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## ATOMIC ENERGY PROJECT

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Health and Biology

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# QUARTERLY TECHNICAL REPORT

July 1, 1951 thru September 30, 1951

It should be noted that the Quarterly Technical Reports of The University of Rochester Atomic Energy Project do not attempt to describe progress in all of the research programs but only in those in which some significant results have been achieved but which are not sufficiently complete to be written up as final reports.

Submitted by: Henry A. Blair
Director

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EDUCATIONAL AND FELLOWSHIP PROGRAM

# RADIUM ANALYSIS OF MUNICIPAL WATER SUPPLIES

By
J. B. Hursh and A. Gates

Background: The radium analysis of source and tap water obtained from cities in the United States has been continued and additional results have been obtained. For a description of the background of this study and the methods of analysis used, see report UR-152.

Results: The results to date are listed in Table I (page 7). It will be noted that repeat analyses of samples from the first 15 cities show rather poor reproducibility in some cases. During the period in which the first measurements on samples of this group were made, minor improvements in the apparatus and in the measurement technique were introduced. The results of these improvements are evidenced in the better reproducibility of the later repeat measurements, as well as in the third run checks of the divergent values referred to above.

Distilled water blanks gave "radium content" measurements of 0.19, 0.19, 0.22, 0.23, 0.20, 0.15, and 0.21 x $10^{-16}$ grams radium per ml. In order to get net radium content of the public water samples, 0.20 x $10^{-16}$ should be subtracted from the measured values as listed in the table.

Discussion: It should perhaps be pointed out that the radium values listed pertain only to the single sample which was analyzed, and that seasonal variations unquestionably occur, particularly in supplies using rivers as the source.

It may be of interest to note that when the projected analysis of municipal water supplies is complete, the water consumed by 20 per cent of the population of the United States will have been sampled and studied.

TABLE I

Radium Analysis of Municipal Water Supplies

| City | Radium Content in Units of 1 x $10^{-16}$ gm radium/cc* | | | |
|---|---|---|---|---|
| | First Analysis | | Repeat Analysis on Same Water Sample | |
| | Source | Tap | Source | Tap |
| Salt Lake City | 1.58 | 1.06 | 0.43 | 0.64 |
| Bismarck, N.D. | 3.02 | 0.42 | 2.07 | 0.31 |
| Denver, Col. | 0.86 | 0.68 | 0.90 | 0.50 |
| Boise, Idaho | 1.06 | 1.24 | 1.23 | 0.90 |
| Sacramento, Cal. | 0.32 | 0.34 | 0.27 | 0.17 |
| Los Angeles, Cal. | 0.51 | 0.46 | 0.26 | 0.20 |
| San Francisco, Cal. | 0.63 | 0.32 | 0.37, 0.30 | 0.18 |
| Tacoma, Wash. | 0.25 | 0.51 | 0.13 | 0.10 |
| Portland, Ore. | 0.48 | 0.21 | 0.13, 0.21 | 0.09 |
| Phoenix, Ariz. | 0.37 | 0.35 | 0.40 | 0.29 |
| Pierre, S.D. | 1.59 | 2.41 | 0.76, 0.74 | 0.23, 0.32 |
| Wichita, Kan. | 2.47 | 2.00 | 2.62 | 0.86, 0.89 |
| Omaha, Neb. | 18.9 | 1.20 | 16.5 | 0.68, 0.63 |
| Oklahoma City | 1.52 | 2.78 | 1.11 | 0.58, 0.49 |
| Memphis, Tenn. | 3.13 | 2.88 | 2.20, 2.18 | 1.83 |
| Dallas, Tex. | 0.98 | 0.44 | 0.93 | 0.43 |
| St. Louis, Mo. | 11.3 | 0.40 | 10.6 | 0.33 |
| Chicago, Ill. | 0.34 | 0.42 | 0.37 | 0.40 |
| Charleston, S.C. | 2.03 | 1.60 | 1.84 | 1.50 |
| Louisville, Ky. | 0.90 | 0.52 | 1.03 | 0.56 |
| Indianapolis, Ind. | 1.49 | 0.99 | 1.50 | 1.00 |
| Detroit, Mich. | 0.36 | 0.28 | 0.41 | 0.33 |
| Buffalo, N. Y. | 0.46 | 0.42 | 0.48 | 0.41 |
| Cleveland, Ohio | 0.45 | 0.35 | 0.44 | 0.37 |
| Birmingham, Ala. | 0.33 | 0.41 | 0.39 | 0.46 |
| Cincinnati, Ohio | 0.74 | 0.48 | 0.71 | 0.44 |
| New Orleans, La. | 4.08 | 0.30 | 4.56 | 0.30 |
| Atlanta, Ga. | 0.29 | 0.19 | 0.29 | 0.26 |
| Baltimore, Md. | 0.31 | 0.20 | 0.35 | 0.23 |
| Boston, Mass. | 0.25 | 0.27 | 0.28 | 0.31 |
| Charleston, W. Va. | 0.59 | 0.56 | 0.49 | 0.59 |
| Richmond, Va. | 0.44 | 0.36 | 0.47 | 0.36 |
| Raleigh, N. C. | 0.37 | 0.37 | 0.33 | 0.41 |

*Distilled water blanks which average 0.20 x $10^{-16}$ grams/cc have <u>not</u> been subtracted from the values reported above.

# CYTOLOGICAL STUDIES ON THE BLOOD OF SALLY, AN INDIAN ELEPHANT

by

M. Ingram, L. Coonan, J. Jespersen, G. Nielsen, D. Piatt, M. Wright

Background: Studies carried out in this laboratory have indicated that an increased incidence of lymphocytes with bilobed nuclei in the peripheral blood is a sensitive indicator of exposure to extremely small doses of ionizing radiation in dogs and man (1, 2). The abnormal lymphocytes are noted in smears of peripheral blood very infrequently. The "normal" incidence in a group of 111 cyclotron new hires during a two year period ending October, 1950 was as follows: 3.7% of all blood smears examined contained one or more of the abnormal lymphocytes. This corresponds to 0.022 lymphocytes with bilobed nuclei per 1000 leukocytes or 0.07 lymphocytes with bilobed nuclei per 1000 lymphocytes examined. Even an increased incidence may represent very few cells on an absolute basis. For example, in the group of 111 new hires mentioned above, 0.15 lymphocytes with bilobed nuclei per 1000 leukocytes, or 0.5 per 1000 lymphocytes was considered to be a high incidence.

A reference by H. Fox to an unusually high incidence of cells with bilobed nuclei in elephant blood was called to our attention by Dr. W. B. Mason (3). According to Fox, 24% of the leukocytes were of this type and were described as follows:

"The cells called bilobed are unusual and can probably best be accounted for as directly dividing small lymphocytes. The staining properties and shape of the nuclei of the bilobed cells are most closely related to the lymphocytes. They are not always regular, however, but may be almost as irregular as the polynuclears. In practically every instance, however, a connecting isthmus may be found between the spherical nuclear portions. No

mitotic figures seen. The protoplasm is homogenous and pale blue or lilac in relatively large amount. No granules were ever seen."

It was decided to check this remarkable finding if the opportunity could be found, as it was eventually through a suggestion of Dr. W. H. Strain, who suggested that Mr. Strassle of the Seneca Park Zoo might be of assistance in the matter. Mr. Strassle was contacted, and agreed to make the Indian elephant and ex-circus performer, "Sally" available for the undertaking.

Procedure: On June 27, 1951, the entire hematology group accompanied by R. Hay, M. Tyler, and W. B. Mason, set out for Seneca Park Zoo, equipped with several syringes varying in size from 2 cc to 30 cc, powdered heparin, solutions of heparin, procaine, needles ranging from No. 20 to No. 15, and a generous supply of coverslips and blood pipettes. The literature offered little help regarding the matter of bleeding elephants. An experienced zoo veterinarian, however, had indicated that the ear presented the only large superficial readily identifiable veins.

Elephant Handling: At about 9:30 A.M. the group was escorted into Sally's two-room suite at Seneca Park Zoo. The elephant manifested considerable uneasiness at the approach of strangers.* Mr. Strassle energetically and skillfully maneuvered Sally into position so that she stood relatively quiet. This favorable situation was sustained by Mr. Strassle's feeding the elephant large handfuls of candy at regular intervals, the length of the intervals being determined largely by the speed with which Sally could swallow and reopen her mouth. Once or twice the candy supply ran out at crucial moments. Mr. Strassle saved the situation in these instances by thrusting his empty fist quickly into and out of Sally's mouth, after which Sally would swallow contentedly, sans candy. A fresh supply of candy was procured each time before the limits of

*This feeling, it should be noted, was mutual.

Sally's gullibility were reached. It is estimated that the elephant ate one half bushel of candy during the entire procedure.

Bleeding: Inspection of Sally revealed that the veins in the ears were indeed large and superficial, and they would have been ideal for venepuncture except that 1) the ears were in constant vigorous motion; 2) Sally seemed to be quite apprehensive about having people even looking at her ears, and 3) the ear veins could have been reached only with the aid of a ladder. Further inspection suggested that the tail might be a suitable site for obtaining blood, since it was out of Sally's range of vision. Consequently, with one zoo attendant steadying the elephant's tail, 3-4 cc of 1% procaine were injected subcutaneously about half-way up the appendage. No vein could be felt through the extremely thick, tough skin, and after one or two attempts to aspirate blood into a syringe, the idea of venepuncture was abandoned, and blood was obtained by repeatedly sticking the tail vigorously in the procainized area with a No. 18 needle. Each time several large drops of blood flowed freely from the puncture site. The freely flowing drops were drawn up into pipettes and discharged a drop at a time onto coverslips for blood smears. Attempts were also made to obtain routine dilutions for red and white blood cell counts and hemoglobin determinations. The blood tended to clot very rapidly, however, and since smears received priority, not all the routine counts were obtained with desirable accuracy.

The bleeding procedure was complicated only by Sally's attempts to sit down when the needle extended beyond the procainized area. This required critically timed prodding and shouting on the part of the tail attendant, and gave rise to a certain wariness of the entire tail-end crew, but otherwise was not a serious detriment to the experiment.

Results: Attention has been focused mainly on an evaluation of cell types as classified on Wright stained and peroxidase stained blood smears. Results are presented in Tables 1 and 2 (Pages 14 and 15).

At the outset it should be noted that only after peroxidase stained smears had been examined was it possible to classify the cell types. The cells described by Fox were almost certainly polymorphonuclear leukocytes with two-lobed nuclei. These cells on Wright's stained smears very strikingly resemble lymphocytes with bilobed nuclei, for the granulation is not readily apparent and the two lobes of the nuclei appear to be spherical, unlike the usual elongated lobes of the polymorphonuclear leukocyte nuclei. When the two lobes overlap to a large extent, the cells may be difficult to differentiate from lymphocytes with slightly indented nuclei on Wright's stained preparations. The peroxidase stain demonstrates that the cytoplasm of these cells is filled with peroxidase positive granules. Morphologically, elephant polymorphonuclear leukocytes with three or more lobes in the nucleus are not remarkably different from the characteristic cell of this type in man. With Wright's staining, the cytoplasmic granules of the elephant's cells appear slightly more pink than those of man, but are readily differentiated from eosinophiles which possess the usual large globular brilliant pink granules.

A total of approximately 4000 leukocytes were examined on five different peroxidase-stained smears, and no lymphocytes with bilobed nuclei were seen. The lymphocytes in general tend to be relatively large, with abundant cytoplasm. Some of the large peroxidase-negative mononuclear cells which were observed were difficult to classify, although for the most part, they resemble lymphocytes and seem to belong to that series of cells.

A few typical monocytes were observed. These have rounded or very slightly indented nuclei with very fine chromatin structure and finely stippled pale lavender cytoplasm.

Discussion: This study clearly demonstrates the great value of using peroxidase stain in studying blood smears, particularly when there is a question relative to the classification of cells as lymphocytes or granulocytes. The fairly close agreement between initial differential counts of leukocytes on Wright's stained blood smears and the differential counts of elephant blood as reported by Fox, and the mistaken classification of certain cells in both these studies when interpreted in light of peroxidase staining, indicates that the Wright's stained smears may indeed be misleading in this respect.

The presence of lymphocytes with bilobed nuclei in the peripheral blood tends to lead to speculation regarding the significance of their presence. One possibility is that they may be dividing cells. This was one reason for the interest in carrying out the studies presented in this paper. If the incidence of these cells were actually phenomenally high in the elephant, one might entertain the notion that the huge size of the beast plus a very slow heart rate (about 30-40 beats per minute) would result in the lymphocytes being in the peripheral blood stream for relatively long periods, so that they would be obliged to divide in transit. Since the incidence of lymphocytes with bilobed nuclei in the blood of the elephant was found not to be high, this assumption is not substantiated.

Summary: A study of the differential leukocyte count of an Indian elephant was carried out for the purpose of checking a report (published in 1923) of a remarkably high incidence of cells believed to be lymphocytes with bilobed nuclei in the peripheral blood of elephants.

With the aid of peroxidase staining, the cells in question were found to be granulocytes. The differential leukocyte count of the elephant "Sally" is presented in table form and discussed briefly.

## References

1. Ingram, M., and S. W. Barnes, Experimental Confirmation of a Previously Reported Unusual Finding in the Blood of Cyclotron Workers. Science, 113, 32-34, (1951)

2. Ingram, M., The Occurrence of Lymphocytes with Bilobed Nuclei in Cyclotron Personnel, UR-152, (1951)

3. Fox, Herbert, Disease in Captive Wild Mammals and Birds, J. B. Lippincott Company, Philadelphia, London, Chicago (1923)

TABLE I

Differential Leukocyte Counts; Blood of "Sally"

| | Juveniles | Stabs | Polymorphonucleears with 2 lobed nuclei | Polymorphonucleears with 3 or more lobed nuclei | Eosinophiles | Basophiles | Lymphocytes | Monocytes |
|---|---|---|---|---|---|---|---|---|
| Peroxidase 1000 cells | 1.3% | 8.1% | 36.3% | 25.9% | 4.9% | 0.0% | 22.8% | 0.7% |
| Wright's 1000 cells | 1.7% | 9.5% | 33.2% | 25.8% | 1.9% | 0.3% | 25.9% | 1.7% |
| Average Peroxidase and Wright's | 1.5% | 8.8% | 34.75% | 25.85% | 3.4% | 0.15% | 24.35% | 1.2% |

Note: It is interesting that when an initial attempt was made by the same examiner to classify the cells on Wright's stained smears before the peroxidase stained smears had been carefully studied, 26% of 500 leukocytes were classified as polymorphonuclear cells with two-lobed nuclei, 14% as typical polymorphonuclear leukocytes, and 39.2% as lymphocytes. This compares fairly closely with the results of Fox. Many of the cells originally classified as lymphocytes were undoubtedly polymorphonuclear leukocytes with two-lobed nuclei in which the lobes overlabbed to a large extent.

Compare the above results with TABLE II.

Table 2

Differential Leukocyte Counts Reported by H. Fox;
Blood of an Indian Elephant

| | Polymorphonuclears | Small Lymphocytes | Large Mononuclears | Eosinophiles | Bilobe Cells | Basophiles |
|---|---|---|---|---|---|---|
| Wright's Stain | 15.0% | 47.4% | 7.6% | 5.8% | 23.8% | 0.4% |

Figures based upon counts of 200 cells.

# ELECTROLYTE STUDIES IN IRRADIATED DOGS

by

W. B. Mason, M. P. Coulter and F. W. Furth

Serum sodium, potassium, chloride, and carbon dioxide content have been determined serially on dogs receiving single massive whole-body exposures to x-rays. Sodium, potassium, and chloride have also been studied by a balance technique. The salient observations are reported briefly in the paper which follows.

## Balance Studies

Procedure: A total of 10 dogs were studied. These were adult mongrel dogs ( 5 males, 5 females) previously dewormed and vaccinated against distemper. The dogs were housed in individual galvanized metabolism cages and offered a diet consisting of 300 grams (dry weight) Purina laboratory chow mixed with 600 ml. of tap water per 24 hours. This corresponds to an average daily intake of 55 meq Na, 57 meq. K, and 50 meq. Cl. At the end of the 24 hour period (10:00 A.M.) the metabolic residue within the cage (including urine, feces, uneaten food and vomitus, if any) was collected and made uniform in a Waring blender. Aliquots (approximately 1/200 of the entire sample - usually weighing roughly 4 grams) were weighed into clean dry beakers, subjected to wet combustion, and assayed for Na, K, and Cl. Na and K determinations were done by flame photometry. All determinations were done in triplicate. Chloride was determined by a modified Carius procedure, using an open vessel for the wet combustion. This procedure was shown to give chloride recoveries sufficiently accurate for the problem at hand.

Each dog was studied continuously during a period extending from approximately 12 days prior to irradiation until death, or to a maximum of

28 days after exposure. Radiation exposure consisted of 450 r of whole body x-irradiation (250 KV - Al parabolic and 0.5 mm. Cu. filtration) administered at a rate of 7 r per minute.

