ARM2.950150.001

ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 195
28 June 1955

THE USE OF ULTRAFRACTIONATED RADIATION IN THE STUDY OF RADIATION-INDUCED REACTIONS*

*Subtask under Biological and Medical Aspects of Ionizing Radiation, AMRL Project No. 6-59-08-014, Subtask, Effects of ionizing Radiation.

RESEARCH AND DEVELOPMENT DIVISION
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

REPORT NO. 195

THE USE OF ULTRAFRACTIONATED RADIATION IN THE STUDY OF RADIATION-INDUCED REACTIONS*

by

J. G. Kereiakes, R. H. Hodgson and A. T. Krebs

from

Radiobiology Department
ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
28 June 1955

*Subtask under Biological and Medical Aspects of Ionizing Radiation, AMRL Project No. 6-59-08-014, Subtask, Effects of Ionizing Radiation.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

Report No. 195
Project No. 6-59-08-014
Subtask AMRL S-1
MEDEA

ABSTRACT

THE USE OF ULTRAFRACTIONATED RADIATION IN THE STUDY OF RADIATION-INDUCED REACTIONS

OBJECT

To study the effects produced by ultrafractionated radiation and to relate the observed minima in cell damage to the quantitative and/or specific importance of radiation-induced reactions.

RESULTS

Ultrafractionated ultraviolet irradiation did not damage yeast cells to the same extent as continuous irradiation. Definite minima in the per cent of yeast cells damaged were found for single exposure times of 0.024 sec and 0.0025 sec corresponding to radiation pulses of 13.8 per sec and 133.3 per sec, respectively.

CONCLUSIONS

The observed minima in per cent of yeast cells damaged that resulted from ultrafractionated ultraviolet irradiation may be related to the time existence of some radiation induced reactions. The magnitude of these observed minima may be of value in considering the quantitative and/or specific importance of particular reactions in the overall biological effect.

RECOMMENDATIONS

Because of the fundamental value of such studies in understanding the mechanisms by which irradiation effects are produced in chemical and biological systems, the studies should be extended to include various types and qualities of radiations, various chemical and biological systems, various ratios of exposure time to pause time, a range of dose rates, different extremes of exposure temperatures and total body x-irradiation (ultrafractionated).

Washington National Record Center
Office of the Army Surgeon General
Record Group 112

Submitted 19 April 1955 by:

J. G. Kereiakes, Ph. D., Radiobiologist
R. H. Hodgson, PFC
A. T. Krebs, Ph. D., Head, Radiobiology Department

APPROVED: Ray G. Daggs
RAY G. DAGGS
Director of Research

APPROVED: William W. Cox
WILLIAM W. COX
Lt Colonel, MC
Commanding

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
FRC A 1094 (541)

THE USE OF ULTRAFRACTIONATED RADIATION IN THE STUDY OF RADIATION-INDUCED REACTIONS

I. INTRODUCTION

The influence of ultrafractionated x- and beta-radiation on various biological systems, Drosophila pupae and eggs, and B. coli, has been investigated by Witte and co-workers (1-3) and by Hofmann and Muller (4). Differences were observed between ultrafractionated radiation and continuous radiation of the same total dose. In addition, definite minima were observed in the number of cells damaged or the per cent deaths that occurred for certain frequencies (single radiation exposure times) of the ultrafractionated radiation. No definite explanation has been given for these observed phenomena.

This report presents studies on ultrafractionated ultraviolet irradiation of yeast cells with an attempt to relate the minima in cell damage to the time existence of the radiation-induced reactions.

II. EXPERIMENTAL

The experimental arrangement is shown in Figure 1. It consists of a high intensity ultraviolet radiation source (a Hanovia Utility Model Quartz Lamp) operated at 143 volts DC, 1.3 amps, no filter, and source-specimen distance of 7 cm.

The ultrafractionation of the radiation was produced by a strobic disc made of ST-6 aluminum*, 1/8 inch thick, 8 1/2 inches in diameter, circular apertures 7/8 inches in diameter, with aperture centers 3 3/8 inches from disc center. The ratio of the exposure time to pause time for the disc was 1:2 with 8 single exposures per disc revolution. The disc was driven by a Bodine Electric Motor, type NSE-12, 1/7 horsepower. A constant voltage transformer compensated line voltage changes. A powerstat provided regulation of motor speeds up to 2100 rpm. The disc speed was measured with a strobotac.

The test specimen used in these studies was a strain of Saccharomyces cerevisiae**. Stock cultures of yeast were grown for

*Supplied by the Reynolds Aluminum Company, Louisville, Kentucky.

