOSURE

THERE ARE SMALL, FAIRLY DISCREET VESICLES ON THE SECOND AND THIRD FINGERS OF THE LEFT HAND. THESE VESICLES HAD A DEFINITE BLUISH COLOR. THE RIGHT HAND WAS NEVER INVOLVED.

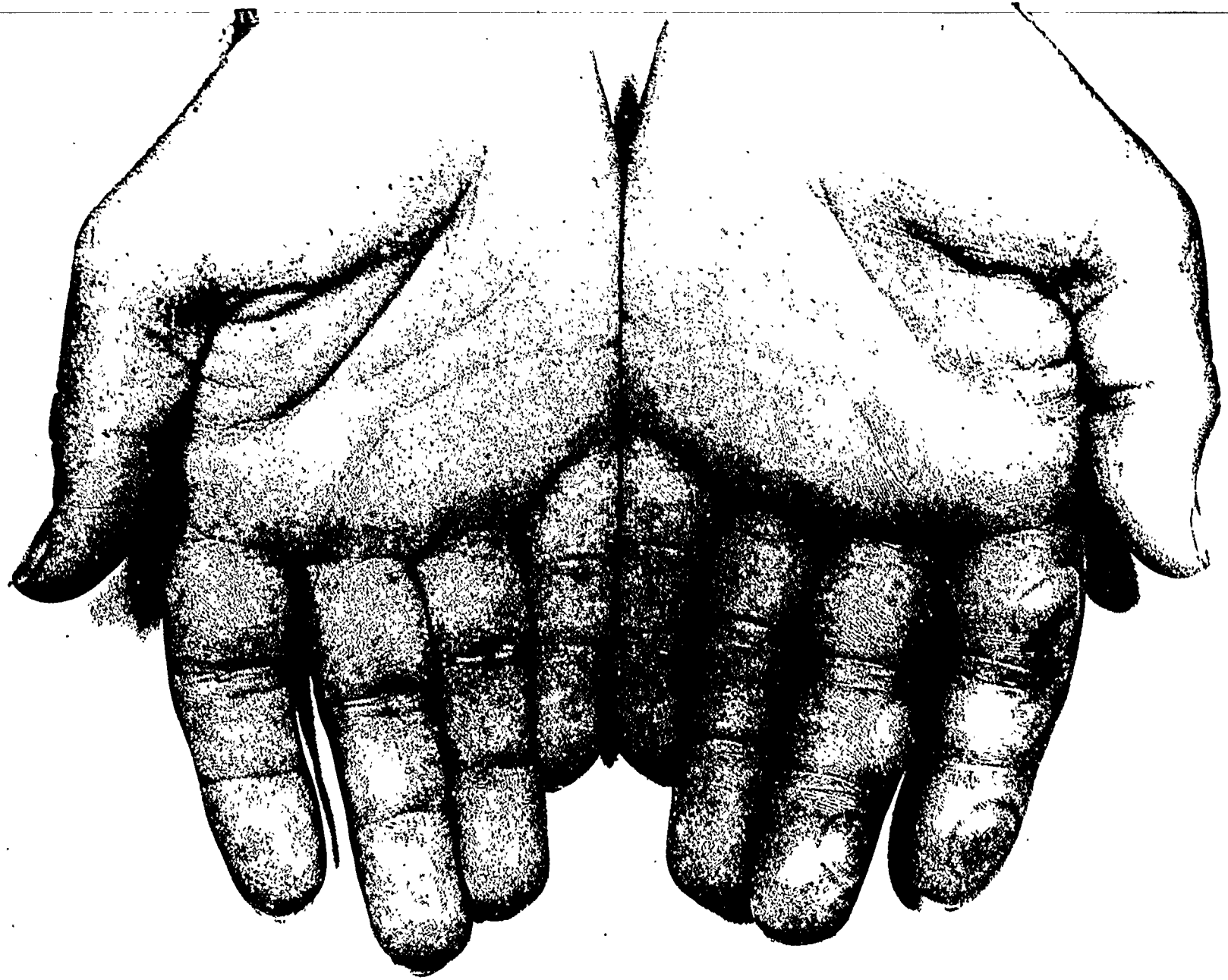


FIGURE 10

CASE III - 67 DAYS AFTER EXPOSURE

THERE ARE FAIRLY DEEP ULCERS SIMILAR TO THAT SEEN IN FIGURE 3 AND CORRESPONDING TO THE POSITION OF THE VESICLES SEEN IN FIGURE 9. THE SUPERFICIAL DESQUAMATION OF THE PALMAR AND DIGITAL SURFACES IS ALSO SHOWN.

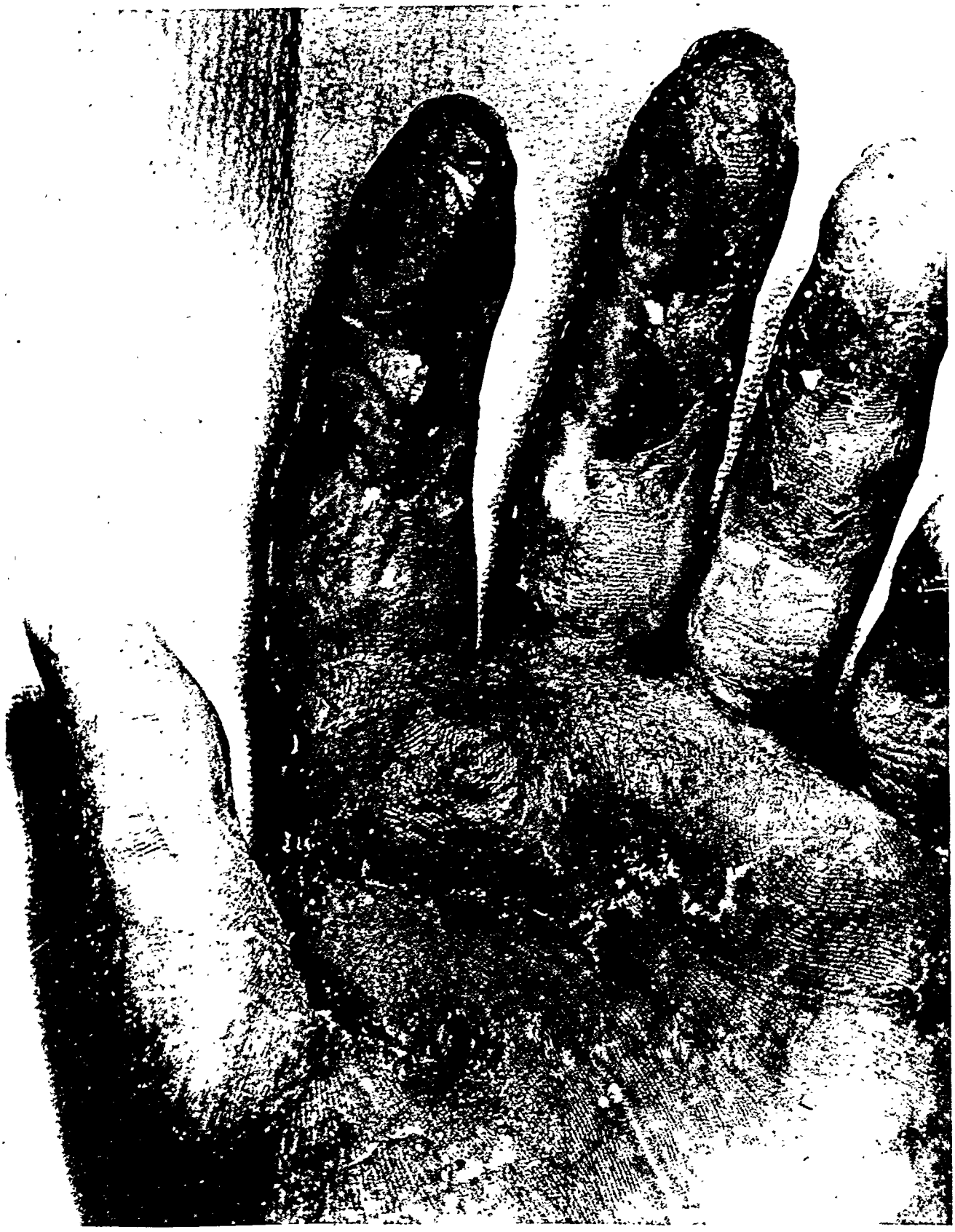


FIGURE 11

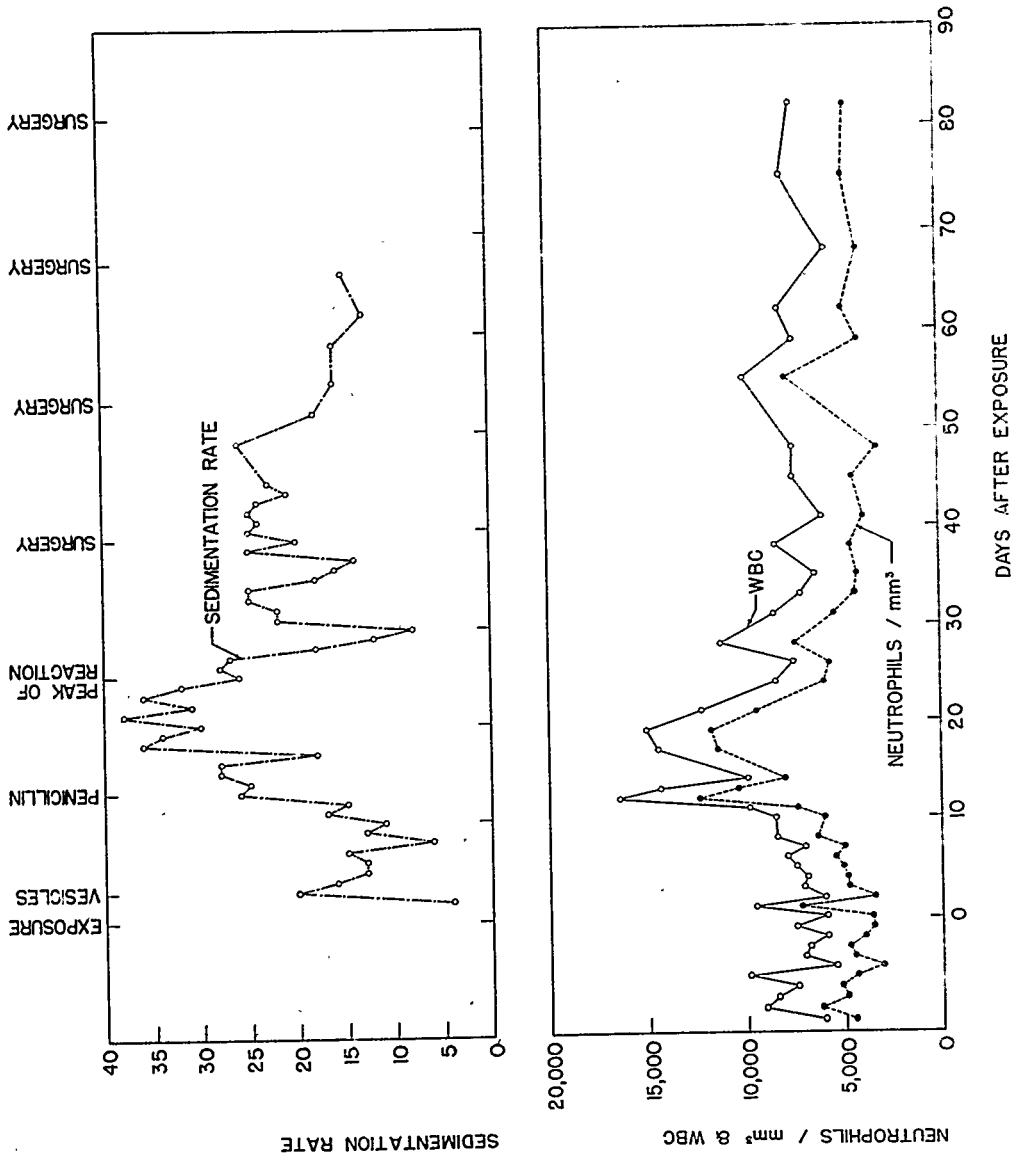
CASE III - 116 DAYS AFTER EXPOSURE

THIS CLOSEUP VIEW SHOWS THE EXTENT OF THE ULCERS DURING THE FOURTH MONTH. SMALL HEMORRHAGES CAN BE SEEN IN THE FIBROTIC GRANULATION TISSUE AND A SHINY TENDON SURFACE IS PRESENT IN THE LESION OVER THE FIRST PHALANGEAL JOINT OF THE INDEX FINGER.

