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**BIOLOGY DIVISION**  
**QUARTERLY PROGRESS REPORT**  
for Period Ending August 15, 1949

Alexander Hollaender, Director

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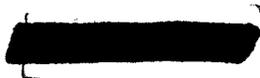
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## BIOLOGY DIVISION

Quarterly Report to August 15, 1949

Alexander Hollaender, Director

*Introduction.* An event of importance to the Division was the arrival of Dr. Jacob Furth on July 1 to assume the leadership of the reorganized Pathology and Physiology Section. Three members of Dr. Furth's former group, a biochemist and two assistants, accompanied him to Oak Ridge. Research plans for this section have been established and extensive experiments in the use of slow neutrons on mice, as well as other projects, have been initiated. During the past quarter two new greenhouses were constructed to provide space for growing material for cytological studies and to make facilities available for the group working in biological synthesis. The administration of the greenhouse has been placed under the direction of a committee whose chairman is Dr. G. R. Noggle. The integration of research between different sections, especially Dr. Furth's group and the Radiobiochemistry Group under Dr. Sheppard's direction, is developing in a highly satisfactory manner.

A number of interesting and promising developments in cytogenetics have taken place in the last few months, the importance of which is at present difficult to estimate. Significant new projects in radiation biology may be opened up through these findings. It has been quite well established that the frequency of induced gene mutations, or point mutations, is proportional to dose, independent of the intensity at which it is given. The linear relationship apparently exists even at extremely low dosage levels. Chromosome breaks also appear to show this linear relationship (see Muller's article "Some Present Problems in the Genetic Effects of Radiations".) As far as is known, no recovery takes place after these changes have been produced. The following new phenomena have been reported which might force modification of these views: (1) The production of reverse mutations by radiation, which give the appearance of possible nullification of mutations originally produced (Giles); (2) The experiments in reactivation, at least as far as ultraviolet light is concerned, which have given highly significant results (Anderson, Kimball, Carlson); (3) A significant reduction in the percentage of chromosome rearrangements in *Tradescantia* after irradiation in an atmosphere of nitrogen rather than air (Giles, Riley); (4) The effect of nitrogen on chromosome translocations in *Drosophila* (Baker). These

developments may upset the previous concept of mechanism of effects of ionizing radiations.

In the work on photoreactivation a number of the individual reports, appearing herein, show the existence of some interesting new developments. (a) Dr. Anderson reports that he has been able to obtain heat reactivation of a certain strain of *Escherichia coli* after ultraviolet irradiation. (b) Dr. Carlson has found that the nucleolus, which is easily damaged by ultraviolet radiation, can recover under the influence of visible light. (c) Reactivation of *Paramecium* after ultraviolet irradiation, by means of visible light, is reported by Dr. Kimball. Intensive work is also being conducted in the effort to discover whether reactivation after X irradiation is possible. It increasingly appears that, whereas the final effects of ultraviolet and X rays might be the same, the effecting mechanism may be biophysically, as well as biochemically, different. Some interesting developments in this field may be anticipated.

Determination of the response of the different structures in the nucleus to micromanipulation, before and after irradiation, is the subject of a study recently initiated by Dr. Carlson. Preliminary work on this problem was completed during the past quarter.

An extensive study of the effects of X rays on embryonic development in the mouse has been completed by the Mammalian Genetics Group, giving results of great significance in the field of radiology.

A reorganization of the Radiobiochemistry group under Dr. Sheppard's direction has diverted the work of this group from development of techniques in the handling of radiochemistry problems to specific studies of the use of radioisotopes in animal experimentation.

The biological synthesis group is now ready to produce ~~the~~ first few grams of the  $C^{14}$  sugar which is essential for some other studies which are planned in the Division. The biochemistry studies on the chemical and enzymatic degradation of ribonucleic acid have developed very satisfactorily. This work has now progressed to the point of planning a symposium on radiation studies of nucleic acid for the spring of 1950. The microbiology group under Dr. Carson's direction has made good progress in studies with the use of  $C^{14}$  and function of propionic acid bacteria. The organic chemistry group, of the Chemistry Division, under Dr. Collins' leadership moved into this building in the last quarter. It is now well prepared to proceed on research problems. Dr. H. I. Kohn's group is in the process of completing its work. Dr. Kohn will leave this laboratory by September 15.