**Culture supplied by Joseph E. Seagram and Sons, Inc., Louisville, Kentucky and is listed as No. 4125 in the American Type Culture Collection Catalog.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
22 A 1094 (841)

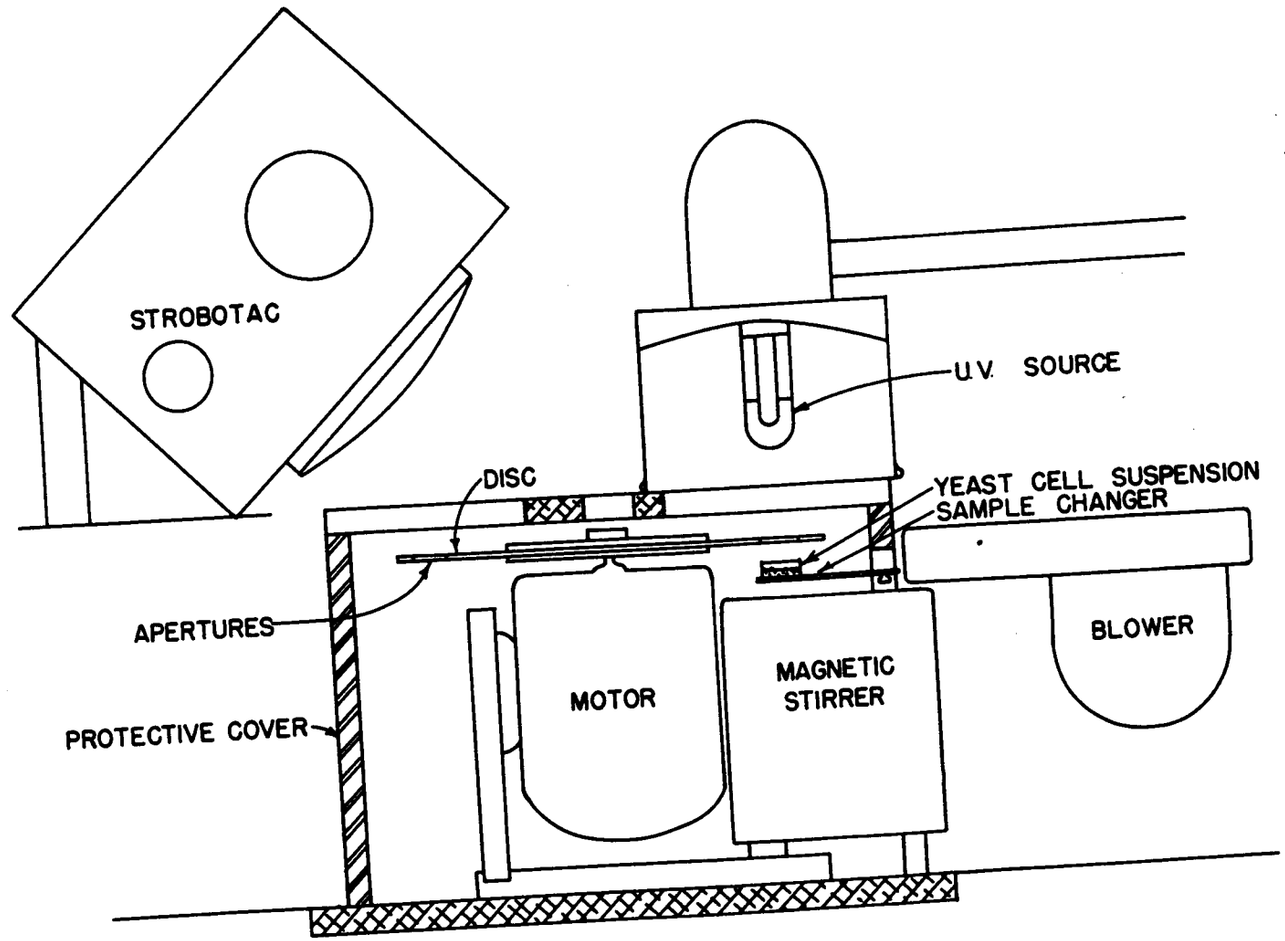

FIG. 1. EXPERIMENTAL ARRANGEMENT

a period of one to two weeks on slants having the following composition: 1.0% dextrose, 0.5% yeast extract, 0.5% peptone and 2.0% agar. After incubation at room temperature for 36-48 hours, the fresh slants were stored at 5°C. Cells washed from a stored slant into Sorenson's phosphate buffer at pH 5.4 served as inoculum for the liquid cultures to be used in the experiment. By suitable dilution the inoculum was brought to a standard optical density of 0.60-0.67 (compared to 0.00 for distilled water) as read on a Coleman Spectrophotometer (600 λ). Three 20 ml liquid cultures (media composition: 5% dextrose, 0.5% yeast extract and 0.5% peptone) were inoculated with 0.5 ml of cell suspension and then incubated without shaking at 30°C for 16 hours. After incubation the pooled cultures were centrifuged at 200 g for 5 minutes. The supernate was discarded and the residue resuspended in an equivalent volume of Sorenson's phosphate buffer at pH 5.4. After centrifuging and resuspending twice more, the optical density of the suspension was brought to a standard value between 0.60 and 0.67.