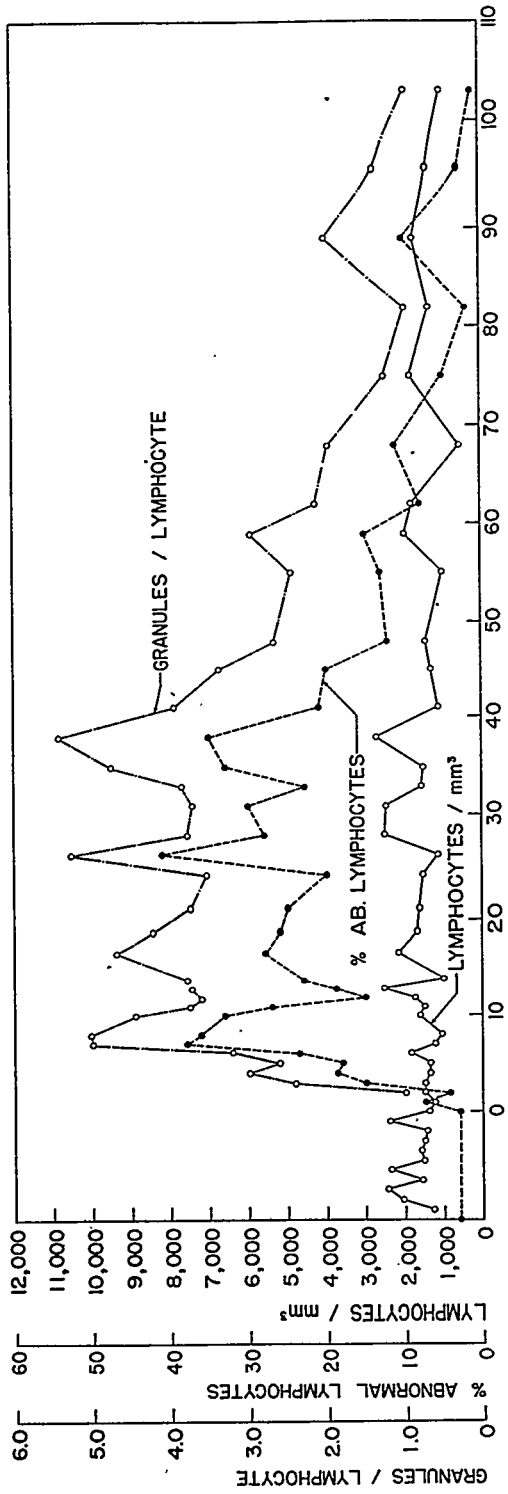
FIGURE 12

CASE III - 165 DAYS AFTER EXPOSURE

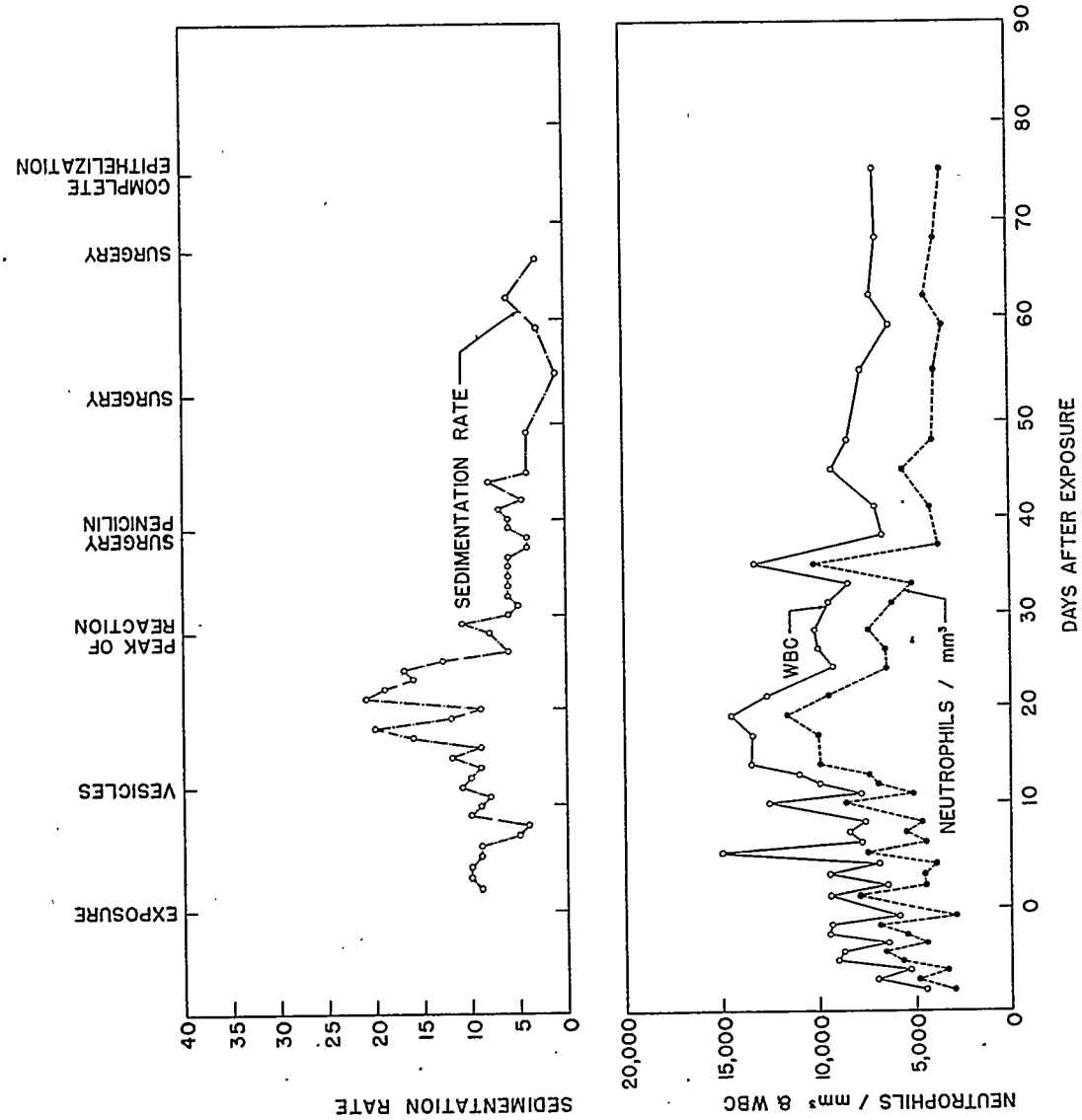
THIS DEMONSTRATES THE DEGREE OF HEALING WHIGH OCCURRED DURING THE 49 DAYS BETWEEN THIS PICTURE AND FIGURE 11. THE TENDON SURFACE IS STILL VISABLE THROUGH A THIN LAYER OF FIBROUS TISSUE.


SEDIMENTATION RATE
EXPOSURE
VESICLES
PENICILLIN
PEAK OF REACTION
SURGERY
SURGERY
SURGERY
SURGERY
SEDIMENTATION RATE
40
35
30
25
20
15
10
5
0
WBC
NEUTROPHILS / mm³
20,000
15,000
10,000
5,000
0
NEUTROPHILS / mm³ & WBC
0
10
20
30
40
50
60
70
80
90
DAYS AFTER EXPOSURE

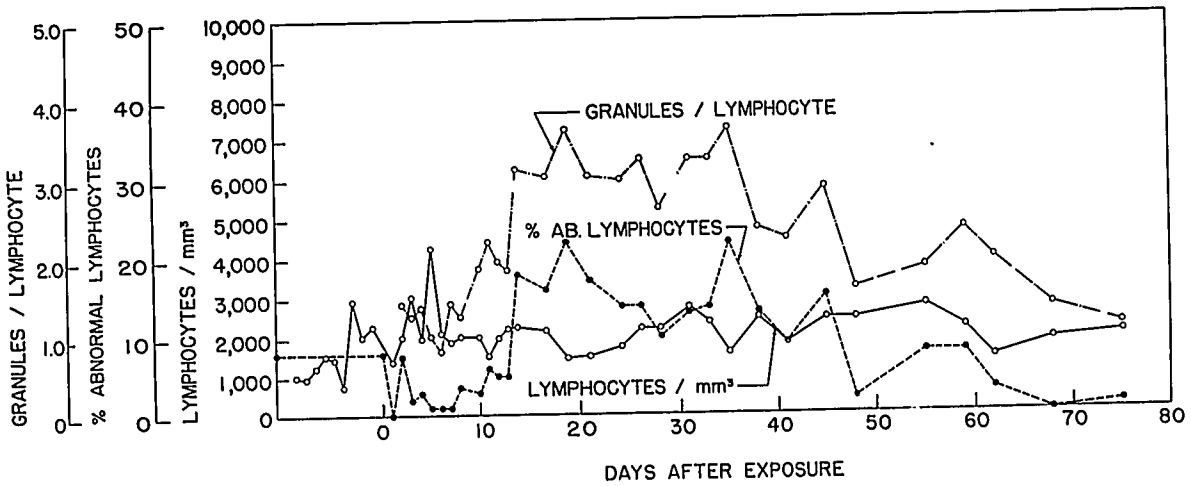
GRAPH Ia


GRANULES / LYMPHOCYTE
% AB. LYMPHOCYTES
LYMPHOCYTES / mm³
DAYS AFTER EXPOSURE
GRANULES / LYMPHOCYTE
% ABNORMAL LYMPHOCYTES
LYMPHOCYTES / mm³