Three visitors from Southern Universities will be leaving during the next few weeks. All three have made significant contributions in their research projects. Dr. H. P. Riley of the University of Kentucky, is completing his research problem on "The Effect of Oxygen on the Frequency of X-ray Induced Chromosomal Rearrangements in *Tradescantia* Microspores" in Dr. Giles' group. (See following list of publications.) Dr. M. G. Whittinghill, of the University of North Carolina, is preparing for publication his work on "Effects of Gamma Rays upon Recombinations and the Process of Crossing Over in *Drosophila melanogaster*". Dr. H. W. Schoenborn, of the University of Georgia, who is spending three months with this laboratory, has finished the preliminary work on studies on "The Induction of Biochemical Mutants in *Astasia*" in Dr. Kimball's group. He will continue these investigations in his own laboratory at the University of Georgia, returning here next spring to complete the work.

Dr. J. W. Foster from the University of Texas has reported to the Division for a six month's period. He will be associated with Dr. Carson's group. Dr. G. S. Rabideau, of the Department of Physiology, University of Texas, plans to arrive here February 1, 1950 to be with Dr. Noggle's group. Dr. A. V. Beatty, Emory University is making arrangements to come to this laboratory in the spring of 1950 for a nine month's period of research on cytological problems.

Work on publications has been rather extensive. Several papers are now being prepared by the investigators and many have reached the final stages. A list of publications by Division members follows. In addition to the listed reports, eleven papers presented at the 1948 Oak Ridge Information Meeting for Biology and Medicine have been assembled and submitted for publication.

PUBLICATIONS BY MEMBERS OF BIOLOGY DIVISION

OAK RIDGE NATIONAL LABORATORY

January - August 1949

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT	PUBLICATION
Anthony, D.S., S.F. Carson, Martin Kuna, E.F. Phares	Mechanisms of the Biosynthesis of Propionic Acid (Abstract)	Federation Proceedings 8 (Pt. 1), 5, 1949		
Arnold, W.A.	Calorimetric Measurement of the Quantum Yield in Photosynthesis, A (Chapter in book).	The Iowa State College Press, "Photosynthesis in Plants", pp. 273-276, 1949.		
Bolomey, R.A. and Leon Wish	Thenoyltrifluoroacetone as a Complexing Agent for the Isolation and Purification of Carrier-Free Radioberyllium	Submitted to journal		AECD-2665
Brauer, Alfred	Localization of Prospective Areas in the Blastoderm of the Pea Beetle, <i>Calosobruchus maculatus</i> , as determined by Ultraviolet (2537 A) Irradiation Injury	Submitted to journal	ORNL-364	
Burnett, W.T., Jr., P.C. Tompkins and Leon Wish	Chemistry and Radiochemistry of Phosphorus-Bakelite Beta-Ray Sources		ORNL-263	
Carlson, J.G., Rachel McMaster	Relative Effectiveness of Different Wave Lengths of Monochromatic Ultraviolet Radiation in Producing Nucleolar Abnormalities, The (Abstract)	J. Tenn. Acad. of Sci., XXIV, No. 3, 173, July, 1949		
Carlson, J.G., M. L. Snyder, A. Hoilsender	Relation of Gamma-Ray Dosage Rate to Mitotic Effect in the Grasshopper Neuroblast	Accepted- J. Cell, & Comp. Physiology	ORNL-348	AECU-243
Carson, S.F.	Design and Interpretation of Carbon Isotope Experiments in Bacterial Metabolism	Cold Spr. Hbr. Sym. on Quant. Biology, 13, 75-80, 1948.	ORNL-96	
Carter, C.E.	Decomposition of Diphosphopyridinenucleotide (DPN) and Adenosinetriphosphate (ATP) by Ultraviolet Light, The (Note)	Accepted - J. Am. Chem. Soc.	ORNL-326	AECU-232
Carter, C.E.	Effects of X Rays on the Metabolism of Nucleic Acids in Hematopoietic Tissues, The (Abstract)	Federation Proceedings, 8 (Pt. 1), 351, 1949		
Carter, C.E.	Paper Chromatography of Purine and Pyrimidine Derivatives of Yeast Nucleic Acid	Accepted - J. Am. Chem. Soc.	ORNL-313	AECU-189
Carter, C.E.	(Abstract, of above title)	Abstracts of March 1949 Meeting of Am. Chem. Soc.		
Carter, C.E.	Effect of Total X radiation on the Activity of Several Enzymatic Systems of Rat Spleen, The		ORNL-316	AECU-231

