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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT
FOR QUARTER ENDING MAY 15, 1949

ALEXANDER HOLLAENDER, DIRECTOR

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BIOLOGY DIVISION

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QUARTERLY PROGRESS REPORT

For Quarter Ending May 15, 1949

7/25/55

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ORNL

Alexander Hollaender, Director

DATE ISSUED

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BIOLOGY DIVISION

QUARTERLY REPORT TO MAY 15, 1949

ALEXANDER HOLLAENDER, DIRECTOR

Introduction. This quarter has been a very busy one for the Biology Division. The annual Information Meeting for Biology and Medicine was held in Oak Ridge on April 11 - 13. The first two days were devoted to open meetings in radiation microbiology and biochemistry. The program of this meeting is given below:

FIRST SESSION--DR. G. W. BEADLE, CHAIRMAN

Radioactive substances and their use in the study of plant viruses	James H. Jensen AEC, Washington, D.C.
Comparison of spontaneous and induced mutations in *Escherichia coli*	Howard B. Newcombe N.R.C., Chalk River, Ont.
The effects of combined radiations on mutation rate in micro-organisms	C. P. Swanson Johns Hopkins University

SECOND SESSION--DR. MAX DELBRUCK, CHAIRMAN

Experiments on photo-reactivation with bacteria and other micro-organisms	Albert Kelner Biol. Lab., Cold Spring Harbor
Mutual reactivation of UV treated bacteriophages	S. E. Luria Indiana University
Experiments on photo-reactivation with bacteriophages	Renato Dulbecco Indiana University

THIRD SESSION--DR. C. E. CARTER, CHAIRMAN

The indirect action of ionizing radiations on aqueous solutions and its dependence on the chemical structure of the substrate	Walter M. Dale Christie Hospital, Manchester, England
One-hit and two-hit survival curves resulting from alpha and X radiation of *Aspergillus* spores	R. E. Zirkle University of Chicago

The relation of ion density to efficiency in production of lethal and mutagenic effects in *Aspergillus terreus*	G. E. Stapleton Oak Ridge National Lab.

FOURTH SESSION--DR. UGO FANO, CHAIRMAN

The energy transfer from ionizing particles to an aqueous medium and its bearing on the interpretation of radiochemical and radiobiological change	L. H. Gray Hammersmith Hospital London, England

About 230 out-of-town visitors, exclusive of University of Tennessee people who are considered local visitors, attended the meeting. A breakdown of the list of visitors is offered.

GENERAL LIST	TOTAL	AEC GRANTS	PROGRAM ORNL	AEC CONTR. INSTAL.	SOUTH. UNIV.	OTHER INDEP.
Abbott Laboratories	1					1
AEC Chicago	1			1		
AEC Washington	6			6		
AFSWP Washington	1			1		
Alabama University	1				1	
Amherst College	1	1				
Argonne National Lab.	14			14		
Brookhaven Nat Lab.	12			12		
Bureau of Ships (Navy)	1					1
Calif. Inst. of Tech.	3	2	1			
Calif. Univ. Rad. Lab	1			1		
Camp Detrick	10					10
C & C C C Charleston	1					1
Carnegie Inst. of Wash.	6					6
Chas. Pfizer Co.	1					1
Chicago University	11	1				10
Children's Hosp., Phila.	1		1			
Cold Spr. Hrbr., Bio. Lab.	1		1			
Colorado University	3	2				1
Columbia University	2			1		1
Cornell University	1					1
Eli Lilly Company	2					2
England	2		2			
Emory University	3				3	
Hanford	3			3		
Harshaw Chemical Co	1			1		
Harvard University	1					1
Indiana University	3		2			1
Iowa State College	2					2

GENERAL LIST	TOTAL	AEC GRANTS	PROGRAM ORNL	AEC CONTR. INSTAL.	SOUTH UNIV.	OTHER INDEP.
Johns Hopkins University	7	1				6
Kansas University	3					3
Kellex Corporation	1			1		
Kentucky University	5				5	
Knolls Laboratory	2			2		
Los Alamos	4			4		
Louisiana State Univ.	1				1	
Mallinckrodt Company	1			1		
Mass. Inst. of Tech.	1			1		
Med. Div., Army Chem. C'r	1					1
Merck & Company	1					1
Michigan University	4	1				3
Mississippi University	6				6	
Missouri University	2					2
Mound Laboratory	4			4		
N.A.C.A.	1			1		
N.B.S.	1			1		
North Carolina State	4				4	
New York Oper. Off.	3			3		
New York University	1					1
N.R.C., Chalk River	3					3
N.R.D.L.	4			4		
Ohio State University	4	1				3
Pennsylvania University	3					3
Purdue University	4					4
Rice Institute	2				2	
Rochester University	7			7		
Sandia Laboratory	1			1		
Signal Corps, Army	2					2
Sloan-Kettering Inst.	1					1
Sou. Illinois University	4					4
Sou. Research Institute	1				1	
Southwestern Med. Col.	1				1	
Squibb, E.R. & Sons	2					2
Texas A. & M.	1				1	
Tonawanda, Linde Co.	1			1		
Tulane University	2				2	
U.C.L.A.	2					2
Union College	1	1				
U.S.D.A.	2					2
U.S.P.H.S.	14					14
Vanderbilt University	1				1	
Vet. Ad. Hosp., Dallas	1					1
Virginia University	1				1	
Western Reserve Univ.	3			3		
Westinghouse	1			1		
Winthrop College	1				1	
Wooster College	1					1
Yale University	1	1				
(W. D. Clause)	1					1
TOTAL	222	9	8	75	30	99

The "Total" column is an accurate count of the number of guests who *registered* at the hotels, the High School sessions or the restricted X-10 sessions. The breakdown is as accurate as could be determined from available records.

It is important to point out that 75 laboratories in the United States, Canada, England, Italy, Switzerland, India and Finland were represented. About 13 per cent of the visitors came from Southern Universities. Open house, attended by 150 guests, was held on Tuesday, April 12. Many individuals came to visit the Laboratory on the days immediately following the meeting.

The Association of Southeastern Biologists, consisting of the Southern Appalachian Botanical Group, the Southeastern Section of the Botanical Society of America, the Kentucky-Tennessee Branch of the Society of American Bacteriologists, and the Southeastern Branch of the Society of American Bacteriologists met in Knoxville on April 14, 15, 16. The Biology Division was host to the members of these societies at a conducted tour of its Y-12 facilities on April 14. More than 200 visitors took advantage of this opportunity of seeing some of the work in progress here. Cooperation was given to the University of Tennessee committee in arranging the symposium on the biological effects of radiation on Thursday, April 14. The program for this symposium is given below:

THE BIOLOGICAL EFFECTS OF RADIATION

Dr. Alexander Hollaender, presiding

The physics of radiation	W. A. Arnold, Biology Division Oak Ridge National Laboratory
The biochemical effects of radiation	C. E. Carter, Biology Division Oak Ridge National Laboratory
The cytological and genetical effects of radiation	J. Gordon Carlson, Department of Zoology & Entomology, Univ. of Tenn.
The physiological effects of radiation	H. I. Kohn, Biology Division Oak Ridge National Laboratory

During this quarter national meetings have attracted a number of Division members. The following persons represented the Laboratory by presenting papers at the societies mentioned:

American Chemical Society, San Francisco, California

Dr. W. E. Cohn	The separation of nucleotides and related substances by ion-exchange reactions

Dr. C. E. Carter	Paper chromatography of purine and pyrimidine derivatives of yeast nucleic acids
Drs. Cohn and Carter	The separation of three naturally-occurring adenine ribonucleotides by paper chromatography and ion exchange

Federation of American Societies for Experimental Biology, Detroit, Michigan

Dr. D. S. Anthony (Dr. S. F. Carson) (Dr. Martin Kuna) (Mr. E. F. Phares)	Studies on the mechanism of the biosynthesis of propionic acid
Dr. C. E. Carter	The effect of X rays on the metabolism of nucleic acids
Dr. A. D. Conger	The cytological effects of slow neutrons
Mrs. L. Russell	X-ray induced developmental abnormalities in the mouse
Dr. C. W. Sheppard (Mr. W. R. Martin)	Isotopic studies of potassium exchange rates between cellular elements and plasma of human and canine blood *in vitro*

Active participation in the Oak Ridge Institute - Oak Ridge Laboratory Traveling Seminar program is continuing. Seminars given by Division members at Southern Universities are as follows:

Dr. Alexander Hollaender at Winthrop College	Practical application of radiation
Dr. Hollaender at Emory University	Cytogenetical effects of low doses of radiation
	General discussion of AEC biology organization
Dr. S. F. Carson at S. C. Academy of Science	The use of isotopes in biochemical research
Dr. W. A. Arnold at Georgia Inst. of Tech.	Photosynthesis

Dr. Arnold at Oglethorpe University	Photosynthesis
Dr. H. I. Kohn at Kingsport Rotary Club	Some of the medical aspects of atomic energy

We have been fortunate in securing the services of several prominent scientists who have given Biology Division seminars during this period. The names of these visitors and the titles of their seminars are listed below:

Dr. W. K. Baker, Zoology Department, University of Tennessee -

The production of translocations by radiation

Dr. C. E. Ford, National Research Council, Chalk River, Ontario -

Effects of gamma rays and nitrogen mustard on *Vicia* root-tip chromosomes

Dr. J. W. Foster, Department of Botany and Bacteriology, University of Texas -

Some problems connected with the physiology and biochemistry of moulds

Dr. Salome Schoenheimer-Waelsch, Genetics Department, Columbia University -

Mutations as a tool in the causal analysis of mouse development

Dr. Jonas S. Friedenwald, Department of Ophthalmology, Johns Hopkins University -

Effects of X rays on the rate of mitosis

Dr. Maurice G. Whittinghill, Department of Zoology, University of North Carolina, has joined this laboratory as a visiting scientist in cooperation with the Oak Ridge Institute of Nuclear Studies.

Research activity in the Biology Division during the three-month period just past has been intense. Many of the investigators are endeavoring to complete work which they plan to publish this summer.

CYTOGENETICS

EFFECTS OF RADIATIONS ON *TRADESCANTIA* AND *NEUROSPORA*

N. H. Giles, Jr. (Leader) A. D. Conger
Dorothy L. Giles Viola Turner
H. P. Riley (Visiting investigator)

Relative reverse mutability of independently produced, allelic, inositolless mutants in Neurospora crassa. (Giles, Giles and Turner) The results to date of studies on differences in reverse mutability among eight independently produced inositolless mutants are being prepared for publication. Further experiments have demonstrated regular segregation for differences in rates of ultraviolet induced reverse mutability among crosses of microconidiating cultures. The results of such an experiment are shown in table 1.

TABLE 1

Comparison of the rates of reverse mutation induced by ultraviolet radiation (2537 A) of microconidia (2000 ergs/mm²) in two allelic inositolless strains and segregates from a cross of the two strains

CULTURE NO.	SURVIVING MICROCONIDIA TESTED	NO. OF REVERSE MUTATIONS	REVERSE MUTATIONS per 10^8 SURVIVORS
37401-CL30-2a (parent 1)	17.0×10^6	65	3.85
89601-CL15-7a (parent 2)	34.4×10^6	0	< 0.03
89601-CL27-5.2	4.5×10^6	0	< 0.2
89601-CL27-5.4	4.0×10^6	0	< 0.25
F_1 segregates			
89601-CL27-5.6	15.3×10^6	52	3.4
89601-CL27-5.7	7.7×10^6	36	4.6

The investigation of the relation between X-ray dosage and reverse-mutation frequency in inositolless strain S-37401 has been resumed. Present data indicate a linear relationship (fig. 1). The analysis is being extended to higher dosages, utilizing new colonial strains producing more abundant microconidia. Results of the genetic analysis of eight X-ray induced reversions selected at random are presented in table 2. The absence of inositol-

less recombinants among the progeny of these crosses indicates that reverse mutation frequency in inositolless strain S-37401 has been resumed. Present data indicate a linear relationship (fig. 1). The analysis is being extended to higher dosages, utilizing new colonial strains producing more abundant microconidia. Results of the genetic analysis of eight X-ray induced reversions selected at random are presented in table 2. The absence of inositolless recombinants among the progeny of these crosses indicates that reverse mutation has occurred in all cases. The linear reversion rate with X-ray dosage provides strong support for this interpretation.

TABLE 2

Results of crosses to wild-type (SY4a) of eight inositol-independent mutants induced by X radiation of microconidia from inositolless strain S-37401

MUTANT NO.	COMPLETE ASCI TESTED		INCOMPLETE ASCI TESTED	
	All cultures inositol-independent	Other segregations	Inositol independent cultures	Inositolless cultures
8743-CL20-16. 1A-R2-65	2	0	60	0
" R3-65	7	0	73	0
" R4-65	10	0	97	0
" R5-65	1	0	21	0
" R6-65	2	0	40	0
" R7-65	6	0	34	0
" R9-65	4	0	67	0
" R10-65	2	0	21	0

The first evidence for a suppressor mutation in an inositolless mutant has been obtained in a stock of S-37401 subjected to ultraviolet. This mutant appeared as a slow-growing type on a minimal agar plate much later than the usual reverse mutations. Further crosses are planned to test the relation of this suppressor to the other inositolless stocks.

FIGURE 1

Relation between X-ray dosage and frequency of reverse mutations to inositol independence in microconidia of inositolless mutant S-37401 (in a colonial, fluffy, microconidiating stock).

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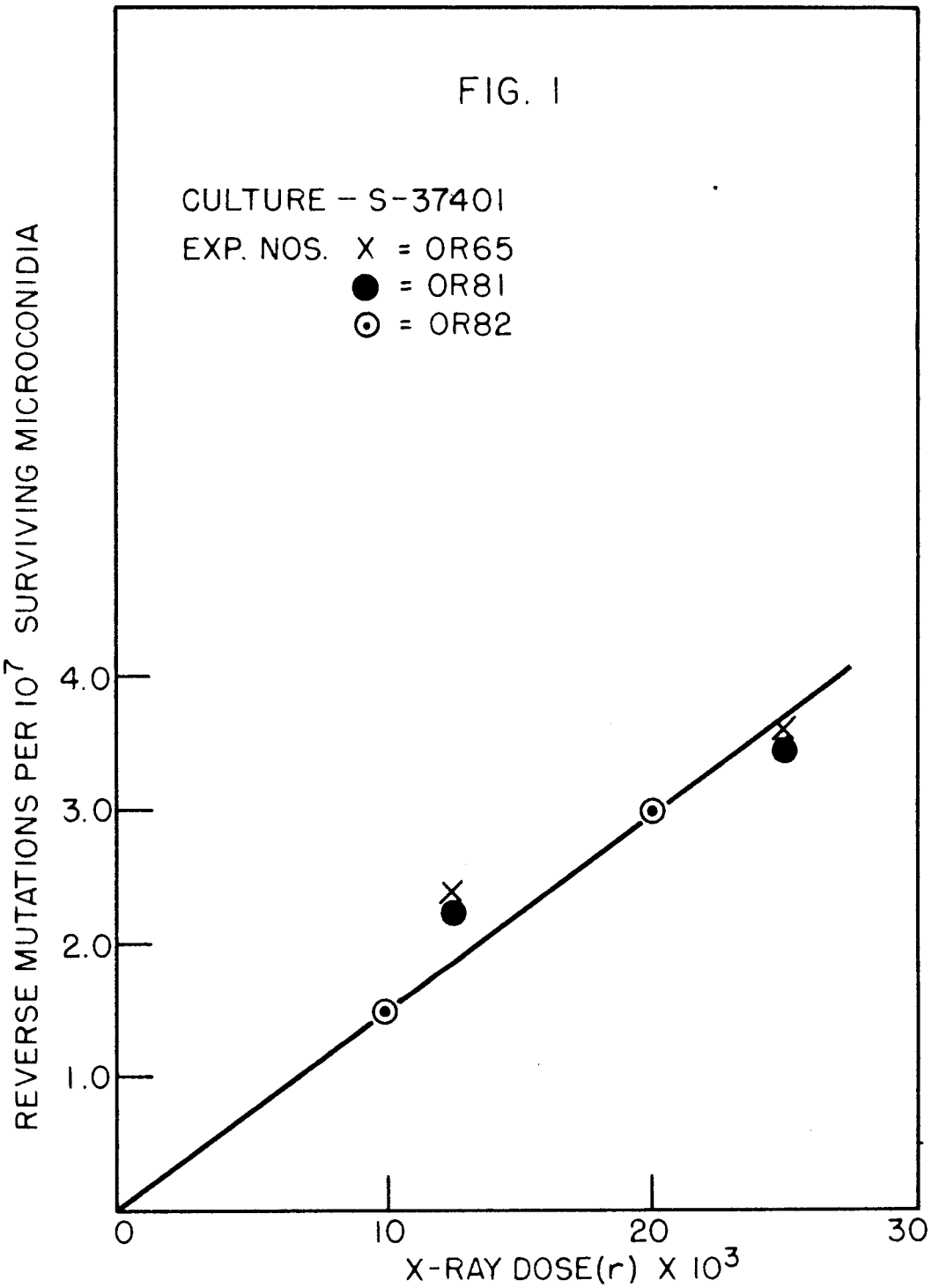

Genetic basis of spontaneous and radiation-induced adaptation in other biochemical mutants in Neurospora. (Giles, Giles and Turner) Studies are continuing of the genetic behavior of induced and spontaneous adaptations in other biochemical mutants in *Neurospora*. The results with methionineless mutant S-4894 are almost complete and show that adaptation results from suppressor mutation rather than from reverse mutation. Present evidence indicates that at least two different suppressors are involved. The genetic behavior of niacinless (Y-17000) and adenineless (S-38701) adaptations is more complicated and requires further analysis.

The effect of anaerobiosis of the frequency of X-ray induced chromosomal rearrangements in Tradescantia. (Giles and Riley) *Tradescantia* inflorescences have been exposed to several different X-ray dosages in air, in nitrogen, and in oxygen and the frequency of chromosomal interchanges determined at the four- to five-day period after exposure. The buds exposed in nitrogen and oxygen were first evacuated in a suction flask and the gas admitted. This procedure was repeated five successive times. The buds were then transferred to a lucite chamber and exposed to X-rays in an atmosphere of the appropriate gas. The same chamber was utilized for making exposures of the control inflorescences in air. The X radiation was given at a constant intensity of 45 r/min. Dosage measurements were made with a 100 r Victoreen thimble ionization chamber placed in the same position as the buds in the lucite box. The results of these experiments are presented in table 3 and plotted in figure 2.

The striking effect of oxygen on radiosensitivity, as measured by the frequency of chromosomal interchanges, is clear from these results. It is not yet possible to decide whether this oxygen effect is due to an increase in the frequency of initial breaks or to an influence on the reunion process, such that recombination is favored over restitution.

Cytogenetic effect of thermal neutrons. (Conger, Suskind) Work on the cytogenetic effect of thermal neutrons on *Tradescantia* chromosomes has been completed and is being written up. The report on this work will be completed during the next quarter.