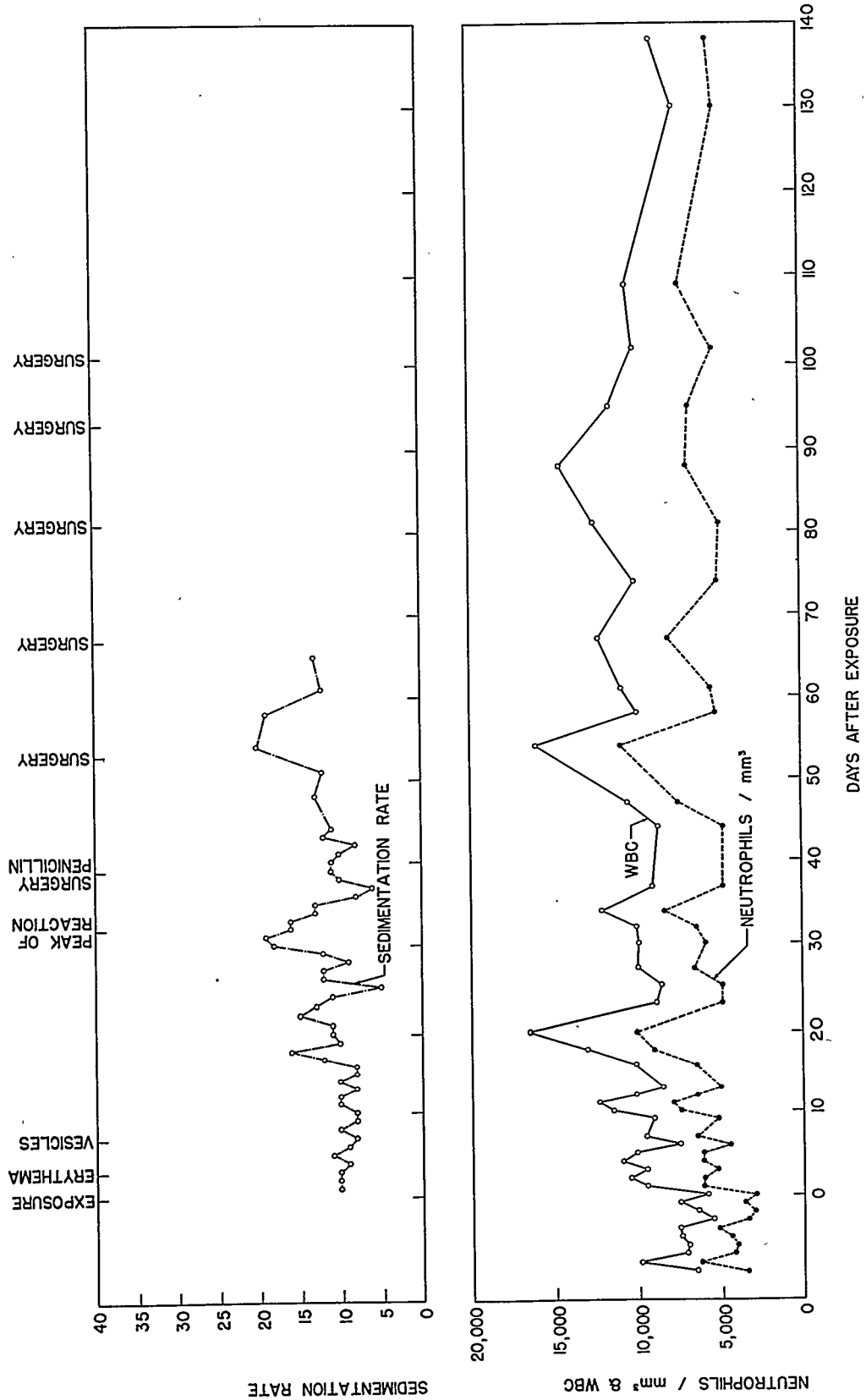
GRAPH Ib


EXPOSURE
VESICLES
PEAK OF REACTION
SURGERY PENICILIN
SURGERY
SURGERY
COMPLETE EPITHELIZATION
SEDIMENTATION RATE
SEDIMENTATION RATE
40
35
30
25
20
15
10
5
0
NEUTROPHILS / mm³ & WBC
20,000
15,000
10,000
5,000
0
WBC
NEUTROPHILS / mm³
0
10
20
30
40
50
60
70
80
90
DAYS AFTER EXPOSURE

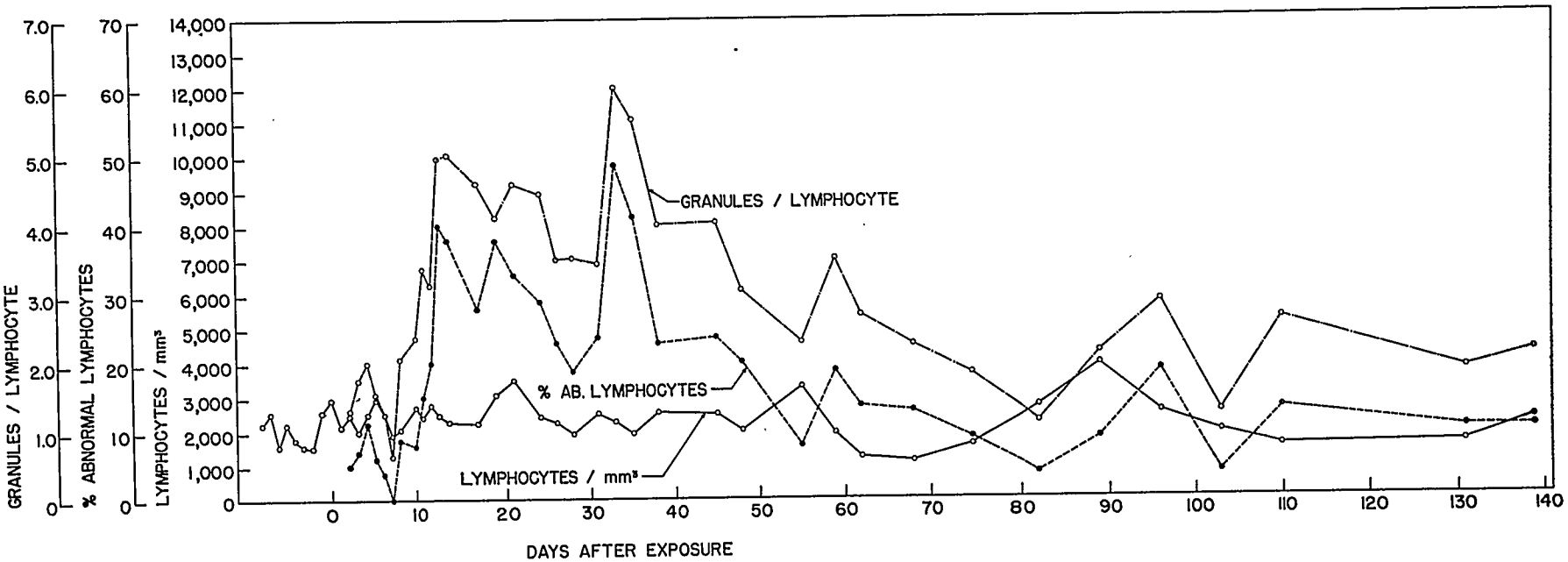
GRAPH IIa


GRANULES / LYMPHOCYTE
% ABNORMAL LYMPHOCYTES
LYMPHOCYTES / mm³
5.0
4.0
3.0
2.0
1.0
0
50
40
30
20
10
0
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
GRANULES / LYMPHOCYTE
% AB. LYMPHOCYTES
LYMPHOCYTES / mm³
0
10
20
30
40
50
60
70
80
DAYS AFTER EXPOSURE

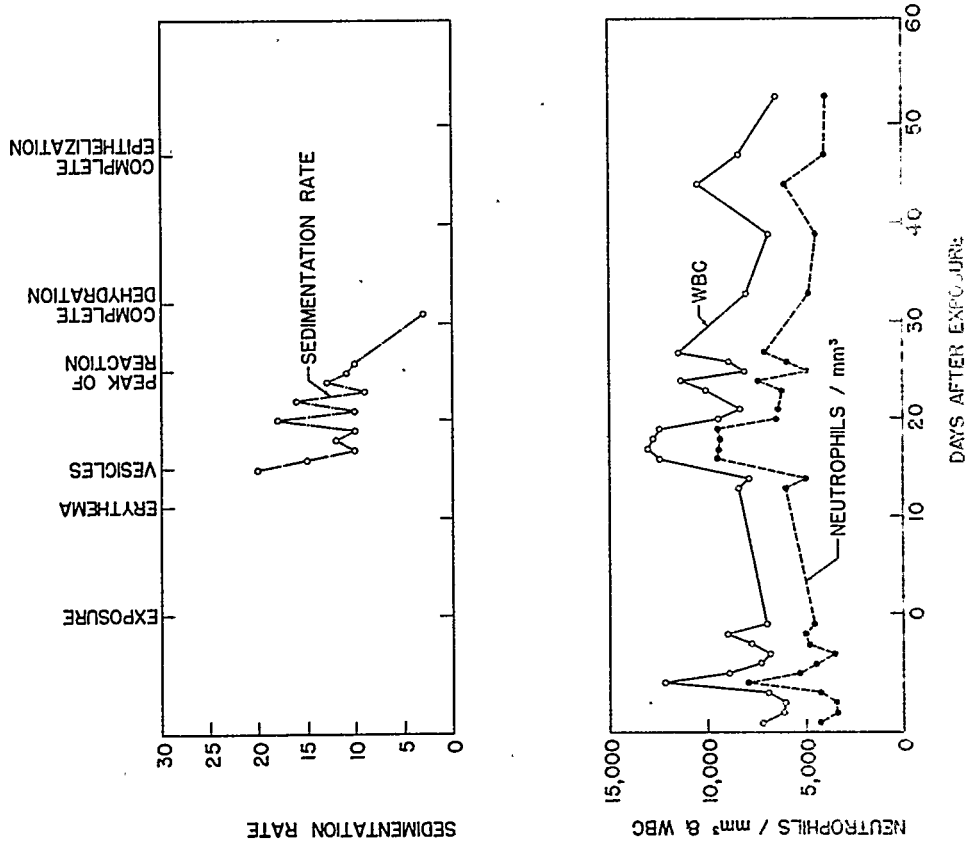
GRAPH IIb


SEDIMENTATION RATE
NEUTROPHILS / mm³ & WBC
DAYS AFTER EXPOSURE
EXPOSURE
ERYTHEMA
VESICLES
PEAK OF REACTION
SURGERY PENICILLIN
SURGERY
SURGERY
SURGERY
SURGERY
SURGERY
SEDIMENTATION RATE
WBC
NEUTROPHILS / mm³
40 35 30 25 20 15 10 5 0
20,000 15,000 10,000 5,000 0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140

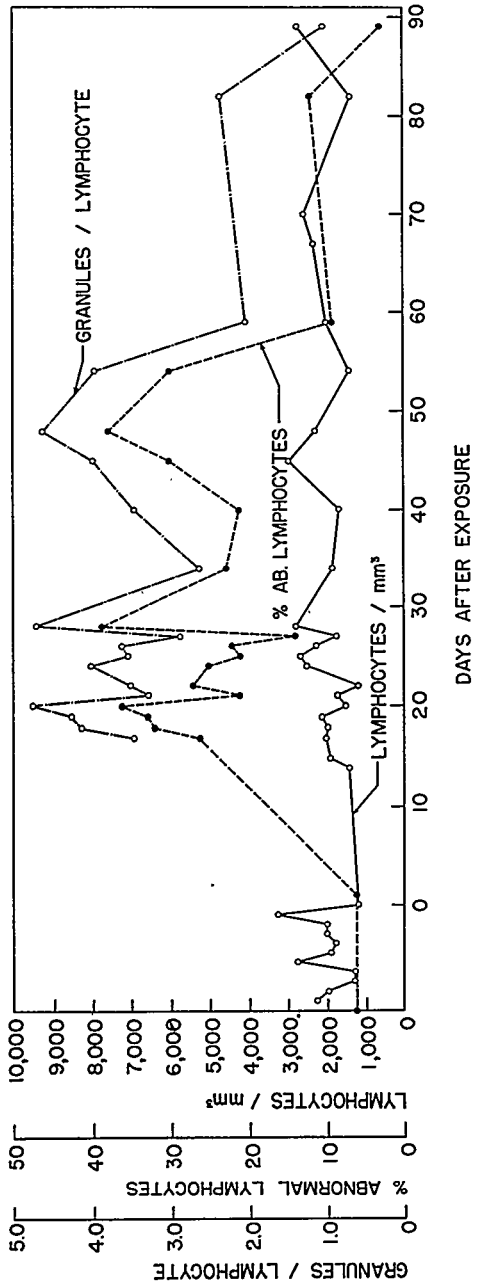
GRAPH III a


GRANULES / LYMPHOCYTE
% ABNORMAL LYMPHOCYTES
LYMPHOCYTES / mm³
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
70
60
50
40
30
20
10
0
14,000
13,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
GRANULES / LYMPHOCYTE
% AB. LYMPHOCYTES
LYMPHOCYTES / mm³
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DAYS AFTER EXPOSURE

GRAPH IIIb


SEDIMENTATION RATE
EXPOSURE
ERYTHEMA
VESICLES
PEAK OF REACTION
COMPLETE DEHYDRATION
COMPLETE EPITHELIZATION
30
25
20
15
10
5
0
SEDIMENTATION RATE
WBC
NEUTROPHILS / mm³
15,000
10,000
5,000
0
NEUTROPHILS / mm³ & WBC
0
10
20
30
40
50
60
DAYS AFTER EXPOSURE

GRAPH IV a


GRANULES / LYMPHOCYTE
% AB. LYMPHOCYTES
LYMPHOCYTES / mm³
DAYS AFTER EXPOSURE
GRANULES / LYMPHOCYTE
% ABNORMAL LYMPHOCYTES
LYMPHOCYTES / mm³

GRAPH IV b

USE OF Na^{24} IN A CIRCULATION STUDY ON A PATIEN BELIEVED TO HAVE IMPAIRED CIRCULATION IN THE LEFT LEG.

Edgar Leifer and John R. Hogness

Since the patient presented a diagnostic problem as to the type of vascular disorder present in his left lower extremity, it was felt that circulation studies with radioactive sodium would be of value in establishing a diagnosis. In view of the projected left lumbar sympathectomy, pre- and post- operative circulation studies were proposed for purposes of evaluation of the effects of operation.