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Carter, C. E. and W. E. Cohn	Separation of Three Naturally Occurring Adenine Ribonucleotides by Paper Chromatography and Ion Exchange. The (Abstract)	Federation Proceedings, 8 (Pt. 1), 190, 1949	
Cohn, W. E.	Anion-Exchange Separation of Ribonucleotides. The	Submitted to journal	ORNL-285
Cohn, W. E.	Separation of Mononucleotides by Anion Exchange Chromatography	J. Am. Chem. Soc., 71, 2275, 1949	
Cohn, W. E.	Separation of Nucleotides and Related Substances by Ion-Exchange Reactions. The (Abstract)	Abstracts of Papers given at March 1949 Meeting Am. Chem. Soc.	
Cohn, W. E.	Separation of Purine and Pyrimidine Bases and of Nucleotides by Ion Exchange. The	Science, 109, 377, 1949	ORNL-327 AECU-208
Cohn, W. E. and C. E. Carter	Preparation of Uridylic and Cytidylic Acids by an Ion-Exchange Procedure. The	In Reviewing Committee	ORNL-377
Conger, A. D. and N. H. Giles, Jr.	Cytogenetic Effect of Slow Neutrons. The	In Reviewing Committee	ORNL-409
Conger, A. D. and N. H. Giles, Jr.	Cytological Effects of Slow Neutrons (Abstract) (Paper in process)	Federation Proceedings 8 (Pt. 1), 27, 1949	
Giles, N. H., Jr. and R. A. Bolomey	Cytogenetical Effects of Internal Radiation	Cold Spr. Hbr. Sym. on Quant. Biology, 13, 104-112, 1948	ORNL-77
Giles, N. H., Jr. and H. P. Riley	Effect of Oxygen on the Frequency of X-Ray Induced Chromosomal Rearrangements in <i>Tradescantia</i> Microspores. The	Submitted to journal	ORNL-376
Henshaw, P. S., R. S. Snider, E. F., Riley	Aberrant Tissue Developments of Rats Exposed to Beta Rays - The Late Effects of P <sup>32</sup> Beta Rays	Radiology, 52, 401-415, 1949	MonH-288 Revised
Hollaender, A.	Cytological and Genetical Effects of Combined Radiations (Abstract)	Proceedings of the 8th International Congress of Genetics, issued as supplementary vol. of <i>Heredity</i> , p. 598, 1949	
Holt, A. S. and W. A. Arnold	Effect of Ultraviolet on Green Algae and Isolated Chloroplasts. The (Abstract)	Will be in Abstracts - J. Gen. Physiology. (June 1949 meeting.)	
Kimball, R. F.	Induction of Mutations in <i>Paramecium aurelia</i> by Beta Radiation. The	Genetics, 34, 210-222, 1949	ORNL-174 AECU-7
Kimball, R. F.	Inheritance of Mutational Changes Induced by Radiation in <i>Paramecium aurelia</i>	Genetics - in press	ORNL-175 AECU-1
Kohn, H. I.	Chemical Changes in the Blood of the Normal, Hypophysectomized, and Adrenalectomized Rat Following Total Body X Irradiation	In Reviewing Committee	ORNL-387