Observations: Both Na and Cl are irregularly lost in small amounts (5-15 meq. per day) during the 3rd week after exposure. These losses correlate well with the reduction of intake of food and water at that time. Beginning 4-5 days after exposure there is a slight increase in the Cl/Na ratio of the metabolic residue. This is maximal during a period from the 8th to 18th days after irradiation, when it amounts to an excess of approximately 5 meq. Cl per day over the Na value. The relative increased loss of chloride parallels a decrease in serum Cl/Na and $CO_2$/Na ratios. Terminally, the Cl/Na ratio of the metabolic residue returns toward normal values.

Although not a certain finding, the data also suggest that there may be potassium retention amounting to some 10 meq. per 24 hours during the latter part of the first week following exposure. This change is overshadowed by irregular potassium losses (amounting to 10-20 meq./24 hours) which occur during the 3rd post-exposure week, and parallel losses in both Na and Cl.

## Serum Electrolyte Studies

Procedure: Six adult mongrel dogs (3 males, 3 females) which served as a control group for therapy studies reported elsewhere were used. These animals were housed individually, and food (Purina laboratory chow) and water were available ad lib. Blood was obtained from the jugular vein and promptly centrifuged. Clean, dry equipment free of Na, K, and Cl was used in all instances. Na and K were determined by flame photometry. Chloride was determined following deproteinization with tungstic acid by the Volhard method using nitrobenzene to sharpen the end-point. The manometric Van-Slyke

technique was employed for determination of $CO_2$ content, using 0.2 ml serum samples. All determinations were done in duplicate, if sufficient serum was available.

Determinations were done on three occasions during the week preceding irradiation, and on post-exposure days 3-4, 7, 10-11, 14, 17-18, 21, 24-25, and 28, if the animal survived to that period. Radiation exposure consisted of 575 r of whole body x-irradiation (1 mev - Pb parabolic filter) administered at a rate of 8.2 r/min.

Observations: (Based on average values for all living animals).

Serum Na remained normal (147 meq./l) during the first week following exposure. During the 2nd and 3rd weeks, however, there was a progressive decrease reaching 135 meq./l in the two animals surviving to the 21st day. The one animal which lived showed a marked rise to a value above normal (161 meq./l) during the 4th week.

Serum K fell progressively, beginning about the middle of the 1st post exposure week, and reached a minimum of 3.6 meq./l (pre-exposure 4.7 meq./l) on the 21st day (2 dogs). By the 28th day, the value had increased to 4.5 meq./l in the one dog which survived. The serum K/Na ratio fell from a pre-irradiation value of 0.032 to 0.028 during the first week, but showed no further change during the remainder of the experimental period.

Changes in serum chloride roughly paralleled those of sodium, and reached a minimum of 101 meq./l 21 days after exposure. The Cl/Na ratio, however, fell steadily during the 3rd and 4th weeks, and by the 28th day had reached 0.72 (pre-exposure 0.76) in the single surviving animal.

Both the serum $CO_2$ content and the $CO_2$/Na ratio showed parallel changes of roughly equal magnitude. A steady fall occurred during the 2nd and 3rd week, and by the 21st day the $CO_2$ content was 19 meq./l (26 meq./l pre-expo-

sure). Both the $CO_2$ content and the $CO_2$/Na ratio returned toward normal during the 4th week, but at the end of the experiment were still below their initial values.

The most striking change in serum electrolyte occurred in the undetermined acid fraction, i.e. the sum of Na + K less the sum Cl + $CO_2$ content. Initially this difference amounted to 15 meq./l serum. During the first week the difference decreased slightly and remained at 12 meq./l from the 3rd to the 7th days. Thereafter, however, there was a steady, almost linear, increase and by the 28th day, the undetermined acid fraction amounted to 26 meq./l in the one surviving dog. There was no indication that a maximum had been reached by the end of the 28 day experimental period.

Discussion: The slight, but probably real, electrolyte changes which have been observed in dogs following single massive whole body exposures to x-rays are in keeping with changes known to occur as a result of dehydration and starvation of moderate degree. Both of these occur following whole body exposure to x-rays. It would appear that dehydration and starvation provide an adequate explanation for the observed changes. Additional studies will be necessary to complete confirmation.

# EFFECTS OF X-RAYS ON YEAST GROWTH AND METABOLISM

By
W. J. Bair and J. N. Stannard

Background: A large store of information is now available concerning the enzymes and metabolic reactions present in yeast cells. Considerably less information is on record concerning the effects of x-rays and other ionizing radiations on yeast metabolism. This is true in part because yeast is not particularly radiosensitive. More recently it has been demonstrated that many vital enzymatic processes in yeast reside on the cell surface (1). The possibility that the presence of these surface reactions might permit some differentiation of "direct" (target) effects of radiation from "indirect" (media) effects led us to undertake background investigations of the effects of x-rays on the metabolic processes in bakers' yeast. The present report contains the first of our observations along these lines.

Methods: Fresh bakers' yeast obtained weekly from Standard Brands, Inc., or yeast seeded from this and cultured 24 hours in Spiegleman's medium (2), was used in these experiments. Yeast removed from the center of the cake was washed twice in distilled water, centrifuged for 2 minutes, and resuspended in M/15 $KH_2PO_4$ buffer (pH 5.6) to the desired concentration (2 to 5 mg/ml) as determined by turbidity measurements with a previously calibrated Rouy-Leitz Photrometer. Yeast grown in Spiegleman's medium was harvested by centrifuging and washing twice with distilled water and resuspended in M/15 $KH_2PO_4$ buffer to the desired turbidity.

For irradiation the suspension was divided into two portions; one being placed in an Erhlenmeyer flask as a control, the other in a 25 ml Petroff flask for irradiation. Moist oxygen was bubbled through both suspensions during irradiation; the control being placed in the x-ray

room behind a 1/2-inch lead shield. The temperature of both suspensions during irradiation was 22°C. The radiation factors were 250 KV, 15 MA, no filter, 2000 r/min. as measured in air; total dose = 90,000 r in 45 minutes*. Following irradiation, the turbidity of both suspensions was checked.

Respiration measurements were made using Warburg-type respirometers. Aerobic studies were made by the direct method of observing $O_2$ uptake and $CO_2$ production. Sufficient glucose solution was tipped in at the beginning of the experiment to make the final concentration 0.05 to 0.075 M. For determining anaerobic $CO_2$ production, $N_2$ was passed through the flask and manometer for 15 minutes prior to tipping in the glucose. Shaker speed was 110/min. and the suspensions contained 2.0 to 2.2 mg. yeast (wet weight) in 2 ml of solution. Temperature of the bath was 26.00° ± 0.30°C. Q values ($Q_{O_2}$, $Q_{CO_2}^{O_2}$, $Q_{CO_2}^{N_2}$) were calculated as µl/mg dry weight/hour. Dry weight was determined to be quite constant at 27 per cent of the wet weight under these experimental conditions.

The rate of glucose uptake was determined colorimetrically in a series of vessels beginning at one hour after the start of irradiation. About 6 mg of glucose was added to each vessel so that the final concentration was 0.015 M. The total volume of yeast suspension was 2 ml. For anaerobic determinations of glucose uptake the vessels were flushed completely with nitrogen and stoppered. They were incubated at 30°C and at predesignated intervals removed for glucose analysis. Following centrifuging to remove the yeast cells the

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*The irradiations and dose rate measurements were performed by Mrs. Florence Van Slyke, Radiation Physiology Section.

supernatant liquid was diluted to 100 ml and the amount of glucose in 1 ml determined by the method of Folin and Malmros (3). At least two standards were prepared at the same time by adding 6 mg glucose to 2 ml. of distilled water. Assuming that the average value obtained for the standards represented the amount of glucose added to the yeast suspensions, the glucose uptake from the medium was calculated.

To study colony formation aliquots of irradiated and non-irradiated cell suspension were removed for counting by the flood plate method, Anderson and Stuart (4), as used by Sherman and Chase (5). The colonies were counted 24 hours after start of irradiation, spending the interim except for the irradiation period, in an incubator maintained at 30°C.

An estimation of cell death due to irradiation was attempted by staining with methylene blue and counting. Two drops of suspension were mixed with two drops of 0.1 per cent methylene blue in M/15 phosphate buffer. After 15 minutes the stained suspension was placed in an improved Neubauer counting chamber and the cells counted.

Results: 1. Inhibition of colony formation vs. catabolic reactions. The relatively high radiosensitivity of certain growth and many other anabolic processes is, of course, well known. To begin our studies, determinations were made in our yeast of the relative dosages required to prevent colony formation as compared with what might be regarded as more characteristically catabolic reactions (such as oxygen uptake, fermentation capacity, etc.). No exhaustive study was made since this point has been amply studied by Sherman and Chase (5). We checked two levels and our results indicated clearly, in agreement with these authors, that colony formation can be completely prevented at x-ray dosages far below those which cause little or no change in over-all gaseous metabolism.

With our conditions and yeast an air dose of 20,000 r units completely prevented the formation of new colonies, while, as will be shown below, even 90,000 r failed to prevent oxygen uptake, $CO_2$ production, or the uptake of glucose by the cells. A difference in radiosensitivity of these processes was expected, but such a wide separation is especially convenient for further experimentation.

2. Glucose uptake: The uptake of glucose by yeast very probably involves the cell membrane in an active process (6). It was thought that this process might be a useful indicator of any differential effects on the cell surface enzyme systems, particularly by substances produced in the medium. Measurement demonstrated, however, that 90,000 r of 250 KV x-rays under our conditions did not alter markedly the disappearance of glucose from the medium. As shown in Tables 1 and 2, this dose, almost five times that necessary to inhibit colony formation, failed to slow or inhibit the uptake of glucose under either aerobic or anaerobic conditions. In fact, the glucose uptake rate in fermentation seemed to be slightly increased (Table 2, page 24). This appears to be the case also under aerobic conditions but is largely limited to the first three or four hours and is followed by some inhibition of questionable significance.

These trends are evidenced by the averages plotted in Figure 1 (page 25).

The method of determination is considered sensitive to at least 10 μg (3) and while there are too few experiments to apply ordinary tests of significance, the differences between irradiated cells and controls are regarded as real.

3. Oxygen uptake: The extraordinary radio-resistance of catabolic reactions in the yeast cell is illustrated by the data summarized in Table 3 (page 26) and Figure 2 (page 27). An air dose of 90,000 r units

TABLE I

AEROBIC GLUCOSE UPTAKE BY YEAST±

| Time* | Exp. # 15 | | Exp. # 16 | | Exp. # 17 | |
|---|---|---|---|---|---|---|
| | Irradiated** | Control | Irradiated | Control | Irradiated | Control |
| hrs. | mg | mg | mg | mg | mg | mg |
| 2 | 0.4 | 0.3 | 1.4 | 0.7 | 1.1 | 0.8 |
| 3 | 1.8 | 1.1 | 3.0 | 2.0 | 2.5 | 2.6 |
| 4 | 4.1 | 3.0 | 4.1 | 4.1 | 4.1 | 3.6 |
| 5 | 4.3 | 3.8 | 4.4 | 3.8 | 4.8 | 4.6 |
| 6 | 6.2 | 6.1 | 5.4 | 5.3 | 4.8 | 5.0 |
| 24 | 7. | 7. | | | | |

± Figures represent mgm. glucose disappearing from the medium at 30° C. There were 5 mg yeast (wet weight) in Exp. 15 and 4.5 mg in Exps. 16 and 17.

* Time after start of irradiation. At one hour 7.1 mg glucose were added in Exps. 15 and 16, 6.2 mg glucose in Exp. 17.

** 90,000 r air dose, 250 KV X-rays.

TABLE II

ANAEROBIC GLUCOSE UPTAKE BY YEAST‡

| Time* | Exp. # 21 | | Exp. # 23 | |
|---|---|---|---|---|
| | Irradiated** | Control | Irradiated | Control |
| hrs. | mg | mg | mg | mg |
| 2 | 0.7 | 0.7 | 3.0 | 2.6 |
| 3 | | | 3.8 | 3.4 |
| 3.5 | 2.8 | 1.4 | | |
| 4 | | | 5.0 | 4.4 |
| 5 | 2.6 | 2.2 | 5.9 | 5.1 |
| 6 | | | 6.2 | 5.5 |
| 6.5 | 3.1 | 2.4 | | |
| 8 | 4.0 | 3.3 | | |

± Figures represent mgm. glucose disappearing from the medium at 30° C. There were 2.1 mg yeast (wet weight) in Exp. 21, 4.2 mg in Exp. 23.

* Time after start of irradiation. At one hour, 6.7 mg of glucose were added in Exp. 21, 7.4 mg glucose in Exp. 23.

** 90,000 r air dose, 250 KV X-rays

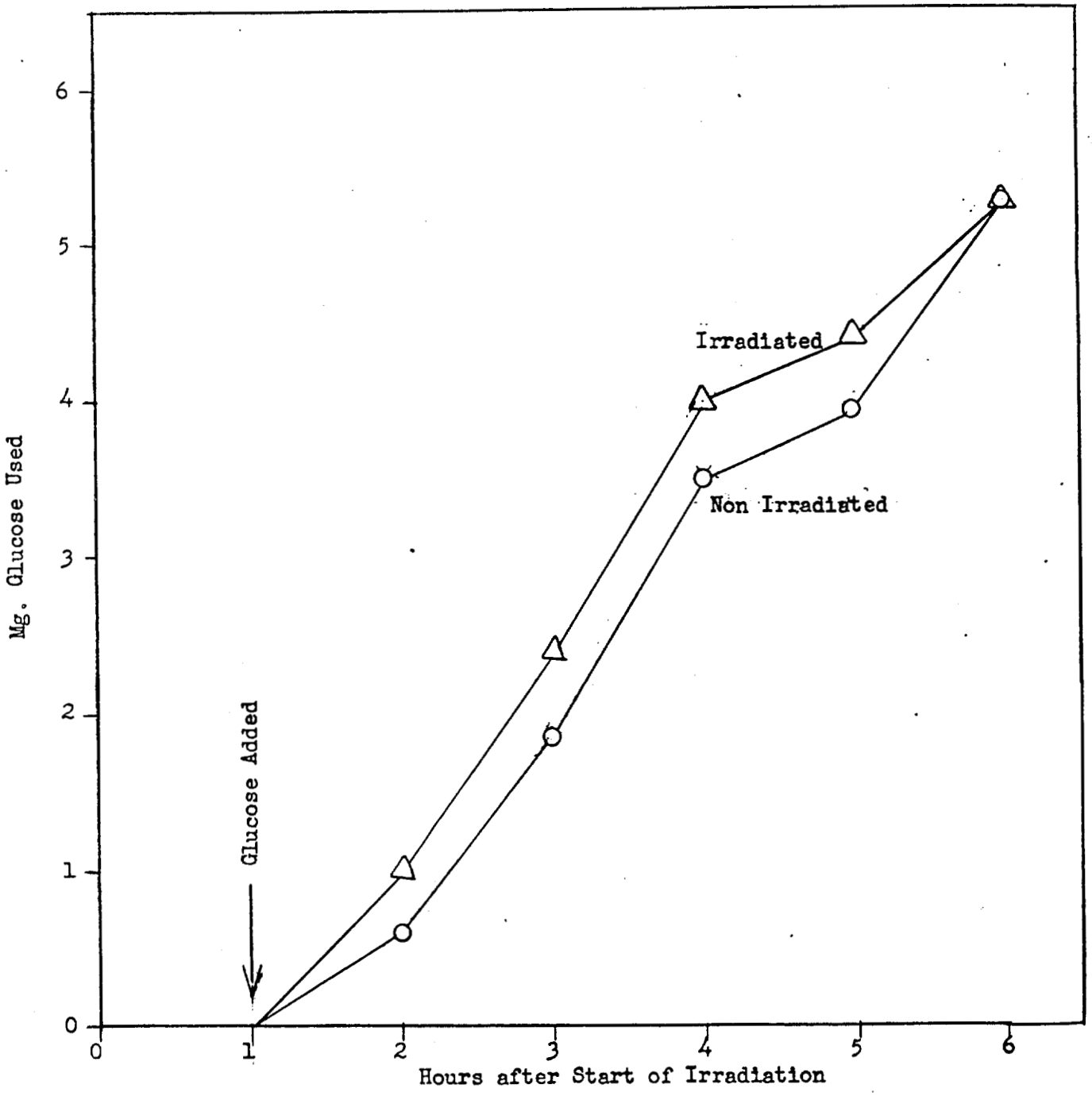



Fig. 1 Aerobic Glucose Uptake by Yeast (averages of three experiments, adjusted to the basis of 4.5 mg of yeast)