One ml aliquots of the cell suspension contained in glass planchets were used for the radiations. The suspension was magnetically stirred during the irradiation to allow a replacement of the top layer of yeast cells and provide a more homogeneous dose to the suspension. A blower served to reduce ozone effects and temperature changes. A one-minute continuous irradiation under the given conditions provided a suitable percentage of damaged cells, as indicated by the uptake of Eosin B (5, 6).

The ultrafractionated irradiation experiments were run for 3 minutes using different disc frequencies. Immediately after the irradiation a drop of cell suspension was placed on each counting area of a hemocytometer slide. A drop of 0.5% aqueous Eosin B was added to each and mixed with the cell suspension. The slide with cover slip was then allowed to stand in subdued light for 10 minutes (this accepted staining time followed by immediate cell counting was a result of preliminary trials). Two hundred cells were counted from each area. Stained and unstained cells were tabulated and percentage of stained cells calculated. The maximum variation encountered between the two 200 cell samples from the same slide was 3%.

III. RESULTS

Table 1 gives, as an example, data for one experiment (Exp. 2) covering single exposure times of 0.1516 sec to 0.0036 sec. These single exposure times correspond to 396 and 16560 single exposures,

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
FC A 1094 (541)

respectively, for the 3 minute total experiment time. The average damage of yeast cells for the one minute continuous radiation was 65.2%. The ultrafractionated irradiation damage is expressed in per cent of continuous irradiation damage. For the above range of single exposure times, the damage of yeast cells compared to the continuous irradiation damage ranged from 95.6% to a minimum of 39.9% at a single exposure time of 0.024 sec. An increase to 97.9% was found with further shortening of single exposure times to 0.0036 sec.

TABLE 1

EFFECTS OF ULTRAFRACTIONATED ULTRAVIOLET RADIATION ON YEAST CELL DAMAGE

Total Time for Ultrafractionated Radiations - 3 minutes

Disc Speed (rev/min)	Radiation Exposures per min.	Exposure Period msec/pulse	Single Exposure Time msec	Single Exposure log(msec)	Damaged Cells %(average)	Damaged Cells (% of continuous radiation damage)
	Staining and manipulation only (average)				1.0	
	1 minute continuous radiation damage (average)				65.2 (64-66)	100.0%
16.5	132.0	454.9	151.6	2.171	62.3	95.6
23.6	188.8	332.0	110.7	2.044	57.3	87.9
36.5	292.0	205.6	68.5	1.836	54.0	82.8
58.4	467.2	128.5	42.8	1.631	47.0	72.1
76.9	615.2	97.6	32.5	1.512	37.3	57.2
96.1	768.8	78.1	26.0	1.415	27.3	41.9
104.0	832.2	72.1	24.0	1.380	26.0	39.9
134.0	1072.2	55.9	18.6	1.270	39.5	60.6
163.0	1304.0	46.0	15.3	1.185	40.5	62.1
208.0	1664.0	36.1	12.0	1.079	41.0	62.9
254.0	2032.0	29.5	9.8	.991	51.0	78.2
305.0	2440.0	24.6	8.3	.914	51.8	79.5
350.0	2840.0	21.4	7.1	.851	53.3	81.8
374.0	2992.0	20.0	6.7	.826	55.3	84.8
430.0	3440.0	17.5	5.8	.763	57.3	87.9
520.0	4160.0	14.4	4.8	.681	59.0	90.5
595.0	4760.0	12.6	4.2	.623	62.5	95.9
690.0	5520.0	10.9	3.6	.556	63.8	97.9

Figure 2 shows the results of 5 individual experiments covering single exposure times from 0.1516 sec to 0.0012 sec. Starting at a single exposure time of 0.1107 sec (right side of figure), there was a decrease in the per cent damage with decreasing exposure time. A minimum in the per cent damage, averaging 36.7% of the continuous irradiation damage, was found at an average single exposure time of 0.024 sec. When the single exposure times were shortened further, the per cent damage increased to approximately 95% of the continuous irradiation value. A second minimum of less magnitude, averaging 77% of the continuous irradiation damage, occurred at an average single exposure time of 0.0025 sec. For further decrease of the single exposure time, the per cent damage increased again to approximately 96.5% of the continuous irradiation damage. The straight line at 100% indicates the damage with continuous irradiation (i.e. an average of 65.2% of total cells damaged - see Table 1).