The *Tradescantia* thermal neutron problem is being continued in an experiment to test the effect of an increased boron content in tissues exposed to thermal neutron radiation. The intent is to observe the effect of the $B^{10}(n,\alpha)\,Li^{7}$ reaction, and it is hoped that a large share of the biologic effect will be due to alpha radiation. Plants have been grown in a boric acid solution for about a month, and the boron content of the anthers followed by spectroscopic analyses made by Cyrus Feldman of the Chemistry Division.

An exploratory exposure of a small group of rats in the thermal neutron facility has been made by Miss Crowell and Mr. Suskind.

TABLE 3

Chromosomal interchanges induced in Tradescantia microspores exposed to X-rays in air, in nitrogen, and in oxygen

	DOSE	NO. OF CELLS SCORED	DICENTRICS	CENTRIC RINGS	TOTAL INTER-CHANGES	INTER-CHANGES PER CELL
			Exposed in air			
EXP. I	90	500	29	11	40	0.080
	180	276	45	20	65	0.236
	270	150	44	10	54	0.360
	360	200	92	29	121	0.605
			Exposed in N_2			
EXPTS. III, IV, and V	90	1150	23	9	32	0.028
	180	400	15	4	19	0.048
	270	1267	107	40	147	0.116
	360	450	59	20	77	0.171
			Exposed in air			
	90	1050	52	30	82	0.078
	180	600	88	44	132	0.220
	270	600	165	73	238	0.397
	360	150	68	25	93	0.620
			Exposed in O_2			
	90	800	50	15	65	0.081
	180	900	165	77	242	0.269
	270	500	175	78	253	0.506
	360	300	157	80	237	0.790

FIGURE 2

Relation between dose and frequency of chromosomal interchanges in *Tradescantia* microspores exposed to X radiation in air, in nitrogen and in oxygen. Constant X-ray intensity of 45 r/min.

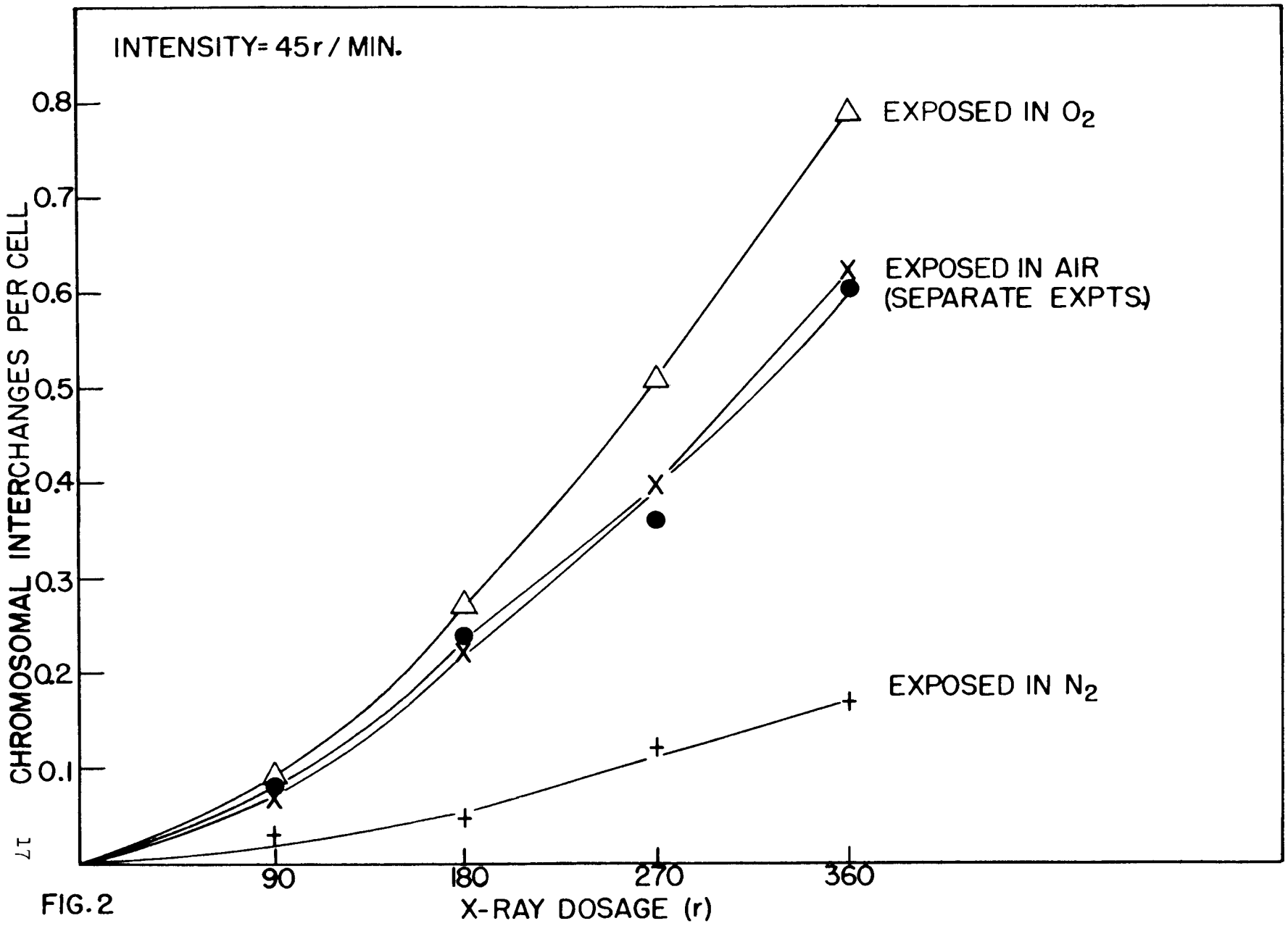

FIG. 2

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EFFECTS OF RADIATIONS ON PARAMECIUM

R. F. Kimball (Leader) Nenita Gaither
Mary K. King

Effects or radiation upon division rate in Paramecium. (Kimball, Gaither, King) The effect or radiation on rate of cell division upon which preliminary data was reported in the last report has been intensively investigated during this quarter. Most of the work has been done with ultraviolet light from a germicidal lamp. One experiment with X rays has been done, but the high doses required can be obtained with the present X-ray machine only under unsatisfactory conditions. Therefore, further work with X rays is being deferred until the new equipment now on order is available. The preliminary work has suggested that the effects of X rays are sufficiently like those of ultraviolet to justify the belief that the results and techniques from studies with the latter will be, in considerable part, of value in future investigations with X rays.

The procedure with ultraviolet was as follows: A group of animals were exposed to the radiation, and a number of animals isolated each into a separate container. In the earlier experiments, 30 irradiated animals were isolated for each treatment, in later ones 72. Unirradiated animals from the same clone were isolated as controls. The day after irradiation the number of animals produced by the division of each irradiated and control animal was recorded, and one animal from each was isolated to continue the line of descent. This procedure was repeated on each succeeding day until complete recovery of the normal division rate had occurred.

From the daily counts, the number of divisions undergone during each day was known for each line. From these data, the mean number of divisions per day could be calculated for any one treatment. However, a more meaningful quantity from the point of view of the biology of the animals would be the mean time between any given pair of divisions after irradiation. Ideally this should be obtained by watching the animals continuously and recording the time at which division actually occurred. Since it would have been difficult to carry any large numbers of animals and make observations more frequently than once a day, a less accurate method was used. It was assumed that the divisions occurring in a given one-day period were equally spaced within the period and that the last division occurred at the end of the period. While these assumptions introduce some error into the calculations, it is believed that the error is not great except for the first two divisions after irradiation with very low doses. In this case, a delayed first division followed rapidly by the second division would be overlooked since both would occur on the same day. In all save the dosage studies, rather large doses were used and two divisions during the first day happened only occasionally.

The average time between divisions for a typical experiment is shown in the form of a histogram in figure 1. The part of each bar representing the control fission interval is the average over the control data for all divisions and all days. The part of each bar above this represents the excess in time over the controls. The conclusions of the previous quarterly report concerning distribution of delay to various division intervals is, in general, confirmed but made more precise. There is considerable delay in the first division after irradiation. The time between the first and second divisions is distinctly longer than for the controls but relatively short when compared to that for the first, third or fourth intervals. Recovery is rapid after the fourth interval and, as far as can be seen, complete.

This is the typical picture for the averages over a number of lines of descent. How far do the individual lines follow this average picture? The first two division intervals correspond quite well in almost all lines though an occasional line shows an especially long second interval associated with short succeeding intervals. The picture is quite different from the average for the next two intervals. Four classes in respect to these intervals can be recognized. (1) The total delay over the controls is small and due to more or less equal and small delays in the first four to five intervals. (2) The third and fourth intervals are relatively short but associated with a relatively long second or fourth interval. (3) The third interval is quite long but the other intervals through the fourth or fifth are relatively short though longer than the control. (4) The fourth interval is long and the others relatively short. Classes (1), (3), and (4) are relatively numerous. Class (2) is rare.

In summary, the time from irradiation to the first division is longer than normal in all lines and in most cases longer than the time from the first to the second division. Many, though not all, lines of descent show a rather long cessation of division which falls in some one division interval, most commonly the third or fourth but occasionally the second or fifth. In addition, all lines of descent divide more slowly during the first five to six division intervals than do the controls.

Relation to dose were briefly mentioned in the last report but the data were not analyzed in detail. The same data have now been analyzed using the total time between irradiation and a given division after it as the quantity measured. The time for the controls to reach the same division is then subtracted to give the delay over the controls. These delays for five different doses are plotted against the division after irradiation in figure 2. The curves rise steeply during the first few divisions and then approach an upper

FIG. 1

TIME BETWEEN DIVISIONS AFTER ULTRAVIOLET IRRADIATION

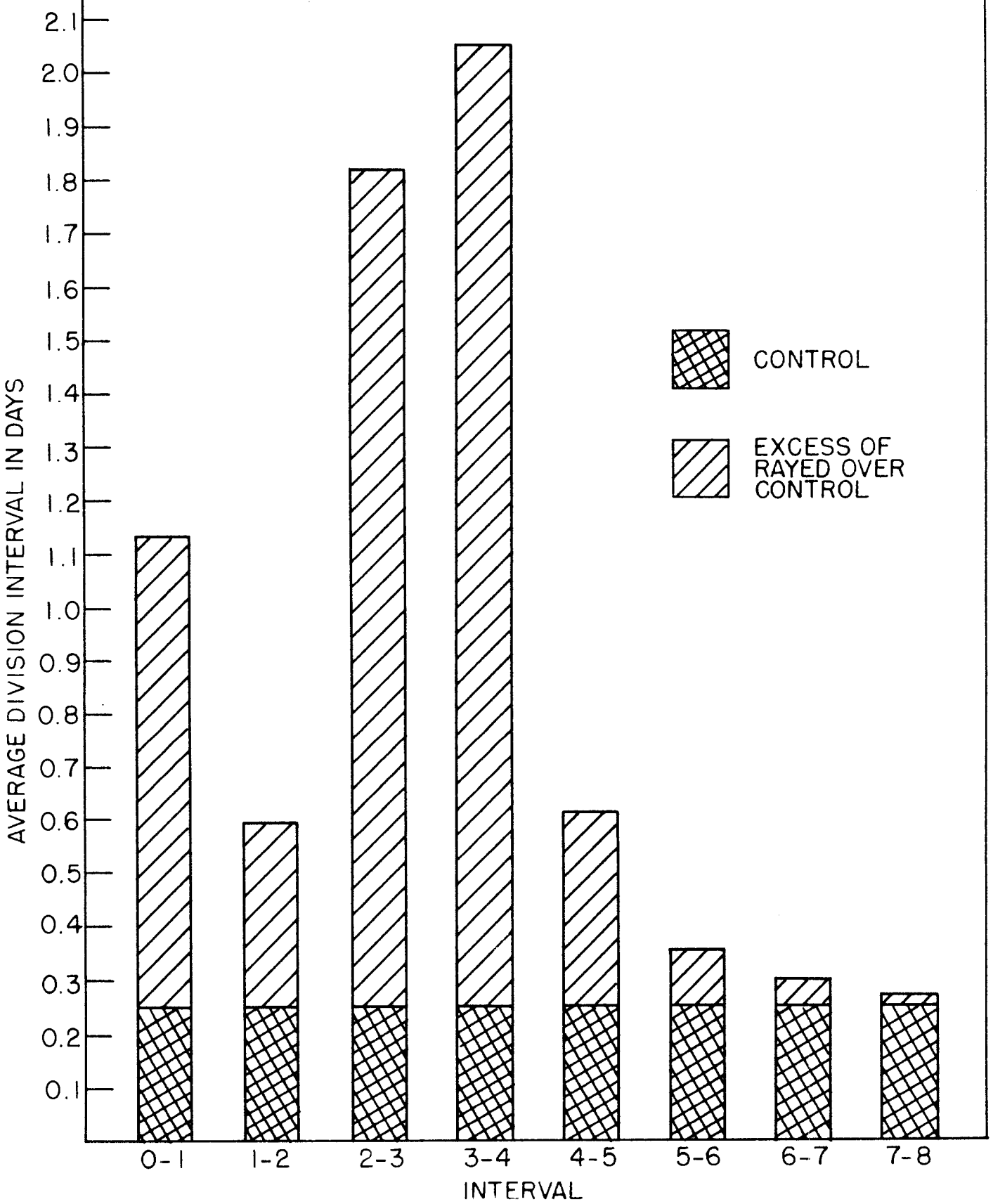
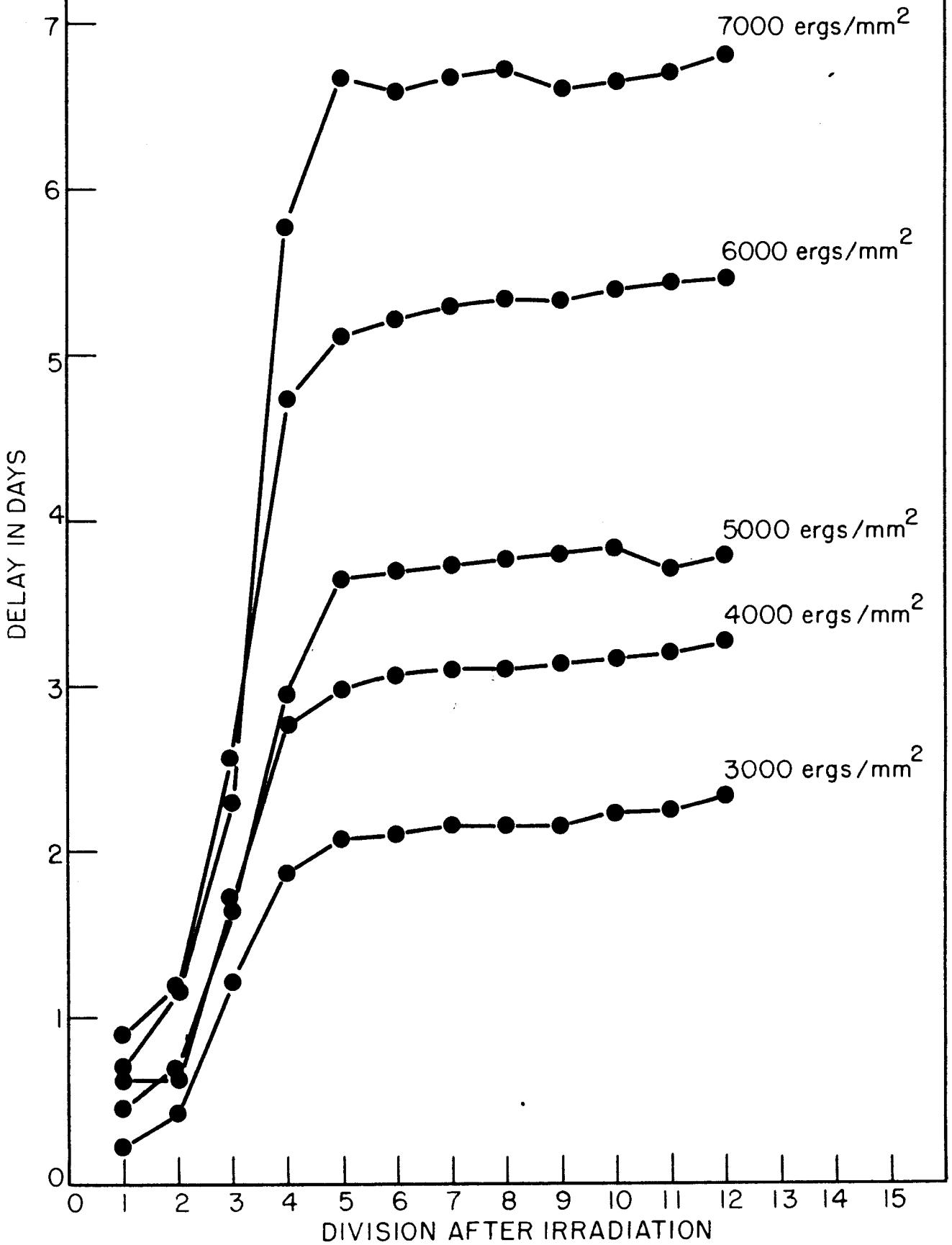


FIG. 2

DAYS DELAY OVER THE CONTROL IN A GIVEN DIVISION PLOTTED AGAINST DIVISIONS AFTER IRRADIATION



limit as recovery of the normal division rate occurs. This upper limit is the total delay due to the summation od delays in all division intervals. Examination of the curves suggests that the increment of delay added between a given pair of divisions is a constant proportion of the total delay independent of dose. That this is probably true is shown in figure 3 in which the delay to a given division is plotted as a per cent of the delay to the twelfth division which is essentially equal to the total delay.

The results plotted in figures 2 and 3 are all from a single experiment and a single clone. The same method of calculation as used for figure 3 has been applied to the data of four other experiments in each of which only one dose was given. These experiments were carried out at different times, with different clones, and with different doses. The results are in good agreement with figure 3.

This means that the delay over the controls in the time taken to reach any given division should all give the same kind of measure of the dosage relation. It also means that we have available a measure independent of dose which can be used to study possible qualitative differences between different kinds of radiation and different auxiliary treatments.

The dosage data are, so far, meager and any conclusions from them as to the form of the dosage curve are purely tentative. However, it is of interest to see what the available data suggest. Figure 4 shows the delay in the twelfth division plotted against dose. The points scatter considerably but a linear relation is possible.