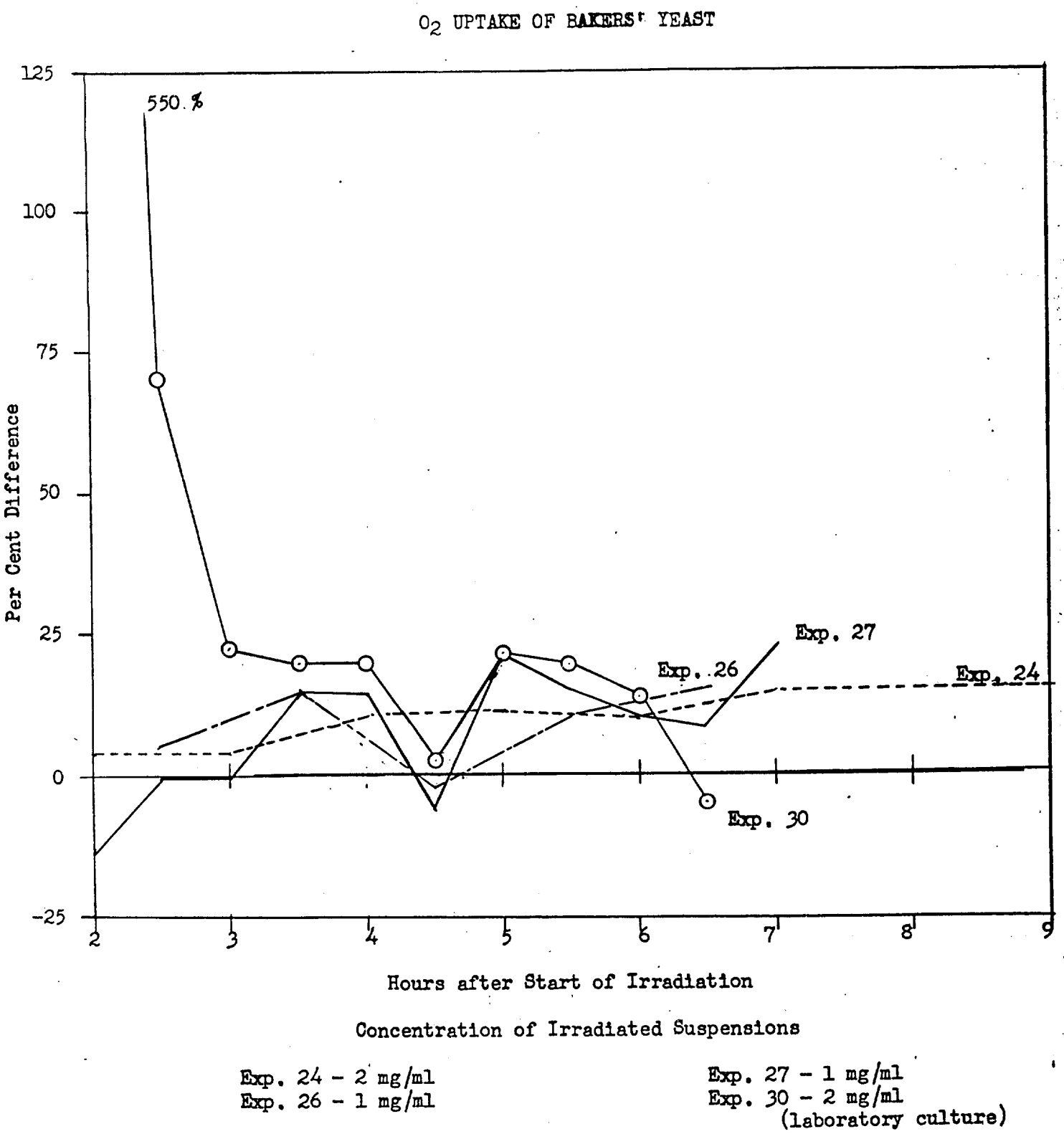
TABLE III

OXYGEN UPTAKE BY BAKERS' YEAST, EXPRESSED AS $QO_2$ (µl/mg dry weight/hour) : Dosage, 90,000 r, 250 KV X-Rays

| Time* hrs. | Exp. # 24 | | Exp. # 26 | | Exp. # 27 | | Exp. # 30** | |
|---|---|---|---|---|---|---|---|---|
| | Irrad. | Control | Irrad. | Control | Irrad. | Control | Irrad. | Control |
| 1 | | | | Glucose | added | | | |
| 2 | 69.4 | 67. | | | 44.4 | 51.6 | 14.7 | 2.3 |
| 2.5 | | | 74.1 | 70.0 | 64. | 64.4 | 13.1 | 7.7 |
| 3 | 74.7 | 72.2 | | | 68.2 | 68.6 | 6.96 | 2.1 |
| 3.5 | | | 66.9 | 59.2 | 66.6 | 58.7 | 11.6 | 9.7 |
| 4 | 67.2 | 61.0 | | | 61.5 | 54.3 | 12.3 | 10.4 |
| 4.5 | | | 82.5 | 85.1 | 58.2 | 62.1 | 17.7 | 17.2 |
| 5 | 57.7 | 51.8 | | | 58.4 | 48.6 | 15.4 | 12.7 |
| 5.5 | | | 62.4 | 57. | 56.3 | 49.0 | 18.5 | 15.7 |
| 6 | 60.5 | 54.8 | | | 52.9 | 47.7 | 24.6 | 21.7 |
| 6.5 | | | 64.2 | 56.2 | 54.3 | 50.2 | 14.7 | 15.7 |
| 7 | 54.9 | 48.0 | | | 52.3 | 42.5 | | |
| 7.5 | | | | | | | | |
| 8 | 56.9 | 49.8 | | | | | | |
| 8.5 | | | | | | | | |
| 9 | 50.6 | 44.1 | | | | | | |
| 10 | | | | | 42.8 | 41.0 | | |
| 13.5 | | | 64.8 | 48.4 | | | 35.5 | 28.9 |

* Hours after start of irradiation

** 24 hour culture in Spiegleman's medium




Concentration of Irradiated Suspensions

Exp. 24 - 2 mg/ml
Exp. 26 - 1 mg/ml
Exp. 27 - 1 mg/ml
Exp. 30 - 2 mg/ml (laboratory culture)

Fig. 2 Changes in Oxygen Uptake after Irradiation

of 250 KV x-rays failed to inhibit the over-all oxygen uptake of bakers' yeast respiring in 0.05 M glucose. In fact, it appeared to produce a small but consistent stimulation. The stimulation appeared whether the cells were cultured in the laboratory (experiment 30) or used directly from the block of commerical bakers' yeast (see contrasting effect on $CO_2$ production below). The figures for the first three hours in experiment 30 indicate a very large stimulation, but are too erratic to be considered significant without confirmation.

4. Anaerobic $CO_2$ production: Comparable data for anaerobic $CO_2$ production are shown in Table 4 (page 29) and Figure 3 (page 30). Yeast used directly from the original pound block showed a decreased rate of fermentation after irradiation in only two instances (hours 2-4 of experiment 23). Otherwise, this dosage produced varying degrees of stimulation. However, an inhibitory action is seen in experiment 29 employing yeast cultured in this laboratory. This may, if confirmed, indicate an effect of cultured conditions. Sherman and Chase (5) found uniform inhibition of anaerobic $CO_2$ production by this dosage of x-rays only in yeast grown in Reader's medium and emphasized the role of previous culture conditions on the susceptibility of this particular process to radiation.

5. Dilution: The concentrations of yeast were chosen to be within the more sensitive range demonstrated by Sherman and Chase (7). No effect of concentration was noted over the range presented herein, but more formal studies of the dilution phenomenon are in progress.

Discussion: The preliminary experiments reported here illustrate the ease with which frankly anabolic reactions such as colony formation can be prevented by x-irradiation without obvious damage to the over-all catabolic reactions. This is an expected result. Stimulation of catabolic reactions

TABLE IV

ANAEROBIC $CO_2$ PRODUCTION BY BAKERS' YEAST

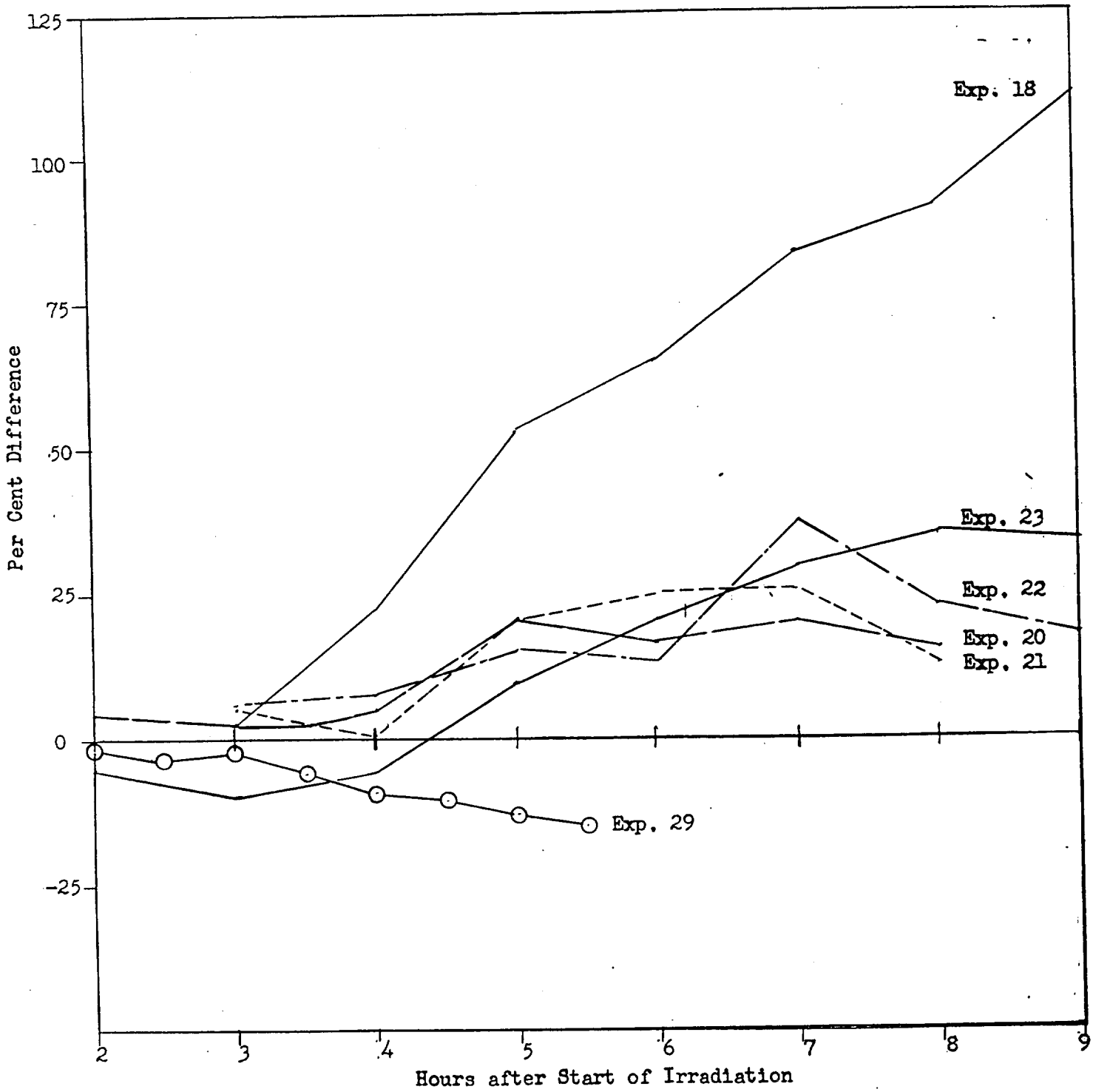
Expressed as $Q^{N_2}_{CO_2}$ (µl/mg dry weight/hour) : Dosage, 90,000, 250 KV X-rays

| Time* | Exp. # 18 | | Exp. # 20 | | Exp. # 21 | | Exp. # 22 | | Exp. # 23 | | Exp. # 29** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hours | Irrad. | Control | Irrad. | Control | Irrad. | Control | Irrad. | Control | Irrad. | Control | Irrad. | Control |
| | | | | | | Glucose | Added | | | | | |
| 1 | | | | | | | | | 226.3 | 237.5 | 168.4 | 171.7 |
| 2 | | | 241.4 | 231.2 | | | | | | | 219.1 | 227.5 |
| 2.5 | | | | | | | | | | | 211.7 | 214.3 |
| 3 | 259. | 253. | | 278.2 | 324.2 | 311.6 | 246.9 | 231.8 | 226.6 | 249.6 | 224.7 | 238.0 |
| 3.5 | | | 257.9 | 253.4 | | | | | | | 197.8 | 216.2 |
| 4 | 246. | 201. | 230.2 | 229.8 | 244.1 | 243.6 | 322.9 | 301.2 | 183.4 | 196.8 | 197.3 | 220 |
| 4.5 | | | | | | | | | | | 183.4 | 212.3 |
| 5 | 199. | 130. | 163.2 | 137.8 | 194.5 | 162.3 | 274.2 | 240.2 | 162.1 | 149.7 | 176.3 | 207.5 |
| 5.5 | | | | | | | | | | | | |
| 6 | 181. | 110. | 127.3 | 109.9 | 154.0 | 122.6 | 233. | 206.0 | 110.3 | 91.9 | | |
| 6.5 | | | | | | | | | | | | |
| 7 | 203. | 111. | 129.4 | 108.8 | 134.7 | 106.6 | 171.1 | 124.5 | 117.1 | 89.7 | | |
| 7.5 | | | | | | | | | | | | |
| 8 | 195. | 101.5 | 146.9 | 126.2 | 193.3 | 170.0 | 128.4 | 104.5 | 121.2 | 89.6 | | |
| 8.5 | | | | | | | | | | | | |
| 9 | 200. | 94.5 | | | | | 114.9 | 97.6 | 115.6 | 85.8 | | |
| 24 | | | | | | | 170.2 | 120.6 | | | | |

* Hours after start of irradiation

** 24 hour culture in Spiegelman's medium

ANAEROBIC $CO_2$ PRODUCTION OF BAKERS' YEAST




Concentration of Irradiated Suspensions

Exp. 18 - 4 mg/ml
Exp. 20 - 5 mg/ml
Exp. 21 - 2.1 mg/ml
Exp. 22 - 2.1 mg/ml
Exp. 23 - 2.1 mg/ml
Exp. 29 - 1.9 mg/ml (laboratory culture)



Fig. 3 Changes in Anaerobic $CO_2$ Production after Irradiation

in yeast by x-rays has not been reported to our knowledge, although this effect is not unexpected either. In fact, Giese (8) has found, under certain conditions, rather large stimulations of the endogenous respiration of yeast by ultra-violet light, and Fenn and Latchford (9) reported a fairly consistent increase of muscle respiration (about 30 per cent) following large doses of unfiltered x-radiation. More recently, Richmond, Altman, and Salomon (10) noted stimulation of the oxygen uptake of bone marrow after irradiation.

The mechanism of these effects is probably complicated and indirect. However, in view of their similarity to the effects of chemical agents such as azides and dinitrophenol where uncoupling of high energy bound phosphorylations occurs, the increase in over-all catabolic reaction rates at x-ray dosages sufficient to prevent many anabolic reactions may be indicative of some inter-relationship.

The fact that glucose uptake was not inhibited by 90,000 r of x-rays somewhat negates the hope of separating "direct" from "indirect" effects of x-rays by a study of reactions at the yeast cell surface (presuming glucose uptake involves the cell surface directly). However, a more formal study of dilution phenomena and of other reactions shown to occur at the cell surface (1) is planned before dismissing these possibilities.

Summary:

1. X-ray dosage levels have been established for complete inhibition of colony formation by bakers' yeast without inhibition of over-all metabolism. A convenient level for this situation is 20,000 r, 250 KV x-rays. This confirms the results of Sherman and Chase (5).

2. Under our conditions 90,000 r, air dose, of 250 KV x-rays usually produced a small but consistent stimulation of both oxygen uptake and anaerobic $CO_2$ production at this dosage level. These stimulation phenomena

have not been described previously under these conditions.

3. Glucose uptake was measured as a reaction involving the cell surface. An x-ray dose almost five times that sufficient to produce complete inhibition of colony formation did not slow the rate of glucose uptake by the cells. There was a slight stimulation of glucose use under anaerobic conditions.

4. These preliminary experiments are being extended.

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# THE OZONOLYSIS OF SUBSTITUTED MALEIC ACID IMIDES AND THEIR USE IN THE DEGRADATION OF PORPHYRINS

By
J. E. Richmond and K. I. Altman

Background: The need for a suitable method for the degradation of tetrapyrrols, such as protoporphyrin and chlorophyll derivatives, for the purpose of studying the $C^{14}$ distribution after incorporation of $C^{14}$-labeled precursors, has led to the development of a method for the degradation of the oxidative breakdown products of tetrapyrrols. The method here to be reported permits the isolation of fragments of the pyrrol nuclei in good yields. Although the oxidative degradation products of porphyrins have been further degraded by other means (1), these procedures have not been accompanied by good yields.

Methods: The method to be described involves the preparation of crystalline ozonides of methyl ethyl maleimide and hematinic acid imide. The preparation of one of the ozonides, namely methyl ethyl maleimide, has been described briefly by Fischer and Deželić (2). Ozonization of methyl ethyl maleimide and hematinic acid imide obtained from chromic acid oxidation of protoporphyrin IX (3) was carried out by passing ozone (1.8 meq./min.) from a high-voltage type ozone generator for 30 minutes through a chloroform solution containing the respective substituted maleimide in concentrations of 100 mg. per 100 ml. chloroform. Both ozonides could be brought to crystallization by concentrating the chloroform solution to a small volume in a vacuum desiccator. The products were recrystallized several times from chloroform-petroleum ether. These ozonides, in contrast to the ozonide of unsubstituted maleic acid, are quite stable and could be characterized by melting point, elementary analysis and infrared and ultra-

violet spectra.