Washington National Record Center
Office of the Army Surgeon General
Record Group 112

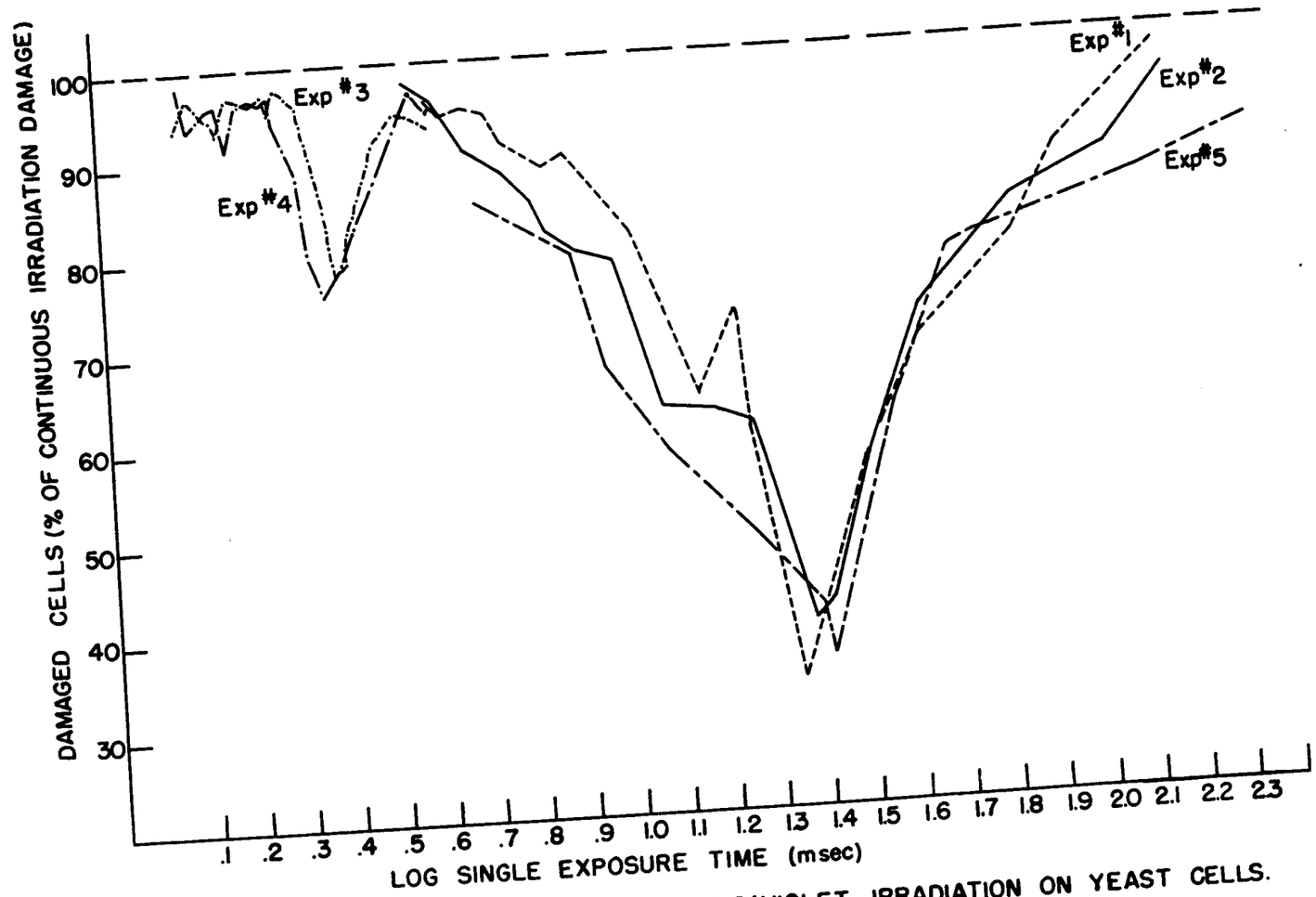

FIG.2. EFFECTS OF ULTRAFRACTIONATED ULTRAVIOLET IRRADIATION ON YEAST CELLS.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
SG A 1094 (841)

IV. DISCUSSION

The results of this study show that definite differences in the per cent of yeast cells damaged occurred when the cells were irradiated with ultrafractionated ultraviolet as compared to continuous radiation. Two definite minima in the per cent damage, 36.7% and 77.0% of the continuous irradiation damage, were observed for single exposure times of 0.024 sec and 0.0025 sec. The experimental curves compare favorably with the observations of Witte (1,2,3) and Hofmann and Muller (4) on the ultrafractionated x- and beta-irradiation of Drosophila pupae and eggs, and B. coli. Witte found that ultrafractionated x-irradiation of Drosophila pupae with a dose rate of 86.5 r per min and single exposure times of 0.01 sec resulted in a decrease in the number of pupae deaths. Witte used a disc ratio of exposure time to pause time of 1:2. A minimum in the curve, 47.3% of continuous irradiation deaths, occurred with single exposure times of 1/3000 sec. If the single exposure times were further shortened, the number of pupae deaths increased again. In more recent investigations, Witte used other ratios of single exposure time to pause time of 1:4 and 1:9 and other dose rates. The location of the minimum in number of pupae deaths was not influenced greatly in these studies, but a second smaller minimum was observed. Hofmann and Muller investigated the dependence of the biological effect (i.e., survival of Drosophila eggs) under conditions of ultrafractionated beta rays of radium. For exposure to pause ratios from 1:2.2 to 1:23 and exposure frequencies from 0.41 to 1166 per second, they observed a definite dependence of the location and magnitude of the minima in per cent deaths of eggs on the exposure to pause time ratio. Huber's investigations (7) with cathode rays, irradiation pulses of 10^{-6} sec, show different effects from those obtained for continuous irradiation with equal doses. Huber's extremely large doses (30-40,000 r in 10^{-6} sec) make it difficult to compare his observations with those obtained with the lower dose rates usually used.