It is planned to study the effect of various auxiliary conditions upon the radiation effect. So far only one has been investigated in sufficient detail to warrant a report. This is the effect of low temperature after irradiation. Figure 5 shows the result of an experiment in which a single group of irradiated animals was divided into two parts after isolation. One part was kept at the usual 26° C while the other was kept at 14° C for one day and then put at 26° C for the remainder of the experiment. The results are plotted as days delay in a given division over the 26° C control. The delay to the first and second divisions is somewhat greater for the low than for the high temperature group probably reflecting the general slowing down of division rate by low temperature. However the delay to later divisions is less for the low than for the high temperature group. It may be noted that the first, and occasionally the second division interval, was spent in whole or in part at low temperature while all later intervals were spent entirely at high temperature. The general result is that recovery from the radiation effect is considerably

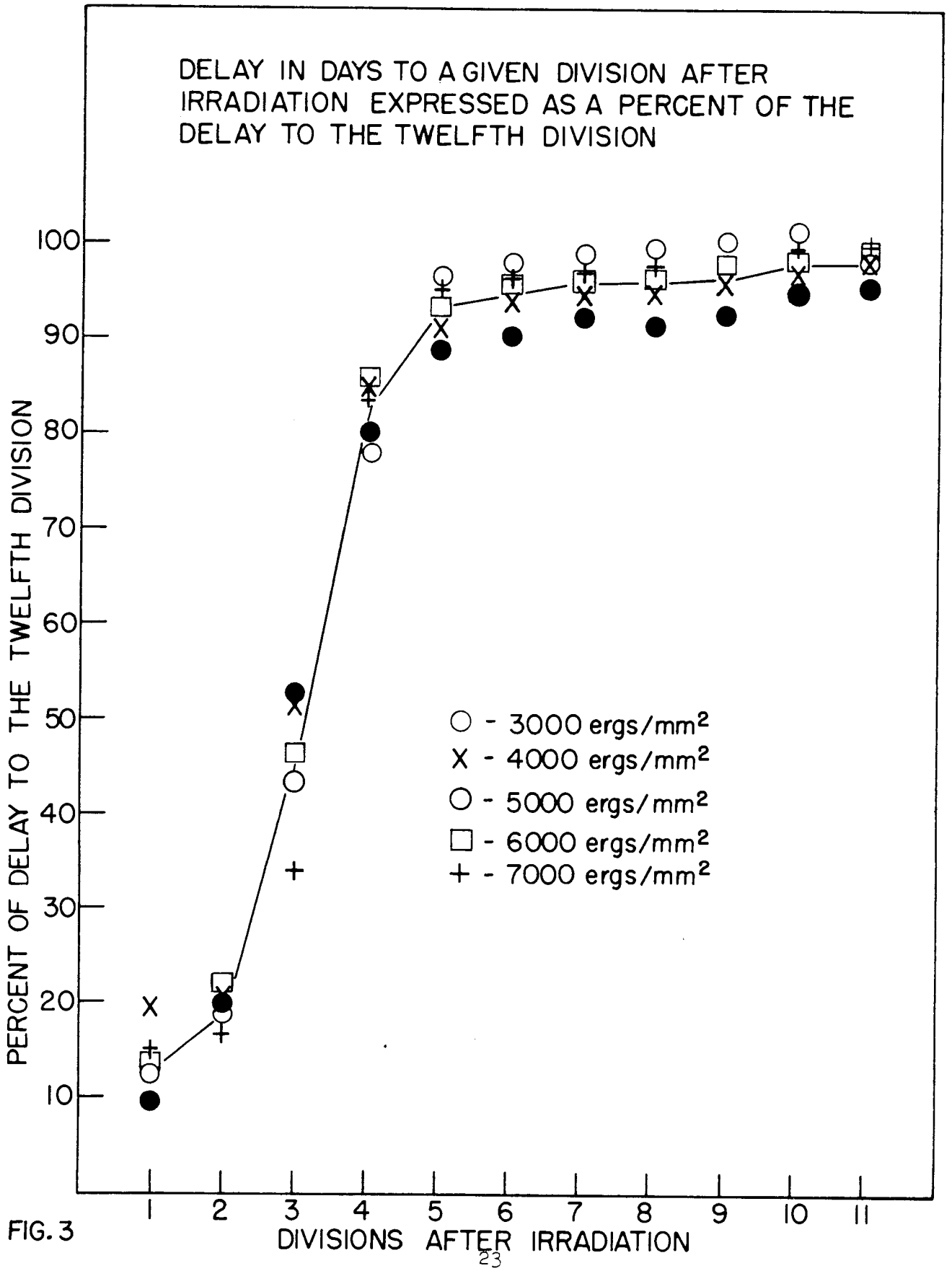
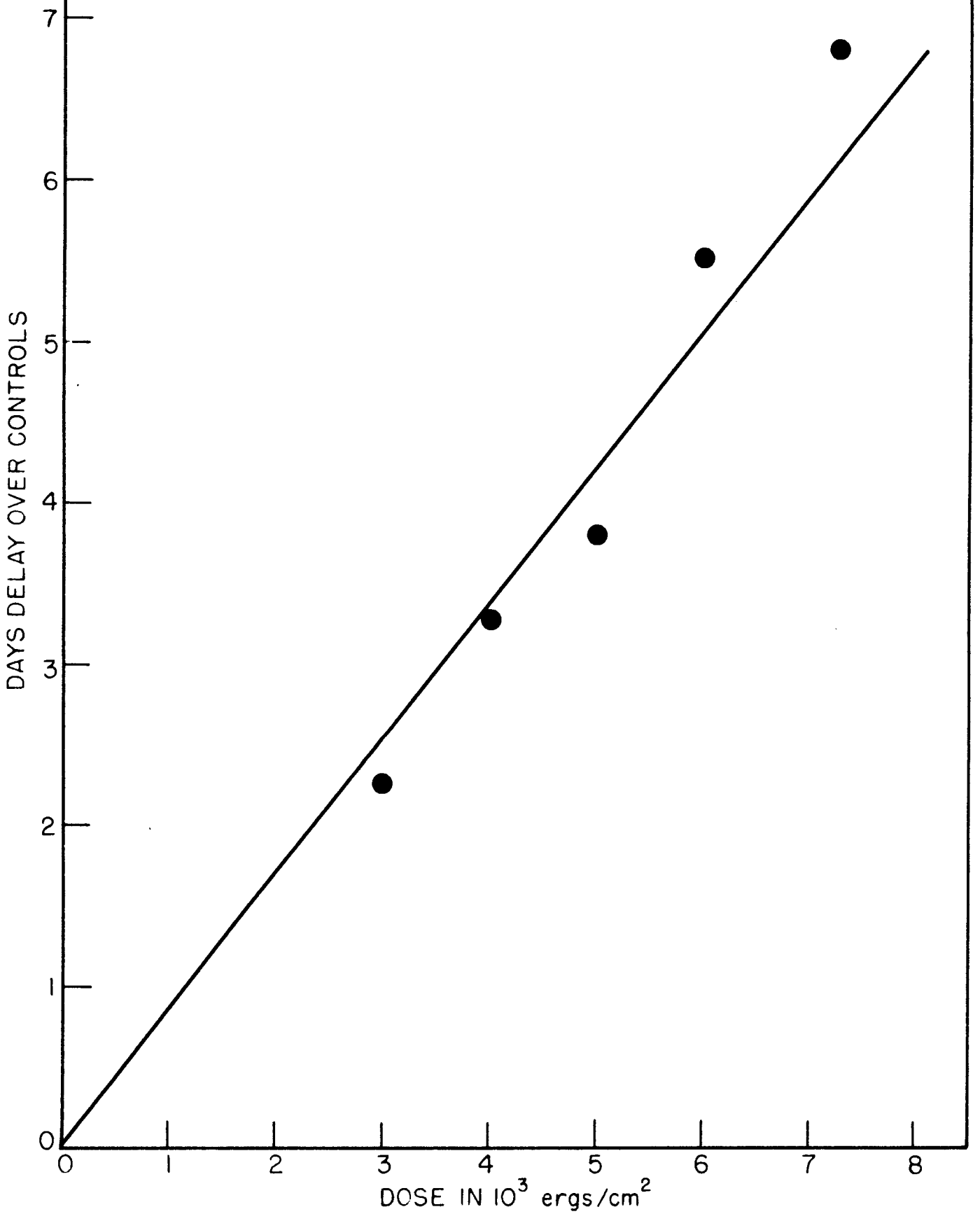

DELAY IN DAYS TO A GIVEN DIVISION AFTER IRRADIATION EXPRESSED AS A PERCENT OF THE DELAY TO THE TWELFTH DIVISION
PERCENT OF DELAY TO THE TWELFTH DIVISION
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
DIVISIONS AFTER IRRADIATION
○ - 3000 ergs/mm²
X - 4000 ergs/mm²
○ - 5000 ergs/mm²
□ - 6000 ergs/mm²
+ - 7000 ergs/mm²

FIG. 3

FIG. 4

DAYS DELAY TO THE TWELFTH DIVISION PLOTTED AGAINST DOSE OF ULTRAVIOLET RADIATION



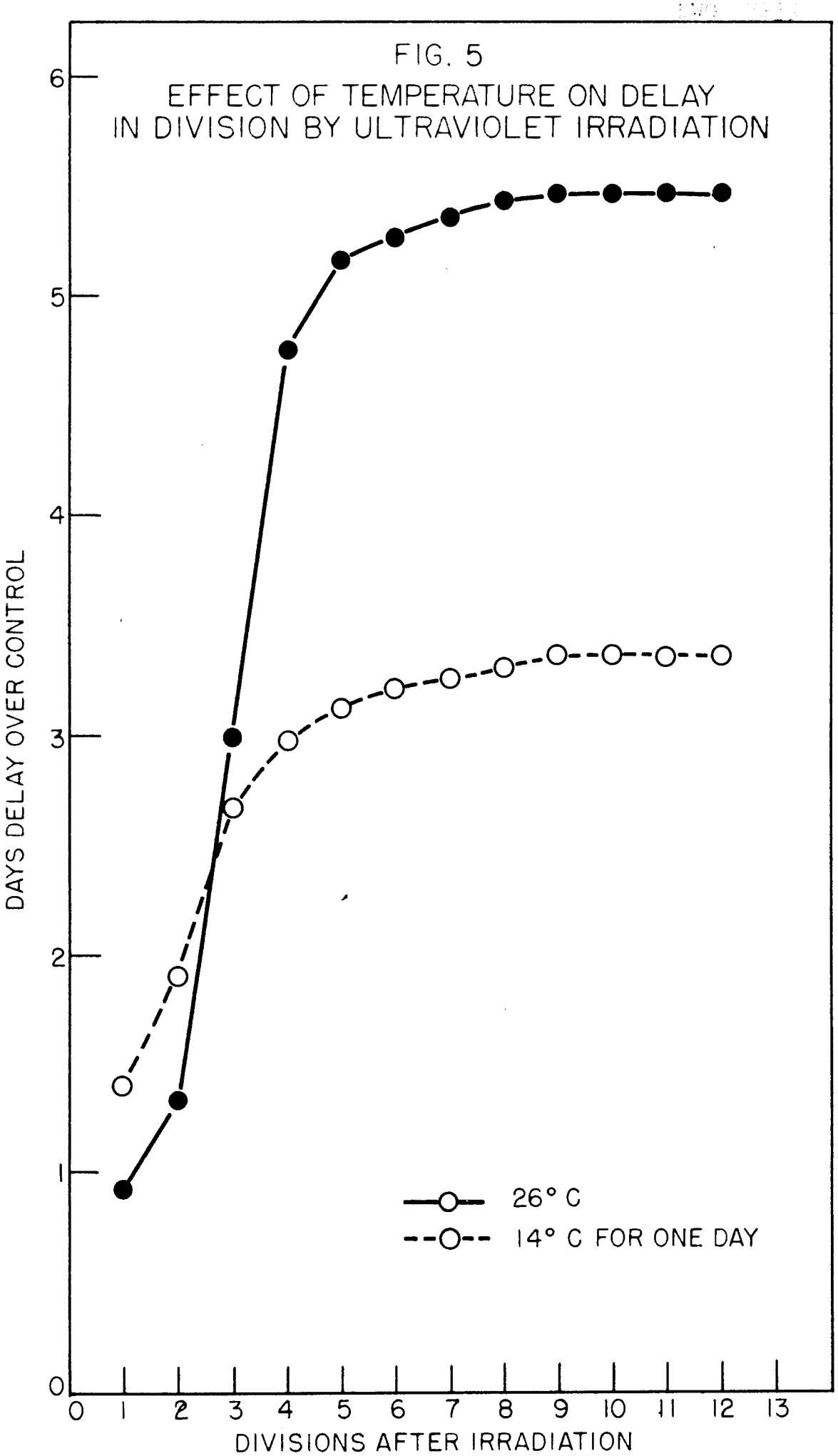


FIG. 5
EFFECT OF TEMPERATURE ON DELAY
IN DIVISION BY ULTRAVIOLET IRRADIATION
DAYS DELAY OVER CONTROL
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DIVISIONS AFTER IRRADIATION
26° C
14° C FOR ONE DAY

more rapid if the animals are held at low temperature for a time after irradiation.

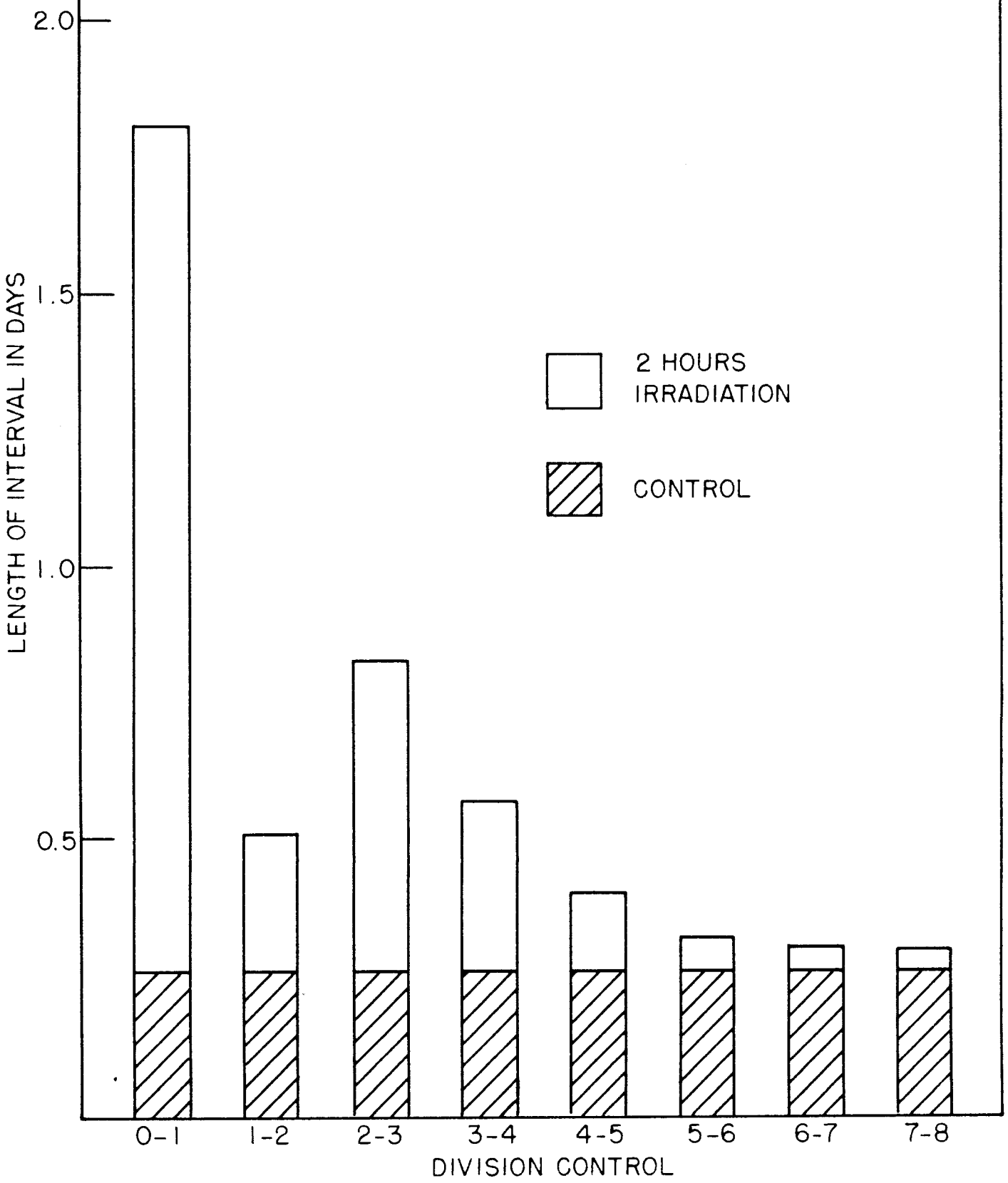
In another experiment the following irradiated groups were set up for comparison: (1) 20° C for one day and then 26° C; (2) 14° C for one day and then 26° C; (3) 14° C for two days and then 26° C; (4) 14° C throughout the experiment; (5) 26° C throughout the experiment. The first group did not differ greatly from the fifth, if anything, recovery was somewhat slower. The second and third groups did not differ greatly from each other but recovered distinctly more rapidly than the fifth. In the fourth group about 60 per cent of all lines died, mostly after the third but before the fourth division. In all other groups the death was around 10 per cent. Because of the large amount of death, no adequate comparisons can be made as to the rate of recovery. It would seem that there must be a rather sharp temperature gradient in the recovery effect below 20° C; that the recovery effect is produced primarily by some action taking place shortly after irradiation; and that continuous exposure to low temperature not only does not hasten recovery but actually prevents it if the large amount of death can be described as a failure to recover.

It has already been mentioned that one experiment was done with X rays. The conditions were unsatisfactory in several respects. The animals had to be placed very close to the tube in a position in which nothing except a very crude estimate of dose could be obtained. Even at this position an exposure of two hours had to be made to get an appreciable effect as compared to about 15 to 20 minutes for ultraviolet. Finally the temperature at this position rose considerably during the exposure and was probably not much below the lethal temperature at the end. However, the results justify the statement that effects similar in a number of respects to those with ultraviolet can be gotten with X rays.

The experiment was carried out in the same manner as the ultraviolet experiments. The two-hour exposure was estimated to have given a dose in the neighborhood of 400,000 r. The average time between each pair of divisions was calculated and is plotted in figure 6 as a histogram. As in the ultraviolet, the time between the first and second divisions appears to be somewhat shorter than that between the second and third, and third and fourth, but the difference is not as striking. The time to the first division is much longer in proportion than with ultraviolet. Nonetheless there are enough similarities between the effects of the two kinds of radiation to justify the belief that the effects have, in part, a common basis. There is the same apparent approach to a normal division rate in the second interval followed by a secondary decrease of the rate in the next two intervals and then fairly rapid recovery.

FIG. 6

DELAY IN DIVISION PRODUCED BY X-IRRADIATION



In summary, the experiments on the effect of radiation on the rate of cell division, have demonstrated a number of regularities from which limited generalizations can be made. At this time it would be premature to go further and attempt to develop a single hypothesis to unite these generalizations.