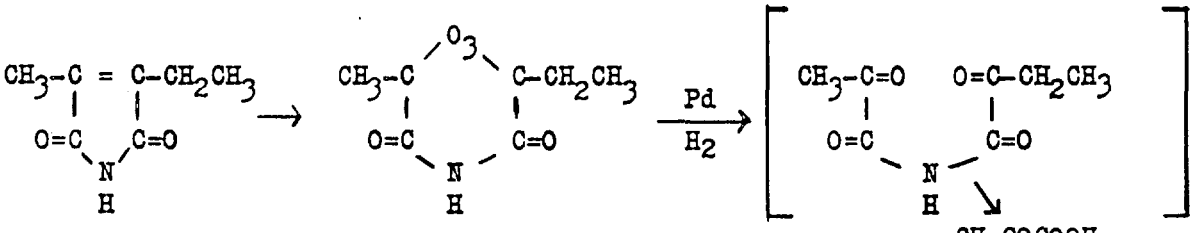
Some efforts have been made to characterize the ozonides. First of all, elementary analyses have been carried out with the following results:

Methyl ethyl maleimide ozonide

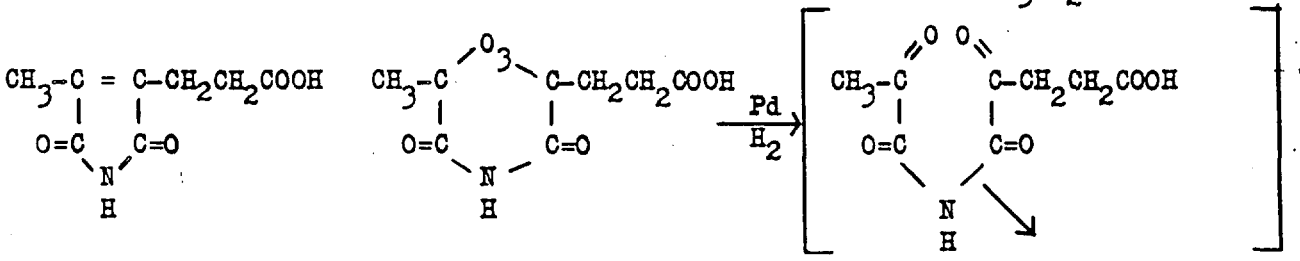
| | Per cent C | Per cent H | Per cent N |
|---|---|---|---|
| Found | 45.42 | 5.11 | 7.86 |
| Calculated | 45.00 | 4.83 | 7.50 |

Infrared absorption spectra were measured by depositing the ozonides in question on AgCl plates*. The spectra are shown on the accompanying figures 1 and 2 superimposed upon the parent compound, that is, the respective substituted maleic acid imides.

Since the decomposition of these ozonides by the usual, mild procedures proved unsuccessful, it was necessary to resort to more vigorous methods. Thus, the catalytic hydrogenation of the ozonides according to the method of Fischer, Dull, and Ertel (4), using $Pd-CaCO_3$ as catalyst, led to decomposition of these stable ozonides and to the isolation of the expected decomposition products in good yield. The following decomposition products were finally isolated from the two ozonides.

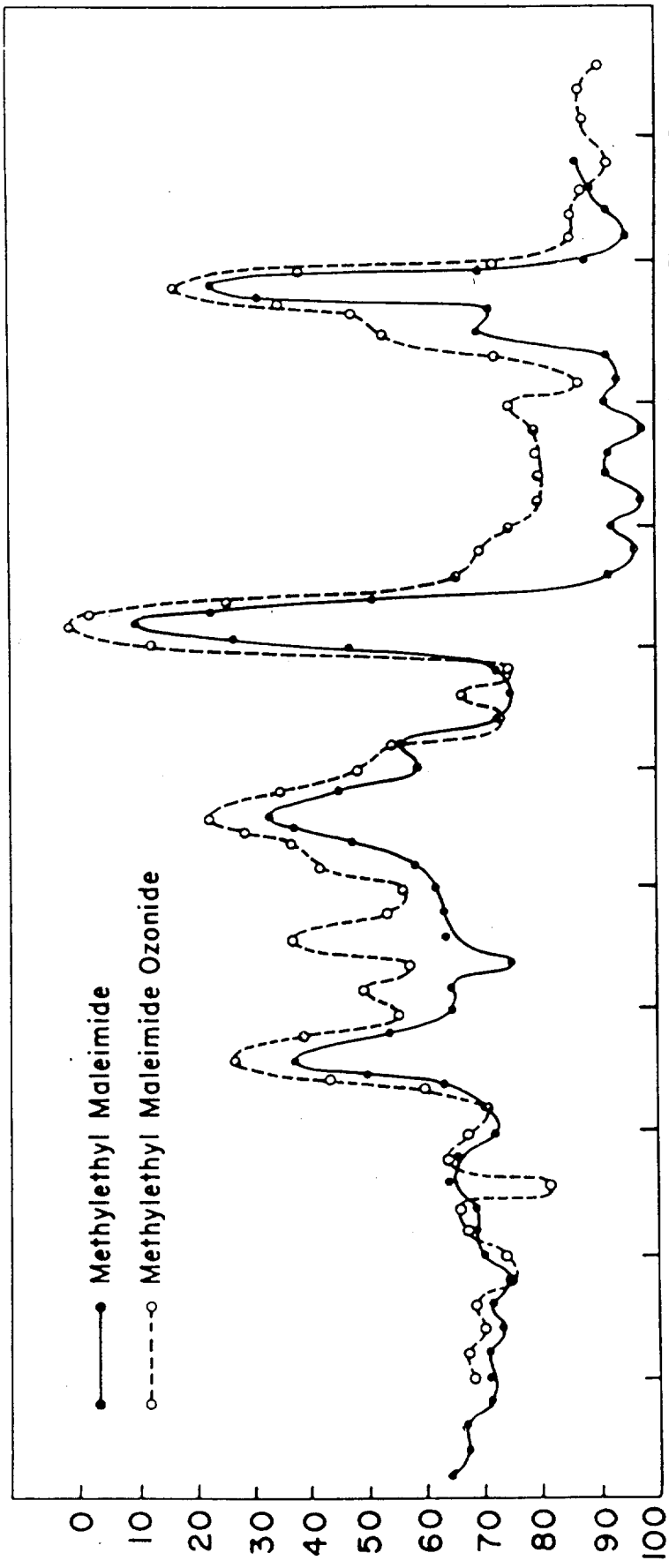



Methylethylmaleimide

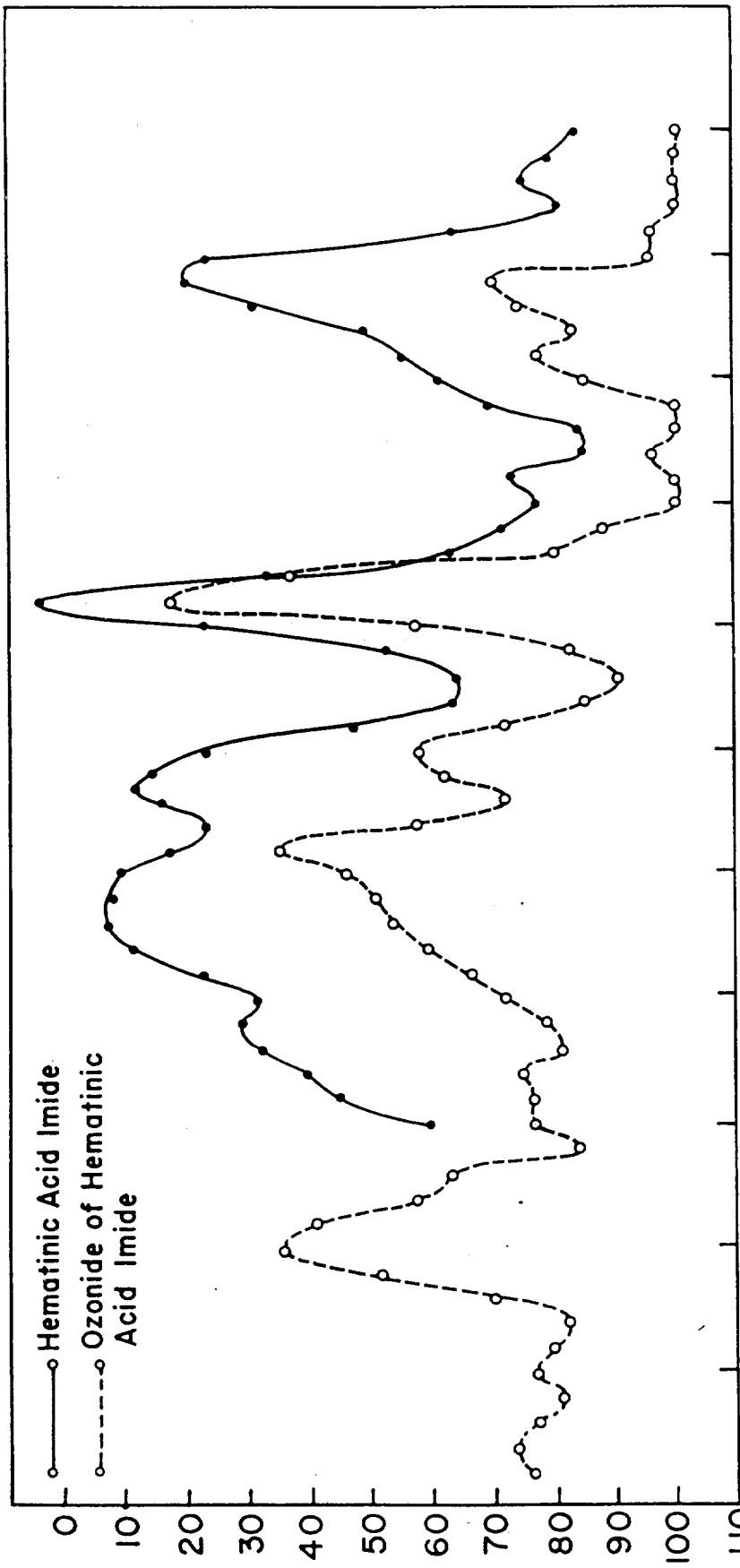



Hematinic Acid Imide

*We are indebted to Dr. Steadman and Mr. Levy for these measurements.




Infrared Absorption Spectra of Methylethyl Maleimide and Methylethyl Maleimide Ozonide.




Infrared Absorption Spectra of Hematinic Acid Imide and the Ozonide of Hematinic Acid Imide

Twenty milligrams of the ozonide were taken up in 50 ml. of dry ethyl acetate and 10 mg. of Pd-$CaCO_3$ were added. Hydrogen gas was bubbled through the reaction mixture at atmospheric pressures and at 25°C for one hour. The reaction mixture was then centrifuged and the supernatant removed and extracted several times with ethyl acetate. The ethyl acetate solution was evaporated to dryness and the oily, unsymmetrical imide transferred to a sealed-tube system for hydrolysis with 50 ml. of saturated barium hydroxide. The sealed tube was shaken continously at room temperature for 24 hours, the contents acified, and the carboxylic acids extracted with ether. After removal of the ether in vacuo the alpha-keto acids were converted to their corresponding 2,4-dinitrophenylhydrazone derivatives for the purpose of chromatographic separation. The 2,4-dinitrophenylhydrazone derivatives of the resulting alpha-keto acids were chromatographed on supercel, essentially according to the method of LePage (5) with slight changes in the relative proportions of the solvents used for adsorption and development of the chromatogram. The phenylhydrazone derivatives were eluted from the supercel column, recrystallized several times, and were then characterized by correctness of the melting point. The phenylhydrazone derivatives could then be degraded further by one of several known methods leading to the isolation of single-carbon fragments.

Conclusion: A feasible method for the degradation of porphyrins of the protoporphyrin IX type is described. This method is applicable to studies of isotope distribution among the carbon atoms of the porphyrin skeleton since it permits eventual isolation of the individual carbon atoms and, therefore, measurement of the $C^{14}$ activity of an individual carbon atom contained in the prophyrin ring. This method will find its application

in future studies with various precurosrs of hemin and chlorophyll.

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# SERUM AND URINARY PLASMIN ACTIVITY RELATED TO THE HEMORRHAGIC DIATHESIS IN IRRADIATED DOGS

By
J. Colgan, E. Gates, and L. L. Miller

Fibrinolysis has been the subject of speculation as a factor in the pathogenesis of the post-irradiation hemorrhagic syndrome in goats (1), but no study of the relation of large dose whole-body irradiation to changes in fibrinolytic enzymes has come to our attention.

We have observed a close association between a rise in the urine and total serum plasmin activity and the development of the hemorrhagic syndrome in dogs after exposure to 450-700 r of whole-body irradiation. The change in the activity of the urinary enzyme is more pronounced quantitatively and approximates a fifty to one hundred fold increase of control base line activity.

Of perhaps greater significance is the fact that the fibrinolytic activity increases markedly 24 to 48 hours before the dogs manifest gross hemorrhagic lesions and become clinically very ill. The enzyme activity remains abnormally high in the dogs that go on to die with hemorrhage as an outstanding, if not primary, cause of death.

Methods: Healthy adult dogs of predominantly beagle strain maintained on the stock kennel diet were kept in metabolism cages for the collection of urines which were preserved by freezing in dry ice-acetone. Blood was drawn daily in amounts of 5 to 8 cc to provide serum for testing.

The methods of assaying serum and urine were essentially those of Cristensen (2) and Ungar (3) respectively. The level of the urine enzyme activity is assessed as the maximum dilution of urine to which an added standard amount of beef fibrinogen (4) fails to form a clot after incubation for exactly 30 minutes at 37° C, when hemostatic globulin Lederle is added.

The serum enzyme activity is reckoned as that dilution of serum which within exactly one hour at 37° C completely lyses a standard fibrin clot formed at the outset of the test by mixing a standard amount of beef fibrinogen with the streptokinase activated serum in the presence of hemostatic globulin.

In the case of the serum, the plasminogen is thus fully activated with streptokinase (generously supplied to us by the Lederle Company, Pearl River, N. Y., as a streptokinase-streptodornase mixture) and the resulting activity corresponds to the total of serum plasmin plus plasminogen. Our attempts to fractionate dog serum quantitatively to remove natural serum inhibitors as described by Milestone (5) and Cohn (6) have failed to give clean-cut fractions. For this reason we have chosen to study the plasmin activity in the presence of the natural serum inhibitors.

As already noted by MacFarlane (7) we have found that the urinary enzyme is excreted in the fully activated state, and its activity is therefore measured without streptodornase activation.

Although each dog served as its own control, the base line data obtained in the two to four days preceding radiation show very closely similar activities in both plasma and urine of all dogs.

Radiation factors for the X-ray machine used are 250 kilovolts, 15 milliamperes, aluminum parabolic filter plus 0.5 mm of copper, half-value-layer equivalent to 2.15 mm copper, and skin target distance 36 inches.

Results: Six animals have been studied to date and the results are without exception very closely similar. However, for the purpose of this report the results of only two experiments will be described in detail.

Table I presents the results of observation on Dog D2 and Table II

TABLE I

Dog D-2 Female Beagle

| Time in Days Before and After Radiation | Urine Fibrinogenolytic Titre | Serum Fibrinolytic Titre | Remarks |
|---|---|---|---|
| 4 | Undiluted urine | 1-1500 | Normal |
| 3 | shows partial | 1-1700 | " |
| 2 | destruction of | 1-1500 | " |
| 1 | fibrinogen after 30 min. at 37° C. | 1-1500 | " |
| | 700 r Whole Body Radiation | | |
| 1 | | 1-1600 | Vomited after X-Ray |
| 2 | | 1-1500 | Dog eating poorly |
| 3 | | 1-1600 | Slight diarrhea, no food |
| 4 | 1-7 | 1-2000 | Slight diarrhea, food started |
| 5 | 1-9 | 1-2000 | Diarrhea persisting, food intake normal |
| 6 | 1-9 | 1-1900 | Stools solid. Unremarkable |
| 7 | 1-4 | 1-1900 | " |
| 8 | 1-5 | 1-1700 | " |
| 9 | 1-4 | 1-1500 | " |
| 10 | 1-5 | 1-1600 | " |
| 11 | 1-4 | 1-1500 | " |
| 12 | 1-5 | 1-1600 | Slight diarrhea |
| 13 | 1-5 | 1-1700 | Unremarkable |
| 14 | 1-15 | 1-2000 | Anorexic. RBC sedimentation rate grossly increased |
| 15 | 1-18 | 1-2400 | Injected 250 ml Locke's solution IV. Dog drooling from mouth. Ate little |
| 16 | 1-25 | 1-2500 | Anorexic. Dog drooling and weak |
| 17 | 1-50 | 1-2500 | Dead |

Autopsy Findings in Dog D-2

Multiple scattered hemorrhages in thoracic viscera, lymph nodes, GI tract, skin and adrenals. Pneumonia, right lower lobe. Pulmonary edema.