At present, only suggestions have been made for the unexpected effects observed with ultrafractionated radiation as compared to continuous radiation. In discussing his results, Witte has given special emphasis to the space and time distribution of the radiation-produced ions. He has introduced the concept of "time of instability" during which the radiation-induced disturbances exist in the irradiated volume element. The proper relation of the single exposure time to this "time of instability" determined the observed minimum in per cent death of x-irradiated Drosophila pupae. Hofmann and Muller attribute their observed effects to reciprocal actions between the radiation impulse and the following "restitutional" pause.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

and the total time associated with numerous reactions the "total sensitive time." This "total sensitive time" concept is similar to Witte's quite general "time of instability" of the irradiated volume. It is then possible from radiochemical considerations to break down these general time concepts, that is, to apply the sensitive time concept not on a general basis but in terms of "sensitive times" of specific radiation-induced reactions. By so doing it may then be possible to correlate these specific reaction times to certain exposure frequencies (single exposure times) of the incident radiation, as the following illustrates. Let t_1 be the "sensitive time" for a specific "reaction." For single exposure times much greater than t_1, there will be no difference in observed biological effect between constant and fractionated irradiation. As the single exposure time decreases (by increasing disc speed) and approaches t_1, the effect will reach a magnitude which may be detected biologically. If the single exposure times are made smaller than t_1, but with pause times still remaining greater than t_1, then the decrease in the biological effect becomes significant. The minimum will occur when the sum of the single exposure time and pause time is equal to t_1. With further shortening of single exposure times, pause times also decrease and the sum of single exposure time and pause time will become less than t_1. When this is the case two exposures will occur in time t_1 and the effect will increase again. This decrease, minimum, and following increase in biological effect with decreasing single exposure time is shown in Figure 2. Further decrease in single exposure times will result in other observed minima as "sensitive times" of other reactions of the complex biological system are reached. Figure 2 shows at least 2 such "sensitive times" for the range of single exposure times used in this study on ultraviolet irradiation of yeast. The magnitude of the observed minima may indicate the quantitative and/or the specific importance of the particular reaction in the overall biological effect.

The location of these minima may depend to some extent on such variables as the ratio of exposure time to pause time, dose rate, type and quality of radiation, and biological test material. The radiation of the biological specimens at extreme temperatures may also alter the location of the observed minima due to a dependence of various reaction times on temperature. Further detailed studies on these points are indicated.

The considerations presented here can be extended to the field of radiation therapy as indicated by Witte. It may be that malignant tissue and normal tissue will respond differently to ultrafractionated radiation of certain frequencies. This could be due to a difference

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #. 58 A 1094 (541)

A possible interpretation of the observed phenomena may be based on radiochemical concepts involving the "lifetime" of the radiation-induced reactions. It is known that for ionizing radiation these reactions follow the production of ion pairs, believed to occur in about 10^{-12} sec (8); the formation of radicals (activated water products) in about 10^{-9} or 10^{-8} sec (8, 9); and initial chemical reactions in about 10^{-2} sec (10). For ultraviolet radiation the production of the radicals can result from processes such as dissociation and internal conversion. If the oscillational energy of the molecule is greater than its binding energy, it will dissociate directly into radicals after a single vibration. For internal conversion the excitation energy of the electronic system is transferred to oscillational energy of the atomic constituents of the molecule. The molecule will then undergo reactions similar in some respects to a molecule at high temperatures and may break up into radicals. After their production these radicals may recombine, become involved in chemical actions or diffuse through the medium. Gross estimates of the "lifetime" of the radiation-induced reactions have been gained from studies with cysteine and trypsin, and show short-lived as well as long-lived reactions (11-13).

More exact measurements of the lifetimes of the reactions have been found in photochemical and radiochemical studies using rotating sector methods to produce the intermittent radiation. The experiments of Briers et al (14) showed the mean life of the catalyst to be 0.03 sec when a solution composed of potassium oxalate, iodine, and potassium iodide was illuminated with white light. Allmand and Style (15) applied the intermittent light technique for the decomposition of hydrogen peroxide. They postulated that the hydroxyl free radical was the catalyst present, with a mean life of 1 second. Hart and Matheson (16) investigated the mechanism of the Co^{60} gamma-ray-induced decomposition of hydrogen peroxide. This decomposition is a chain reaction in which water, oxygen, and a small amount of hydrogen are the products. More recently, Hummel et al (17), using Co^{60} gamma-ray bombardment, found an average free radical chain lifetime of approximately 0.1 sec in a chloral hydrate solution and approximately 1 sec in a chloroform system.