EFFECTS OF RADIATIONS ON RATE OF MITOSIS

J. Gordon Carlson (Leader)* Rachel McMaster
Jane E. Crowell Mary E. Gaulden*
M. G. Whittinghill (Visiting investigator)

Effect of low intensity gamma radiation in the grasshopper neuroblast. (Crowell and Carlson) The eggs of the grasshopper, *Chortophaga viridifasciata,* containing embryos at an age equivalent to 14 days, are still undergoing exposures to gamma radiation at low intensities. A previous report of no recovery following exposure to 192 r (at four r per hour intensity) may be revised, due to better controlled conditions of the embryos during treatment. The conclusion reached is that recovery with slight overshooting of mitosis occurs four hours later. The latter investigation was undertaken when results of 384 r, at the same intensity, showed an early recovery and subsequent overshooting two and one-half hours following exposure. It is hoped that favorable conditions for the embryos may be worked out in order than an exposure of 576 r can be given.

Studies at approximately one-fourth of the intensity referred to above with a total dose of 47 r, indicate that recovery from mitosis inhibition is occurring at a fairly steady rate. There seems to be no significant difference from control embryos. Studies at the same intensity with a higher dose are in progress.

Effects of gamma rays and neutrons on crossing over in Drosophila melanogaster. (Whittinghill) Studies on effects of gamma rays and of neutrons upon the process of crossing over in *Drosophila melanogaster* have begun. The first phase of the study is with gamma-ray treatments.

The experiments now under way consist of 15 separate families in which egg laying will proceed over a period of 20 days (five successive cultures of four days each) and the offspring will be classified for 17 days from the initiation of each culture. Three control test crosses were started in advance of twelve other control and treated families. These controls were fertile in single pair matings from the very start, and they seem to be furnishing interesting examples of nonrandomness of recombinations which may contribute some-

*Consultants

thing to our knowledge of spontaneous crossing over. The twelve families started later consist of four from heterozygous females exposed to 4000 r of gamma radiation in gelatin capsules within a dummy thimble chamber at the tantalum source, three exposed to 2000 r during one-half of the interval of the above, and five which were stored in gelatin capsules at 24° C for the duration of the treatment of their sisters. Then each female was mated to a single male. Sterility was encountered in the first four-day cultures in both control and treated females, but with the addition of more males most of the females are now fertile. It will be easy to detect the effects of gamma treatment, but the simultaneous analysis for the background of decreasing crossovers with increasing age of the female will probably yeild little data of value at this time. Culture conditions were tested in the sterile bottles, revealing the trouble to be in the relative infertility of the males from both of the *rucuca* stocks used for the test crosses. The *rucuca* stock, consisting of eight recessive markers in the third chromosome of *D. melanogaster*, is difficult to keep in homozygous form, and it is unobtainable at the two leading stock centers. However, its mutant genes mark the chromosome nicely from end to end and in the vicinity of the spindle attachment, a most variable region. The characters produced are classifiable quickly and accurately, so that data extensive enough to be useful can be amassed at one time.

EFFECTS OF RADIATIONS ON MUTATION OF FUNGI

Alexander Hollaender (Leader)
G. E. Stapleton Frances M. Langford

Lethal and mutagenic action of ionizing radiations on Aspergillus terreus. (Stapleton and Langford) The report for last quarter summarized some of the results obtained in comparison of the lethal and mutagenic action of sparsely ionizing electrons and the densely ionizing heavy particles. The preliminary experiments, with protons from the N^{14} (n, p) C^{14} reaction with thermal neutrons, indicated an efficiency factor of an order of magnitude greater than heretofore demonstrated. It was suggested, at that time, that an indetermined heavy-particle reaction was occurring on thermal neutron bombardment, which could account for the apparent efficiency. The suspected element was boron, which has an 18 per cent isotopic concentration of B^{10}, which has a capture cross section of 715 barns. The reaction B^{10} (n, α) Li^{7*} yeilds a 2.4 Mev. alpha particle. Spectroscopic analysis of a large batch of spores, indicated that boron was present in a concentration of 175 p.p.m. or 175 micrograms per gram of spores.

This concentration of boron is sufficient to produce about 25 rep-minute of alpha particles, which is twelve times that dose calculated on the basis of the nitrogen-proton reaction. The relative lethal efficiency of the heavy particles to X rays is reduced by a similar factor, with a resulting alpha/X-ray ratio of efficiency in killing of 2.5:1.

A series of experiments has been made using fast neutrons from uranium fission. The radiation resulting from bombardment of spores with fast neutrons is chiefly recoil hydrogen nuclei or protons. These particles would be expected, because of their relatively high ion density, to have about the same efficiency as the alpha particles described above. The dose measurements of fast neutrons were also made with a 100 r Victoreen thimble chamber, and the dose in "r" units converted to rep by the much-used factor of 2.5.

The technique used in culturing the spores was described in previous reports. The results follow.

Comparative lethal effects were determined thus : A large number of experiments were made using 250 Kvp. X rays and the curves relating survival to dose indicate a second order effect, which probably means that several electrons passing through the sensitive volume produces the lethal effect. An attempt was made to determine the hit number suggested by the curves obtained. The current analysis is based on that suggested by Curie, which suggests that the probability of a number of hits occurring relative to dose is given by the expansion of the Poisson expression:

$$\bar{P}_n = \frac{x^n e^{-x}}{n!}$$

Where n = number of hits 1, 2, 3, 4--------n

and x = some constant times dose.

$$\text{Survival or } \frac{N}{No} = e^{-x} + \frac{x\ e^{-x}}{1} + \frac{x^2\ e^{-x}}{2!} + \text{------} + \frac{x^n\ e^{-x}}{n!}$$

$$\text{or } \frac{N}{No} = e^{-x}\ (1+x) + \frac{x^2}{2!} + \text{----------} + \frac{x^n}{n!}$$

Graphic analysis of the X-ray survival curves indicates about three hits are necessary to kill.

The survival curves for the heavy particles, both alpha particles and protons are exponential suggesting that a single particle produces the lethal

effect. Calculation of the sensitive volume for the heavy particles indicates that this volume has the approximate dimensions of the spore nucleus.

A comparison of the exponential curves relating survival to dose for the heavy particles shows a greater efficiency for protons than for alpha particles. The ratio of effectiveness is about 1.5 to 1.

A comparison of the lethal dose (50 per cent) for X rays, alpha particles and protons is tabulated below.

X RAYS (250 kv)	ALPHA PARTICLES	PROTONS
Dose in rep 2.2×10^4	8×10^3	6×10^3
Efficiency 1	2.75	3.65

It is difficult to imagine that the protons would be more efficient than alpha particles in producing the lethal effect since the average spacing of ions along the tracks is about 90×10^{-5} μ for protons and 20×10^{-5} μ for the alpha particles. In other words, practically every atom along the track of either of these particles is ionized. It is more reasonable to assume the dose measurement for the fast-neutron exposures is in error by the factor stated for the relative efficiencies of the two heavy particles.

The method employed for determining the mutation rate was described in previous reports.

The yield of visible mutations for the densely and sparsely ionizing particles is proportional to dose over a wide range of doses, up to 60,000 r. The relative efficiency of the three radiations in producing mutation is strikingly different from that obtained for the lethal effect, X rays being the most efficient and alpha particles the least efficient. The ratio of efficiency in production of viable mutants is as follows:

X RAYS	PROTONS	ALPHA PARTICLES
1	0.66	0.40

The reduction of efficiency with increasing ion density is indicative of the single-event type of effect often suggested for the production of mutation. It is supposed by most investigators that single ionizations may produce gene alterations which appear as mutations. This idea is further borne out by some experiments which showed the effect to be independent of the intensity at which a dose of X rays was administered.

A further study is being made of the frequency of production of several typical mutants, the desired information being a determination of the sensitive volume for these effects, which might be correlated with the lethal effect. The results of the study on killing do not appear inconsistent with the idea that several gene mutations would result in the inability of spores to germinate and form colonies. If such a process is involved in killing it might be inferred that the mutant forms would have an increased sensitivity to radiation. Some information on this point is being accumulated, but is too meager for reporting at this time.

MAMMALIAN GENETICS

GENETICAL EFFECT OF RADIATION ON MICE

W. L. Russell (Leader)
J. C. Kile
Louis Wickham
Liane B. Russell
Josephine S. Gower

Determination of mutation rate at certain chosen loci. (Russell, Gower, Wickham) Multiplication of the necessary stocks of mice has comprised the bulk of the work on this project. This phase of activity will continue to be of prime importance for some time.

The first part of a pilot experiment has been started. The purpose of this experiment is to determine the optimum dosage for the large scale program. Various factors will have to be investigated and balanced against one another in order to find the most economical way of operating the project. Thus, fractionation of dose, with the possibility of giving a higher total dose, must be balanced against the extra labor and the extra time and maintenance costs involved. It is too early, as yet, to report any results of this pilot experiment except a confirmation of the findings of Snell and Hertwig that litter size is reduced in offspring sired by treated males in the period immediately following irradiation.

X-ray induced developmental abnormalities. (L. Russell and W. Russell) The collection of material in this experiment, which was begun about eight months ago, is now completed except where a few details need elucidation. We have obtained 405 newborn mice, irradiated in fetal life at stages differing by 24-hour intervals and ranging from a half-day to 13½ days after copulation. Control newborns, numbering 307, of the same genetic constitution and in most cases by the same mothers (from the litter previous to the irradiated one) were also observed for the same characters. These characters are: (a) weight and all externally recognizable features and (b) the entire ossified skeleton at birth. The study of the latter is now under way.

Our aim has been twofold: (a) the extablishment of sensitive periods for certain characters and (b) the comparison of results obtained with different doses at certain suitable stages (9½ - 13½ days inclusive). For the former, 200 r was used in general (a few females treated with 100 r); for the latter, we compared 200 r, 300 r, and 400 r.

With the 200 r general survey dose, the pattern of abnormalities obtained at each stage not only occurs with high frequency, but is characteristic for

each stage. In general, in an attempt to correlate the sensitive period for a certain character with a known physical event in the morphogenesis of that character, one finds examples of various probable mechanisms of the radiation effect. It may occur at the time of fastest growth (eye defects, oligodactyly); or before the time of *obvious* fastest growth (polydactyly); or changes may be correlative (sternal defects secondary to rib disturbances).

In the dosage comparisons, the criteria of: (a) relative numbers of animals affected, and (b) relative degree of effect with different doses in individuals and the relation of (a) and (b) during successive stages may be used to answer a variety of questions. For instance, it seems that most primordia can respond in a graded way some are capable of only one response. A chronological pattern of height of sensitivity can also be obtained: in general, sensitivity seems to arise rather suddenly and diminish slowly. Detailed results of this experiment will soon be published.

Artificial insemination. (Logan and Kile) Miss Logan left during the quarter to continue her graduate studies. Dr. Kile recently has taken over this problem. The following report summarizes the work completed by Miss Logan. This material is being prepared for publication.

For artificial insemination to be successful, it appears that it must occur within a short time of ovulation. It is therefore, necessary to be able to determine the time of ovulation as accurately as possible. For the present work, it was decided to approach this problem through the fact that post-partum females ovulate within 24 hours after delivery. Preliminary observations were directed toward determining ovulation time in these mice under the conditions to be used, and ascertaining the frequency of post-partum mating. Mating of artificially inseminated females with sterile males would probably be necessary in most cases in order to insure (activation of the corpora lutea and) implantation. The mice used were CFW mice obtained from Carworth Farms, their offspring, and the first and second generation offspring from miscellaneous matings of ABC mice obtained from the Jackson Laboratory. Except where definitely stated, no noteworthy differences appeared between the two types of mice, and they are considered together. The lighting in the mouse room was controlled by an electric time switch. The lights were on from 7 P.M. until 7 A.M. and off from 7 A.M. until 7 P.M., except for short periods for observation and care of the mice. Pregnant females were isolated, with males in most cases, and were checked for litters six times a day, at 3, 7 and 11 o'clock. The phrase "delivery time" is used to indicate the middle of the four-hour period in which the first young were born. The following results include only

those mice having at least half the litter in the period in which delivery started. Following delivery, the females were checked for vaginal plugs at irregular times. At the time of autopsy, nine to nineteen hours after delivery, the ovaries and oviducts were dissected out, placed in Locke's solution, and examined under the dissecting microscope. When ovulation had occurred, the swelling of the ampulla of the oviduct was evident, and in many cases, the ova themselves were visible within the ampulla. The ampulla was then torn, and the ova, which were rapidly expelled, were counted. One or more ova in the oviduct were counted as a positive result. The uterus was examined for implantation sites, in order to determine whether the number of young recorded represented the full number in the litter. In some cases the ovaries were preserved and serial sectioned for a check on the progress of ovulation.

The results given in table 1 were obtained from 76 females. The positive results include eight in which the total number of ova from both oviducts was less than four, and one (9 P.M. group, eleven hours) in which no ova were found, but young corpora lutea were present in both ovaries. With the number of mice used, none of the differences between delivery groups can be demonstrated to be significant. However, there is an indication that ovulation may occur slightly later in the 5 P.M. and 9 P.M. groups than in the others. The results of all groups taken together give 13 - 14 hours after delivery as about the middle of the ovulation period.

Mating occurred in 46 of the 68 cases where males were present with the females. Twenty of the mice which had mated had not ovulated at the time of autopsy. Although the relation between mating time and ovulation time was not accurately determined, it appeared that some of the mice mated several hours before ovulation.

There were discrepancies between the number of young observed and the number of implantation sites in 29 of the 72 mice examined. A total of 531 implantation sites were observed, 475 of these represented by living young and nine by dead young. The percentage (8.8) of implantation sites not represented in the litters recorded is considerably higher than the 2.5 per cent reported by Merton (1938). This may be a result of disturbing the females in checking for litters.

Inseminations were performed between 12.5 and 16 hours after delivery. In general, the insemination time for each group was chosen as about the middle of the ovulation period for that group as indicated in table 1. There was, however, considerable variation in the actual insemination time within groups. Spermatozoa were suspended in distilled water solution of the following

TABLE 1

Occurrence of ovulation in post-partum mice

DELIVERY TIME		HOURS AFTER DELIVERY										
		9	10	11	12	13	14	15	16	17	18	19
9 AM	+			0			4	2	1			
(7 AM - 11 AM)	-			1			1	0	0			
1 PM	+			1		3						2
(11 AM - 3 PM)	-			0		2						0
5 PM	+			0			1	4	0		1	
(3 PM - 7 PM)	-			2			3	1	1		0	
9 PM	+			1			1	3		2		
(7 PM - 11 PM)	-			2			6	1		1		
1 AM	+			1			4	4				
(11 PM - 3 AM)	-			5			3	0				
5 AM	+	0			0		6					
(3 AM - 7 AM)	-	3			2		1					
All groups	+	0		3	0	3	16	13	1	2	1	2
	-	3		10	2	2	14	2	1	1	0	0

composition: NaCl 0.9 per cent, KCl 0.042 per cent, dextrose 0.72 per cent. Sperm stripped from the vasa deferentia of one male were suspended in 0.3 or 0.5 cc of the solution. In the uterus and oviducts of the post-partum female motility was maintained by such sperm up to at least six hours. Fresh suspensions, not more than one-half hour old, were used for all inseminations. Motility was checked before and after the inseminations. A glass speculum held in a clamp attached to the desk, a head mirror and a light made it possible to see the cervix and to introduce the sperm suspension from an 0.5 cc tuberculin syringe. A 19-gauge, $1\frac{1}{2}$-inch needle was used. The point was blunted and the needle was bent at a right angle in such a way that when it was attached to the syringe held at a convenient angle, the bevel of the needle was in a vertical position. Two needles were used, with the bevels on opposite sides, for insertion into the right and left horns of the uterus. Approximately 0.05 cc of the sperm suspension was injected into each horn. Following insemination, the females were placed with vasectomized males so chosen that, in case they were fertile, their offspring could be distinguished from those of the males used for insemination. Checks were made for vaginal plugs at intervals following insemination.

In all, 110 inseminations were made, with the results given in table 2. The proportion of plugs which had litters is significantly greater in the ABC group than in the CFW group and, in general, the results with the ABC group appear to be better than those with the CFW mice. No litters were obtained from the 1 A.M. group until the time of insemination was changed from 12.5 - 13.5 hours after delivery to 14 - 15 hours post-partum. Approximately equal numbers of mice were inseminated at the two times. Both of the pregnancies in the 5 A.M. group resulted from insemination at more than 15 hours after the time of delivery.

TABLE 2

Artificial insemination

	MICE	PLUGS (certain)	PLUGS (doubtful or unknown)	LITTERS	LITTER SIZE
ABC	42	36	1	15	3 - 10
CFW	68	37	5	7	2 - 6
9 AM	12	7	0	2	
1 PM	14	7	0	5	
5 PM	18	11	2	2	
9 PM	25	20	1	7	
1 AM	22	18	2	3	
Estimated*	19	11	1	3	
TOTAL	110	73	6	22	

* Delivery time known within 8 hours

MICROBIOLOGY

TRACER STUDIES ON METABOLISM

S. F. Carson (Leader)
D. S. Anthony Martin Kuna
E. F. Phares W. J. Jefferson
L. A. Nutting Mary V. Long

Potentiometric titrations with the hydrogen and the glass electrodes. (Kuna) Quartz vessels were used for the determinations. The vycor vessels used previously were not very versatile because of the lack of ground glass joints. This applies to the titrations with the hydrogen electrodes.

A pH 9.95 commercial buffer which was standardized in January was found to be only 9.82 in May. This buffer gave the following voltages with time in the new quartz vessel at 25° C:

MINUTES	MILLIVOLTS
8	821.30
12	821.18
16	821.15
22	821.15
30	821.00
43	821.03
88	821.06
120	821.13

A reading of 823.00 millivolts was obtained after an overnight passage of hydrogen through the solution. Thus, there is no drift towards the lower pH values in quartz as there is in Pyrex.

Using the quartz vessel, some fractions of adenylic acids were titrated with the following results:

	pK'2	MOISTURE	M.W.	PER CENT
Adenylic acid B-3	6.03	10.5	367	106
" " B-1	5.98	16.8	333	96
" " A-1	6.21	4.1	364	105

The values reported previously (in a vycor cell) were:

Adenylic acid A	6.21
" " B	6.06
Adenosine-5-phosphoric acid	6.49

In the titrations with the glass electrode, a siphon constant temperature circulation was found to be much better than the circulating pump arrangement used previously. The variations of the recording pen were cut down enormously. A new calibration of the Beckman alkali glass electrode was made with buffers which were checked with the hydrogen electrode. A straight line was obtained with the Brown recorder with ball point pen set at a zero of 60.

pH 4.02	=	22.6
" 7.02	=	52.0
" 9.82	=	79.7

The rate of addition of alkali by the one rpm motor from a 0.1 cc Gilmont microburette was checked and found to be $4.174 \pm .005 \times 10^{-5}$ cc/sec. (±0.12 per cent).