TABLE II

Dog D-3 Female Shepherd Mongrel

| Time in Days Before and After Radiation | Urine Fibrinogenolytic Titre | Serum Fibrinolytic Titre | Remarks |
|---|---|---|---|
| 6 | Undiluted urine | 1-1900 | Normal |
| 5 | some fibrinogen | 1-1900 | " |
| 4 | destruction in 30 min. | 1-2000 | " |
| 3 | Undiluted urine completely destroyed fibrinogen in 30 min. | 1-1900 | " |
| 2 | | 1-1900 | " |
| 1 | | 1-1900 | " |
| | 450 r Whole Body Radiation | | |
| 1 | 1-3 | 1-1900 | Unremarkable |
| 2 | 1-3 | 1-2000 | Slight diarrhea |
| 3 | 1-5 | 1-2000 | Unremarkable |
| 4 | 1-11 | 1-2200 | " |
| 5 | 1-10 | 1-2100 | " |
| 6 | Contaminated with feces beyond use | 1-2200 | Diarrhea |
| 7 | 1-3 | 1-2300 | Stools solid. Unremarkable |
| 8 | 1-2 | 1-2300 | " |
| 9 | 1-3 | 1-2300 | RBC Sedimentation rate grossly increased |
| 10 | 1-4 | 1-2200 | Diarrhea |
| 11 | 1-3 | 1-2200 | Stools solid |
| 12 | 1-25 | 1-2200 | Stools solid |
| 13 | 1-35 | 1-2200 | Diarrhea |
| 14 | 1-40 | 1-2500 | Conjunctival hemorrhage. Ulceration of lip with edema |
| 15 | 1-70 | 1-2600 | Edema of mouth increasing. Dog very weak |
| 16 | 1-75 | 1-2500 | Ulcer and edema severe. Dog failing |
| 17 | | | Dead |

Autopsy Findings in Dog D-3

Massive pulmonary hemorrhages in left upper, lower and middle lobes, and in right lower and middle lobes. Hemorrhages of skin, diaphragm, intestines, lymph nodes, and conjunctiva. Infected ulcer of lower lip.

presents those on Dog D3. Both dogs showed some clinical disturbance manifest by slight malaise and diarrhea in the first few days after radiation; this was reflected in both the urinary fibrinolytic activity which reached a peak about the fifth to seventh day after radiation, decreased somewhat without returning to normal, and then increased sharply around the twelfth to fourteenth day after radiation until the time of death three or four days later. The changes in serum fibrinolytic activity in Dog D2 resembled qualitatively the urine activity changes. In Dog D3 the early rise in serum fibrinolytic activity was not as large, and the increased level of activity was sustained until three days before death when the level finally reached was about 25 per cent above the base line control. The final level of serum fibrinolytic activity in Dog D2 was about 50 per cent above the base line level.

It is remarkable that the pronounced changes in enzyme activity were noted 24 or 48 hours before the dogs' clinical condition deteriorated and that widespread grossly recent hemorrhagic lesions were noted in both dogs. Most of the hemorrhages in Dog D2 were small and the immediate cause of death was apparently a pneumonia. In Dog D3, however, there was massive interstitial pulmonary hemorrhage with terminal bleeding from the mouth, as well as widespread small hemorrhages into almost all organs.

Discussion: It now appears that survival of dogs from whole-body irradiation at the 500 r to 750 r dose level is conditioned by the twin hazards of microbial infection and hemorrhage. Thus, while microbial infection may be controlled or eliminated with antibiotics, the danger of serious or fatal hemorrhage remains to be dealt with.

The pathogenesis of the hemorrhages resulting from radiation injury have been analyzed in terms of (a) the loss of platelets from the circulating

blood, (b) damage to capillary endothelium with resultant increase in permeability or actual capillary rupture, (c) diminished coagulability of the blood presumably related by some to "heparinoid" substances in the blood. As a result of our observations, it seems reasonable to strongly suspect that abnormal fibrinolytic activity is fundamentally related to the development of tissue hemorrhage as a result of radiation injury. Tissue factors capable of locally converting the inert plasminogen to the fibrinolytic enzyme plasmin have been described (8). In Permin's report lung has the highest plasminogen activating capacity and this coincides with the fact that pulmonary hemorrhages are very common in the post-radiation hemorrhagic diathesis.

Largely unknown are the origin, function, and fate of serum plasminogen and plasmin, and their relation to the urine fibrinolytic enzyme which has been described to have properties slightly different (7) from the serum enzyme.

In view of the demonstrated in vitro (9) and in vivo (10) action of crystalline soy-bean trypsin inhibitor in the inhibition of serum plasmin activity, it seems highly desirable to test this material as a therapeutic agent for preventing or minimizing the hemorrhages incident to radiation injury.

Summary:

1. The urinary and serum fibrinolytic activity has been measured in dogs after 450-700 r of whole-body radiation.

2. Three or four days before death both the urine and serum fibrinolytic activity increase very significantly reaching maximum values just before death.

3. The relation between the enhanced fibrinolytic enzyme activity

and the hemorrhagic lesions noted at autopsy is discussed.

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# THE EFFECT OF SPLEEN SHIELDING ON X-IRRADIATION MORTALITY IN DOGS

by
M. P. Coulter and F. W. Furth

Background: Jacobson and his associates have reported a 77.7% survival of mice exposed to 1025 r of whole body x-irradiation with lead shielding of the exteriorized spleen (1). A rapid recovery from hemapoiesis was demonstrated in the surviving mice (2). This effect has been shown not to be species specific by their other studies on spleen shielding of rats and guinea pigs (3, 4). It became of interest to determine whether spleen shielding during x-irradiation would alter the survival rate and hasten the hematopoietic recovery in the dog.

Methods: In the initial study when methods were being worked out, 14 dogs which had previously received 450 r whole body x-irradiation (6 weeks prior to this study) were used (Group A). Following this, a second group (B) of 4 normal adult dogs were studied.

The dogs were operated upon under intravenous nembutal anesthesia. The spleen was mobilized and exteriorized through a left lateral abdominal incision. One-half of the dogs had their spleens shielded with one-eighth inch lead during the x-irradiation. The spleens of the control dogs were exteriorized but not shielded. Immediately following operation the anesthetized dogs received total body x-irradiation under the following conditions: 250 KV 15 ma plano-convex aluminum filter with 0.5 mm. copper, target skin distance 40 inches at a rate of 7.15 r per minute. The first group of 14 dogs (A) received 450 r and the second group of 4 dogs received 500 r (B).

Hematological studies including hematocrit, counts of the white blood cells, platlets, reticulocytes and smears were carried out twice prior to

irradiation and twice weekly following irradiation. Bone marrow aspirations were made prior to irradiation and at varying periods post-irradiation by Dr. Marylou Ingram. The results of the bone marrow studies will be reported later.

Results: The hematological data are shown in Graphs I - IV. In Group A, as shown in Graph I, the white blood cell count remained at a higher level in the spleen shielded dogs than in the control dogs and showed a trend toward recovery at an earlier period. The leukocyte count fell to equally low values in shielded and control dogs in Group B, as shown in Graph III, but again an earlier recovery was evidenced. The hematocrit values as shown in Graphs II and IV also indicate that the red blood cell counts began to return to normal values at an earlier period in the shielded than in the control dogs. There was no significant difference in the platelet counts between the shielded and control dogs, except that after the 11th day in Group A dogs the value began to rise and return toward normal values in the shielded animals. The reticulocyte counts did not show a response in the shielded dogs during the 28 day post-irradiation period of study.

In Group A, the first death in the control dogs occurred on the 8th day post-irradiation while the first death in the shielded group occurred on the 14th day post-irradiation. The 28 day mortality was 100% in the control dogs; 44% in the shielded dogs. Neither of the shielded dogs in Group B died. The first control dog in this group died on the 11th day post-irradiation and the second one on the 18th day post-irradiation.

The wounds of the control dogs all became necrotic a few days prior to death. Necrosis of the abdominal wound was noted in only two of the shielded dogs. One of these died while the other healed his wound and lived.

Gross pathology studies did not reveal any significant differences between the control and shielded dogs that died. The major derangements were pulmonary hemorrhage and edema, and gastro-intestinal hemorrhage.

Only the spleens of those dogs in Group A that died have been studied microscopically. The spleens were uniformly hypoplastic and no difference was noted between spleens from shielded and control dogs. However, it should be noted that all of these animals had received 450 r of x-irradiation 6 weeks prior to this present study.

Conclusion: Lead shielding of the exteriorized dog spleen during x-irradiation appears to be an effective means of reducing x-irradiation mortality. Of 14 previously irradiated dogs, 100% of the control animals died while 44% of the spleen shielded dogs died. Of the 4 normal dogs studied, the 2 controls died while the 2 spleen shielded animals survived. Peripheral hematological studies of the blood smears and the bone marrow studies are at present incomplete so that no definite statement can be made regarding an early recovery of hematopoietic tissue in the spleen shielded dogs. However, the peripheral white blood cell counts and hematocrits indicate that this may occur. It is realized that an evaluation of the hematopoietic tissue of the spleen would involve a sacrifice experiment in order to compare the tissue from living and dying animals at the same period, post-irradiation. This type of study is more easily carried out with small animals.

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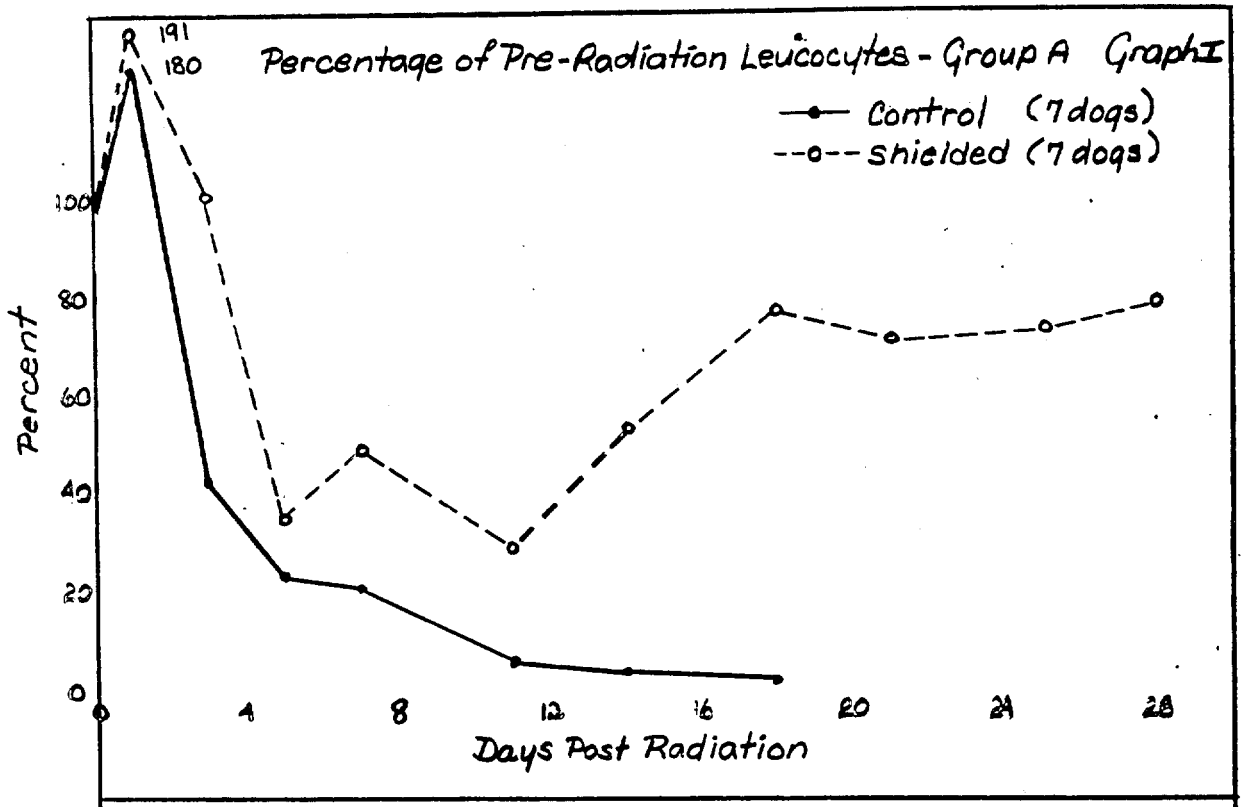
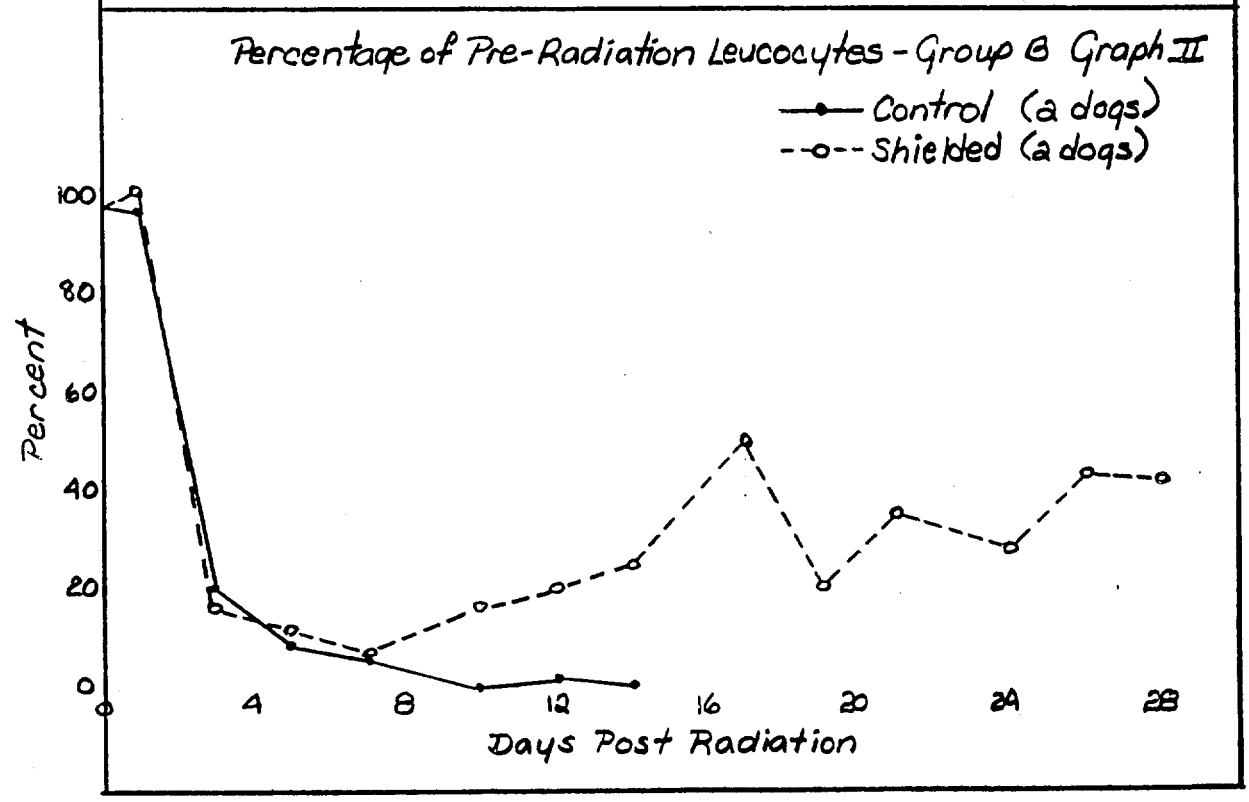
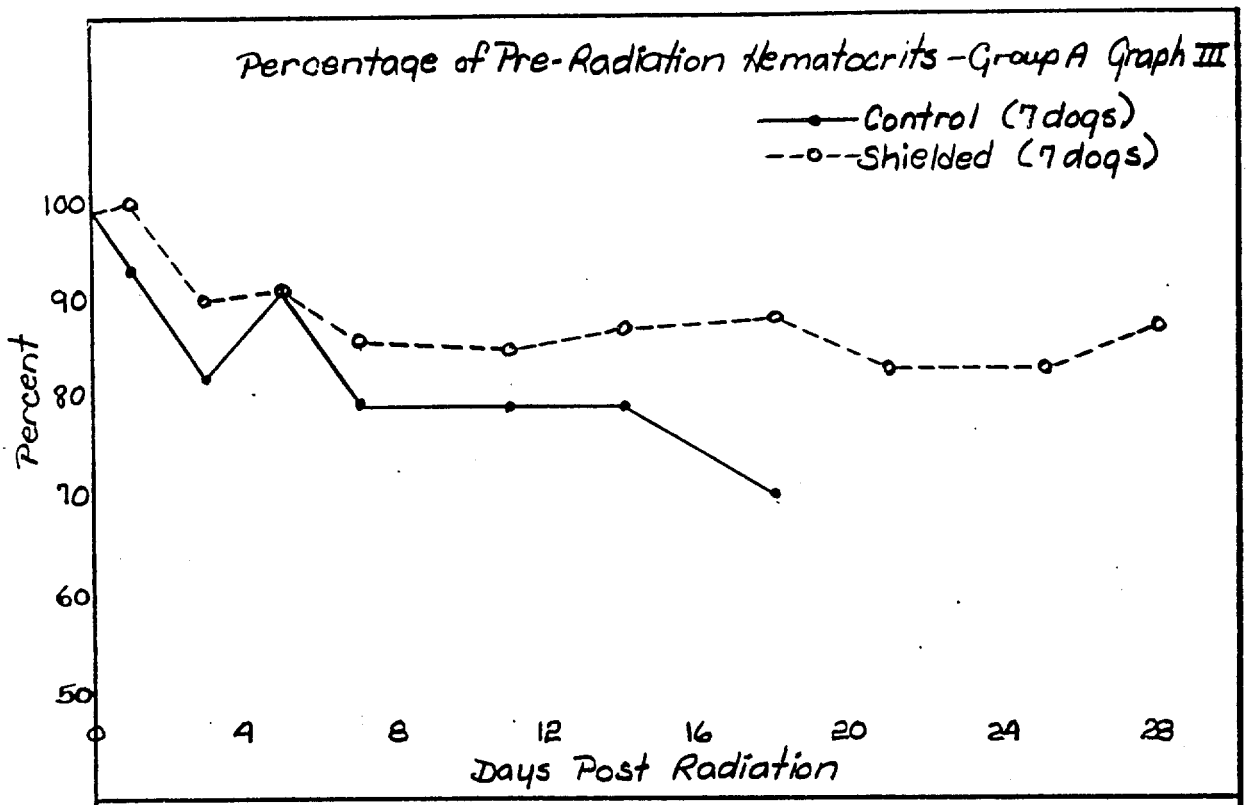
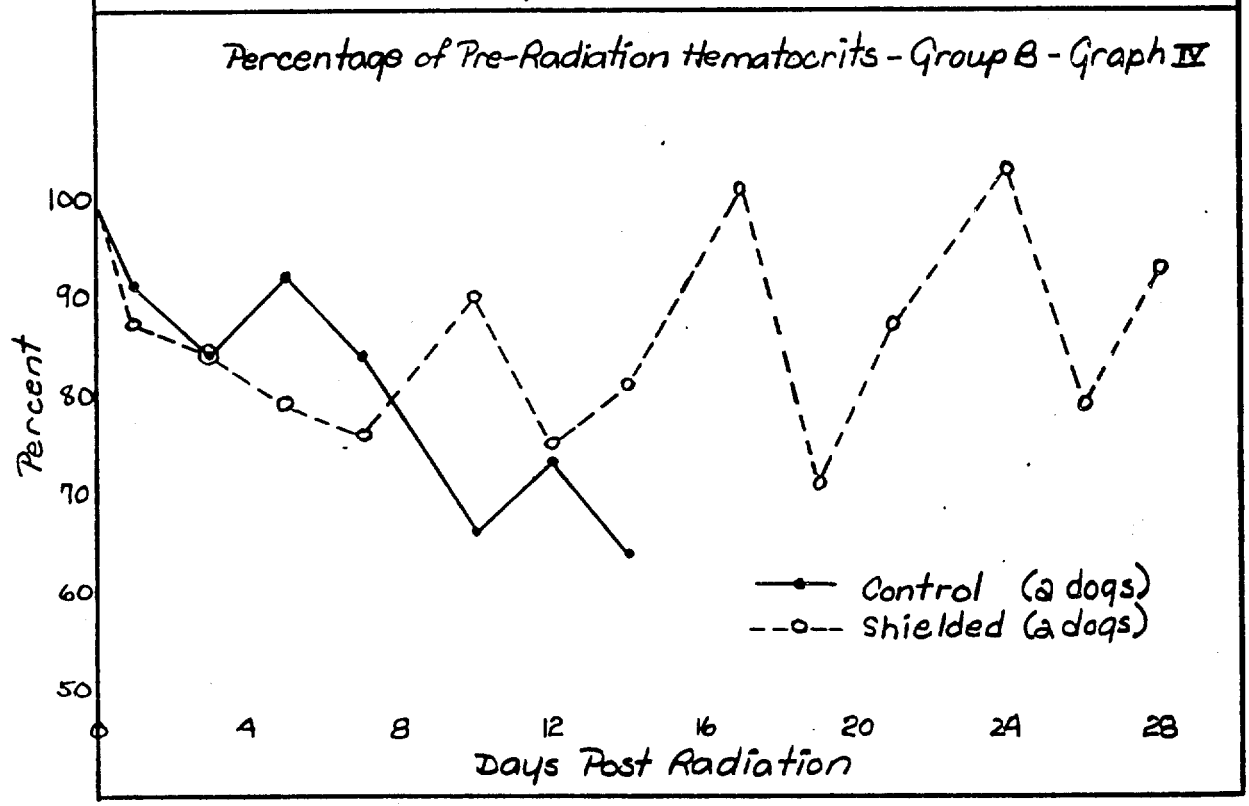