Five cc. of normal saline containing 50 microcuries of radioactive Na were injected into the left antecubital vein. Radioactivity was determined at the soles of both feet for 30 second periods, alternately, until the counts had levelled off and the exchange of Na had apparently reached equilibrium. (See accompanying graph). Observed radioactivity in both feet was found to be equal within experimental error. Counts in both feet levelled off at the same time (about 30 minutes) comparable with the normal observed by Smith & Quimby (Radiology: Vol. 45, No. 4, Page 335 - 1945.)

Circulation time from arm to foot was measured and was found to be 30 seconds in both extremities, which is within normal limits.

After the counts at the soles of both feet had reached equilibrium, radioactivity was determined at various points up both

legs to the sacro-iliac regions. (See accompanying chart). These counts were found to be almost equal for both right and left lower extremities at all points, except just above the knee. In this area there was a variation, the left (diseased) extremity recording a higher count by about 200 counts/30 sec. than the right. The estimated experimental error is about 100 counts/30 sec. (repeated determinations at any one point agreed within 50 counts/30 sec.). The apparent difference between the two extremities above the knee is of border line significance.

One hour after injection a sample of blood was withdrawn from the patient's arm and the amount of radioactive sodium present in the blood and plasma was determined. Using this value, the Na space was calculated to be 13.9 liters. No radioactive Na was found in the erythrocytes.

Urine and feces were collected for 24 hours after injection and analyzed for radioactivity. It was found that 8.1% of the total body Na is excreted in 24 hours in the urine. Similarly, 1.0% of the Na was found in the two stools excreted by the patient in the 24 hours after injection.

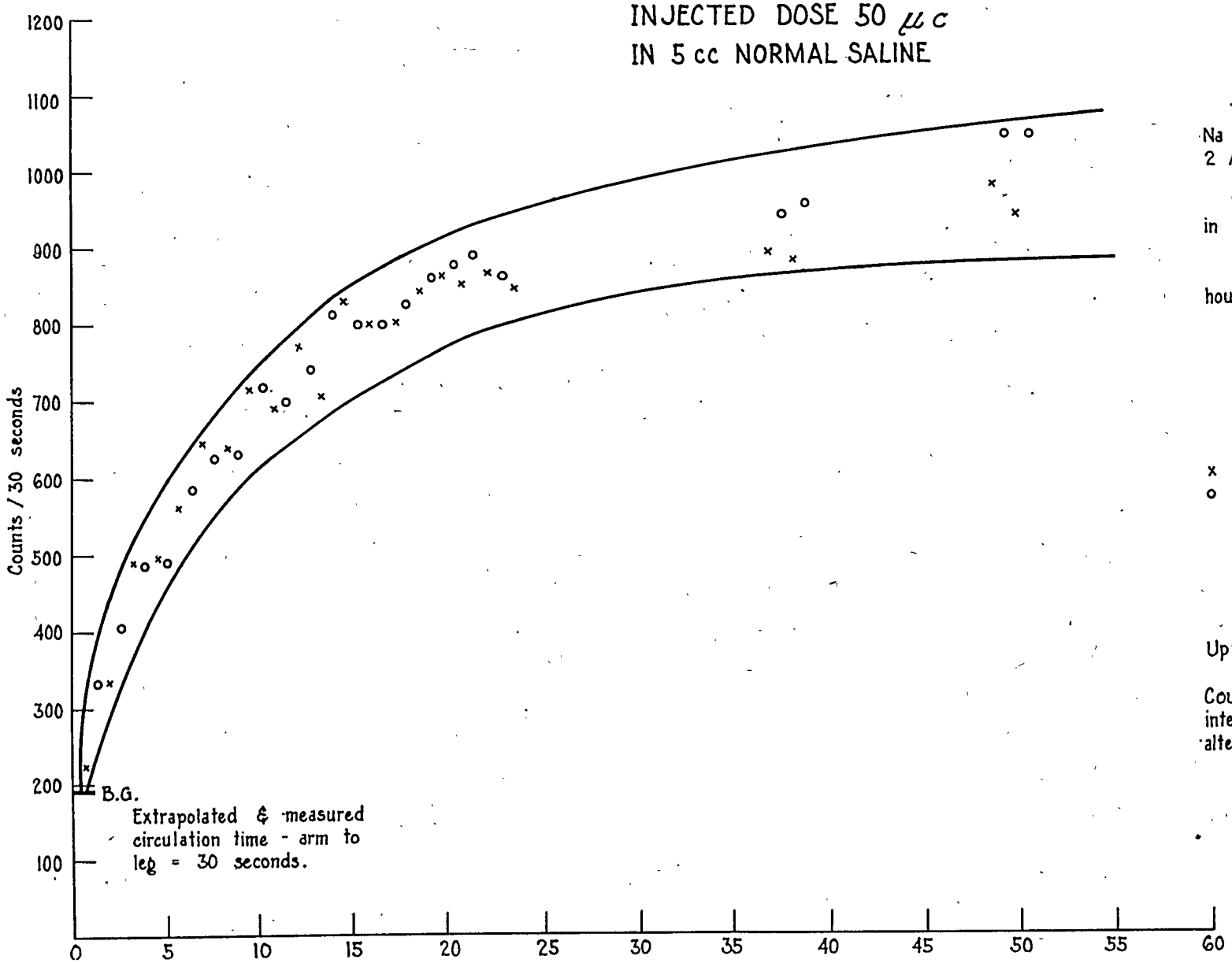
CONCLUSIONS:

1. The lack of difference between the readings obtained at the soles of both feet after injection is evidence against the diagnosis of active thromboangitis obliterans.

2. The time at which equilibrium was reached in both feet, as well as the circulation time is within the range reported by Smith and Quimby in their series of normals. This tends to

confirm a normal circulation by the sodium test with the extremities horizontal and at rest.

3. The difference in the readings above the knee (left (diseased) extremity greater than the right) is at the borderline of significance and may indicate the existence of collateral circulation about the left knee with possible obstruction of the popliteal artery.



7.6 % of injected radioactive Na excreted in urine from 1845 2 April 1948 to 1800 3 April 1948.

1.0 % of radioactive Na excreted in feces in same period.

Na turnover 8.1 % per 24 hours.

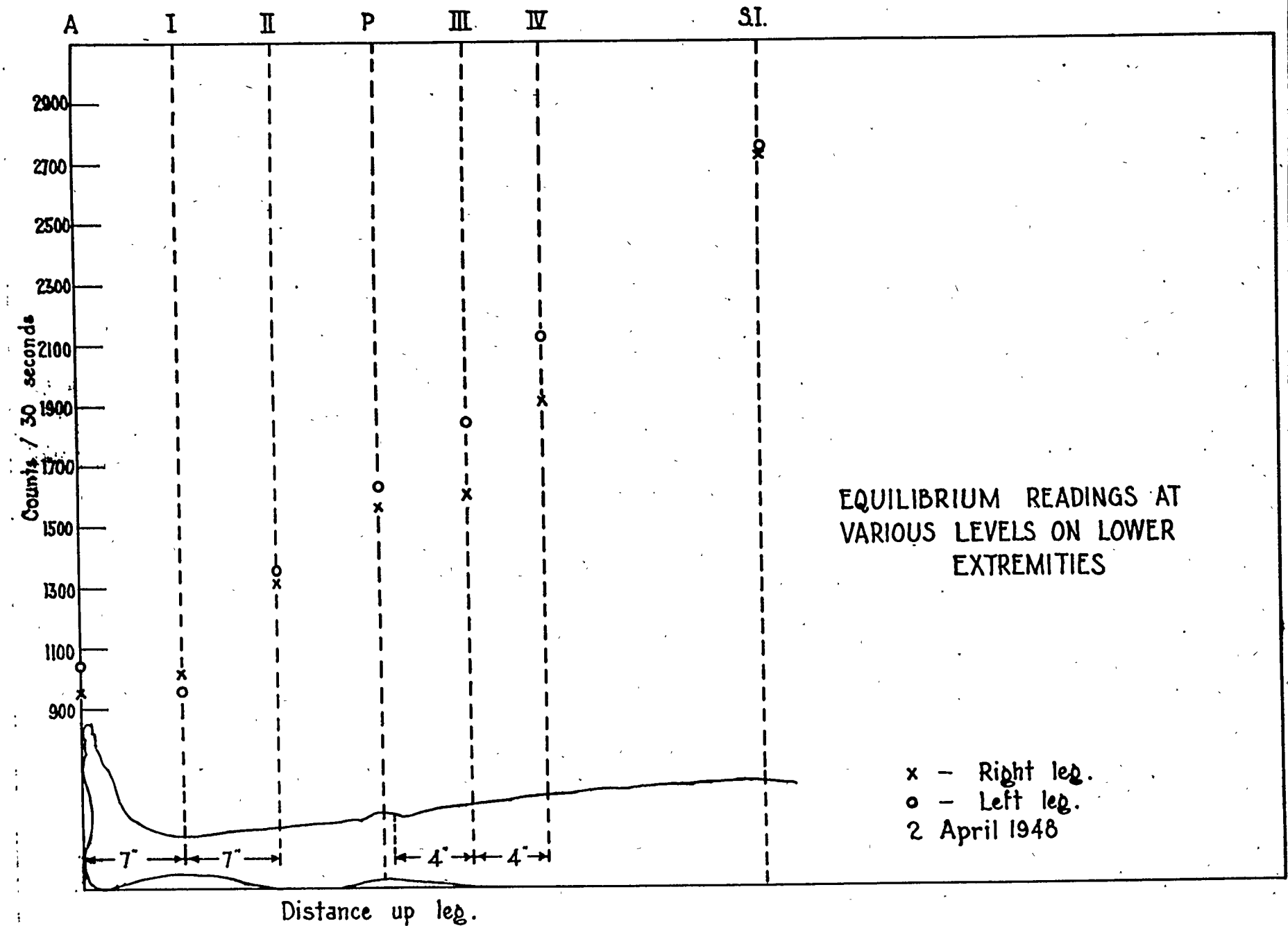
Na space 13,880 c.c.

x - Right foot.
o - Left foot.

Uptake of radioactive Na in extremi

Counts determined for 30 second intervals at soles of right & left alternately.

2 April 1948


A
I
II
P
III
IV
S.I.
2900
2700
2500
2300
2100
1900
1700
1500
1300
1100
900
Counts / 30 seconds
7"
7"
4"
4"
Distance up leg.
EQUILIBRIUM READINGS AT VARIOUS LEVELS ON LOWER EXTREMITIES
x – Right leg.
o – Left leg.
2 April 1948

TREATMENT OF A CASE OF THYROTOXICOSIS WITH IODINE^{131}

Edgar Leifer, Guy Schlaseman & John Hogness*

Patient [redacted] gave a history of having hyperthyroid symptoms extending over a period of about five years. The diagnosis was first made while the patient was serving in the Armed Forces. At that time, according to the patient, his BMR was above + 100. At that time operative removal of part of the thyroid was advised, but the patient refused. The patient was then put on Lugol's solution with some remission of symptoms.

After being discharged from the Army, the patient was examined by another physician who said the diagnosis of thyrotoxicosis was not justified, and Lugol's was discontinued. The patient later moved to Santa Fe and consulted another physician, and the diagnosis of hyperthroidism was again made. Treatment with thiouracil was instituted. After a period of this treatment the patient developed agranulocytosis with multiple abscesses of the neck and perineum. Thiouracil was then discontinued and for some time thereafter the BMR remained low. Gradually symptoms of a hyperactive thyroid reappeared and the patient went back to the use of Lugol's solution with some relief. The patient did not wish operation, so this was deemed a good case for treatment with Iodine^{131}.

All iodine therapy was discontinued for more than a month prior to the estimated date of treatment. The Iodine^{131} was ordered from Oak Ridge and arrangements were made for the patient's admission to the Los Alamos Hospital where patient was to be studied and the treatment given.

Studies made prior to treatment showed BMR's of + 55 and + 39 on consecutive days. The blood cholesterol was 183 mg. % and the cholesterol ester was 82 mg. %.

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* The authors gratefully acknowledge the assistance of certain Clinical and Laboratory personnel of the Los Alamos Hospital for their part in collecting clinical and laboratory data.