These studies on the lifetime of the radiation-induced reactions, along with the ideas of Witte, suggest the following interpretation of the observed minima. Considering the incidence of radiation on a biological system, there will occur, depending on the complexity of the system, several radiation-induced "reactions" leading to some observable effect. The total volume of the system influenced by this radiation incidence may be called the "total sensitive volume"

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

and the total time associated with numerous reactions the "total sensitive time." This "total sensitive time" concept is similar to Witte's quite general "time of instability" of the irradiated volume. It is then possible from radiochemical considerations to break down these general time concepts, that is, to apply the sensitive time concept not on a general basis but in terms of "sensitive times" of specific radiation-induced reactions. By so doing it may then be possible to correlate these specific reaction times to certain exposure frequencies (single exposure times) of the incident radiation, as the following illustrates. Let t_1 be the "sensitive time" for a specific "reaction." For single exposure times much greater than t_1, there will be no difference in observed biological effect between constant and fractionated irradiation. As the single exposure time decreases (by increasing disc speed) and approaches t_1, the effect will reach a magnitude which may be detected biologically. If the single exposure times are made smaller than t_1, but with pause times still remaining greater than t_1, then the decrease in the biological effect becomes significant. The minimum will occur when the sum of the single exposure time and pause time is equal to t_1. With further shortening of single exposure times, pause times also decrease and the sum of single exposure time and pause time will become less than t_1. When this is the case two exposures will occur in time t_1 and the effect will increase again. This decrease, minimum, and following increase in biological effect with decreasing single exposure time is shown in Figure 2. Further decrease in single exposure times will result in other observed minima as "sensitive times" of other reactions of the complex biological system are reached. Figure 2 shows at least 2 such "sensitive times" for the range of single exposure times used in this study on ultraviolet irradiation of yeast. The magnitude of the observed minima may indicate the quantitative and/or the specific importance of the particular reaction in the overall biological effect.

The location of these minima may depend to some extent on such variables as the ratio of exposure time to pause time, dose rate, type and quality of radiation, and biological test material. The radiation of the biological specimens at extreme temperatures may also alter the location of the observed minima due to a dependence of various reaction times on temperature. Further detailed studies on these points are indicated.

The considerations presented here can be extended to the field of radiation therapy as indicated by Witte. It may be that malignant tissue and normal tissue will respond differently to ultrafractionated radiation of certain frequencies. This could be due to a difference

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
TS A 1094 (541)

in the chemical composition of the cells or media and/or a difference in the diffusion rates of the reactions in malignant and normal tissues. It would thus be possible to ultrafractionate the therapeutic dose at such a frequency as to result in maximal damage to malignant tissue with little damage to surrounding normal tissue.

V. SUMMARY

Results are reported which show differences in the per cent of yeast cells damaged by ultrafractionated ultraviolet irradiation as compared to continuous irradiation. Definite minima in per cent of yeast cells damaged were found for single exposure times of 0.024 sec and 0.0025 sec corresponding to radiation pulses of 13.8 per sec and 133.3 per sec, respectively. It is suggested that these minima may be related to the time existence of some radiation-induced reactions. The magnitude of these observed minima may indicate the quantitative and/or specific importance of particular reactions in the overall biological effect.

VI. RECOMMENDATIONS

These studies using ultrafractionated irradiation techniques should be extended to include:

1. The use of various types and qualities of radiation and of various chemical and biological test systems.

2. The influence on the observed minima of various ratios of exposure time to pause time and of various dose rates.

3. The influence of extreme temperatures on the observed minima of the biological effect.

4. The determination of the biological effect for ultrafractionated total body x-irradiation. This study should include pre-irradiation injection of protecting agents such as cysteine, glutathione, etc., in an attempt to identify the reactions responsible for the minima.

VII. BIBLIOGRAPHY

1. Witte, E. Ultrafraktionierung. I. Mitteilung. Strahlentherapie, 82: 209, 1950.

2. Witte, E. and R. Sigmund. Ultrafraktionierung. II. Mitteilung. Strahlentherapie, 88: 384, 1952.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

3. Witte, E. Experimentelle untersuchungen uber die wirkung intermittierend verabfolgter ionisierender strahlen, 89: 586, 1953.

4. Hofmann, D. and K. Muller. Mitteilung uber erweiterte untersuchungen des biologischen effektes ultrafrakionierter bestrahlungen. Strahlentherapie, 96: 403, 1955.

5. Fraser, C. G. The action of methylene blue and certain other dyes on living and dead yeast. J. Phys. Chem. 24: 741, 1920.