Percentage of Pre-Radiation Leucocytes - Group A Graph I
Control (7 dogs)
Shielded (7 dogs)
191
180
Percent
100
80
60
40
20
0
4
8
12
16
20
24
28
Days Post Radiation


Percentage of Pre-Radiation Leucocytes - Group B Graph II
Control (2 dogs)
Shielded (2 dogs)
Percent
100
80
60
40
20
0
4
8
12
16
20
24
28
Days Post Radiation


Percentage of Pre-Radiation Hematocrits - Group A Graph III
Control (7 dogs)
Shielded (7 dogs)
Percent
100
90
80
70
60
50
0
4
8
12
16
20
24
28
Days Post Radiation


Percentage of Pre-Radiation Hematocrits - Group B - Graph IV
Control (2 dogs)
Shielded (2 dogs)
Percent
100
90
80
70
60
50
0
4
8
12
16
20
24
28
Days Post Radiation

# EFFECT OF AUREOMYCIN AND WHOLE BLOOD, SINGLY AND IN COMBINATION, ON THE X-IRRADIATED (575 r) DOG

by

Frank W. Furth, Molly P. Coulter, Robert W. Miller and Scott N. Swisher

This report is a brief preliminary summary of the results of an experiment on 96 dogs that received 575 r of 1 mev whole body x-irradiation (rate 8 r/min.), and that were treated with Aureomycin and/or whole blood transfusion. A complete report is in the process of preparation.

Methods: Following a control observation period to irradiation, the dogs were separated into 4 equal groups of 24. One group received no medication whatever and served as controls. One group received one 250 mg. capsule (approximately 50 mg./kg.) of Aureomycin twice daily at 12 hour intervals starting 24 hours after irradiation, and continued for 28 days post-irradiation. Another group received Aureomycin in an identical fashion, and in addition received typed, cross-matched, compatible whole blood whenever the individual dog's hematocrit fell below 80% of the pre-radiation value. The remaining group of 24 dogs received no Aureomycin but were given whole blood on a "demand" basis as outlined above. The blood had been drawn from the donor dog into standard ACD solution at least 48 hours before administration, stored at 4° C and was administered in 125 cc. amounts into the hind leg vein over a period of 10 to 12 minutes.

Results:

1. Mortality

The mortality rate and final mortality in each group is summarized in the table on the following page.

TOTAL DOGS DEAD

| Days Post-Radiation | 10 | | 15 | | 20 | | 25 | | Final Mortality (40 days) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| Controls | 7 | 29 | 18 | 75 | 20 | 83 | 21 | 88 | 21 | 88 |
| Aureomycin | 1 | 4 | 13 | 54 | 18 | 75 | 21 | 88 | 22 | 92 |
| Aureomycin + Blood | 1 | 4 | 9 | 38 | 17 | 71 | 18 | 75 | 20 | 83 |
| Blood | 4 | 16 | 16 | 67 | 21 | 88 | 22 | 92 | 22 | 92 |

Note: Six dogs in the blood group and one dog in the blood and Aureomycin group did not receive a blood transfusion because death occurred before the hematocrit fell below 80% of normal.

2. Hematology

The leucocyte count fell rapidly, post-irradiation, in an exponential manner and at the identical rate in each experimental group. The platelet count decreased in a more linear fashion and no significant difference could be detected among the groups. The hematocrit decreased in a direct linear fashion with all the groups paralleling each other until the 9th to 10th post-irradiation day when the average level had reached 80% of normal. By means of blood transfusions the average hematocrit in the two groups receiving transfusions was between 80 and 85% of the pre-radiation value during the entire post-irradiation period. In the control and Aureomycin groups the hematocrit continued to decrease until it had reached an average level in the surviving dogs of approximately 55% by the 18th post-irradiation day.

3. Blood Transfusions

All the dogs that were to receive blood transfusions were typed for the presence or absence of the dog "A" Factor. The A positive dogs received A positive or A negative blood, whereas the A negative dogs were given only A negative blood. In addition, a minor and major cross-match was done immediately before each transfusion. A donor colony of 25 large dogs was maintained. Only dogs having a

hematocrit of 42 or above were used for donors. A total of 140 transfusions of 125 cc. each were administered to 40 dogs during the course of the experiment - an average of 3.5 transfusions or 438 cc. per dog (approximately 50% of the estimated normal blood volume). The dogs treated with Aureomycin and blood received on the average more transfusions per dog - 4.1 transfusions or 513 cc. per dog - than those receiving blood alone. The dogs receiving blood alone were given 2.8 transfusions or 350 cc. per dog on the average. This difference may be explained by the fact that more of the Aureomycin and blood treated dogs lived into the post-irradiation period of severe anemia and bleeding. Six dogs in the blood treated group and one dog in the blood and Aureomycin treated group died before their hematocrit dropped below 80% of the pre-radiation value and therefore received no transfusion. One Aureomycin and blood treated dog that survived had no hematocrit level below 80% during the post-irradiation period and was not transfused. No other surviving dogs maintained their hematocrits above 80% of normal pre-irradiation value. The following table shows the number of dogs living in each group and the number of transfusions given on each of the post-irradiation days.

| Days Post-Irradiation | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 20+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blood Only | | | | | | | | | | | | | | | |
| No. Dogs Living | 23 | 22 | 20 | 20 | 16 | 14 | 12 | 9 | 8 | 6 | 5 | 3 | 3 | 3 | 2 |
| No. Transfusions | 5 | 2 | 1 | 5 | 7 | 7 | 6 | 8 | 5 | 5 | 0 | 0 | 0 | 0 | 0 |
| Blood + Aureo | | | | | | | | | | | | | | | |
| No. Dogs Living | 24 | 24 | 24 | 23 | 21 | 19 | 18 | 16 | 15 | 13 | 13 | 10 | 8 | 7 | 6 |
| No. Transfusions | 0 | 4 | 2 | 4 | 7 | 4 | 7 | 11 | 14 | 7 | 10 | 2 | 2 | 4 | 12 |

No hemolytic, allergic, or other transfusion reactions were observed in any of the transfused dogs.

4. Gross Pathology

A complete autopsy was performed on each dog that died to determine the principal cause of death. Sections were taken for microscopic examination and the results of these examinations will be reported later. The following table shows the percentage of the dogs that died in each group which exhibited the lesions listed.

| | Controls | Aureomycin | Aureomycin + Blood | Blood Alone |
|---|---|---|---|---|
| Severe Pulmonary Hemorrhage | 29% | 18% | 40% | 50% |
| Severe Gastro-Intest. Hemorrhage | 43% | 32% | 50% | 50% |
| Severe Myocardial Hemorrhage | 5% | 23% | 0% | 18% |
| Severe Urinary Bladder Hemorrhage | 10% | 14% | 5% | 9% |
| Duodenal Ulceration | 38% | 45% | 15% | 23% |

Some of the variation in the incidence of the lesions listed above may be due to differences in time post-irradiation when the dogs in the separate groups died. However, the increased incidence of severe pulmonary hemorrhage in the groups receiving blood transfusions appears to be significant. A more detailed description of the gross pathology will be given in a later report.

5. Aureomycin Blood Levels

Aureomycin blood levels were done on a few dogs post-irradiation at one hour intervals following the administration of the single 250 mg. capsule of Aureomycin. The results of these studies show that the maximum blood level achieved was 8 mcg./cc. three hours after the administration of the drug, and that the level was above 2 mcg./cc for only a maximum of 4 hours. Beyond the 10th day post-irradiation and in dogs appearing severely ill, the level did not exceed 2.5 mcg./cc. and was above 2 mcg/cc. for only 2 hours. The dogs which showed the low blood levels had no evidence of gastro intestinal hemorrhage.

# A CADMIUM SULFIDE CRYSTAL DETECTOR MOUNTED IN HYPODERMIC NEEDLE FOR IN VIVO RADIATION STUDIES

By
Samuel G. Zizzo

This report summarizes progress in the construction of CdS-type crystal radiation detectors with hypodermic needle mounting suitable for in vivo use in animal experiments. The use of one of these units for circulation time measurements in rats is described.

A limited number of CdS crystals have been grown with some difficulty at this laboratory by the method of crystallization from the gaseous phase as briefly described by Frerichs (1). Due to the difficulties encountered, the technique for growing crystals is being further investigated.

Both flat and cylindrical crystals have been mounted. A flat crystal mount is shown in figures 1 and 2. A mounting method for hollow cylindrical crystals is shown in figure 3. Only the cylindrical mounting will be further described here because its greater sensitivity makes it the more promising.

For the hollow cylindrical crystal an internal electrode was made by allowing a thin aquadag suspension to be drawn internally by capillary action. The end of the wire was then inserted and the process repeated. The outer surface of the crystal was then aluminized by evaporation and a wire lead attached with aquadag. The leads were insulated by concentric glass tubing. The crystal had an outside diameter of 0.5 mm. and internal diameters at the ends of 0.2 and 0.3 mm. The crystal was supported on leads mounted with wax at the end of a hypodermic needle as shown in figure 3 (page 60). A glass envelope covered the crystal assembly. A potential across the crystal of 60 volts caused continual discharge.

For counting, a slightly lower voltage was used.

For the detection of beta particles a preamplifier and linear amplifier, Atomic Instrument Model No. 205 and 204-C respectively were used. One crystal electrode was capacity coupled to the preamplifier, the other grounded. Microphonics originating at the crystal-preamplifier stage have not yet been reduced to an entirely satisfactory level. A maximum pulse height of 700 μ volts was measured at the preamplifier output when the detector was immersed in a $P^{32}$-containing solution; in an $I^{131}$ solution the maximum pulse was 230 μ volts. Under the best conditions the amplifier noise level was 10 μ volts.

A $P^{32}$ solution of 0.15 μc/cc gave a counting rate of 200 counts per minute, 50 times the background rate of 4 counts per minute.

Biological Application

An experiment was conducted on 400 gram rats which were anesthetized with sodium barbitol injected intraperitoneally. Blood circulation time studies were made by injecting radioactive-labeled $H_3PO_4$ into the left femoral vein and detecting the radioactivity of the blood as it flowed through the right femoral vein. Incisions were made along each leg thereby exposing the femoral vessels, such that the cylindrical counter could be placed along side the one vein and the radioactive dose injected into the other. Voltage pulses produced by betas after the injection of the radioactive dose were amplified, fed into a counting rate circuit, and recorded by a graphic ammeter. A reproducible record is given in Figure 4 (page 60). The time of injection has been labeled by T = 0 and peaks labeled 1, 2, and 3 represent detection of the administered dose of 0.1 cc of 2 millicuries $P^{32}$ per cc as it was diluted by the blood. In repeating this experiment

ten times three definite peaks were observed after $P^{32}$ was injected and the average circulation time between peaks was found to be 3.5 seconds.

In three cases continuous records were taken for five to nine minutes after the $P^{32}$ injection. From the data obtained, it was found that the average $P^{32}$ concentration in the blood decreased 13 per cent per minute after the first half minute.

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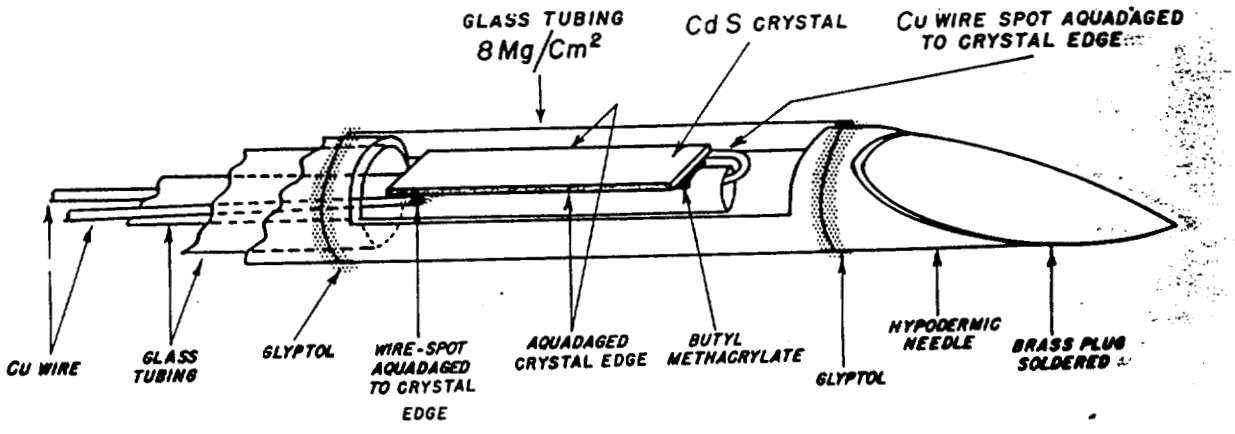



Fig.1 — A Mounted Flat CdS Crystal.