Blood examination showed a relative lymphocytosis, otherwise negative. Other preliminary studies showed a roentgenologically normal chest, a negative C. C. Floculation, normal glucose and galactose tolerances, a zero Thymol turbidity, and a normal EKG except for sinus tachycardia. The patient was then reevaluated and considered a good case for treatment with Iodine^{131}.

Upon receiving the Iodine^{131} an assay was made and checked against the Oak Ridge assay. The two checked favorably. The patient's thyroid was examined and estimated to weigh 50 grams. On the basis of treatment consisting of giving 100 microcuries per gram of thyroid, the patient should receive 5 millicuries of Iodine^{131}. Being conservative, however, the patient was given only 4.1 millicuries of Iodine^{131} contained in 2 ml. of solution. The 2 ml. were put in a beaker and diluted to 15 ml. with water which the patient then drank. Then 15 ml. of water were put in the beaker and the patient drank this. This procedure was repeated and then the patient rinsed out his mouth with water and this was discarded. In less than a minute, counts were picked up in the thigh indicating absorption of the radioactive iodine from the stomach and its reaching the thigh via the circulation. Counts over the regions of the stomach and the thyroid showed a gradual decrease in counts over the former and a more marked increase in counts over the latter.

Urinary excretion studies of the radioactive iodine were done. The values of the amount excreted were calculated back to the time of administration of the iodine so that the percentage of the administered dose excreted could be calculated. These results are given in Table I.

TABLE I:--Urinary Excretion of Iodine^{131}

Days After Treatment	Per Cent of Dose Excreted
0 - 1	36.2%
1 - 2	3.5%
2 - 3	1.2%
3 - 4	1.3%
4 - 5	1.1%
10 - 11	0.7%
11 - 12	1.3%

The progression of the basal metabolic rate is shown by the data in Table II.

TABLE II:--Progression of the Basal Metabolic Rate After Administration of Iodine^{131}

Date	BMR
August 31	+ 55
September 1	+ 39
TREATMENT (September 2)	
September 7	+ 29
September 15	+ 46
September 22	+ 37
September 29	+ 35
October 13	+ 25
October 27	+ 24
November 24	+ 12

Blood cholesterol and cholesterol ester levels were determined and are shown by the data in Table III.

TABLE III:--Blood Cholesterol and Cholesterol Ester Levels Following the Administration of Iodine^{131}

DATE	CHOLESTEROL	CHOLESTEROL ESTERS
August 31	186 mg. %	84 mg. %
September 2	183 mg. %	82 mg. %
TREATMENT (September 2)		
September 7	135 mg. %	108 mg. %
September 15	204 mg. %	-
September 22	195 mg. %	-
September 29	180 mg. %	-
October 27	140 mg. %	-
November 24	180 mg. %	-

The blood picture remained essentially unchanged following treatment. It remained negative except for a slight relative lymphocytosis which was also noted

prior to treatment.

Routine urine examinations remained negative throughout.

The patient has had a gradual remission of such symptoms as nervousness, tremor, and restlessness. He has been gradually gaining weight, sleeps soundly at night, and has noticed a marked improvement in his personality. Now, almost three months after treatment, the patient, to use his own words, feels better than he remembers having ever felt in the past. The only symptom which has not shown improvement to present is his exophthalmos. This has remained essentially unchanged.

CONCLUSION:-- A patient with thyrotoxicosis was treated with Iodine^{131}, the patient receiving 80 microcuries per estimated gram of thyroid. Results three months following treatment are excellent with no evidence of toxic complications from the radioactive iodine.

ELIMINATION OF URANIUM OXIDE (U_3O_8) FROM THE LUNG OF THE RAT FOLLOWING TRACHEAL INSUFFLATION

Guy Schlaseman, James Dutli and Gerold Tenney

As a preliminary experiment to possible later investigation of the fate of uranium oxide inhaled by humans, it was thought advisable to first study animals which had received intrapulmonary uranium oxide. Adult rats were chosen for this purpose. Intratracheal insufflation was decided upon as a convenient means of administering the uranium oxide dust. Following administration of the material its mode and rate of elimination from the respiratory tract was followed qualitatively by means of periodic soft x-ray examination of the lungs and abdomen. Post mortem examinations were made also for possible correlation with the x-ray examinations.

MATERIALS AND METHODS

Finely ground uranium oxide (U_3O_8) dust was obtained and examined under the microscope. The particle size was found to vary considerably, some particles being greater than ten microns in diameter. In order to obtain a dust consisting of particles of two microns or less (optimum size for entering the alveoli) several grams of the original powder were placed in a tall measuring cylinder containing 1000 cc. of distilled water. The cylinder was shaken vigorously and the U_3O_8 allowed to settle for fifteen minutes. At that time the top 250 cc. of the resulting suspension were decanted and filtered by suction. The volume in the cylinder was then restored to 1000 cc. with distilled water and the procedure repeated several times. The filter paper containing the dust was dried in an oven, and the fine powder was scrapped off. The material was then shaken in a flask with glass beads to break up the clumps. The particles were examined microscopically and found to have a much more even size distribution. A rough estimate showed the majority to lie between one and three microns in diameter. A more accurate determination of particle size was not thought necessary for this experiment.

Preliminary work showed intratracheal insufflation to be a relatively simple procedure. Anesthesia with ether was found unsatisfactory because of excess mucous formation. Nembutal was found to be better in this respect, and was used, despite the fact that as deep a stage of anesthesia could not be obtained with it as with ether. Atropine sulfate was used at the dosage of one-quarter grain per 100 gram rat to reduce mucous formation. Mucous formation proved to be an important factor, and will be discussed later.

A midline incision was made in the neck, the fascia was cut with scissors, and the soft tissues and muscles were separated by blunt dissection to reveal the trachea. A short section of the trachea was freed and elevated. A transverse incision in the trachea was then made with a razor blade. It was found that bleeding was minimal if the incision was made in the middle of one of the tracheal rings. A weighed amount of the prepared sample of U_3O_8 dust was placed in a small caliber glass tube bent in such a fashion that its tips (which was drawn out to capillary dimensions) could be inserted into the opened trachea. The tube was then inserted into the tracheotomy incision with its opening towards the lungs. A sudden gush of air, supplied from a 10 cc. syringe attached to the tube sprayed the dust into the lower respiratory tract. Later examination showed this to be an effective means of getting the particles into the alveoli. Care was taken in the insufflation so that the inserted tube did not occlude the trachea. The tip of the tube was of such dimensions so that when it was inserted there was still sufficient passage space in the trachea for air to leave the lungs. This precaution lessened the possibility of traumatic rupture of the lung from the sudden influx of the 10 cc. of air used in the insufflation. During the insufflation, the tip of the tube was placed as near the biforcation of the trachea as could be estimated. The trauma of the operation, the insertion of the

tube, and the insufflation of 10 cc. of air (no uranium oxide dust) produced no mortality in control animals.

During the actual administration of the uranium oxide, it was found that excess mucous formation caused immediate death. The reason for this was that the mucous immediately collected the insufflated dust particles and gathered them into a clump which was large enough to occlude the trachea causing the animal to suffocate. This difficulty was overcome by giving atropine sulfate to cut down on mucous formation and by having a smaller opening in the tip of the tube which gave greater dispersion of the dust particles.

OBSERVATIONS

During the first few hours after insufflation the particles which had been deposited along the bronchial tree were moved along by ciliary action and often collected together into a viscous mixture of dust and mucous in the mid portion of the trachea. This mass was sometimes sufficiently large to occlude the trachea and cause suffocation. During this stage forceful artificial respiration was sometimes sufficient to disperse the mass and save the animal. At this time and for a day or so later, a nasal discharge of U_3O_8 dust mixed with mucous was often observed.

Animals which survived the early critical stage did fairly well for periods ranging from four days to three weeks, averaging about a week, following which they then developed what appeared clinically to be a pneumonitis and died. Post mortem examinations at this time revealed congestive lungs compatible with a pneumonic process.

Of the insufflated animals receiving follow-up x-ray examinations, Rat #9 is a typical example. This animal received approximately 50 milligrams of prepared uranium oxide dust intratracheally. Selected X-ray films taken of

this animal are presented in Figs. 1-9.

Figure No. 1 was taken forty minutes following insufflation. The proximal half of the trachea and a great portion of the bronchial tree have been made radiopaque by the U_3O_8 dust adhering to the surfaces. There is irregular distribution along the main bronchi suggesting that partial elimination has already taken place. In the mid portion of the left lung field and in the right base are seen patches of increased density where dust particles have entered the lung tissue proper.

Figure No. 2 was taken one and one-half hours following insufflation. At this time breathing was irregular and a black nasal discharge was noted. There is now an appreciable breaking of continuity of the dust film in the trachea and left bronchus indicating elimination of the U_3O_8 by ciliary action. This process is much slower in the right bronchus. The fine distribution of particles in the mid portion of the left lung field is better seen in this figure.

Figure No. 3 was taken three and one-half hours following insufflation. There is now almost complete elimination of the dust from the trachea and from the left bronchus except at the base. Little change has taken place in the major portion of the right bronchus. The densities previously seen in the lung tissue of the right base have become finer and more numerous. In the mid portion of the trachea are seen two accumulations of dust in the process of being moved upwards. A small amount of U_3O_8 which has been completely removed from the trachea and swallowed is now seen in the stomach.

Figure No. 4 was taken twelve hours following insufflation. The dust has been almost completely eliminated from the trachea and major portions of both bronchi. This material has been swallowed and is seen in the stomach. Distention of the stomach is also noted at this time. The distention is probably