The alkaline solutions from the hydrogen electrode titrations were transferred to a lucite beaker and titrated with 3.48 N HCl. The results were as follows:

	pK'2
Adenylic acid B-3	5.86
" " B-1	5.85
" " A-1	6.14
" " A-1	6.13

Separation of acetic and propionic acids. (Phares and Long) Separation of acetic and priopionic acids by methods not involving the use of organic solvents were still not successful. Those tried included counter-current extraction with a silicone-water system, differential solubility of basic lead salts in hot water, distillation methods, and ion exchange.

A partition chromatography method described by Peterson and Johnson (J. Biol. Chem., 174:775-789, 1948) involving only benzene as the organic solvent, on wet celite, appeared promising. A survey showed that carbon impurities in the organic salt after the solvents had been evaporated, including carbonate, picked up in the process, amounted to about three per cent, about the order of the counting accuracy. It was decided to accept this error for the sake of clean separation.

A celite column of the same dimensions as that described by Peterson and Johnson, 60 × 10 mm, was used. However, as a substitute for celite No. 545, No. 535 washed free of fine material, was used to support the immobile solvent water. The column was packed as a wet paste of celite under benzene and washed with benzene under pressure until any excess water was removed.

In order to apply the sample, consisting of a few tenths of a millimole of total acids, to the column it was necessary to extract most of the acid with no more than three ml of benzene. The solution of salts was evaporated in the presence of some celite. This was moistened with two or three drops of water, rubbed around the flask walls, and transferred to a twelve-mm fritted-glass filter. This was repeated with more celite and then with 0.5 to 0.7 g anhydrous sodium bisulfate. These were mixed in the filter and extracted with six 0.5-ml portions of dry benzene directly onto the column. In this manner 85 - 90 per cent of the propionic acid and 80 - 85 per cent of the acetic acid was extracted.

The column was first developed with water-saturated benzene, the propionic acid coming off in about 20 ml in approximately one hour. Samples of two to five ml were titrated after adding water. When the propionic acid was completely off, two three-ml portions of water were applied, removing the acetic acid completely.

With this column, some overlapping of bands was observed. However, better than 99 per cent separation was obtained by discarding approximately five per cent of the recovered acids in the intermediate fractions. By adding only one- to two-ml samples to the column, clean separation of the entire mixture was obtained. However, this was not practical for the problem at hand.

The separation was checked using labeled acids. The results are shown next.

MIXTURE	ACTIVITY OF SEPARATE ACIDS (cts/sec)	
	Propionic	Acetic
Labeled propionic Unlabeled acetic	103	1
Unlabeled propionic Labeled acetic	1	151

Preparation of carbonyl-labeled pyruvamide. (Kuna, Phares, Anthony, Long) $C^{14}O_2$ from 32.0 mM $BaCO_3$ containing 3.0 millicuries of activity was passed over the surface of a methyl magnesium iodide solution which was stirred magnetically in two tandem flasks at -20° C. Small recording ion chambers (two μc threshold) were used before and after the reaction flasks to assist in control of the flow of CO_2. The addition required about forty minutes. The Grignard

was hydrolized in sulfuric acid and ice, and the acetic acid was steam distilled after precipitation of iodide with silver sulfate. The yield of acid for three such runs averaged 89 per cent, calculated from the titration. Sixty per cent (five mc) of the total aqueous sodium acetate was evaporated to dryness and distilled from phosphoric acid, yielding 4.0 g of acetic acid containing 14 per cent water. Addition of 3.06 g of acetic anhydride gave a total of 7.0 g of glacial carboxyl-labeled acetic acid.

In preparation of acetyl bromide the glacial acetic acid was distilled from three equivalents of PBr_3 giving 8.0 ml (100 mM) of acetyl bromide. An additional 15-ml amount of inactive acetic acid was distilled with the residue from the first distillation, yielding 14 ml of slightly active acetyl bromide.

The procedure for the preparation of pyruvamide was that of Anker (J. Biol. Chem., 176:1333 - 1335, 1948). The acetyl bromide was converted to pyruvonitrile by allowing it to stand in a sealed tube four days with cuprous cyanide, wet with an ether-cyclohexane mixture. The nitrile was extracted with ten 15-ml portions of ether. The ether solution was saturated with dry HCl in a jacketed fritted-glass filter, cooled with ice water. After addition of 1.35 g (75 mM) of water, the HCl stream was continued for one hour. The pyruvamide was filtered off, washed, and dried in a stream of nitrogen. Yield: 3.65 g pyruvamide, specific activity, 300 cts/sec/mg,* m.p. 129°.

The low activity bromide was similarly converted to the nitrile and extracted with 300 ml ether. One-half of this was converted to the amide. Yield: 2.52 g, 12 cts/sec/mg, m.p. 125 - 129°.

The remaining portion of the ether solution was combined with the filtrate and washings from the high activity preparation. The mixture yielded 5.13g amide, 36 cts/sec/mg, m.p. 128 - 129°.

The filtrates and washings from all three preparations were combined and saturated with dry HCl, being cooled with an acetone-ice mixture. Yield: 0.75g pyruvamide, 54 cts/sec/mg, m.p. 125 - 129°.

A sample of the low activity (twelve counts) pyruvamide was hydrolyzed with hydrochloric acid on a steam bath for 90 minutes and determined colorimetrically with salicylaldehyde, KOH reagent, against a known standard. The sample was determined to be virtually 100 per cent pyruvamide.

Intermediates in biological synthesis of acetate. (Carson, Anthony, Long, Jefferson) Experiments on the propionic acid fermentation were made with carbonyl-labeled pyruvate as substrate. The end-products, propionic and acetic acids, were degraded by methods outlined in preceding report. It was

* Solid sample counting as ~ $BaCO_3$ 2 mm below a mica end-window counter (window of 1.3 mg/cm^2).

indicated that propionate is formed from succinate, and that succinate is formed by at least two routes. Details were presented by D. S. Anthony at the recent meeting of the Society of Biological Chemists in Detroit, Michigan.

More recent experiments indicate that some components of the C_6-acid cycle may also be implicated in this fermentation. Tracer experiments have given clear evidence that acetate is formed from pyruvate by a mechanism other than, or in addition to, the usual oxidative decarboxylation of pyruvate. This is interesting in view of the fact that this latter mechanism is the only one so far known for the synthesis of acetate from carbohydrate derivatives. In view of the great importance which has been recently demonstrated for the participation of the acetate radical in biological syntheses, this second possible mode of formation of acetate is being followed carefully.

RADIATION BACTERIOLOGY

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Recovery of Escherichia coli from ultraviolet irradiation inactivation. (Anderson, Whittle, Hill) Microorganisms which have been exposed to ultraviolet light lose their ability to divide. These cells are then said to be inactivated or nonviable in that they are incapable of forming colonies when plated on a suitable solid medium. Under certain conditions of chemical treatment and storage after irradiation, a small percentage of so-called inactivated cells may become reactivated or regain their ability to divide and form colonies when plated on solid medium. Recently Kelner has reported that illumination with visible light induces the reactivation of a very large percentage of ultraviolet inactivated spores of *Streptomyces griseus* and *Penicillium notatum* and cells of *Sachromyces cereviseae* and *Escherichia coli* strain B/r.

During the past quarter the radiation bacteriology group has been investigating the reactivation of ultraviolet irradiated cells of *E. coli*. It has been found that *E. coli* strains B and B/r both respond to reactivation with visible light. Preliminary experiments to determine the wave length dependency of photoreactivation for this material indicate that the effective wave lengths lie below 5400 Å.U. It is planned to carry out further studies to determine more accurately the wave lengths responsible for photoreactivation for these two strains of *coli*.

In studying the effect of temperature on photoreactivation it was found that heat treatment alone can cause a reaction of *coli* strain B. In these experiments 18-hour cultures of the organism, diluted in nonabsorbing buffer solution, were exposed to ultraviolet intensities giving a dark survival ratio of approximately one per cent when plated on agar and incubated 48 hours at the "normal" incubation temperature of 37° C. Duplicate plates made in the same manner incubated at 40° C gave a ten- to thirtyfold increase in survival ratio over that obtained at plate incubation temperatures of 37° C. Other experiments indicate that temperature reactivation and light reactivation may be independent factors.

BIOCHEMISTRY

BIOCHEMISTRY OF NUCLEOPROTEINS AND ENZYMES

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The isolation and characterization of spleen ribonucleic acid. (Volkin and Carter) Several investigations have dealt with the analytical composition of mammalian ribonucleic acids of unknown molecular homogeneity (1), but the preparation and characterization of high molecular weight ribonucleic acids from mammalian tissues have received little attention. In view of the apparent dependence of the composition of ribonucleic acids upon the method of preparation as well as the source (2,3,4), certain criteria appear necessary to the assessment of physical and chemical descriptions of ribonucleic acid preparations. It is necessary to present; first, the preparative procedure and source; second, a statement of the physical-chemical homogeneity and particle size of the ribonucleic acid; third, a description of the degradation of the ribonucleic acid by ribonuclease in order to determine how many susceptible bonds are present in the molecule; and fourth, a statement of the purine and pyrimidine composition of the ribonucleic acid. A preparation of ribonucleic acid obtained from beef spleen by a procedure involving the separation of the ribonucleic acid guanidine salt has been studied with respect to these criteria.

Although the ribonucleic acid isolated by Cohen and Stanley[2] from tobacco mosaic virus exhibited a molecular weight or 300,000, and ribonucleic acids of large particle size have been isolated from bacterial surfaces by Stacey et al. (5), in general, the preparations which have been studies by physical-chemical techniques have been characterized by smaller particle size. The estimated molecular weight of yeast ribonucleic acid reported by several investigators[3,4] is about 17,000 while an alkaline extracted sample of tobacco mosaic virus ribonucleic acid was found to have a molecular weight of 35,000. The native molecular character of ribonucleic acid is presumably not reflected in the latter descriptions because of the degradation involved in the preparative procedures.

Ribonucleic acid isolated from beef spleen by the procedure described below is essentially homogeneous in the analytical ultracentrifuge, and exhibits

a sedimentation constant ($S_w^{20°}$) of 3.09 which, in the absence of diffusion data, indicates a molecular weight of approximately 50,000. The difference between the molecular weight of the virus nucleic acid prepared by Cohen and Stanley[2] and beef spleen ribonucleic acid may be due to inherent structural differences or to factors introduced in the isolation procedure.

Ribonuclease liberates 1.5×10^{-6} equivalents of titratable acid per mg of spleen ribonucleic acid under conditions which in similar experiments with commercial yeast ribonucleic acid yields 7×10^{-7} equivalents of titratable acid per mg. Presumably the spleen ribonucleic acid preparation has approximately twice as many linkages susceptible to nuclease degradation as yeast ribonucleic acid. The mononucleotide products of ribonuclease degradation of yeast ribonucleic acid have been determined by ion exchange and chromatographic procedures to be predominantly uridylic and cytidylic acids (Carter and Cohn, 6), in agreement with the data of Schmidt (7). By chromatographic procedures[6] the mononucleotide product of the nuclease degradation of spleen ribonucleic acid has been found to be uridylic and cytidylic acids, although small amounts of adenylic and guanylic acids would escape detection under the conditions employed. It has been found that ribonuclease liberates approximately 30 per cent of the cytidylic acid and 20 per cent of the uridylic acid as free mononucleotides from spleen ribonucleic acid.

The composition of spleen ribonucleic acid has been determined by methods of quantitative paper chromatography. In comparison with several preparations of yeast ribonucleic acid which have been examined by these techniques, spleen ribonucleic acid contains a much higher molar proportion of pyrimidine nucleotides and a reversal of the uridylic acid to cytidylic acid ratio. Although the composition of yeast nucleic acid has been found in this laboratory to vary with the method of preparation, the following molar proportions determined by the analytical scheme described below for a "purified" preparation are fairly typical; adenine, 2.04; guanine, 1.54; uridylic acid, 0.85; and cytidylic acid, 0.70. These results are not in agreement with those of Vischer and Chargaff (8). Spleen ribonucleic acid has a molar proportion of adenine 1.54, guanine 1.55, uridylic acid 0.87, and cytidylic acid 1.31. In view of the finding that ribonuclease liberates predominantly pyrimidine nucleotides from ribonucleic acid, the more extensive degradation of spleen ribonucleic acid as compared with yeast ribonucleic acid may be attributed to the higher proportion of pyrimidine nucleotides in spleen ribonucleic acid as well as to the higher molecular weight (more intact susceptible linkages).

Since the classical statement of the "statistical" tetranucleotide structure of yeast ribonucleic acid by Levene (9) several investigators have presented evidence which was not in agreement with such a simplification. Gulland et al. (10) through titrimetric and elementary analysis concluded that a tetranucleotide structure was untenable, and Vischer and Chargaff[8], employing techniques of quantitative paper chromatography, found molar proportions of the basic constituents of ribonucleic acids which did not support a tetranucleotide structure. Although the evidence presented in this paper for the composition of spleen ribonucleic acids is at variance with the equimolecular proportions of the nucleotides required by the Levene hypothesis, judgment regarding the ultimate validity of the tetranucleotide structure of ribonucleic acids might be withheld until the influence of preparative methods on the analytical composition of the nucleic acid has been further studied.

Experimental work on this problem is herein reported. The method of isolation of ribonucleic acid from a spleen homogenate consists in (a) the removal of desoxyribonucleic acid as a nucleic acid protein complex (11), (b) the solubilization of the protein in the presence of a high concentration of guanidine hydrochloride at 38° C, (c) precipitation of the guanidine ribonucleate salt in the cold, and finally (d) further purification of the ribonucleic acid by chloroform extraction (12).

This procedure has been found applicable to a number of mammalian tissues, the results of which will be presented in another publication. The method of preparation of ribonucleic acid from calf spleen is presented below in detail:

Frozen calf spleen (200 g) is cut in small pieces and blended for eight to ten minutes with two volumes per gram tissue of 0.15 M NaCl-0.03 M phosphate buffer, pH 6.8. The homogenate is filtered through four thicknesses of cheese cloth, then centrifuged at 3000 g for sixty minutes. By this process the desoxyribonucleic acid is removed in the form of an insoluble nucleic acid-protein complex as described by Mirsky and Pollister[11]. All operations are carried out between 2 and 6° C.

To the supernatant solution, enough solid guanidine hydrochloride is added, with rapid stirring, to make the solution four molar with respect to guanidine hydrochloride. The solution is then placed in a 38° C water bath for one hour. Under these conditions the proteins of the splenic extract are made soluble by the high concentration of guanidine hydrochloride, while the ribonucleic acid is split from its protein linkage and combines with the guanidinium ion. After heating at 38° C the solution is immediately chilled

at 0 - 2° C and allowed to stand at this temperature for one hour. The guanidine ribonucleate precipitates and can readily be centrifuged out at 3000 g for 20 minutes. The precipitate is washed with a solution of four M guanidine hydrochloride in pH 6.8 saline-phosphate buffer, then dissolved in water. The somewhat turbid solution is allowed to dialyze exhaustively against water after which some insoluble denatured protein is removed by centrifugation. The supernatant solution containing the ribonucleic acid is slightly yellow colored, and further purification is achieved by adjusting the acidity of the solution to pH 4.2 and precipitating the nucleic acid with two volumes of cold ethanol. Upon redissolving in water, additional protein remains insoluble and can be removed by centrifugation.

The final traces of protein can be removed by extracting the aqueous solution of nucleic acid with chloroform-octyl alcohol[12]. A solution of nucleic acid in water is shaken with an equal volume of a 5:1 chloroform-octyl alcohol mixture for one hour. The mixture is centrifuged and the upper aqueous layer containing the nucleic acid removed. The contaminating protein remains as a precipitate at the interface. The ribonucleic acid is then reprecipitated by adjusting the solution to pH 4.2 and adding two volumes of alcohol. The precipitate is washed with alcohol and ether and dried in a vacuum desiccator.

The dried ribonucleic acid is a white powder which readily dissolves in water to give a water-clear solution. From 200 g of whole spleen a yield of about 500 g of purified ribonucleic acid is obtained. The nucleic acid preparation gives an intense orcinol reaction (13) while even the most concentrated solutions failed to give a reaction with diphenylamine reagent (14), indicating that all of the nucleic acid is of the ribose type.

The spectrophotometric assay procedure is described. In figure 1 are shown the ultraviolet absorption spectra of (a) nucleic acid, precipitated once with alcohol but not purified by chloroform extraction, and (b) the final product, purified by chloroform extraction. It can be seen that the absorption curve of the purified product is quite characteristic of a nucleic acid. The presence of protein in the impure material can be readily detected by the markedly increased absorption at wave lengths below 240 mμ.

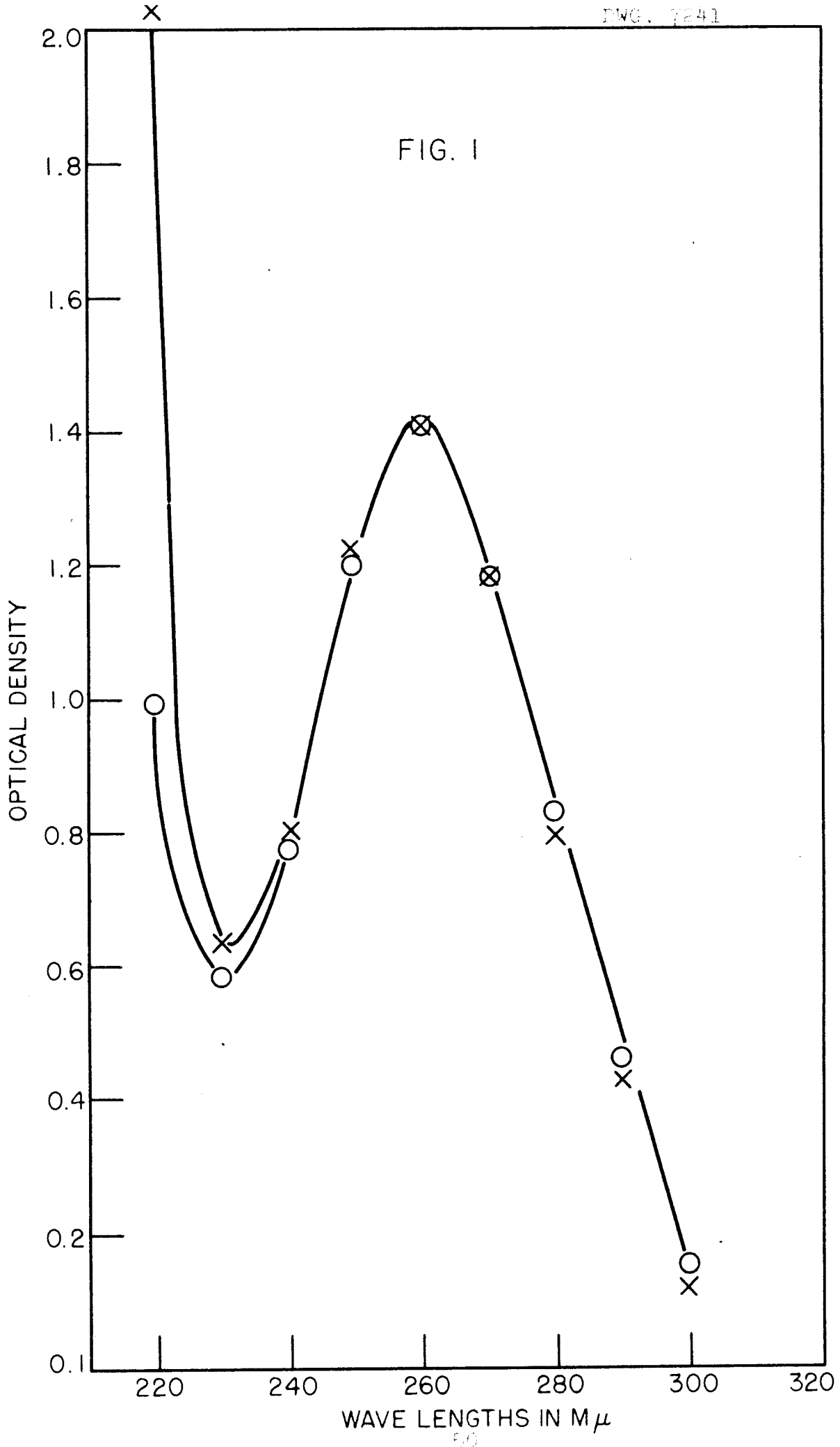
The degree of molecular homogeneity of the ribonucleic acid preparations was determined by means of the analytical ultracentrifuge. Figure 2 shows the sedimentation-velocity photographs of the nucleic acid preparations described above, before purification by chloroform extraction (A), and after chloroform

FIGURE 1

The ultraviolet absorption curves of spleen ribonucleic acid preparations.