6. King, D. W., S. R. Paulson, N. C. Hannaford and N. L. Puckett. The effect of injury on Ehrlich tumor cells. AMRL Report No. 170, Fort Knox, Kentucky, 8 May 1955.

7. Brasch, A., W. Huber, V. Friedemann and F. B. Traub. Action of high intensity electrons on biological objects. Proc. Rudolph Virchow Med. Soc. 8: 3, 1949.

8. Symposium on Radiobiology. John Wiley & Sons, Inc., New York, 7-8, 1952.

9. Basic Mechanisms in Radiobiology. II. Physical and Chemical Aspects: Nuclear Science Series. Ed. by John L. Magee, Martin D. Kamen and Robert L. Platzman. Publ. 305, National Research Council, Committee on Nuclear Science, Washington, D. C., 1953.

10. Lea, D. E. Actions of Radiations on Living Cells. The MacMillan Co., New York, 52, 1947.

11. Patt, H. Protective mechanisms in ionizing radiation injury. Physical Rev. 33: 35, 1953.

12. Bellack, S. and A. T. Krebs. Protection of single cells and small cell groups against radiation. AMRL Report No. 60, Fort Knox, Kentucky, 10 July 1951.

13. McDonald, M. R. The effects of x-rays on dilute solutions of crystalline trypsin: continued inactivation after termination of irradiation. Brit. J. Rad. 27: 62, 1954.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

14. Briers, F., D. L. Chapman and E. Walters. Influence of the intensity of illumination on velocity of photochemical changes. The determination of the mean life of a hypothetical catalyst. J. Chem. Soc. Part 1: 562, 1926.

15. Allmand, A. J. and D. W. G. Style. The photolysis of aqueous hydrogen peroxide solutions. I. Experimental methods. J. Chem. Soc. 596, 1930.

16. Hart, E. J. and M. S. Matheson. Mechanism and rate constants of the gamma-ray induced decomposition of hydrogen peroxide in aqueous solutions. Disc. Faraday Soc. 12: 169, 1952.

17. Hummel, R. W., G. R. Freeman, A. B. Van Cleave, and J. W. T. Spinks. Rotating sector method applied to reactions induced by Co^{60} gamma rays. Science, 119: 159, 1954.

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

DISTRIBUTION LIST FOR AMRL REPORTS

Category (A)

ARMY	Copies
The Surgeon General Department of the Army Main Navy Building Washington 25, D. C. ATTN: Chief, Research and Development Division	10
Director Armed Forces Institute of Pathology Walter Reed Army Medical Center Washington 12, D. C.	1
Army Library Room 1A 522, The Pentagon Washington 25, D. C. ATTN: National Defense Review	1
Headquarters Army Medical Service Graduate School Walter Reed Army Medical Center Washington 12, D. C.	1
Chief, Human Relations & Research Branch Military Personnel Management Division Office of Assistant Chief of Staff Department of the Army Room 2C724, The Pentagon Washington 25, D. C.	2
Office Assistant Chief of Staff, G4 Department of the Army The Pentagon Washington 25, D. C. ATTN: Research & Development	1
Commanding Officer Chemical Corps Medical Laboratories Army Chemical Center, Maryland ATTN. Chief, Technical Information Branch	1
Army Environmental Health Laboratory Building No. 1235 Army Chemical Center, Maryland	1
Commandant Marine Corps Schools Quantico, Virginia ATTN: Library & Record Section, MCTG	1
Medical Field Service School Fort Sam Houston, Texas	2
Library, Army War College Carlisle Barracks Pennsylvania	1

NAVY	Copies
U. S. Naval School of Aviation Medicine U. S. Naval Air Station Pensacola, Florida	1

ARMY	Copies
Supreme Headquarters of the Allied Powers in Europe Medical Section Rocquencourt, France	2
Office, Chief of Army Field Forces Fort Monroe, Virginia	1
Walter H. Moursund, Jr., Colonel GS Office of the Army Attache, Box 36 U. S. Navy 100 Fleet Post Office New York, New York	2
Commanding General Headquarters, QM R&D Command QM R&D Center, U. S. Army Natick, Massachusetts	3
Commanding Officer Medical Nutrition Laboratory 3937 TU U. S. Army Fitzsimmons Army Hospital Denver 7, Colorado	1
Headquarters 406th Medical General Laboratory APO 500, c/o Postmaster San Francisco, California	1
Headquarters Camp Detrick Frederick, Maryland ATTN: Technical Library	1
Scientific Publications and Reports Office QM Food and Container Institute for the Armed Forces 1819 West Pershing Road Chicago 9, Illinois	1
Commanding Officer Arctic Test Branch, OCAFF (AAU 8576) APO 733, c/o Postmaster Seattle, Washington	1
Research and Development Division Office, Quartermaster General Room 2102, Building 'A' Washington 25, D. C.	1
Armed Forces Staff College Norfolk, Virginia	1
Library, Quartermaster School Fort Lee, Virginia	1