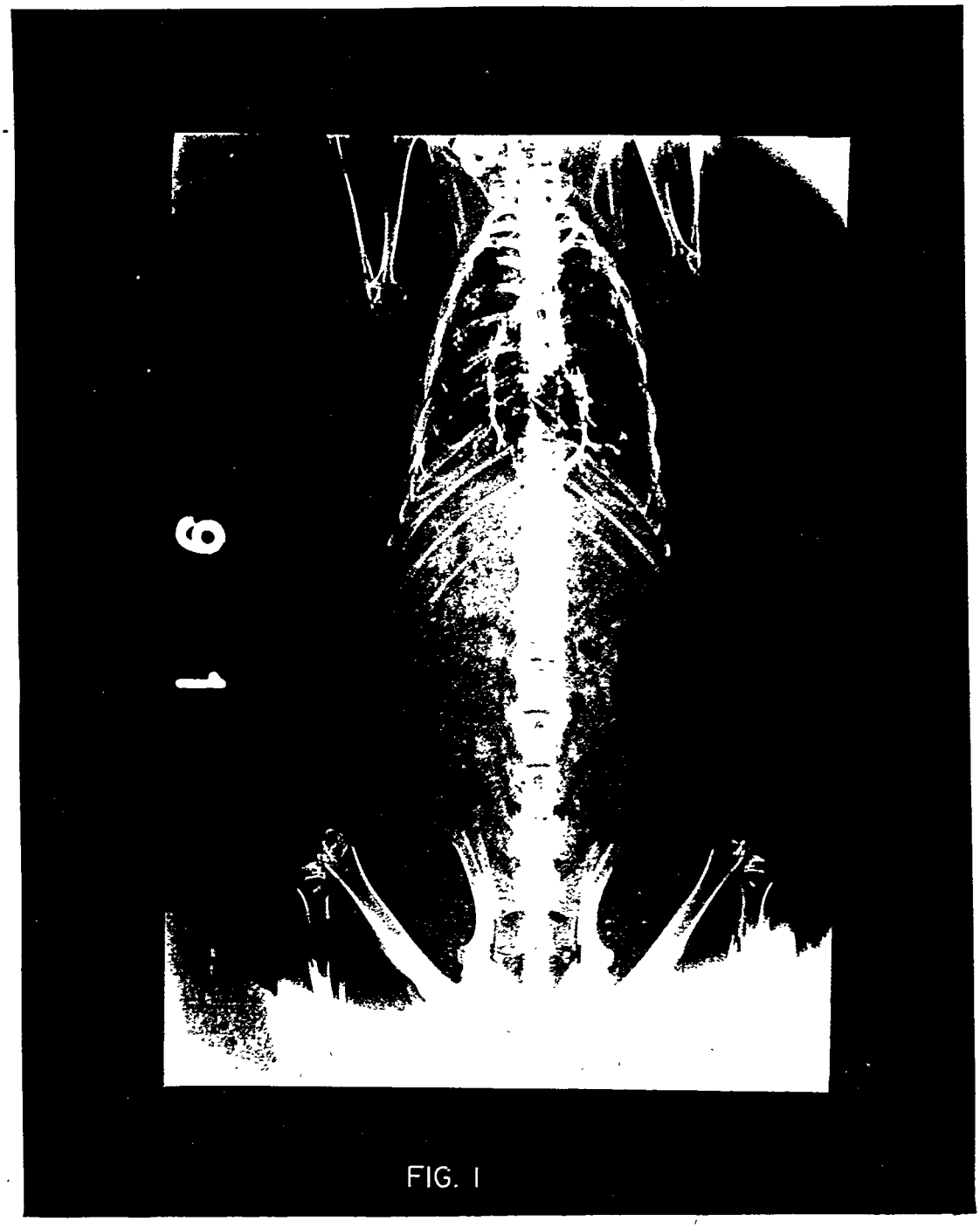

FIG. 1

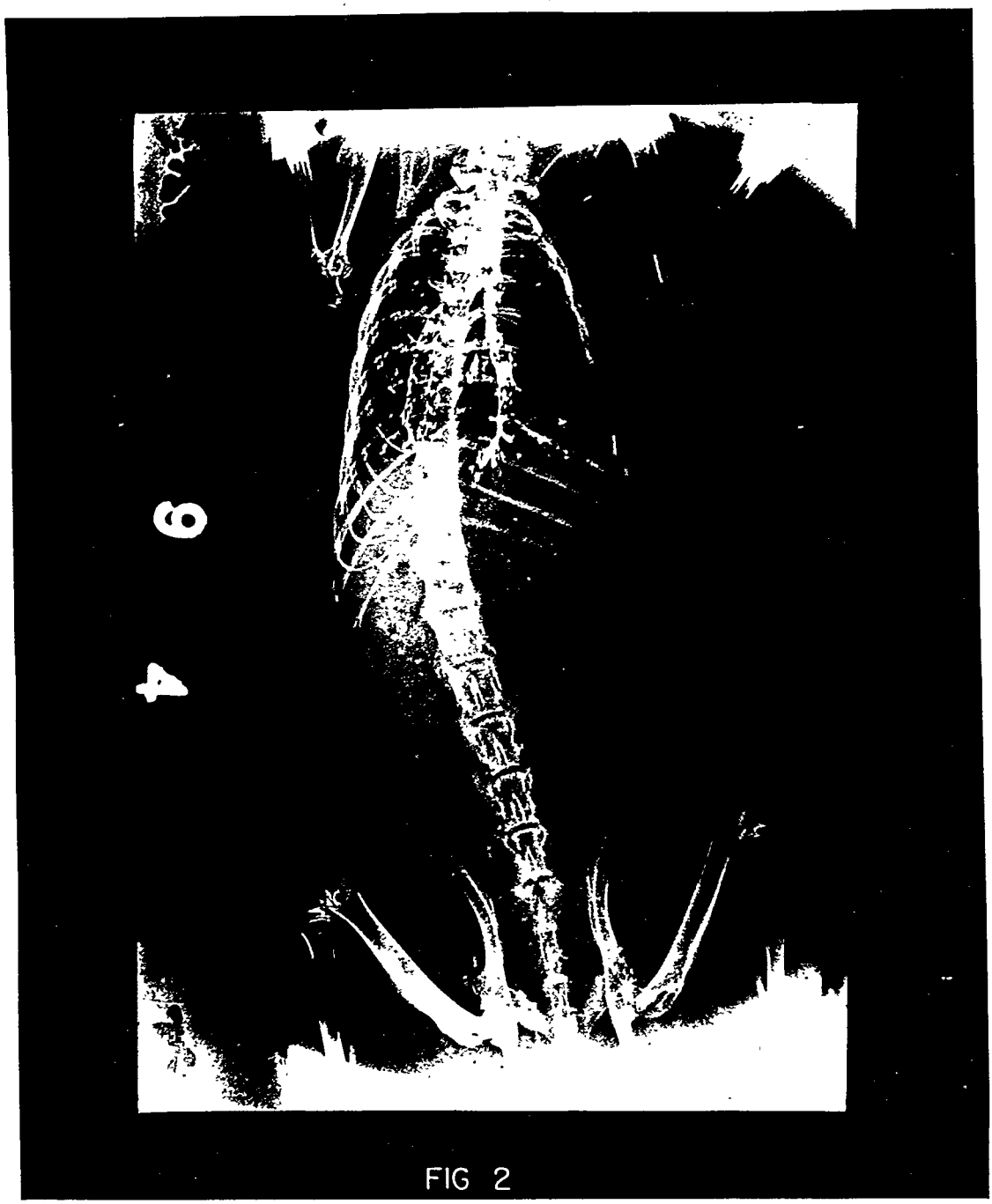

FIG 2

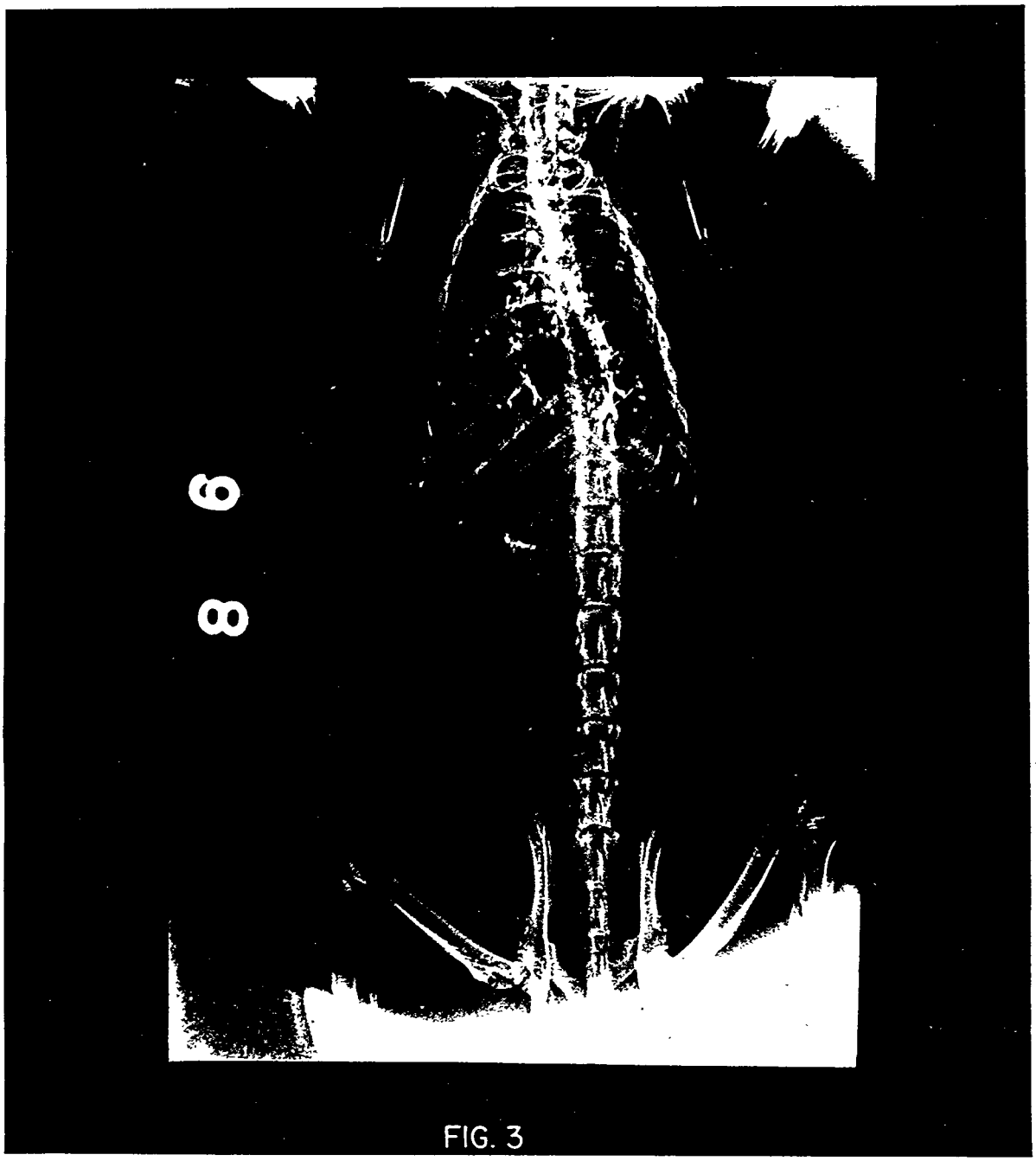

FIG. 3

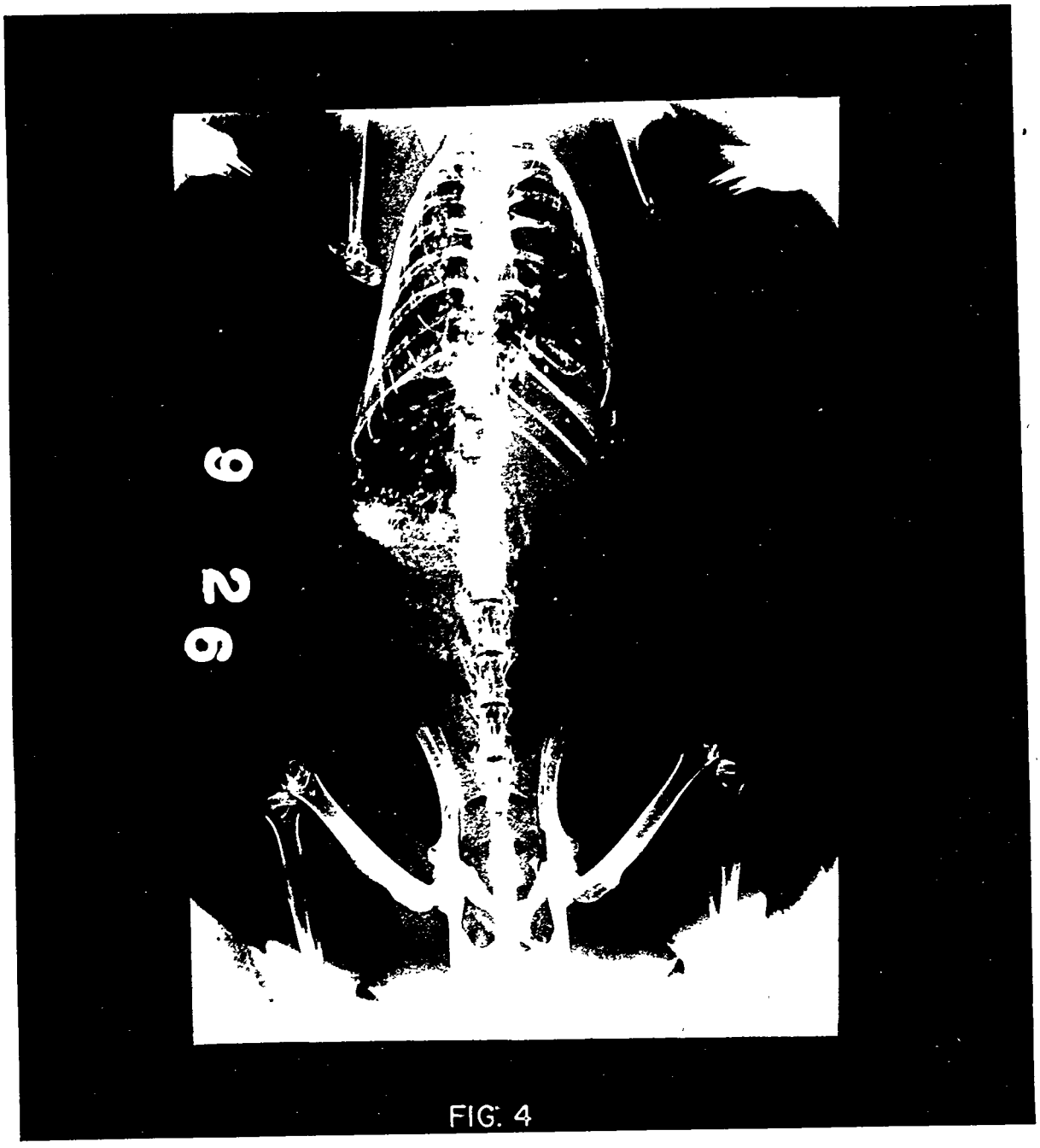

FIG. 4

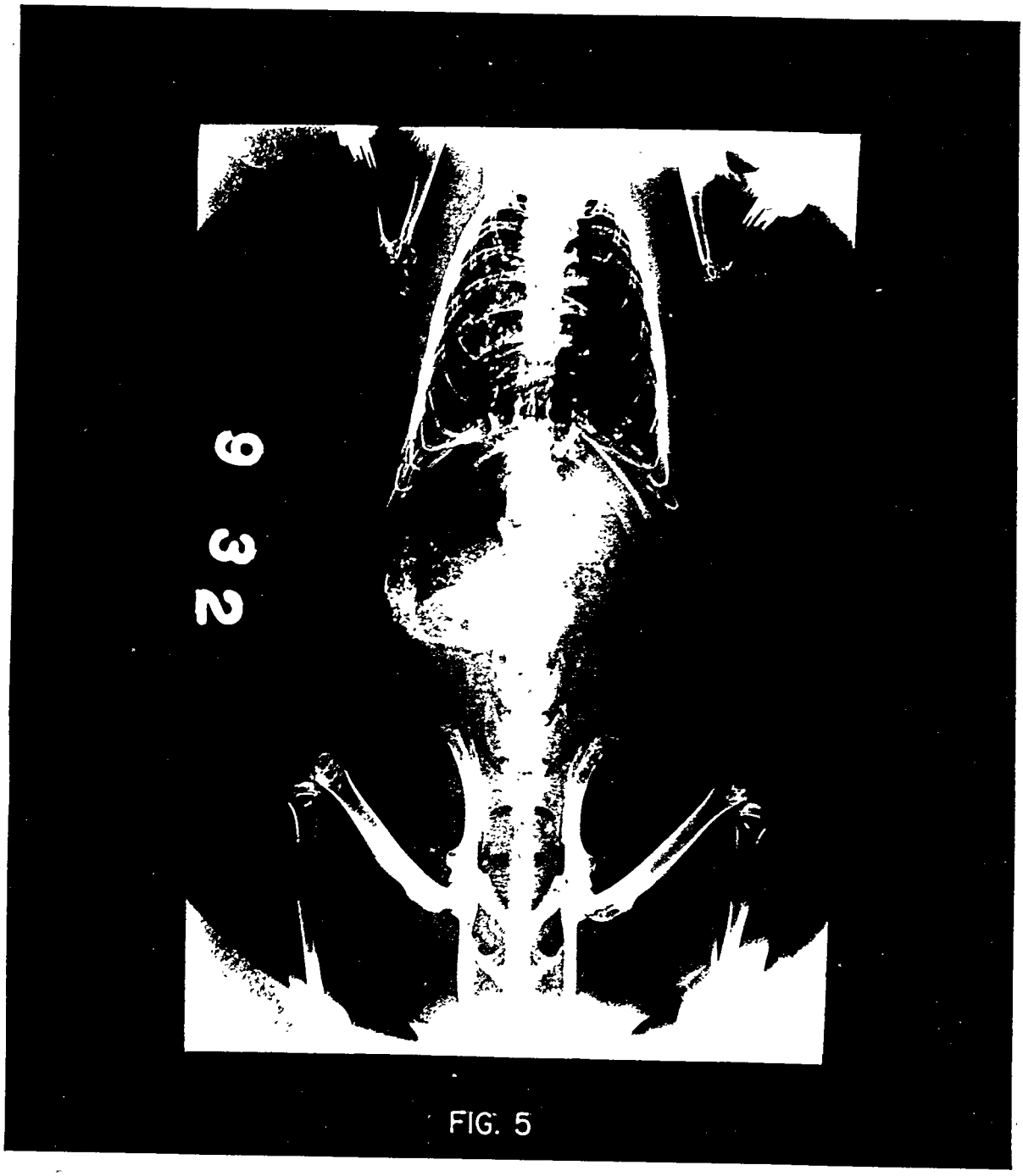

FIG. 5

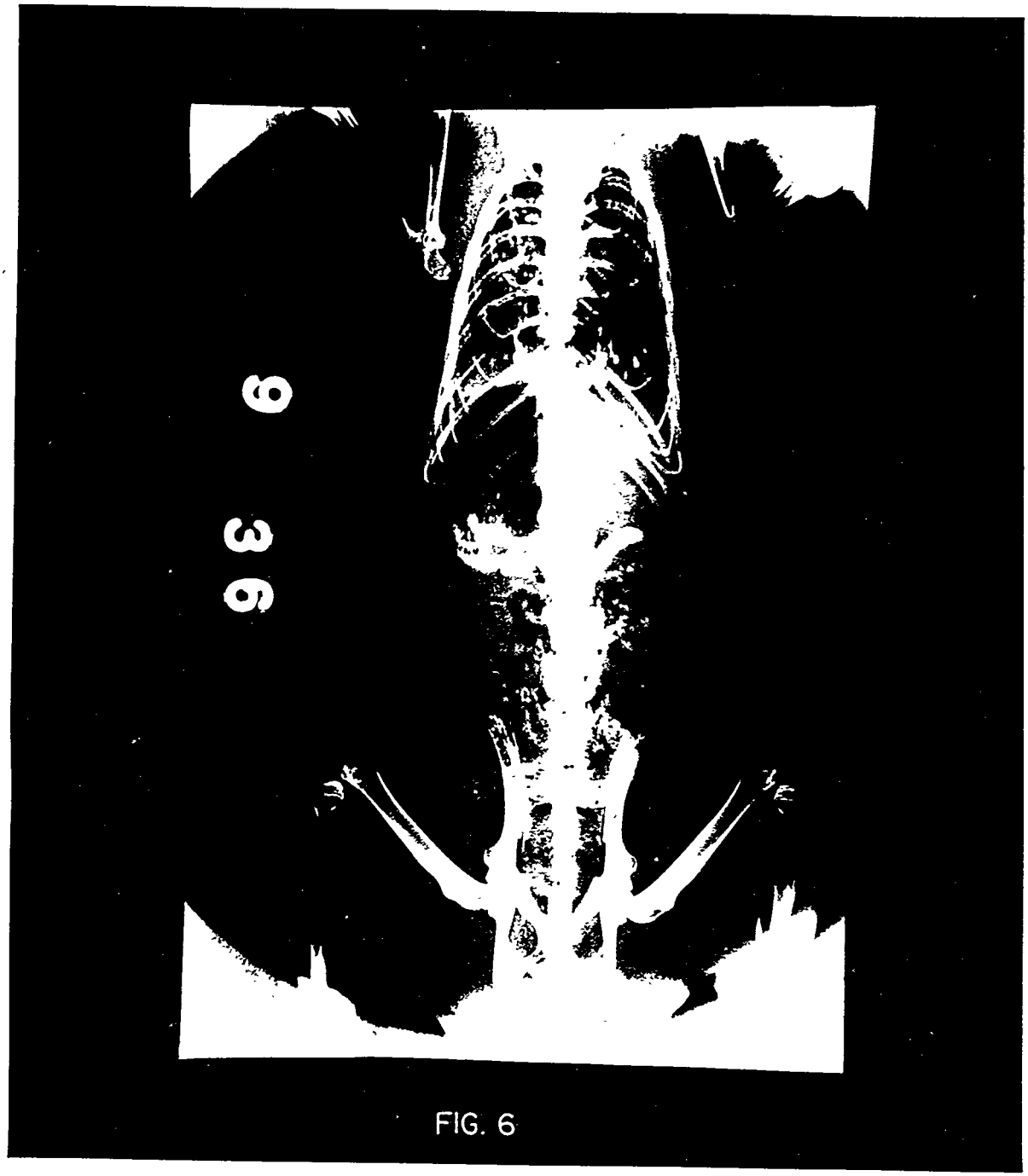

FIG. 6

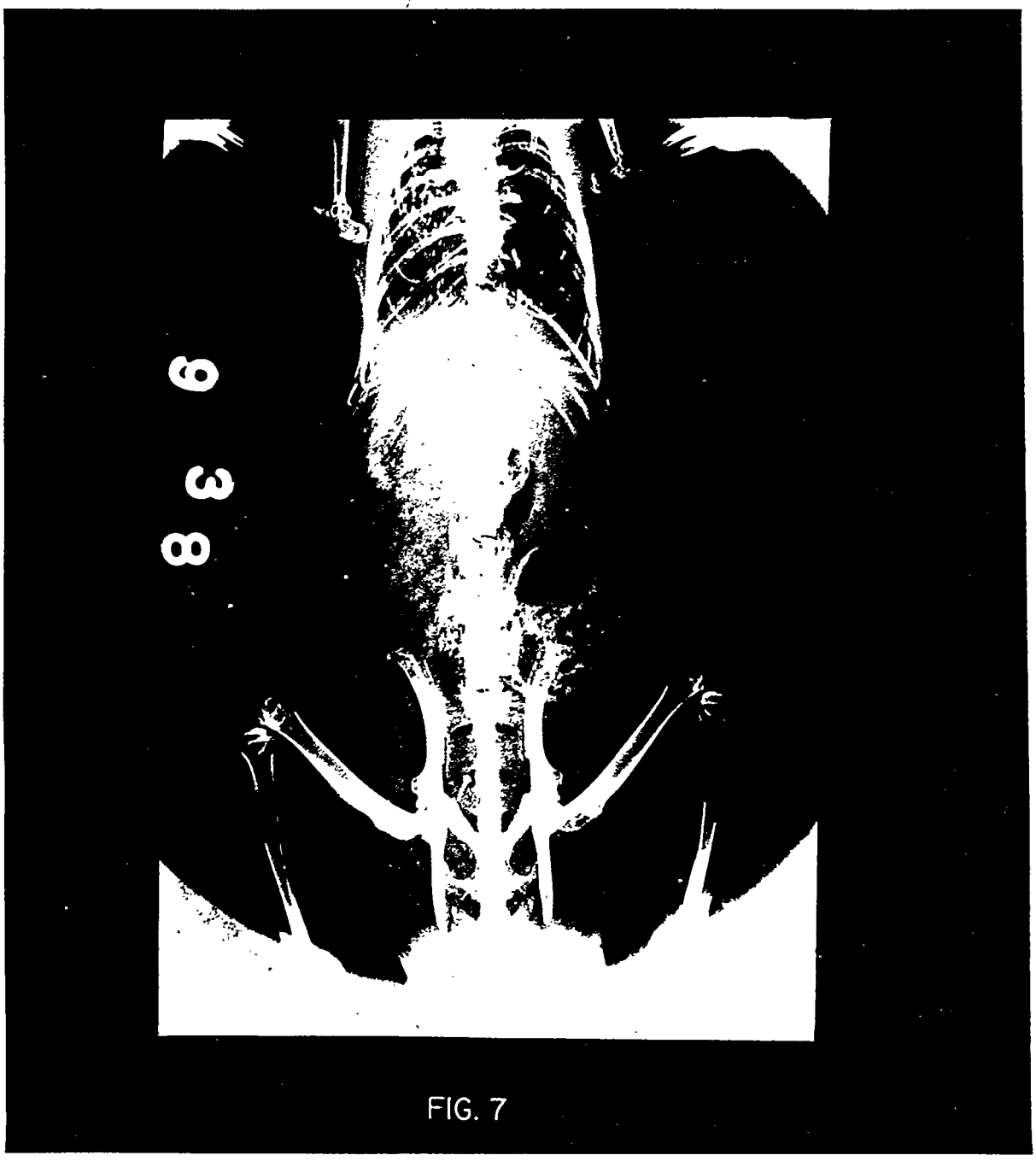

FIG. 7

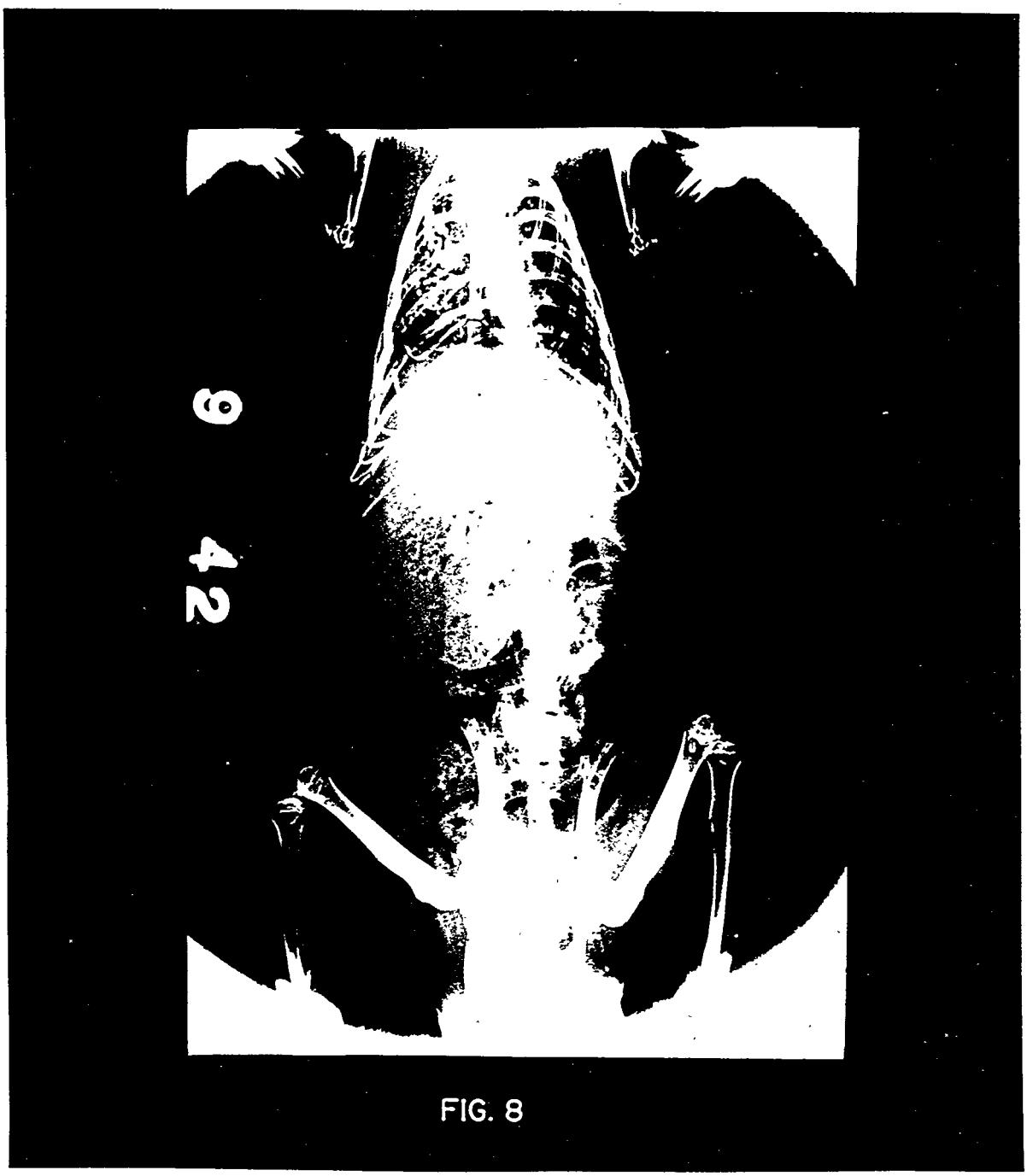

FIG. 8

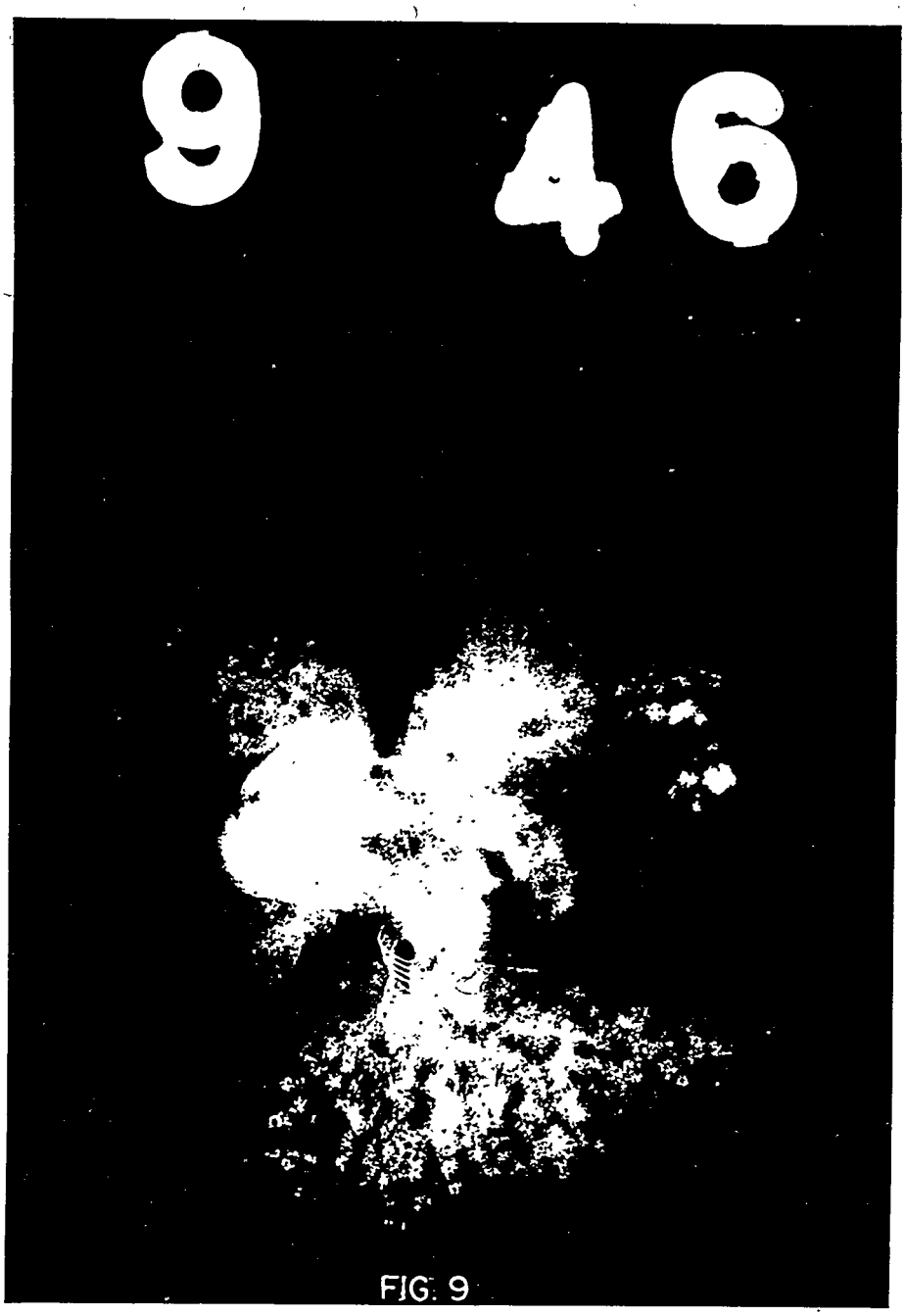

FIG. 9

due to the use of ether as an anesthetic to sedate the animals during filming. There has been little removal of particles from either base. Apparently some elimination did take place in these regions only to be stopped by plugs of mucous and dust in the lower bronchi. In the right upper lung field of Figure No. 3 was a suggestion of clouding which is more definite in this figure. Apparently this region has also been invaded to some degree by the dust particles.

Figure No. 5 was taken twenty-four hours following insufflation. At this time breathing was quite noisy. There is little change noted in the lung picture. The plugs remain in both bases, and dust may be seen in the lung tissue of the right base, the right upper lobe, and the mid portion of the left lung field. The particles which were removed from the lung and swallowed are now seen leaving the stomach.

Figure No. 6 was taken thirty-one hours following insufflation. There is no appreciable change in the lung picture. The material which has been swallowed is now seen in the stomach and scattered along the small intestine.

Figure No. 7 was taken fifty-five hours following insufflation. At this time the clumps of material in both bases have undergone partial resolution and elimination. There is still some U_3O_8 remaining in the stomach. The major portion of it, however, is now in the colon. The animal was sacrificed on the thirteenth day.

Figure No. 8 was taken immediately following sacrifice. The clumps of material which were seen in the right base are now broken up and mostly eliminated. Those previously seen in the left base have been completely removed. Pulmonary involvment is striking in the mid portion of the left lung field and suggested in the upper portion of the right lung field. The U_3O_8 is now completely eliminated from the gastro-intestinal tract.