X = Crude preparation, before chloroform extraction.
Concentration = 0.08 mg per cc.

O = Purified preparation, after chloroform extraction.
Concentration = 0.07 mg per cc.


DWG. 7241
FIG. 1
OPTICAL DENSITY
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.1
220
240
260
280
300
320
WAVE LENGTHS IN Mμ

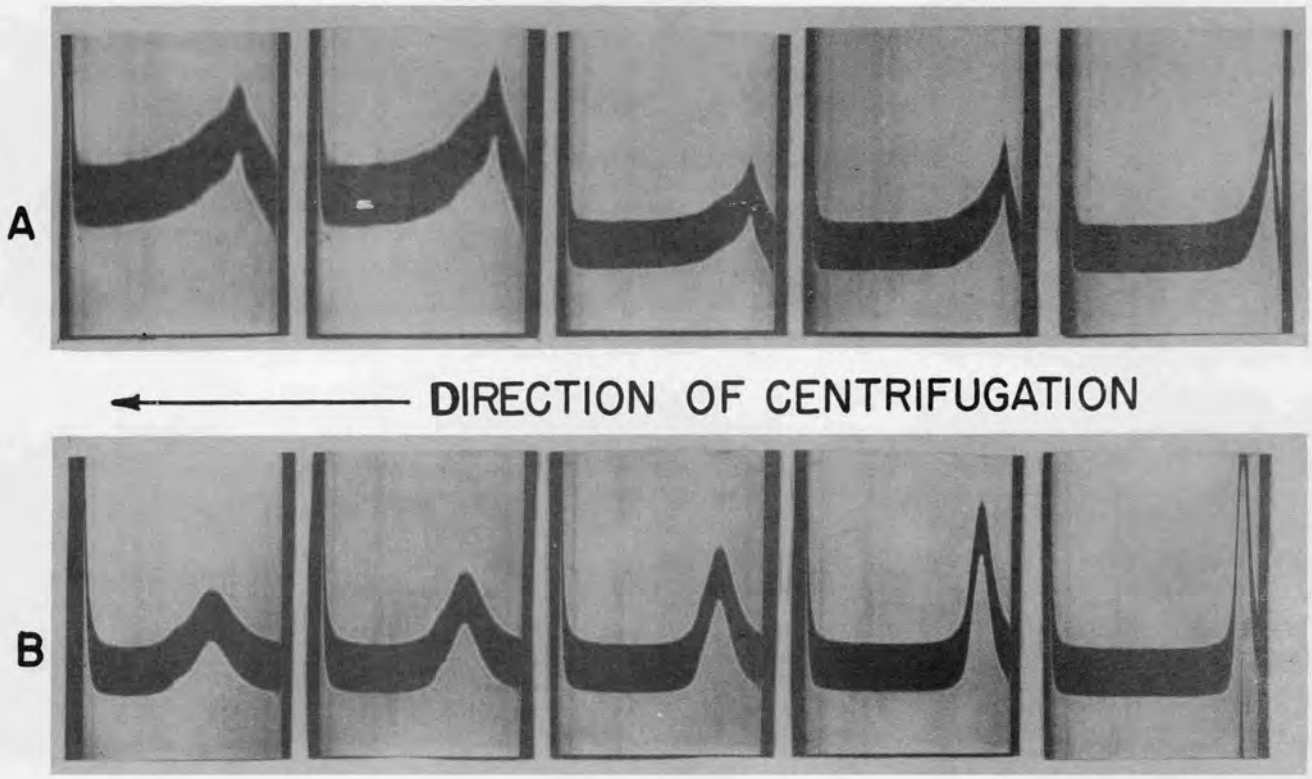


FIG. 2

Analytical Ultracentrifuge Photographs of Spleen Ribonucleic Acid Preparations

A. This mean ultracentrifugal field was 185,500 g. Photographs were taken at 8-minute intervals. The inclined bar in the Philpot-Svensson optical system was at a 65° angle for the first three pictures and at a 45° angle for the last two. The concentration was 12 mg per cc, dissolved in 0.2 M NaCl 0.05 M phosphate buffer, pH 6.7.

B. The mean centrifugal field was the same as in A, while the photographs were taken at 16-minute intervals and the angle of the inclined bar was 65° throughout. The concentration was 15 mg per cc, dissolved in the same buffer as A.

extraction (B). The conditions of the experiments are given in the text of the figure. The photographs of the impure nucleic acid (A) show the presence of a major component with a sedimentation constant $S_{20,w} = 3.11$, along with about 40 per cent of faster sedimenting material. Since the spectrophotometric assay of this material indicates the presence of about 85 per cent ribonucleic acid it may be inferred that the faster moving components are protein-nucleic acid complexes. Photographs of the purified material (B) indicate that only about five per cent of the nucleic acid preparation is a faster moving component. The sedimentation constant of the major component is $S_{20,w} = 3.09$. In the absence of diffusion data, it may be estimated that the molecular weight of the ribonucleic acid is of the order of magnitude of 50,000.

The composition of spleen ribonucleic acid has been determined using methods of quantitative paper chromatography similar to those by which Vischer and Chargaff[8] and Hotchkiss (15) first demonstrated that purines and pyrimidines could be resolved on a paper chromatogram, eluted and determined in the ultraviolet spectrophotometer, thereby providing a quantitative analytical procedure for the basic derivatives of nucleic acids. The conversion of nucleic acids to free purines and pyrimidines is rendered difficult by the resistance of the pyrimidine nucleotides to hydrolysis. Mild acid hydrolysis converts ribonucleic acid to adenine, guanine, uridylic and cytidylic acids. It has been found that an alcohol-citrate solvent system (pH 3.5) resolves these components which result from acid hydrolysis and that they may be detected by fluorescence and quantitated by ultraviolet spectrophotometry as previously described (16).

The solvent system employed consists of a mixture of 200 ml of ethanol and 40 ml of a solution of citric acid adjusted to pH 3.5 with concentrated ammonium hydroxide. The chromatogram is developed by ascending capillary flow of the solvent in an atmosphere saturated with butanol vapor in a constant temperature room for a period of about 20 hours. The components were detected by fluorescence[16], adenine and guanine eluted in 5.0 ml of 1.0 N hydrochloric acid, and uridylic and cytidylic acids in 5.0 ml of 0.01 N hydrochloric acids. By these procedures standards were recovered to the extent of 94 - 97 per cent. The samples for analysis were also chromatographed in solvent systems previously described[16] to exclude the possibility of formation of hydrolytic products other than those indicated in the alcohol-citrate system.

For analysis, a 50-mg sample of spleen ribonucleic acid (not critically dry) was hydrolyzed with 1.0 ml of 1.0 N sulfuric acid in a sealed tube at

100° C (air) for one hour. The hydrolysate was then adjusted to pH 2.5 and to a volume of approximately 5.0 ml. From this solution two 1.0-ml aliquots were taken for nitrogen and phosphorus analyses, and three 0.025-ml aliquots for chromatographic analysis. Longer hydrolysis periods result in the formation of easily detectable amounts of hypoxanthine and uracil. Although the recovery of components from the chromatogram calculated as nitrogen equivalents is adequate for an analytical procedure, it should be emphasized that a failure to recover three per cent nitrogen might give strikingly erroneous conclusions if this were all represented by one pyrimidine nucleotide.

Although data of the type recorded in table 1 contribute to an elucidation of the structure of nucleic acids, the nucleotide composition is not reflected completely, for it has been shown that ribonucleic acid from yeast contains two adenylic acid isomers[16]. Spleen ribonucleic acid was subjected to hydrolysis by 1.0 N NaOH at 37° C for 15 hours. The nucleotides were then isolated as the barium salts and regenerated in sulfuric acid. This solution was chromatographed in an isoamyl alcohol-disodium phosphate solvent system[16] and the two adenylic acids in approximately equimolar proportions, corresponding to those present in yeast ribonucleic acid, were detected.

In figure 3 the time course of the enzymatic degradation of purified beef spleen ribonucleic acid by crystalline ribonuclease is shown. The enzymatic hydrolysis of spleen ribonucleic acid liberates a total of 1.5×10^{-6} equivalents of H^+ ions per milligram of nucleic acid, which is twice the amount found when the enzyme acts on commercial yeast ribonucleic acid[6]. This difference may be attributed to either the inherent structural differences of the two nucleic acids or to the fact that spleen ribonucleic acid by virtue of its higher molecular weight and presumably less degraded state possesses more susceptible bonds per milligram of substrate.

After the enzymatic reaction had gone to completion (120 minutes) the digest was analyzed for the mononucleotide components by the method of paper chromatography. This analysis revealed that uridylic and cytidylic acids are split from spleen ribonucleic acid by ribonuclease. Approximately 30 per cent of the cytidylic and 20 per cent of the uridylic acid contained in the substrate molecule in polynucleotide linkage are liberated as mononucleotides during the course of enzymatic degradation. These data, in table 2, are essentially in agreement with the finding of Carter and Cohn[6] regarding the products of the enzymatic degradation of yeast ribonucleic acid by ribonuclease.

In summary, a new method of preparation of ribonucleic acids from mammalian tissue involving their precipitation as guanidine salts is described.

With this technique a purified ribonucleic acid from calf spleen has been prepared which is essentially homogeneous in the analytical ultracentrifuge

TABLE 1

Quantitative paper chromatographic analysis of spleen ribonucleic acid

COMPONENT	RF	Micrograms	SPLEEN RIBONUCLEIC ACID 0.025 ml HYDROLYSATE ANALYZED Moles x 10^{-7}	Nitrogen equivalent
Adenine	0.60	20.8		
		20.4		
		21.3		
		Av. = 20.8	1.54	10.7
Guanine	0.41	25.0		
		21.8		
		23.4		
		Av. = 23.4	1.55	10.9
Uridylic	0.31	26.7		
		28.1		
		33.1		
		Av. = 29.0	0.87	2.52
Cytidylic	0.17	41.2		
		43.4		
		Av. = 42.3	1.31	5.54

Total nitrogen put on paper (0.025 ml hydrolysate) = 31.4

Total nitrogen recovered = 29.66
per cent recovery = 94.00

Adenine/Guanine = 1.0

Uridylic/Cytidylic = 0.66

FIGURE 3

The enzymatic degradation of spleen ribonucleic acid by crystalline ribonuclease

Forty mg of ribonucleic acid was dissolved in 2.5 cc of water and the solution adjusted to pH 7.5 with dilute NaOH. Then 2 mg of solid crystalline ribonuclease was added to the solution under stirring. As the reaction progressed the solution was maintained at pH 7.5 by the addition of 0.01 N NaOH from a 1.0-ml burette graduated in divisions of 0.01 ml. The additions of 0.01 N NaOH with time are shown in the figure.

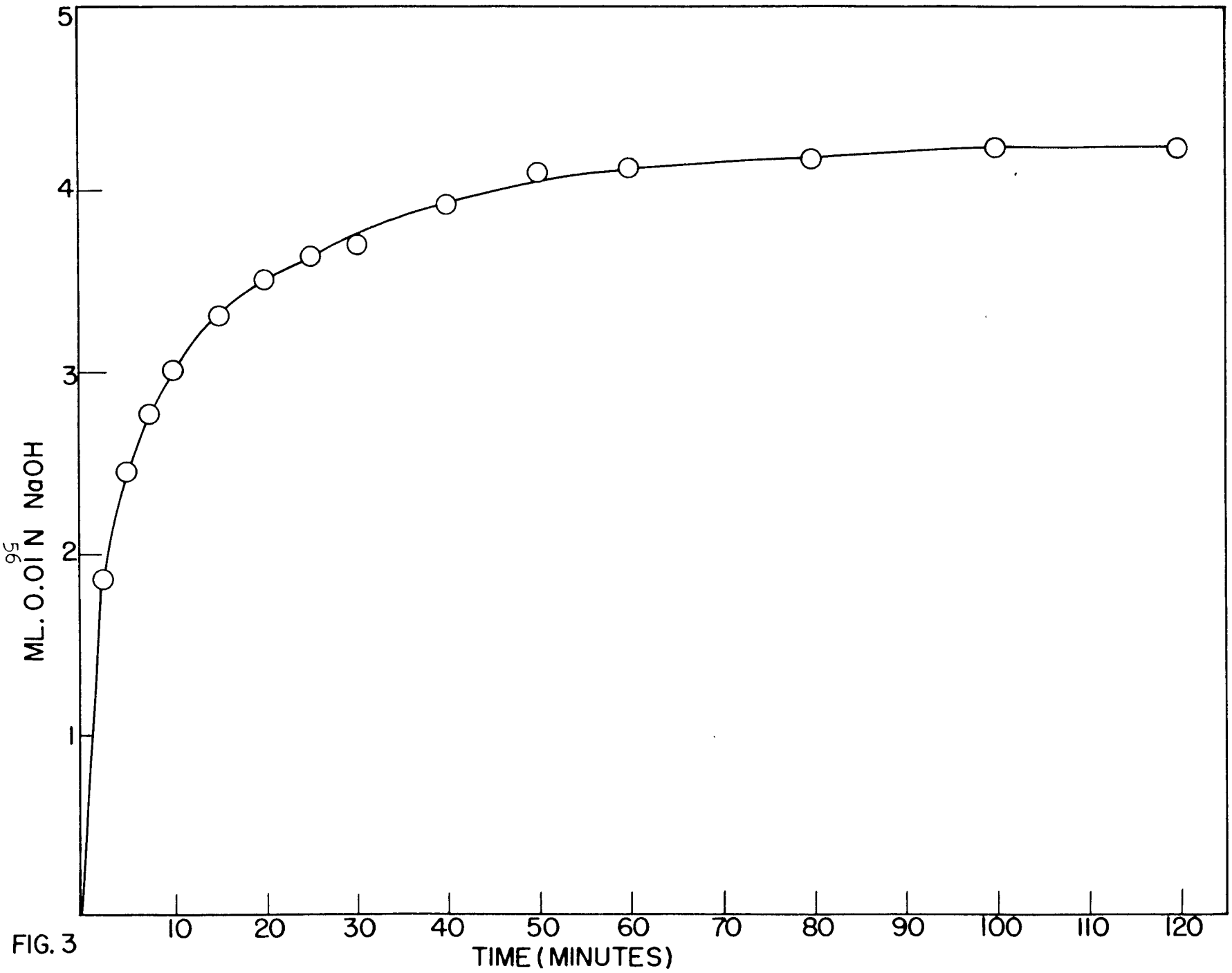


FIG. 3

NOT CLASSIFIED
DWG. 7242

TABLE 2

Quantitative paper chromatography of the degradation products of spleen ribonucleic acid by ribonuclease(1)

COMPONENT	RF (2)		Ratio optical density $\frac{2800\ A}{2600\ A}$ (3)			Per cent (4) Hydrolyzed
	Std	Digest	Std	Digest	Amount	
URIDYLIC	.31	.31	.33	.31	8.2 γ	20.1
CYTIDYLIC	.17	.17	1.8	1.85	19.0 γ	30.6

(1) Experimental conditions described in legend to figure 3.

(2) RF values are referred to a bright fluorescent line which appears below the solvent front.

(3) The ultraviolet absorption spectra of the components eluted from the chromatogram were determined and the ratio of the optical density at 2800 A and 2600 A recorded. This value which is characteristic for each nucleotide is necessary for the identification of the component in the cytidylic region of the chromatogram, for adenylic and guanylic acids also appear in this region. If small amounts of the latter two nucleotides were present the 2800/2600 A ratio would be below 1.0. As shown in the table, the ratio 1.85 indicates that the spot is exclusively cytidylic acid. A chromatogram run in butanol, where only free bases and nucleosides move, showed that none of these components were present.

(4) A 0.06 ml sample of the digest was chromatographed which is equivalent to 358 micrograms ribonucleic acid containing 58.8γ cytidylic acid and 40.8γ uridylic acid.

and which exhibits a sedimentation constant of 3.09, indicating a molecular weight of about 50,000.

The analytical composition of the spleen ribonucleic acid was determined by a method of quantitative paper chromatography. It was found that the basic constituents were present in the molar ratio; adenine 1.54, guanine 1.55, cytidylic acid 1.31 and uridylic acid 0.87.

The enzymatic degradation of the spleen nucleic acid by crystalline ribonuclease resulted in the splitting off of approximately 30 per cent of the cytidylic and 20 per cent of the uridylic acids contained in the substrate molecule.

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Investigation of the equilibria involved in the formation of enzyme substrate complexes. (Vaslow and Doherty) An investigation of this nature requires high activity in the labeled substrates and therefore should be carried out close to the source of active material. Bromine has been selected as the labeling substance because its gamma emission can be measured directly. The system to be studied is the synthesis of peptide anilides and the hydrolysis of the corresponding peptide amides. The bromoanilines are to be used as the labeled substances, therefore conditions for their preparation on a microscale are being investigated. A new series of meta- and parabromo amino acid anilides has been prepared demonstrating that bromine on the phenyl ring does not interfere with synthesis by papain.