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT	PUBLICATION
Kohn, H.I.	Chemical Changes in the Blood of the Rat During Ether and Barbiturate Anesthesia	In final preparation		
Kohn, H.I.	Effect of Total-Body X Irradiation upon the Immunologic Response of the Rat to Sheep Erythrocytes, The	In Reviewing Committee	ORNL-412	
Kohn, H.I.	On the Modification of the Toxicity of X Rays by Immunization	In Reviewing Committee	ORNL-410	
Lowrance, P.B. and C.E. Carter	Effect of Nitrogen Mustards on Nucleic Acids of Bone Marrow	Submitted to journal	In Biol. Div. Quar. Rep't - ORNL-318	AECU-395
Morse, M.L. and C.E. Carter	Effects of Ultraviolet Irradiation on the Synthesis of Nucleic Acid by <i>Escherichia coli</i> B. The (Abstract)	J. Bact., Abstracts of papers 49th Gen. Meeting Soc. Am. Bact., p. 14, 1949		
Morse, M.L. and C.E. Carter	Synthesis of Nucleic Acids in Cultures of <i>Escherichia coli</i> , Strains B and B/r, The	J. Bact., 58, 746-753, 1949		
Morse, M.L. and C.E. Carter	Synthesis of Nucleic Acids During the Lag Phase of Cultures of <i>Escherichia coli</i> B. The I. Normal Cultures		ORNL-317	AECU-180
Russell, L.B.	X-Ray Induced Developmental Abnormalities in the Mouse (Abstract)	Federation Proceedings, (Pt. 1), 367, 1949		
Sheppard, C.W.	Distributed Type Source for Exposing Biological Materials to Gamma Rays, A			AECD-2566
Sheppard, C.W.	Construction and Calibration of Equipment for Measuring the Surface Exposure of Phosphorus-Bakelite Beta-Ray Sources	In final preparation	ORNL-265	
Sheppard, C.W. and A.S. Householder	Interpretation of Experimental Investigations of Transfers within a Two-Compartment System, Using Isotopic Tracers, The	In Reviewing Committee	ORNL-411	
Stapleton, G.E. and A.D. Conger	New Thermal Neutron Treatment Chamber for Biological Materials, A (SECRET - LIMITED DISTRIBUTION)			ORNL Central Files Number 48-10-79
Stapleton, G.E., W.D. Gude, H.I. Kohn, J.C. Klie and P.C. Tompkins	Autoradiography and the Particle Problem			ORNL-352
Teas, H.J.	Mutants of <i>Bacillus subtilis</i> that Require Threonine or Threonine Plus Methionine	Submitted to journal	ORNL-369	
Tompkins, P.C.	Preparation of $H_2B^{10}O_3$ from $B^{10}$		ORNL-249	AECU-318

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Tompkins, P. C.	Some Aspects of Handling Radioactive Material in Animal Farms	In Reviewing Committee	ORNL-281
Tompkins, P.C. and O.M. Bizzell	Radioactive Decontamination Properties of Laboratory Surfaces: I. Glass, Stainless Steel and Lead	Submitted to journal	ORNL-381
Tompkins, P.C., O.M. Bizzell and C. D. Watson	Radioactive Decontamination Properties of Laboratory Surfaces: II. Paints, Plastics, and Floor Material	Submitted to journal	ORNL-382
Tompkins, P.C., L.B. Farabee, J. X. Khym	Procedure for the Radiochemical Analysis of Barium, Strontium and the Rare Earths in Human Urine (CONFIDENTIAL)		ORNL-368
Tompkins, P.C., Leon Wish	Estimation of P <sup>32</sup> by Measurement of Bremsstrahlen in an Ionization Chamber		ORNL-314
Tompkins, P.C., Leon Wish, W.T. Burnett, Jr.	A Method of Estimation of Beta Activities from Bremsstrahlung Measurements in an Ionization Chamber	Submitted to journal	ORNL-276 Revision of ORNL-314
Volkin, Elliot and C. E. Carter	Isolation and Characterization of Spleen Ribonucleic Acid, The	Submitted to journal	In Biol. Div. Quar. Rept.- ORNL-244
Wish, Leon and R. A. Bolomey	Spectrophotometric Studies of Beryllium Thenoyltrifluoroacetone	Submitted to journal	AECD-2669
Wish, Leon, W.T. Burnett, Jr., P. C. Tompkins	Radiation Intensities of Phosphorus-Bakelite Beta-Ray Sources as Calculated from their Specific Activities		ORNL-264
Sheppard, C.W. and W.R. Martin	Isotopic Studies of Potassium Exchange Rates Between Cellular Elements and Plasma of Human and Canine Blood <i>in vitro</i> (Abstract)	Federation Proceedings, 8 (Pt. 1), 145, 1949	

# CYTOGENETICS

## EFFECTS OF RADIATIONS ON *TRADESCANTIA* AND *NEUROSPORA*

N. H. Giles, Jr. (Leader)  
Dorothy L. Giles                   A. D. Conger  
Seymour Pomper                   Lucille McMichael  
S. R. Suskind                   Jean H. Hemmerly  
H. P. Riley (Visiting investigator)

*Relative reverse mutability of independently produced, allelic inositol-less mutants in Neurospora crassa.* (Giles, Giles and Hemmerly) The extensive data already obtained on spontaneous and ultraviolet induced reverse-mutation rates are being prepared for publication.