The lungs of the animal were then removed and X-rayed. This is shown in Figure No. 9. There is diffuse involvement of the left lung where dust particles got into the alveoli. In the right base there are still some unresolved clumps and minimal involvement of pulmonary tissue proper. There is also a slight involving of the right upper lobe.

Periodic X-ray examinations of other animals were essentially similar with minor differences. In two other series the distribution of the particles was a little more localized. The majority of the dust, outside of that remaining in the trachea and bronchial tree, was concentrated in part of one or the other lower lobes. The pattern of elimination of the particles was similar in all cases. The only differences noted were quantitative as referred to the distribution within the lungs. Thus, the elimination as outlined in the discussion of rat number 9 (Figs. 1-9) may be taken as typical of what was observed in this experiment.

Pathologic examinations of the lung were done at various stages. Animals which died immediately after or within several hours following insufflation showed a large viscous clump in the trachea which consisted of U_3O_8 dust mixed with mucous. There was sufficient accumulation to block the trachea and cause death by suffocation. There were small dust particles seen trapped in the alveoli and distributed along the bronchial tree. As was indicated by the X-ray films, the distribution of particles was more or less localized, which was no doubt due to the method of administering the material. At this early stage those particles which were in the alveoli were seemingly lying free. Most of the alveoli that contained particles were in one localized section of the lung.

Examinations done on the lungs at later dates were too limited to warrant