Chemical synthesis and identification of new nucleic acids. (Doherty) Two nucleic acids differing from those previously reported in the literature have been isolated by Drs. Carter and Cohn using the techniques of paper chromatography and ion exchange resins. A few degradation studies performed by Dr. Carter seem to indicate that the difference resides in the position of attachment of the phosphate residue to the ribose molecule. Chemical proof of this will be established when the new acids have been both degraded to known compounds and synthesized by unequivocal methods.

As the most probable isomer, barring ring shifts or alpha-beta isomerization, is adenosine 2-phosphate, attention is being devoted to its synthesis. Benzylidene adenosine has been prepared and will be phosphorylated in the near future. The only hydroxyl free to react in this compound is the 2 OH, so that phosphorylation will yield only the 2-phosphate. Benzylidene guanosine has also been prepared and will be phosphorylated.

Degradation of the new nucleic acids should yield ribose 2-phosphate if the above suppositions are right. Therefore it is desirable to prepare this compound by other methods for comparative purposes. Four methods are theoretically available; from the borate or orthoacetate complexes of ribofuranose, from D arabinose through a Walden inversion, from D arabonic acid through epimerization, and from D xylose through a Walden inversion. Work is in progress on all four methods. D arabinose has been synthesized from calcium gluconate and converted successively into methyl arabinoside, monoacetone methyl arabinoside and monoacetone, 2-tysyl methyl arabinoside. D xylose has been converted to diacetone xylose, monoacetone xylose and 5-benzoyl monoacetone xylose.

RADIOBIOCHEMISTRY

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Leon Wish Maryann Huddleston
Gertrude Beyl

Determination of the disintegration rates of Co^{60} and P^{32}. (Wish, Burnett and Tompkins) The purpose of this investigation is to compare the best known present methods for the determination of absolute activities with those as used by the Experimental Biology Section of the University of Chicago during and immediately after the war. It has been known for some time that the absolute disintegration rate of a source as a function of the counting rate as determined with the standard Geiger-Muller tube is given by the follow- relation:

Equation 1.

$$d/m \quad \frac{c/m}{G\ f_W\ f_A\ f_B\ f_H\ f_S}$$

where f_W is the correction factor for the apparent beta particle absorption by the window of the Geiger-Muller tube and by the air gap between the window and the source; f_A is the factor for the scattering of the particles by the air into the counter; f_B is the factor for the backscattering effects due to the material on which the source is mounted; f_H is the effect of the scattering by the walls and support which house the counter; f_S is the effect of the absorption and backscattering arising from the mass of the source itself, and G is the solid angle subtended by the source and the window of the tube. G is often referred to as the "geometry" and merely signifies the fraction of the particles emitted by the source which enter the tube.

No extensive study was made during the war to evaluate the above mentioned factors and therefore it was necessary to make several assumptions in determining the absolute disintegration rates of various radioactive isotopes. With this in mind we have undertaken this work in order to check on the validity of their methods since Zumwalt and coworkers have now thoroughly investigated the actual values of the various factors involved in equation 1. At the same time the performance of our instruments can be checked and calibrated using isotopes of known decay schemes, and emitting both beta and gamma radiations. The disintegrations rates can then be measured independently on the Geiger-Muller counter and an ion chamber properly calibrated for the gamma radiation.

The work of Peacock et al. was repeated with Co^{60}. Our chamber was checked against a similar one previously calibrated by Overman and Jones. The disintegration rate of the Co samples was then determined using Zumwalt's pro-

cedure for mounting the sample and his method of extrapolation of the observed counting rate. The counter geometry, G, was determined by means of a RaD-RaE standard mounted on Al. The disintegration rates thus measured independently differed by ± three per cent based on the average of four determinations.

The counter efficiency was then determined by separating UX_2 from a known quantity of uranium and mounting it in a Coors No. 0 porcelain capsule. The absorption curve was then extrapolated to zero absorber by a straight line running through the 48 mg/cm² added Al absorber in the manner previously used in Chicago. The activity of a carrier free P^{32} solution was then measured by comparing an aliquot mounted in a porcelain capsule with the UX_2 standard mounted in the same way. Another aliquot was measured by the Zumwalt procedure against both the RaE standard and a UX_2 standard which had also been mounted as a point source on polystyrene. Other aliquots were submitted to the Bureau of Standards. The results of these measurements as well as the value originally placed on this sample by the Operations Division of this Laboratory are shown in table 1. The point source method gave a value seven per cent lower than that obtained by either the Bureau of Standards (source mounted on Ag and compared with RaE assuming that both have the same saturation backscatter) or the Chicago extrapolation using a spread source mounted on porcelain. We feel the lower value to be more nearly correct since the counter geometry obtained by Zumwalt's procedure is essentially independent of energy whereas the other procedures assume that both RaE and P^{32} radiations will vary in the same way and by the same amount. Also the disintegration rate measured by Zumwalt has been shown to be only two per cent above that determined calorimetrically (2). Our measurements indicate that values obtained by the Bureau and Chicago should be ten per cent too high since we have demonstrated that we could reproduce Zumwalt's results on Co^{60}. Therefore, we can assume that we have also reproduced his results with P^{32}. The same seven per cent difference for the point source method, incidentally, was obtained during an inter-Laboratory check on P^{32} which has just been conducted by the Bureau.

TABLE 1

Comparison of P^{32} absolute activities

1.	Zumwalt Method	2.39
2.	Bureau of Standards	2.56
3.	Chicago Method	2.58
4.	Operations Division, ORNL	2.40

The next isotope checked was I^{131}. The I^{131} samples for beta determinations were prepared with one or ten mg KI/ml and aliquots of each were evaporated on polystyrene. The ion chamber efficiency for I^{131} was calculated from the revised decay scheme of Metzger and Deutsch. The results are shown in table 2.

TABLE 2

Method	*Efficiency of ion chamber*	
Overman and Jones	0.375	4 per cent
Beta determination	0.362	7 per cent
Calculated (M & M Scheme)	0.388	

A low value for the chamber efficiency as determined from beta counting reflects a higher μc value for I^{131}. The large uncertainties in the internal conversion factors reported by Metzger and Deutsch make the calculated efficiency subject to a possible error of several per cent. This error will give a disintegration rate higher than the true value and hence a lower efficiency. Since the value listed by Overman and Jones, 0.375, was determined by coincidence counting, it should agree with the beta determination, and should certainly be within ten per cent of the correct value.

A sample sent to the Bureau of Standards was reported back as 36.0± six per cent μc/ml. We obtained 34.8 μc/ml from the gamma measurement and a 0.388 efficiency, 36.0 from a 0.375 efficiency and 36.9 from the beta determination. This is considered satisfactory agreement under the circumstances since the ± six per cent range set by the Bureau bracketed the complete range of our possible values.

The work was then extended to the measurement of S^{35} using preliminary measurements on Cb^{95} which had a 0.15 Mev beta ray and 0.758 Mev gamma ray. The disintegration rate of a sample of pure Cb^{95} provided by Ira Whitney was determined through the ion chamber. Aliquots of this sample were then measured on different counters with different window thicknesses using both the RaE standard and Co^{60} standard to check the geometry of the counter. The absorption curve of the Cb^{95} was extrapolated graphically to zero absorber since Zumwalt's equations do not hold for 0.15 Mev beta radiation. The results of table 3 show that empirically, the 0.15 Mev beta absorption curve could be graphically extrapolated to zero absorber thickness as it is assumed that both S^{35} and C^{14} samples mounted in the same way would do likewise. An unknown

quantity of high specific activity $BaC^{14}O_3$ carbonate was then sprinkled on a polystyrene tape and sealed on with colodion. A sample of carrier-free S^{35} was then prepared in the usual way on polystyrene. The absorption curves for Cb^{95}, S^{35}, and C^{14} were found to superimpose at absorber thicknesses less than 10 mg/cm² Al equivalent. Therefore, the S^{35} curves, and C^{14} curves were extrapolated in the same manner as the Cb^{95}, and a value for the disintegration rate of the S^{35} established from the known disintegration rate of the Cb^{95} with which it was compared. In the same way, several of the C^{14} samples were determined to be equivalent to an established number of μc of S^{35}.

TABLE 3

Beta determinations mc/cc	*Gamma determinations mc/cc*
Counter 1	
37.4	37.0
37.7	"
35.7	"
34.2	"
Average = 36.3	
Counter 2	
36.4	"
38.8	"
36.3	"
35.1	"
Average = 36.7	

The C^{14} samples now provide secondary standards for S^{35}, and the absolute activity of any properly mounted S^{35} sample can be measured under any conditions where the C^{14} and S^{35} absorption curves superimpose by direct comparison of counting rates.

The activity of S^{35} sources as measured by direct comparison with the C^{14} secondary standard under different conditions is shown in table 4.

TABLE 4

S^{35} Activity determinations on different G.-M. counters using C^{14} standards

	COUNTER 1	COUNTER 2		COUNTER 3
Window Thickness (mg/cm^2)	2.0	1.6	3.0	2.8 + aquadag
μc used	0.268	0.067	0.134	0.067
μc determined				
Shelf 1	-	0.067	-	0.068
Shelf 2	0.255	0.067	0.136	0.067
Shelf 3	0.259	0.068	0.134	0.069
Shelf 4	0.270	0.074	0.143	0.069

BIOLOGICAL SYNTHESIS

G. R. Noggle (Leader) R. A. Bolomey

Isolation and purification of C^{14}-labeled carbohydrates from sugar beets. (Noggle and Bolomey) The biosynthesis program has been concerned with the separation and purification of glucose, fructose, sucrose and starch from the sugar beet plant. The separation of glucose and fructose has been attained through the use of Magnesol as an adsorbent and ninety-five percent ethanol or ethanol-benzene mixtures for the development of flowing chromatograms. Indiscriminately C^{14}-labeled glucose and fructose have been prepared from sugar beet leaves and are to be separated and purified by the above outlined method.

As a preliminary to the growing of sugar beets in artificially enriched atmospheres of $C^{14}O_2$, a comparative study of four sugar beet varieties has been initiated. Rates of growth, leaf size, sugar and starch content of leaves and roots and certain other physiological aspects of greenhouse plants are being investigated. It is hoped that from this study will come information regarding the best time to introduce the plants into the artificial environment chambers so as to attain a maximum yield of C^{14}-labeled compounds.

The induction of X-ray induced mutations in peanuts. (Noggle) The irradiation of peanuts for the North Carolina project of producing X-ray induced mutations has been completed. Preliminary irradiations of 1,000 seed lots at

2,500 r, 5,000 r, 10,000 r, 20,000 r, 40,000 r and 80,000 r were given. The X-ray tube burned out before the 40,000 r and 80,000 r levels could be treated. In order to expediate the problem, the tantalum gamma source was used. The gamma dose was determined with the vibrating reed electrometer by Dr. C.W. Sheppard. Five hundred seed lots of peanuts were irradiated with gamma rays at 2,500 r, 5,000 r, 10,000 r, 20,000 r, 40,000 r and 80,000 r. Both the X-ray and the gamma-ray treated peanuts were sent to the North Carolina State Experiment Station at Raleigh for germination and greenhouse tests. On the basis of the initial tests, Dr. W. C. Gregory, of the Raleigh Station, decided that 40,000 peanuts (approximately 100 pounds) were to be irradiated with 10,000 r of X-rays and another 40,000 peanuts with 15,000 r of X rays. A comparison of the X-ray and the gamma-ray data indicated that the gamma rays were more lethal (10,000 r of gamma rays were roughly equivalent to 20,000 r of X rays). It is hoped that this aspect of the problem can be more fully investigated. The final irradiations were all based on Victoreen measurements of X-ray beam. The 10,000 X rays were given with the Biology Division machine; the 15,000 r X rays were given with the Y-12 million-volt X-ray tube through the cooperation of F. E. Clark, V. C. Jackson and H. D. Whitehead.

BIOPHYSICS

PHOTOSYNTHESIS

W. A. Arnold (Leader and Section Chief)
A. S. Holt Imogene A. Brooks

Inhibition of chloroplasts by ultraviolet as a function of wave length. (Holt, Brooks, Arnold) Further work was done with the photometric apparatus to define more precisely the optimum conditions for assaying chloroplast activity. This was required because close to 10^4 ergs per mm^2 at 2537 A are needed from an ultraviolet monochromator to cause a measurable inhibition. In making such an exposure the activity loss of the chloroplasts with time must be a minimum in order to accentuate the inhibition by irradiation, and the absorption by the chloroplasts of the incident radiation must be not more than 15 per cent. This requires the use of extremely dilute preparations whose activity assays are close to the limits of measurement. With chlorophyll concentrations between 0.0023 to 0.0092 mg per cc the rate of dye reduction is proportional to the chloroplast concentration using 1.5×10^{-5} M 2,6 dichlorophenol 3^1-chloroindophenol. Such a dye concentration has an O_2 equivalent of 0.3 mm per ml. Unless the amount of chloroplast material needed for an assay can be reduced to near 0.0002 mg per ml it appears that an action spectrum cannot be obtained. The use of fresh leaves from other species in nature as sources of chloroplasts is planned in an attempt to obtain more highly active preparations.

It has been found that doses which completely inactivate dye reduction cause little or no inhibition of the catalase activity associated with chloroplasts.

Effects of ultraviolet radiation (2537 A) on algae. (Holt, Brooks, Arnold) A small Warburg apparatus has been installed which allows the quartz tube to be kept in a fixed position. This makes the radiation procedure independent of the apparatus used for photosynthesis measurements.

Results obtained with *Scenedesmus* show that the survival ratios of photosynthesis after radiation are independent of the cell concentrations used during the photosynthesis measurement. Aliquots containing 60, 12, 6 mm^3 of cells from a single radiated suspension show survival ratios all close to 36 per cent. This means that the ultraviolet inhibition of photosynthesis is the same of all light intensities.

Experiments were also undertaken to further elucidate the mechanism in photosynthesis that is inactivated. This consisted of finding the concentrations of HCN and NH_2OH required to inhibit photosynthesis 50 per cent in non-irradiated algae, and applying the same concentrations to radiated algae whose rate of photosynthesis had also been reduced to 50 per cent.

The preliminary results obtained with Scenedesmus showed that both of the poisons inhibited the irradiated suspensions by an amount close to that of the nonirradiated suspensions. This would seem to indicate that the inactivation per cell is a complete process and does not occur in a stepwise or gradual manner.

BIOPHYSICAL STUDIES ON SINGLE CELLS

C. W. Sheppard (Leader) W. R. Martin

Electrolyte disturbances in mammalian erythrocytes. (Sheppard, Martin) It has been shown by earlier investigators (Harris, Danowski) that when human blood is kept at about 5° C the potassium leaks from the cells into the plasma. We have investigated human blood at 6° C using the radioactive isotope. The exchange rates which were equal and opposite at physiological temperatures now differ. The transport from plasma to cells is practically zero, whereas the movement from cells to plasma is still appreciable which explains the earlier findings. In irradiated blood this movement from cells to plasma is accelerated. Electrolyte disturbances have been found by Ting in irradiated human blood at 6° C. So far we have been unable to detect any disturbance of electrolyte exchange in irradiated blood at 37° C, i.e., physiological temperature.

ULTRAVIOLET MICROSCOPY

R. W. Koza

Assembly and testing of equipment. The Daniel and Heidt lamp tank and control circuits were completed and put into use. Considerable time was spent in working out a satisfactory method for assembling the lamps, when the usual method proved unsatisfactory in this equipment.

Preliminary work on a suitable indicating instrument for use with the photocell has been carried out.

SPECIAL PROBLEMS

Seymour Benzer (Leader)

Ultraviolet irradiation of bacteriophage during intracellular growth. (Benzer, assisted by M. B. Hill and R. W. Whittle) Infection of a bacterium (*E. coli*, strain B) by a single particle of bacteriophage T_2 initiates a sequence of events culminating in lysis of the cell after a latent period of about 20 minutes (at 37° C) with simultaneous release of around 100 replicas of the original phage particle. Luria and Latarjet (1) had the idea that it might be possible to find the number of phage particles at a given stage in the latent period by exposing the infected bacteria to ultraviolet light and determining the fraction of infected bacteria which retain their ability to release viable phage. If the intracellular phage is inactivated in the same way as free phage (which has an exponential survival curve), then as the number of particles within the cell increases, there should be a transition from an exponential single-hit survival curve to nonlinear curves with shapes characteristic of the number of particles.

The actual results obtained by Luria and Latarjet were not readily interpretable according to this scheme. In the first few minutes after infection the survival curve remained exponential but the resistance to radiation increased very rapidly. At later times, a peculiar upswing developed in the survival curve and only toward the end of the latent period did the shape become similar to the expected multiple-target curve. Latarjet (2) repeated these experiments, using X rays, with very similar results.

Since then, two types of reactivation of ultraviolet inactivated phage have been discovered. Luria (3) found that if a single bacterium is infected with two or more irradiated phage particles, each alone incapable of producing viable phage, cooperation can occur leading to lysis with liberation of active phage (multiplicity reactivation). Evidently this must play an important role in experiments where infected bacteria are irradiated during the latent period. Dulbecco (4) has also found that ultraviolet inactivated phage, when singly adsorbed on bacteria, may be photoreactivated by light in the near ultraviolet and visible range. The role of light in the Latarjet and Luria experiments is therefore open to question.

In these experiments illumination was provided by a yellow light tested and found to produce very little photoreactivation. Bacteria were obtained from a two-and-one-half-hour-old culture in broth (1×10^8/ml), concentrated ten times by centrifugation to 1×10^9/ml in order to provide rapid adsorption

of phage. A phage sample (T_2) containing about one phage particle for every hundred bacteria was introduced (at 37° C) and one minute allowed for adsorption. The mixture was then heavily diluted in broth to stop further adsorption and a sample centrifuged, and the supernatant plated to determine the unadsorbed phage fraction (about ten per cent). The diluted culture was aerated at 37° C. At a particular time a sample was withdrawn, diluted in buffer to make the ultraviolet adsorption of the medium negligible, and irradiated with a germicidal lamp. The irradiated infected bacteria were then plated with a large excess of normal bacteria so that each bacterium in which the infection has survived irradiation produced a plaque. (The unadsorbed phage particles also form plaques, but corrections for these are easily made).

Figure 1 illustrates typical curves for survival of infection versus dose of ultraviolet at different times after infection. These results are very similar to those of Latarjet and Luria and it is seen that light is not primarily responsible for the peculiar behavior of the survival curve.

In order to specifically test for the effect of light in infected cells at different stages, experiments were run in the same way, placing half the plates under white fluorescent lights and keeping the other half in the dark, at room temperature. After an hour, all plates were placed in a dark incubator at 37° C overnight. Figure 2 illustrates some results. Photoreactivation is quite noticeable in the later stages. However, it is surprising to find that there is very little photoreactivation when the irradiation is done early in the latent period since at this time the phage particle would be expected to be not very different from a free phage particle. In figure 3 a comparison is given of the amount of photoreactivation obtained (1) when the phage is irradiated separately and adsorbed on normal bacteria and (2) when normal phage is adsorbed on normal bacteria and the infected cells irradiated six minutes after infection.