Experiments comparing the effects of X rays and ultraviolet light on reverse mutability of certain selected inositolless mutants have been initiated. Present results indicate that there are marked differences among certain mutants in their comparative responses to X-ray and ultraviolet treatment. For example, mutants S-89601 and S-37401 are very different in their response to ultraviolet, strain S-89601 having a very low reverse-mutation rate compared to that of S-37401. With X rays, however, the rates of reverse mutation are quite comparable in the two mutants. This difference between ultraviolet and X rays exists in the two parental types and also in the F<sub>1</sub> segregates of a cross of these two strains.

Further crosses have been made to establish unequivocally the nature of the inositolless suppressor mentioned in the previous report. The initial cross in which the suppressor mutation was detected involves the F<sub>1</sub> segregates of two ultraviolet-induced reversions from experiment OR-35. Subsequent crosses have demonstrated that one of these (R5-35) is a reverse mutation, whereas the other (R6-35) is a suppressor mutation. Tests of the cultures from the four spore pairs of an ascus segregating 6:2 for inositol independence versus inositolless phenotypes have yielded the expected ascus types, as indicated in table 1. Crosses of the suppressor to other inositolless mutants are being made to provide further evidence on the question of allelism.

*Genetic basis of spontaneous and radiation-induced adaptations in other biochemical mutants in Neurospora.* (Giles, Giles and Hemmerly) Additional crosses have been made to establish the behavior of the methionineless (S-4894) suppressor mutations. Analyses of these crosses are almost complete and will furnish conclusive evidence as to the number of different loci involved. Further, the crosses will provide valuable linkage data for a number of the mutants. Analyses of reversions in the niacinless (Y-17000) and adenineless (S-38701) mutants are continuing.

TABLE 1

*Crosses involving an inositolless suppressor mutation*  
(8743-23-2.5a-R6-35)

STOCK NO.	PHENOTYPE	CROSSED WITH	SEGREGATIONS + IN COMPLETE ASCI			
			0+:8	4+:4	6+:2	8+:0
8743-23-2.5a-R5-35	Inositol indep. (normal)	Inositolless	0	7	0	0
8743-23-2.5a-R6-35	Inositol indep. (slow)	Inositolless	0	5	0	0
F <sub>1</sub> of R5 above (8743-CL14-3.5a)		F <sub>1</sub> of R6 above (8743-CL15-5.5a)	0	3	8	2
8743-CL23a-1.1a	Inositolless	Inositolless	6	0	0	0
		Wild type	0	11	0	0
8743-CL23a-1.3a	Inos. indep. (slow)	Inositolless	0	7	0	0
		Wild type	0	0	4	1
8743-CL23a-1.5a	Inos. indep. (normal)	Inositolless	2	1	5	0
		Wild type*	0	0	0	6
8743-CL23a-1.7a	Inos. indep. (normal)	Inositolless	0	6	0	0
		Wild type*	0	0	0	7

\* No inositolless types were recovered in a total of 100 cultures from spore isolations in cross of 1.5a and 139 cultures from 1.7a.

+ Symbol + indicates inositol independence.

*The effect of oxygen on the frequency of X-ray induced chromosomal rearrangements in Tradescantia.* (Giles, Riley and McMichael) Work on the effect of oxygen on the production of chromosomal aberrations in *Tradescantia* microspores by X rays is continuing. Certain aspects have been written up for publication. In that paper it is shown that both interchanges and interstitial

deletions observed in microspores four or five days after irradiation are more frequent when the inflorescences are irradiated in oxygen as compared with air, but very much less frequent when the irradiation is carried out in nitrogen. This was true at radiation dosages of 180 r, 270 r, and 360 r, given at 45 r per minute. Aberration frequencies were also reduced when the buds were in an atmosphere of argon or of helium. These gases were tested at 360 r only. When the gas surrounding the buds was a mixture of nitrogen, argon, or helium with air, the frequency of aberrations was greater than in the pure gas but less than in air. These results indicate that the presence of oxygen is the important factor.

Further experiments in connection with this investigation are under way or being planned. It has been found that the preliminary evacuation of the buds is not as important as the actual gas in the lucite exposure box at the time of irradiation. The aberration frequency was high when the box contained air even though the buds had been previously evacuated and helium introduced, but was low with helium in the box both when the buds had been evacuated in helium, and when they had not. Experimental exposures in oxygen, air, and helium at different radiation intensities are being performed. Experiments designed to test the effect of oxygen at pressures in excess of one atmosphere are under way. A new exposure chamber has been designed to withstand these pressures and also to enable a very rapid exchange of gasses while the chamber is still in the X-ray machine. It is hoped that the intensity experiments and those involving gas exchange will produce evidence to indicate whether the observed effect of oxygen on radiosensitivity is exerted by way of the initial breakage mechanism or the recovery process.