the drawing of definite conclusions, but a tendency could be noted. They indicated a gradual elimination of the foreign matter from the bronchial tree and trachea. As time progressed, less and less of the uranium oxide could be found in these regions with the exception of the areas where most of the dust localized. Apparently in these areas were formed plugs of mucous and dust which blocked early elimination from the smaller bronchioles. As evidenced by the X-ray films, these probably underwent a gradual resolution.

After several days, the particles remaining in the alveoli appeared to become incorporated into the alveolar walls, and at later dates some of the particles were observed in the interstitial tissues of the lungs and also within clumps of lymphoid tissue near the larger bronchi. In many of the involved areas, at about one week and longer after administration, were noted areas of congestion and edema suggesting possibly a foreign body reaction and/or pneumonitis. Throughout the congested areas were scattered particles of uranium oxide. The particles could be seen in sections stained with hemotoxin and eosin. These particles were also demonstrated nicely by dark-field examination of the sections. The particles were identified by placing a portion of the involved lung into a crucible containing sodium fluoride and the mixture then fused at high temperatures. The resultant product fluoresced under ultra-violet light while a control did not, thus identifying uranium in the involved lung.

On one examination done two weeks following administration, a hilar lymph node was sectioned and was found to contain what appeared to be a few particles of uranium oxide. This, with the particles seen in clumps of lymphoid tissue around the larger bronchi, seemed to indicate some elimination of the particles

from the lung via the lymphatic system.

SUMMARY:

A series of rats received intratracheal insufflation of fine particles of U_3O_8. The majority of the particles lay between one and three microns in diameter. Most of the particles were deposited along the trachea and bronchi. These were moved upwards by ciliary action out of the trachea and subsequently swallowed and eliminated through the gastro-intestinal tract. The particles which entered the alveoli were apparently picked up by macrophages and eliminated in part by the lymphatic system.