As Dulbecco[4] found, photoreactivation of ultraviolet inactivated free phage particles does not occur unless they are adsorbed on bacteria during exposure to the reactivating light. It is therefore possible that irradiation of the bacteria, while not interfering strongly with their ability to support growth of active phage (5), has a marked effect on their ability to participate in photoreactivation of irradiated phage.

To test this possibility samples of bacteria were subjected to various doses of ultraviolet light. Then each was infected with the same amount of an irradiated phage (T_2) stock inactivated to a survival of 10^{-2}. The infected bacteria were then plated with a large excess of normal bacteria. Then the

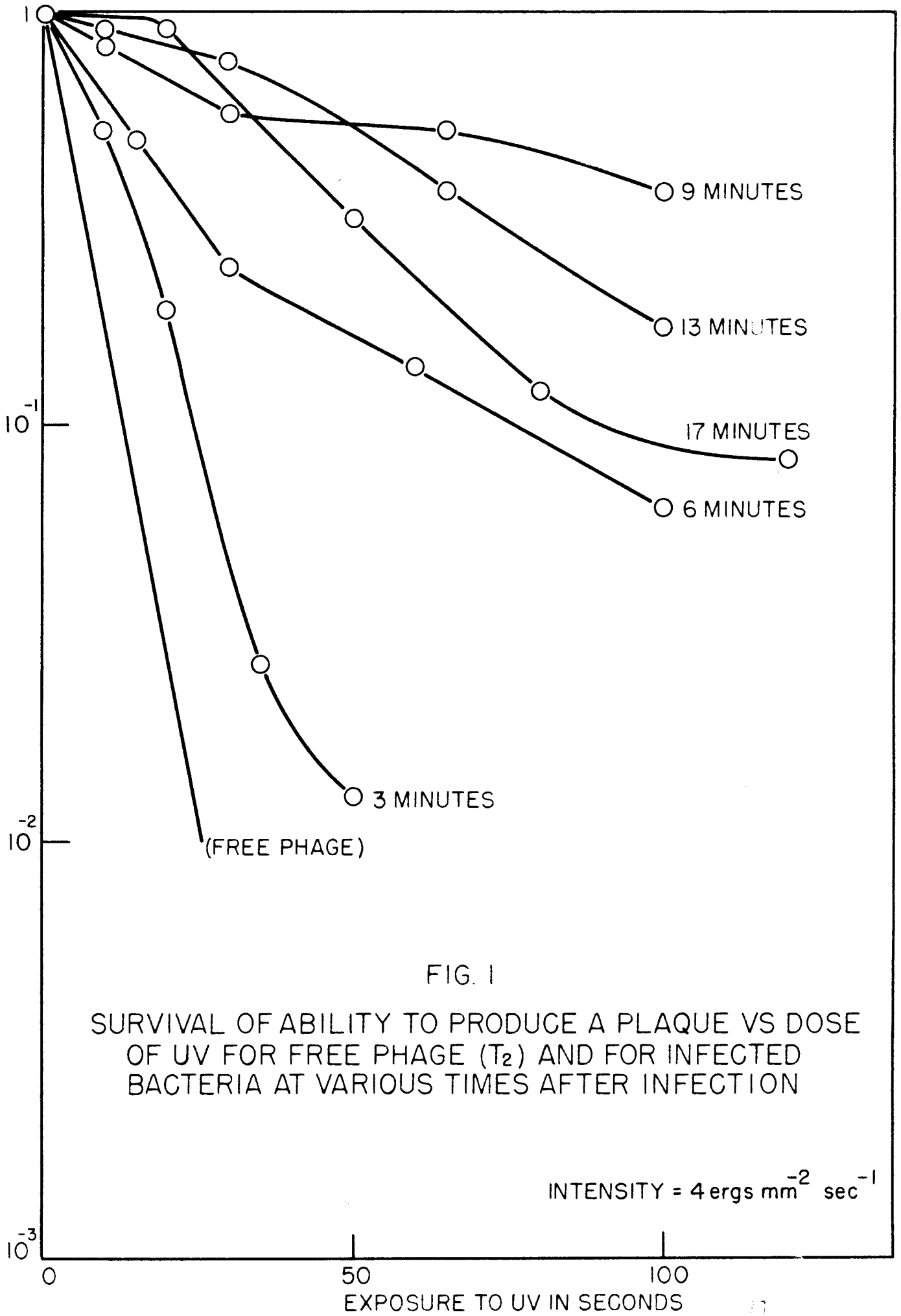

FIG. 1

SURVIVAL OF ABILITY TO PRODUCE A PLAQUE VS DOSE OF UV FOR FREE PHAGE (T_2) AND FOR INFECTED BACTERIA AT VARIOUS TIMES AFTER INFECTION

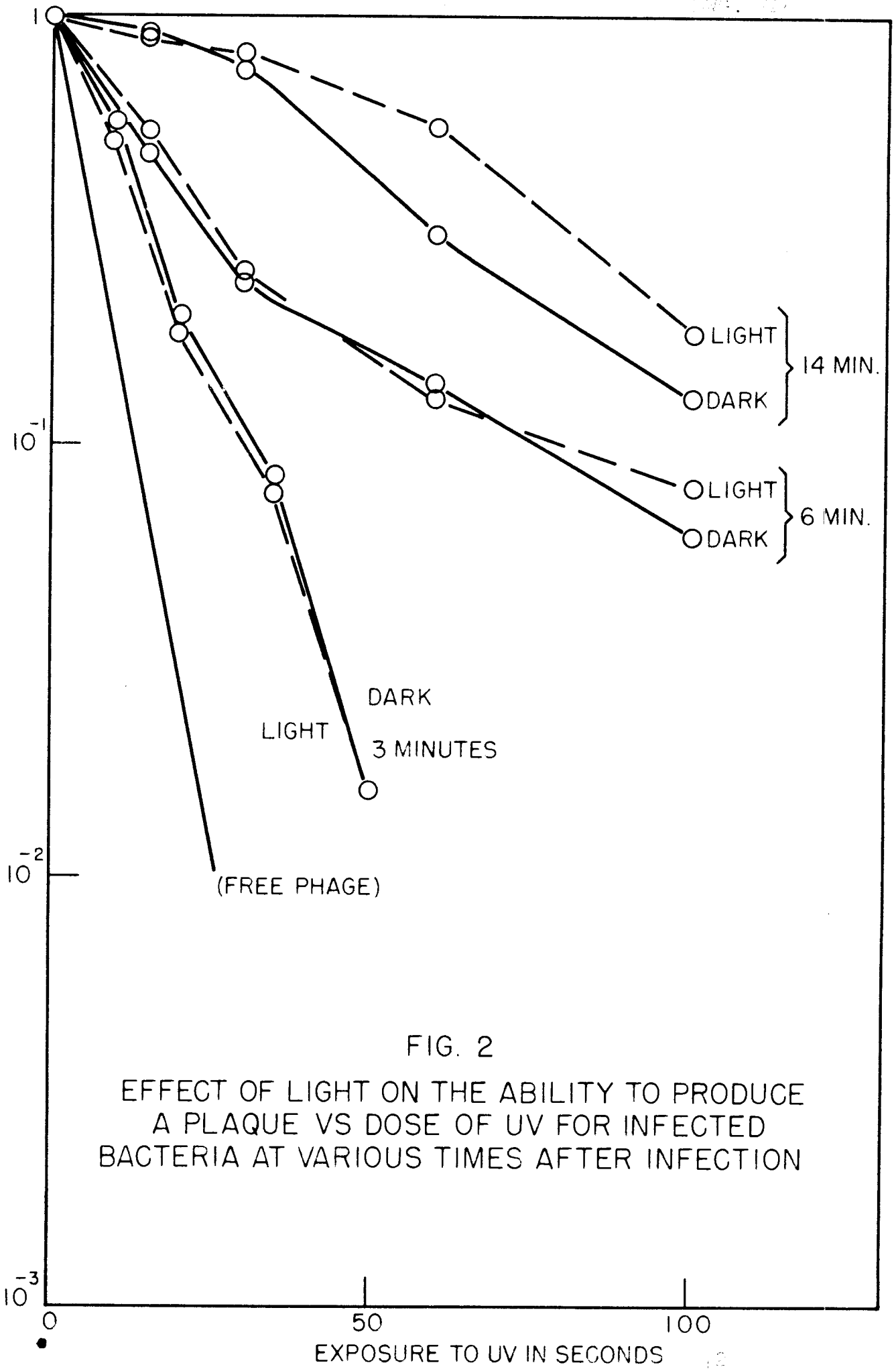

FIG. 2

EFFECT OF LIGHT ON THE ABILITY TO PRODUCE A PLAQUE VS DOSE OF UV FOR INFECTED BACTERIA AT VARIOUS TIMES AFTER INFECTION

plates were split into light and dark, as described above. In figure 4, the ability of the irradiated bacteria to produce photoreactivation of the phage is indicated by the vertical difference between the dark and light curves. Evidently, irradiation of the bacteria very rapidly reduces their ability to function in photoreactivation of irradiated phage and this phenomenon is probably responsible for the very small amount of photoreactivation when infected cells are irradiated. The rate at which the photoreactivation drops with ultraviolet dosage is comparable with the rate of inactivation of the ability of the bacteria to form colonies. This may be merely coincidental, however. Similar experiments using B/r, a radiation-resistant strain of *E. coli* (6), are in progress.

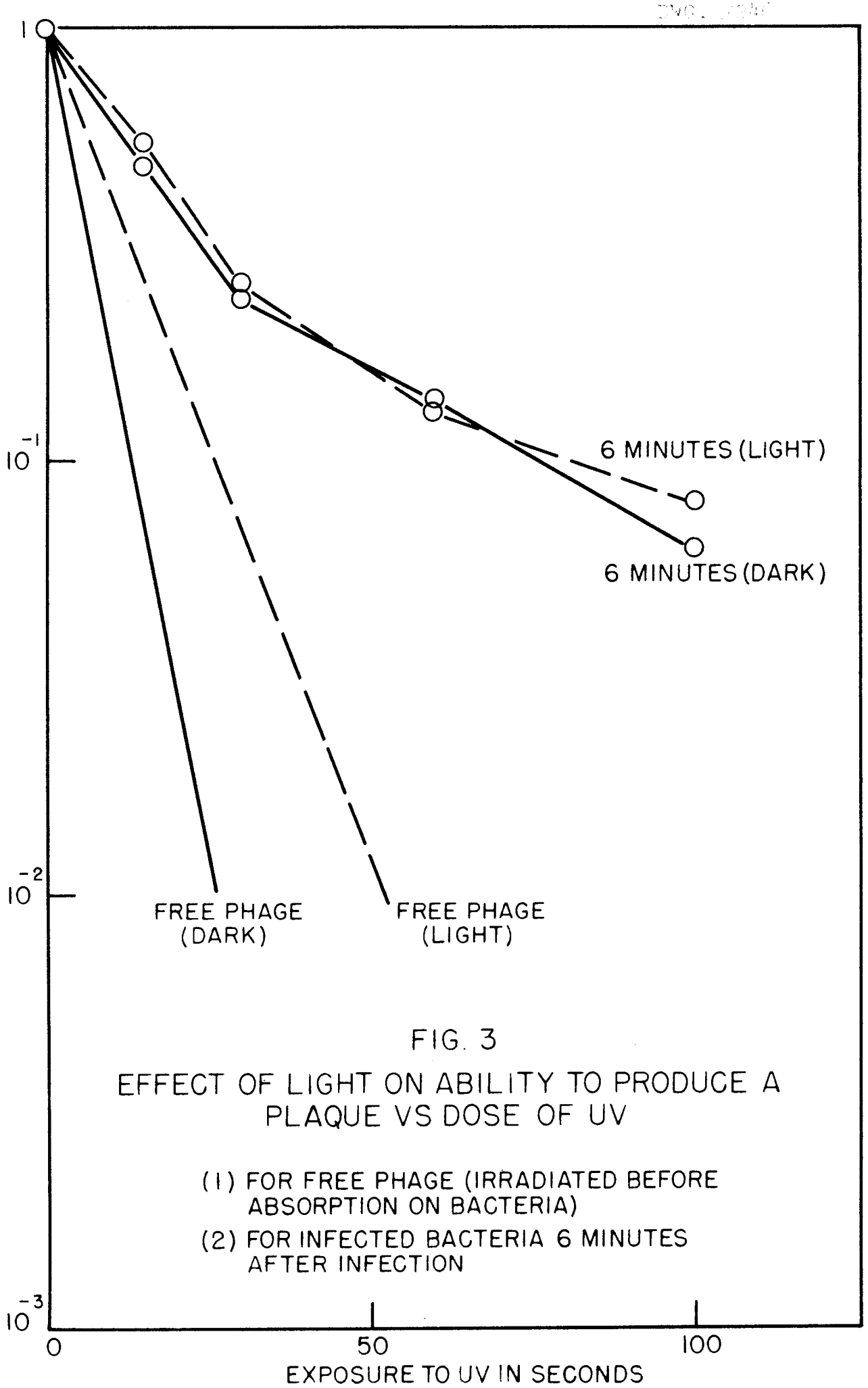

FIG. 3

EFFECT OF LIGHT ON ABILITY TO PRODUCE A PLAQUE VS DOSE OF UV

(1) FOR FREE PHAGE (IRRADIATED BEFORE ABSORPTION ON BACTERIA)

(2) FOR INFECTED BACTERIA 6 MINUTES AFTER INFECTION

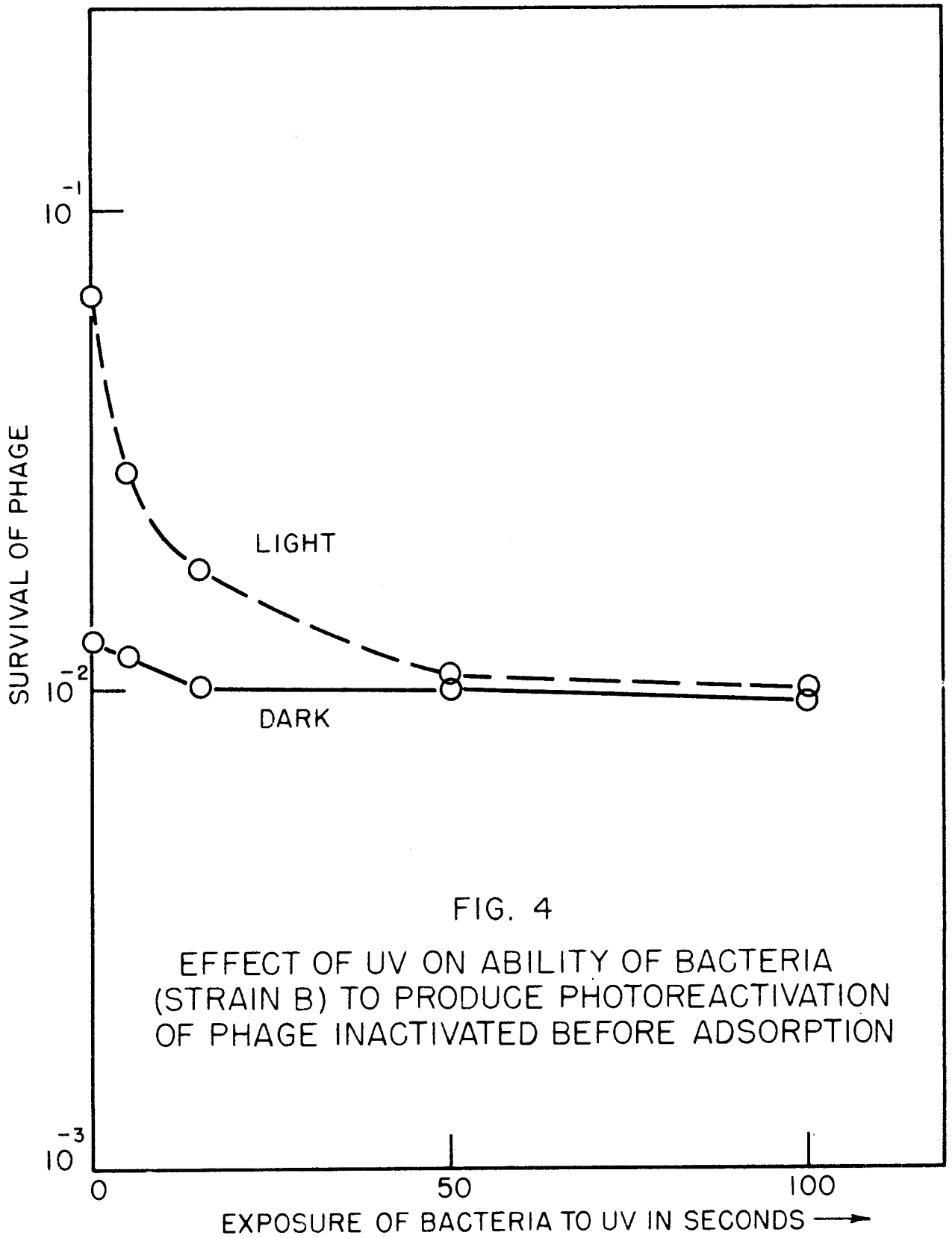

FIG. 4

EFFECT OF UV ON ABILITY OF BACTERIA (STRAIN B) TO PRODUCE PHOTOREACTIVATION OF PHAGE INACTIVATED BEFORE ADSORPTION

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PHYSIOLOGY AND PHARMACOLOGY

RADIATION SICKNESS

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Mammalian nongenetic irradiation injuries. (Kohn, Click, Kirslis, Lane, Ledford, Pigford, Swingley, Robertson, Gude) It was reported previously that a pattern of changes occurred in the plasma of the irradiated rat, involving glucose, NPN, chloride, total protein and A/G ratio. To these may now be added total cholesterol, which in the Sprague-Dawley strain rises from a normal value of about 115 mg per cent to 160 - 190 mg per cent on the third day following exposure to the LD-50 per cent dose of 250 KV X rays, remains elevated for several days, and finally returns to normal. The rise is approximately coincident with the rise in chloride, but is not maintained as long. It is not due to decreased food consumption.

It is now clear that the age at which hypophysectomy is performed influences the result. Mature animals, operated upon and allowed adequate time for the full effects to become manifest, show the same pattern of chemical changes in their blood following irradiation as controls. On the other hand, operated immature animals do not show the change in chloride, and show a reduced change in glucose and NPN. They show a somewhat greater rise in A/G ratio and fall in total protein. This result seems consistent with current knowledge of the adrenal and pituitary glands, if it be recognized that the immature gland is not the equivalent of the mature.

The immunologic studies have progressed sufficiently far to state two conclusions. First, the general impression that irradiation must precede antigen administration by several hours in order to inhibit antibody production, is incorrect for the rat. Second, the protective effect (in terms of raising the LD-50 per cent) of immunization (with sheep cells), is small in the rat, when it is present, and does not have a close relationship to antibody titer.