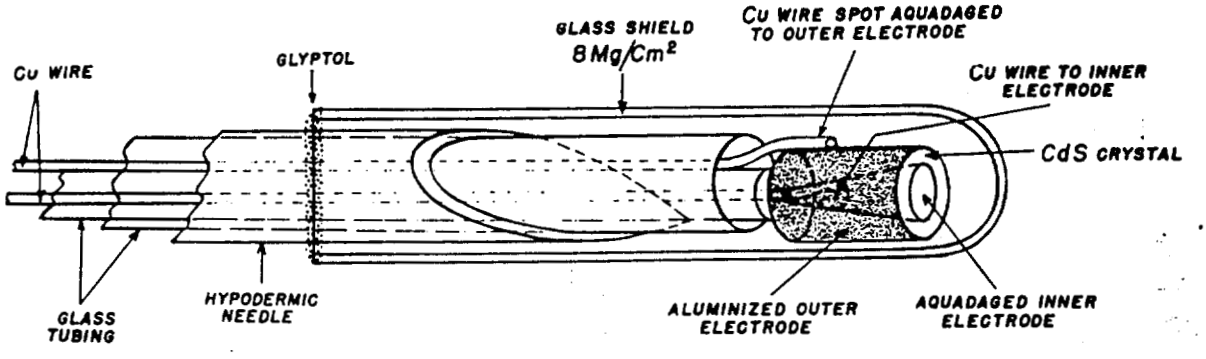



Fig.2 — A Mounted Hollow Cylindrical CdS Crystal.

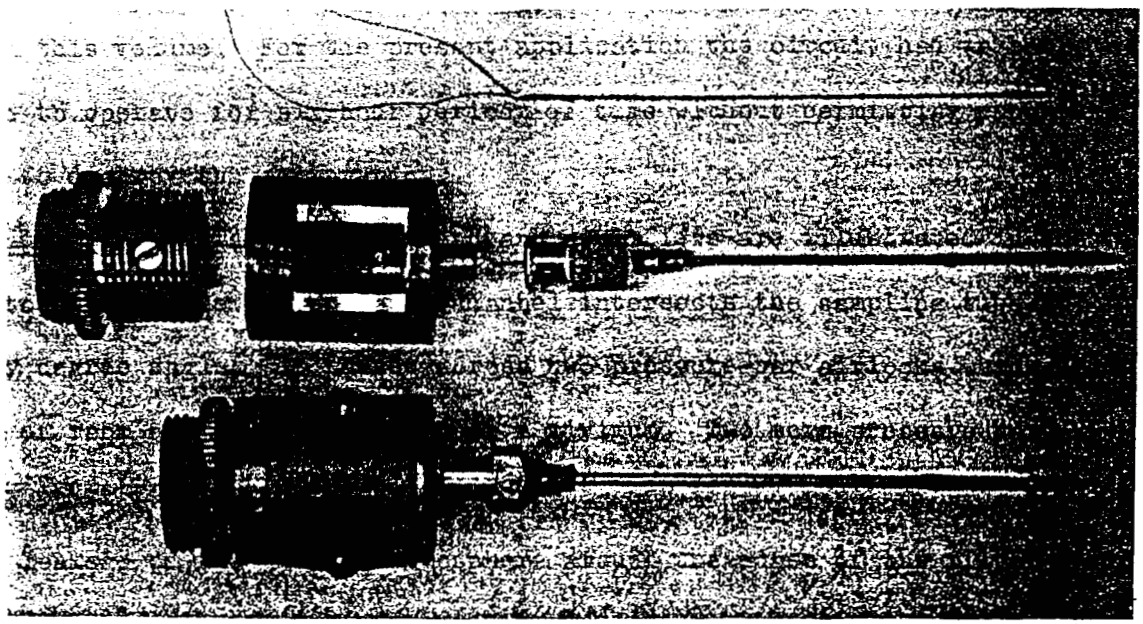


Fig. 3— Detailed Detector Assembly.

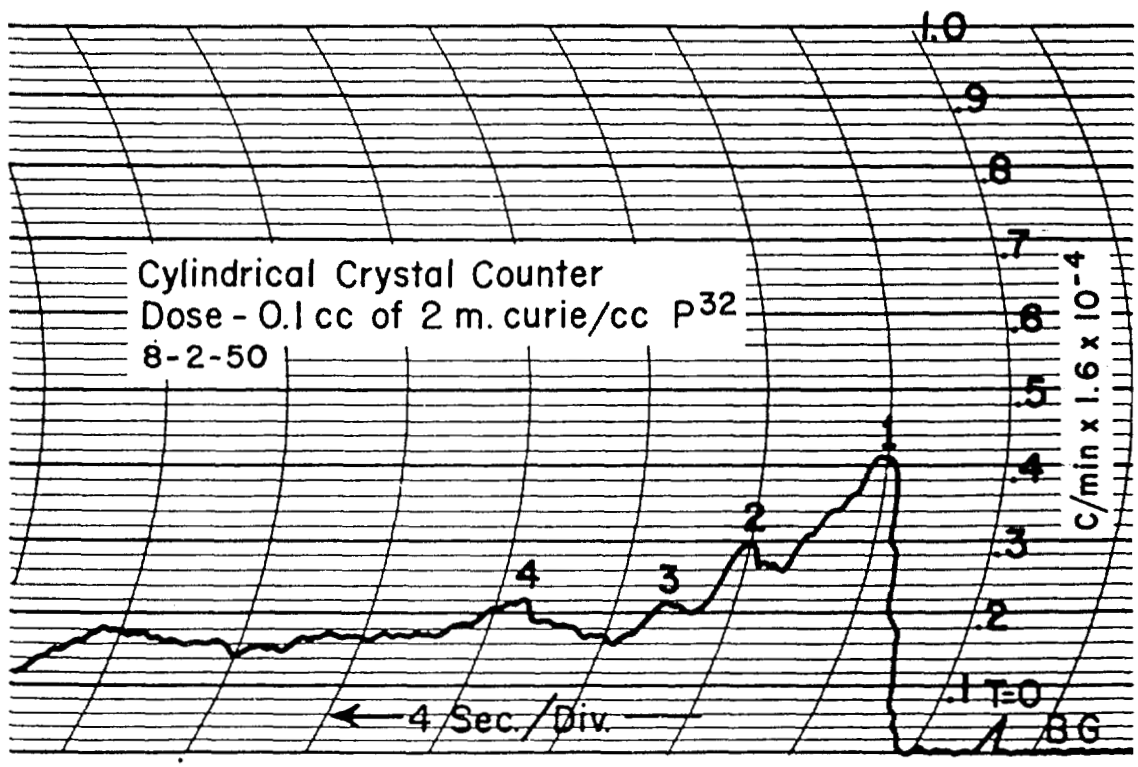



Fig. 4— A Circulation Time Record.

UR 01827

# AN INSTRUMENT FOR CONTINUOUS SAMPLING AND RECORDING OF AIRBORNE DUST CONCENTRATION

by
George A. Simon and Sidney Laskin

Introduction: A next step in animal inhalation exposure chamber technique was foreseen to be the development of an instrument which would continuously withdraw a sample of atmosphere from the inhalation chamber, and measure and record the concentration of airborne particulate matter contained therein. Ultimately the instrument could so control the chamber atmosphere that the concentration of particulate matter would automatically be maintained at some pre-determined level. Such a device, if made available, was considered for use on the proposed chronic $UO_2$ inhalation study to be begun in the near future. It is most desireable in low level studies to be aware of any temporary upward surge in concentration of the aerosol inhaled by the animals, because such a surge when working near the toxicity threshhold values, could easily equal the entire amount of aerosol normally inhaled by the animals over a period of hours. Such surges or concentration peaks are easily missed by any non-continuous sampling method. With this in mind, the instrument described in this report was designed and built.

Brief: A continuous strip of filter paper, one inch wide is pulled at a constant rate through an airlock and across a sampling port, where the aerosol is filtered from a constant flow of exposure chamber atmosphere. The paper tape is then brought out of the sampling head through another airlock. A 931A photomultiplier tube scans the blank filter paper before the aerosol deposit is laid down, and a second photomultiplier tube scans the strip after the deposit is laid down. Each photomultiplier circuit is then permitted to trace a separate curve on a strip chart recorder.

The base curve thus established effectively divorces all variations in filter paper density from actual variation in density of the aerosol deposit. The strip chart recorder is calibrated directly in units of milligrams of aerosol deposit per cubic meter of atmosphere sampled. A continuous, permanent record of aerosol concentration in the chamber is thus obtained. In addition, the filter paper strip containing the deposit is reeled up and saved for future check analyses by chemical methods.

Primary Considerations. Only three instruments were found in the literature which make use of a strip to collect airborne matter, and whose record is continuous. The first is a smoke recorder used by the Socony-Vacuum laboratories (1) to study oil burner operation. Smudges of smoke appear to be filtered by a moving strip of filter paper. There are no details of the recorders' design, and although "photoelectric facilities" are mentioned, it seems that the filter paper tapes are examined visually. The second instrument is one developed by Willis G. Hazard (2). The airborne matter is impinged on a moving strip of film, which is coated with a sticky substance just prior to the impingement. A moving optical wedge supplies compensation for variations in the instrument. The third is a sampling instrument used by the Brookhaven National Laboratories (3). In this instrument a filter paper strip is exposed to the atmosphere. As it passes across a sampling head connected to a vacuum pump, atmospheric dust is filtered out, and deposited on the strip. The paper strip is then scanned by a G-M tube, and the concentration of any radioactive material appearing on it is recorded.

There are other instruments where light is beamed directly through the atmosphere to be analyzed. The amount of absorption or scattering is then measured photoelectrically. None of the instruments mentioned here were

satisfactory for the present application.

Since some feed mechanisms permit a variable concentration which tends to vary about a mean value, too close a scrutiny of variations is not necessary so long as the total mass of material to which the animals are exposed is known. Hence, a sample which is drawn for a period of from one to five minutes, and representing an average of the aerosol concentration during this period, is felt to be acceptable and undesirable. By passing a filter paper strip across a sampling port at a constant predetermined speed, we obtain a strip sample each increment of which represents an average sample over a period of time which is a function of paper speed, port area, and shape of the port.

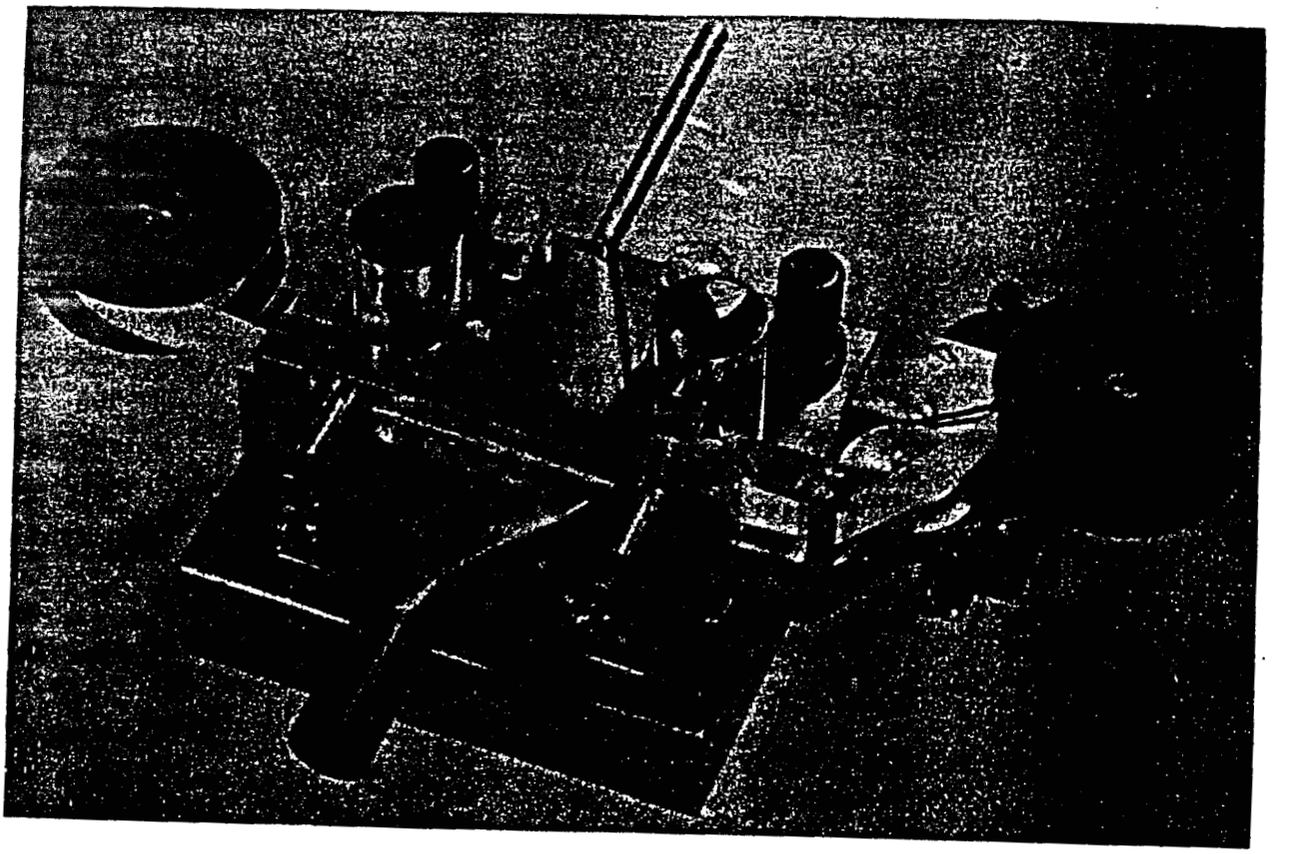
The system must be isolated from atmospheric pressure. Exposure chambers are operated at a slight negative pressure as a safety precaution. This negative pressure must be maintained up to the sampling port where an even greater drop in pressure occurs across the filter paper due to the vacuum pump which draws the sample through the filter. The only alternative would be to place the entire sampling head within the exposure chamber and operate in much the same way as the Brookhaven instrument. This, however, entails loss in flexibility of use of the sampling device, and presents problems in corrosion prevention and decontamination. Furthermore, the filter paper strip must still be introduced into the chamber and removed from it after the sample has been deposited.

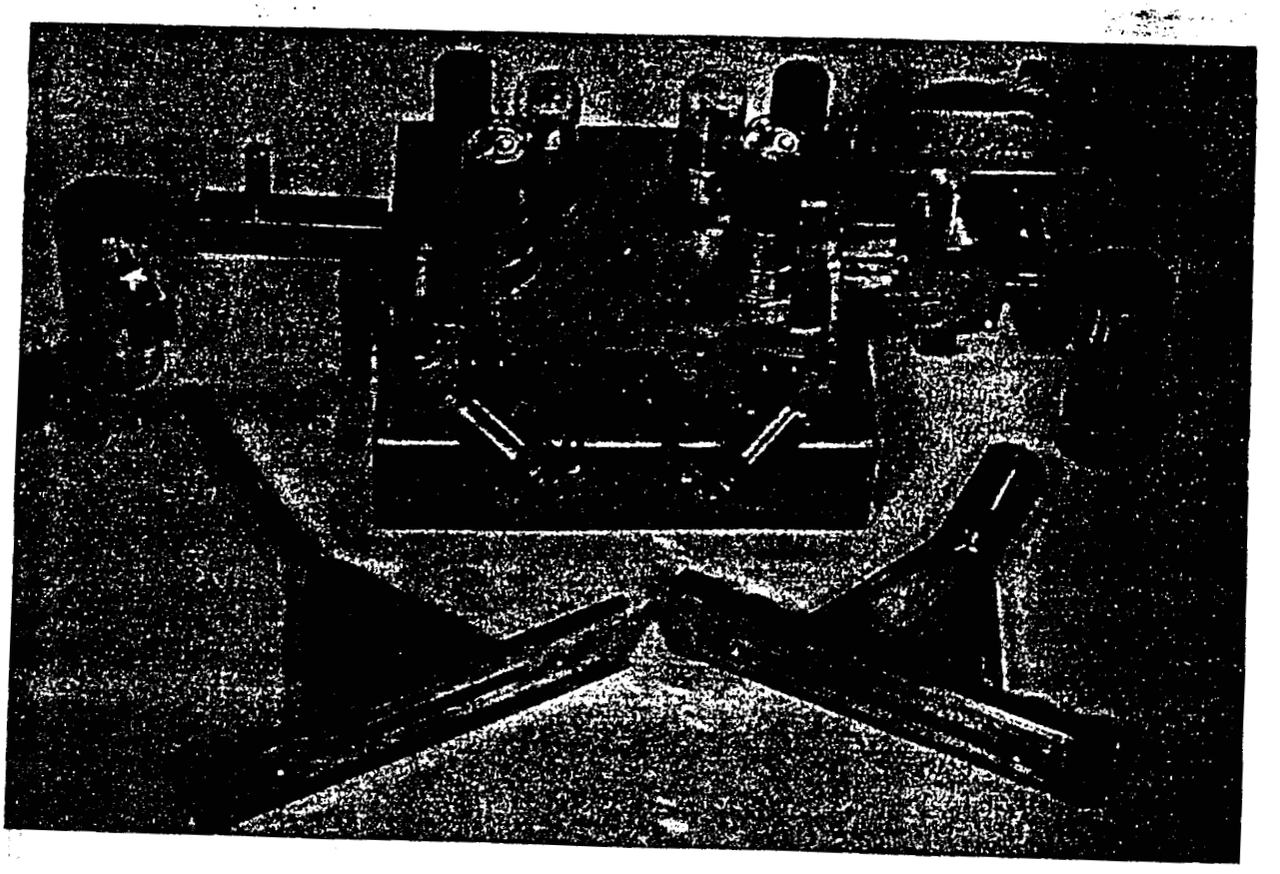
A photoelectric scanning method appeared to be the only practicable method of continuously measuring and recording the concentration of aerosol appearing on the filter tape. In addition to this, once variation in the density of the deposit on the tape has been transformed into variation of electrical current, not only are recording problems simplified, but ultimate control of the chamber through servo mechanisms is within reach.