NAVY	Copies
Commanding Officer U. S. Naval Air Development Center Johnsville, Pennsylvania ATTN: Aviation Medical Acceleration Lab	1

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 58 A 1094 (541)

Category (A) Continued

NAVY	Copies
Commanding Officer Naval Medical Research Institute National Naval Medical Center Bethesda 14, Maryland	1
Chief, Bureau of Medicine & Surgery Department of the Navy Washington 25, D. C.	1
Commanding Officer Naval Medical Field Research Laboratory Marine Barracks Camp LeJeune, N. C.	1
AIR FORCE	
Commandant USAF School of Aviation Medicine Randolph Air Force Base Randolph Field, Texas ATTN: Research Secretariat	1
Commander HQ Wright Air Development Center Wright-Patterson Air Force Base, Ohio ATTN: Aero Medical Laboratory (WCRD) Directorate of Research	1
Director Air University Library Maxwell Air Force Base Alabama	1
OTHER AGENCIES	
Exchange & Gift Division Library of Congress Washington 25, D. C.	1
Director, Armed Forces Medical Library 7th Street & Independence Ave, SW Washington 25, D. C. ATTN: Acquisitions Division	1
Operations Research Office The Johns Hopkins University 7100 Connecticut Ave Chevy Chase, Maryland ATTN: Librarian	1
National Institutes of Health Division of Research Grants Bethesda 14, Maryland	2
FOREIGN ADDRESSEES	
Defense Research Member Canadian Joint Staff (W) 2001 Connecticut Ave, NW Washington 8, D. C.	2
RCAMC Liaison Officer Surgeon General's Office Department of the Army Room 2842, Main Navy Building Washington 25, D. C.	2

NAVY	Copies
Officer in Charge U. S. Naval Medical Research Lab U. S. Naval Submarine Base New London, Connecticut ATTN: Librarian	1
Special Assistant for Bio Sciences Office of Naval Research Department of the Navy Washington 25, D. C.	1
AIR FORCE	
Department of the Air Force Headquarters USAF Director, Research & Development DCS/D Washington 25, D. C. ATTN: AFDRD-HF	1
Arctic Aeromedical Laboratory APO 731, c/o Postmaster Seattle, Washington ATTN: Librarian, AAL	1
Commander Air Research & Development Command ATTN: RDTRH PO 1395 Baltimore 3, Maryland	1
OTHER AGENCIES	
Armed Services Technical Information Agency Document Service Center Knott Building Dayton 2, Ohio ATTN: DSC-SD32	5
National Science Foundation 1520 H Street, NW Washington, D. C.	1
Department of Physiology University of Rochester School of Medicine and Dentistry 260 Crittenden Blvd Rochester, New York	1
National Research Council Division of Medical Science 2101 Constitution Ave Washington, D. C.	1
FOREIGN ADDRESSEES	
Officer in Charge Office of Naval Research Branch Office Navy 100, Fleet Post Office New York, New York	10
THRU: The Foreign Service of the United States of America United States Army Liaison Office American Consulate General Singapore (for Clearance and forwarding to:) The Deputy Director of Army Health Far East Land Forces Singapore	1

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
[illegible] (541)

Category (A) Continued

FOREIGN ADDRESSEES	Copies
Tropical Research Medical Laboratory School of Tropical Medicine University of Puerto Rico San Juan, Puerto Rico	1
Dr. O. G. Edholm Head, Division of Human Physiology Medical Research Council Laboratory Holly Hill, Hampstead London, NW 3 England	1
British Naval & Army Medical Liaison Officer Bureau of Medicine & Surgery Building 4, Room 60A 23rd & E Streets Washington, D. C.	2

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
FY A 1094 (541)

DISTRIBUTION LIST FOR AMRL REPORTS

Category (C)

ARMY	Copies
Commanding Officer Engineer Research & Development Laboratories Fort Belvoir, Virginia ATTN: Technical Documents Center	1
NAVY	
Technical Information Division, Code 3-222A U. S. Naval Radiological Defense Laboratory San Francisco 24, California	1
AIR FORCE	
United States Air Force School of Aviation Medicine Radiation Laboratory The University of Chicago 930 East 58th Street Chicago 37, Illinois	1
OTHER AGENCIES	
Chief, Radiation Branch National Cancer Institute Department of Health, Education & Welfare Bethesda 14, Maryland	1
The University of Rochester Atomic Energy Project Technical Report Control Unit P. O. Box 287, Station 3 Rochester 20, New York	1

ARMY	Copies
Commanding Officer Surgical Research Unit Brooke Army Medical Center Fort Sam Houston, Texas	1
OTHER AGENCIES	
Research Library Brookhaven National Laboratory Associated Universities, Inc. Upton, L. I., New York	1
Argonne National Laboratory P. O. Box 299 Lemont, Illinois ATTN: Hoylande D. Young, Director, Information Division	1

Washington National Record Center
Office of the Army Surgeon General