Such a photoelectric circuit was evolved and the details appear elsewhere in this volume. For the present application the circuit had to be designed to operate for six hour periods of time without permitting phototube fatigue to destroy the calibration.

Instrument: The sampling head and amplifiers are illustrated in the two photographs. The filter paper channel intersects the sampling tube at a ninety degree angle. It passes across two pressure-bar airlocks which hold leakage of room air into the device at a minimum. Two more pressure bars keep the paper firmly pressed against one surface of the paper channel and prevent leakage of the chamber atmosphere around the edges of the filter-strip. The grid-like suction port is designed to support the paper and prevent it from being torn. The sampling port is 1/4 inch wide and has an area of $\pi/4$ square inches. The double fan-shaped sampling tube is designed to conduct chamber atmosphere to the filter strip with no compression or rarefaction. A cross section taken at any point along the tube displays a constant cross sectional area of $\pi/4$ square inches. In this manner impaction effects are held to a minimum and little of the material is found on the tube walls. The two 931A photomultiplier tubes are covered by light tight cans which lock into slots on the sampling head. The two argon light sources are also covered by light tight cylinders. Circular windows cut from glass microscope cover slips provide a light path from the argon lamps to the photomultiplier tubes. These are sealed to the sampling head in order to prevent room air from leaking into the mechanism through the light channels.

The filter paper strip is pulled across the sampling port at a constant speed by means of knurled rollers driven by a constant speed motor. This same motor drives the take-up reel upon which the exposed paper is





stored. At present, a speed of two inches per minute is used. This corresponds to a sampling time of approximately 1 1/2 minutes. This speed of travel of the filter paper is easily varied in order to best sample more dense or less dense concentrations.

Filter Paper: Whatman filter paper No. 4 is used in this instrument. It is obtained in rools 1 1/8 inches wide and approximately 600 feet long. This is subsequently cut down to a width of 1 inch before use. It is essential that the width of the paper be constant in order to prevent excessive leakage at the entry and exit ports. By performing the final paper cutting operation in the laboratory this constancy of width is assured.

It was desired that the paper to be used be as efficient as possible, be obtainable in continuous strips, and have a sufficiently low pressure drop across it so that existing pumps and manometers could be used. In addition to this the paper had to have a cellulose base as opposed to the series of asbestos base papers, so that chemical analyses could be performed when desired. The paper also had to possess sufficient tensile strength to withstand the pulling operation.

Whatman No. 4 filter paper was chosen as the result of a series of tests performed on a variety of papers; this filter paper closely approaches the ideal for the present application.

Since detailed collection efficiency data exists for Whatman No. 41 filter paper (4), a comparison was made between the two papers. Under identical collection conditions, the No. 4 paper seemed to display a slightly higher collection efficiency. A series of ten samples were drawn from a chamber containing a carnotite dust atmosphere. Alternate samples were taken on No. 41 papers. The five Whatman No. 41 papers collected an average of 101.2 milligrams

of dust per cubic meter of atmosphere sampled. The five Whatman No. 4 samples collected an average of 103.6 milligrams of dust per cubic meter. It was known that under the sampling condition of 14 liters per minute, the No. 41 paper had a collecting efficiency of 96%. Hence the No. 4 paper displayed an efficiency as high as this or higher.

It is to be noted that the accuracy of sampling with this filter paper in continuous motion should be higher than when drawing a sample through a stationary filter. In the stationary filter, as the deposit builds up, the pores of the filter become filled and the volume of air sampled diminishes. The sampling rate is a constantly diminishing function of time and must be compensated for by having an operator continuously observe a flow meter and continuously open a valve in the vacuum line in such a manner as to maintain a constant flow rate. This must be done throughout the sampling period. In addition, as the filter accumulates dust, the pressure drop across it rises and both the spot velocity or air through the paper and its collection efficiency rise.

In the continuous sample method, equilibrium is set up after the first few minutes, and flow rate, pressure drop, and efficiency remain constant. There is no need manually to maintain a constant flow rate.

A three foot length was removed from the end of each of twenty rolls of filter paper in order to determine its optical density and the variation in density to be expected. A total of 100 densitometer readings were taken by means of an Ansco-Sweet densitometer. The average density was found to be 1.046, and the standard deviation from this mean is 0.014.

Discussion and Conclusions: At present the amplified output of the photomultiplier tubes can be fed to separate pens on a dual pen strip chart

recorder. An alternative is to feed the outputs in an alternating manner to a single pen recorder by means of a simple electronic switch. In this case, both of the curves will appear although not simultaneously. There is a certain loss of information by using this second method of recording. The original purpose of the base curve of the blank filter paper was to refer any unusual peak or dip in the milligrams per cubic meter curve to a base line. If the peak or dip could be explained by an unusual density or opacity of the filter paper then the unusual concentration curve would be known to be false. The distance between the two curves gives a true measure of the density of the dust deposit. This alternate method of recording permits only a limited amount of back-checking. It must be pointed out, however, that in view of the rather uniform density of the filter paper as noted above, little trouble is expected from this source.

Servo control of the chamber feed mechanism depends on the mechanism to be used. It could be a simple control of the speed of the feed motor in the case of a Wright feed. In the case of an aerosol produced as liquid droplets, control of the amount of air used to move the aerosol from its production and mixing chamber into the exposure chamber seems to be indicated.

In any case, the first and major problem of converting the variation in concentration of the airborne matter into an electrical change has been accomplished. An electrical current whose magnitude varies as the concentration varies is available.

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2. Hazard, W. G., J. Franklin Inst., 217, 571 (1934).

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4. Presented at the American Industrial Hygiene meetings in 1949.

# PHOTOMULTIPLIER CIRCUIT FOR AIRBORNE DUST SAMPLING INSTRUMENT

by
George A. Simon

Introduction: The following circuit was devised for the filter paper strip, sampling and recording instrument described elsewhere in this volume. It consists of two photomultiplier tubes, each of which feeds into a separate amplifier. Each output is then fed to a pen of a continuous strip chart recorder. One curve so traced, represents the optical density of the filter paper before an aerosol deposit is laid down. The other curve is traced through the output of the circuit which scans the filter paper after the aerosol is laid down. The difference between the two curves then represents density of the aerosol deposit, divorced from variations in the filter paper itself.

A fundamental aim was to develop as simple and foolproof a circuit as possible. A minimum of calibration and adjustment was desired. Since the device is to be used for six hour periods of time every work-day for approximately five years, a constant dependable operation was desired with a minimum of calibration.

Photomultiplier Tubes: 931A photomultiplier tubes were selected because of the extremely high amplification ability they possess. By using these tubes all subsequent amplifier circuitry is held to a minimum. Fatigue is the major problem introduced when using such tubes. During operation, if too great a current is drawn through the tubes, the photo-sensitive surfaces become desensitized, and calibration is destroyed. The possibility of fatigue becomes particularly important in the present application, because of the comparatively long, six hour, periods of operation.

This problem was solved simply by decreasing the intensity of the light input and the voltage on the dyhodes, to the point where the current through the photomultiplier tubes was held to one microampere or less;* under these conditions, an eight hour period of operation displays no tube fatigue. It should be pointed out here, that this solution of the problem brings into prominence yet another factor, namely variations in the photomultiplier output due to dark current. In the present application, this problem is not important, since the inertia of the recording pen acts as a damper for these variations.

Light Source: The light sources used are General Electric AR-3 argon lamps. These possess a strong emission band centered about a wavelength of 4000 angstrom units. The 931A photomultiplier tubes likewise possess peak sensitivity at a wavelength of 4000 angstrom units. Hence, the lamp and tube are well matched for optimum performance. In addition, the argon lamps when operated on alternating current, become completely dark 120 times each second and thus present a modulated light source. This is not true for filament lamps. The advantage presented here is that amplifier circuits following the phototubes need not be of the costly, more troublesome, and less flexible direct current type.

The argon lamps have a very low heat output. On a 100 hour test, the temperature of the close fitting metal shield around the lamp never rose more than three degrees above room temperature. This is most important, since the photomultiplier tubes are sensitive to temperature change. If a filament type lamp were used, ventilation and cooling would become problems. A voltage regulation transformer was found to provide sufficient regulation.

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* The voltage per stage is approximately 70 volts.

The luminous output of the lamps does not change appreciably over a six hour period of operation.

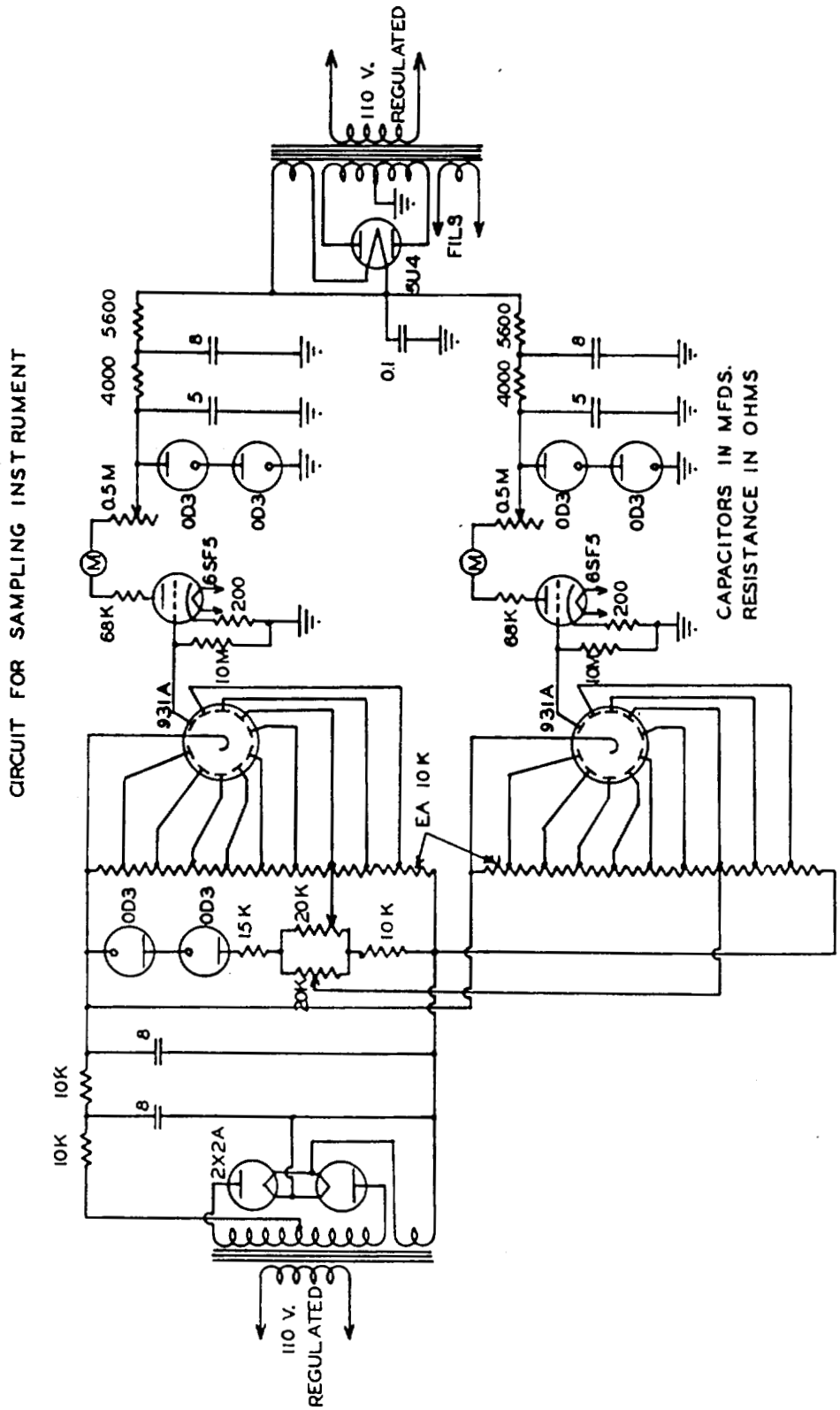
Amplifying Circuit and Recorder: This circuit is a simple application of the 6SF5. This tube was chosen because of low grid current and a one milliampere plate current. The low grid current insures low drain on the multiplier tube, and the plate current permits a recording instrument with a one milliampere movement to be placed in series in the plate circuit.

At present the tube is coupled directly to the photomultiplier as a simple D.C. amplifier. The recorder movement is sufficiently insensitive to the periodic variations in plate current flow, induced by the modulated light source, as to trace a smooth curve.

An Esterline Angus, model A.W. recorder is used.

Power Supplies and Regulation: Regulation of voltages is important and can be carried to great lengths. It was thought desireable, however, to keep such regulation as simple as possible within the requirements of the circuit. The use of a voltage regulator transformer and the VR tubes as shown, was found to be adequate.

The high and low voltage power supplies are conventional.



## EDUCATIONAL PROGRAM

by

J. N. Stannard

During the summer months the A.E.C. Radiological Physics Fellows completed a ten week practical course with the Health Physics Division at the Brookhaven National Laboratory. Our other graduate students spent this period in continuing research on their thesis problems.

On September 20 a new group of twenty A.E.C. Fellows in Radiological Physics registered, three A.E.C. Fellows in Industrial Medicine began their work and several new part-time research associates began work in various divisions of the Project. These latter are registered as graduate students in Biophysics, Biochemistry, Pharmacology or Physiology during the academic year. The total number of graduate students registered in the Department or carrying out their research under its auspices now numbers 81.

A final report summarizing our graduate training activities during the academic year 1950-51 is being prepared (UR-188).

## TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

July 1, 1951 thru September 30, 1951

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|---|---|---|---|
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| UR-167 | A Chemical Dosimeter for Ionizing Radiations (UNCLASSIFIED) Issued: September 24, 1951 | Kanwisher | Health and Biology |
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| UR-172 | The Surface Chemistry of Bone IV. Further Data on Recrystallization (UNCLASSIFIED) Issued: July 23, 1951 | Neuman Mulryan | Health and Biology |
| UR-174 | Quarterly Technical Report April 1, 1951 thru June 30, 1951 (UNCLASSIFIED) Issued: August 21, 1951 | Blair | Health and Biology |
| UR-176 | The Surface Chemistry of Bone V. The Ion-Binding Properties of Cartilage (UNCLASSIFIED) Issued: July 23, 1951 | Boyd Neuman | Health and Biology |
| UR-177 | Acute Toxicity of Inhaled Beryllium IV. Studies of Beryllium Fluoride at Concentration of 10 and 1 mg/$m^3$ (UNCLASSIFIED) Issued: August 2, 1951 | Hall et al | Health and Biology |
| UR-178 | Effects of Irradiation on Embryonic Development. I. X-rays on the 10th Day of Gestation in the Rat (UNCLASSIFIED) Issued: August 21, 1951 | Wilson Carr | Health and Biology |

| Report No. | Title | Authors | Subject Category |
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| UR-179 | Hemoglobin Synthesis in Bone Marrow and Spleen Homogenates as Affected by X-radiation (UNCLASSIFIED) Issued: August 21, 1951 | Richmond Altman Salomon | Health and Biology |
| UR-180 | Use of Commercially Available Portable Survey Meters for Emergency Fission Product Monitoring of Water Supplies (UNCLASSIFIED) Issued: August 23, 1951 | Hursh Zizzo Dahl | Health and Biology |
| UR-181 | Some Ion-Exchange Studies of the Polymerization of Beryllium (UNCLASSIFIED) Issued: September 17, 1951 | Feldman Havill | Chemistry-General |