*Thermal neutron effect on boron-enriched tissues.* (Conger and Suskind)  
An experiment to test the effect of an increased boron content in tissues exposed to thermal neutrons has been completed. The result was as expected, an increased amount of biologic effect (chromosome aberrations in *Tradescantia*) from the same thermal neutron dose, due to the greater amount of alpha radiation resulting from the  $B^{10} (n, \alpha) Li^7$  reaction. Unfortunately, the results were not suitable for any quantitative comparisons with parts not enriched with boron as the boron concentration from bud to bud, judging from the amounts of biologic effect, was decidedly nonuniform. Spectrographic analysis of the boron content of individual buds could not be made as the total amount of boron in a single bud was less than the minimum amount for which analysis could be made.



to 2804 A but not to the A-H5 lamp. All animals were kept in the dark or in low intensity illumination except during the exposure periods. They were isolated in a dim orange light. The results of two experiments are shown in table 1. As a measure of the effect, the time in days required to reach the sixth fission after irradiation averaged over all daily isolation lines was used.

TABLE 1

TREATMENT	AVERAGE DAYS TO SIXTH FISSION		NUMBER OF DAILY ISOLATION LINES	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
none	1.22 days	1.44 days	54	11
A-H5 only	1.26	1.28	50	72
2804 only	9.40	9.16	45	13
2804 and A-H5	1.80	2.20	26	8

It is obvious that exposure to the A-H5 lamp greatly decreases the effect produced by the 2804 A wave length. This result seems quite comparable to the "photoreactivation" reported by several investigators for ultraviolet inactivation or killing of viruses and bacteria.

The results of irradiating during different periods of the division cycle are shown in table 2. The irradiation was by means of a General Electric germicidal lamp having its major output at 2537 A.

TABLE 2

PERIOD OF THE DIVISION CYCLE	TIME TO THE SIXTH FISSION	NUMBER OF DAILY ISOLATION LINES
Recently divided	8.24 days	54
Midway between two successive divisions	9.47	38
Nearly ready to divide	2.30	53
Unirradiated controls	1.38	72

Apparently radiation given in a period just prior to a division is considerably less effective than radiation given at other times. It is interesting to note that it has previously been shown that *Paramecium* in the period just prior to division is relatively resistant to the production of gene mutations by beta radiation. It is difficult to see the possible connection in view of the high improbability that the effect of ultraviolet on cell division is due to effects upon the genes. However this apparent correlation needs further investigation to see whether animals in this same period are also insensitive to other types of radiation.

*Effect of ultraviolet radiation upon survival of paramecia in the absence of autogamy.* (Gaither, Kimball and King) For these experiments, two clones from two different lines of descent were used. Each clone was given four doses of ultraviolet of wave length 2804 A on several successive days. After treatment, 27 animals were isolated from each dose and allowed to multiply for one week. The cultures were then examined for survival. During and after irradiation the animals were kept in the dark as much as possible. Isolations were made in yellow illumination of low intensity. Considerable variation was found from day to day in the 50 per cent lethal dose according to rough approximation from the dosage curves. However, there appeared to be some correlation with time since autogamy, as shown in the following table.

TABLE 3

DAYS SINCE AUTOGAMY	4	5	7	8	9	10	11
Clone #1	-	-	-	2700	2950	3050	4950
Clone #2	1525	2950	3750	-	-	-	-

The numbers in the body of the table are in ergs/mm<sup>2</sup> and represent the 50 per cent lethal dose as roughly approximated from the graphs. It appears that the animals become more resistant to the radiation as the time since autogamy increases.

*Microscopically visible effects of ultraviolet irradiation upon the macronucleus.* (Kimball) Compressed living animals examined with the Zeiss phase microscope show in the macronucleus many small, round bodies which appear dark in comparison with the rest of the macronucleus. The bodies, probably hundreds, appear to be about two microns in diameter. Following exposure of the animals

to ultraviolet light of wave lengths 2650 A or 2804 A, these bodies enlarge, become vacuolated, and coalesce with one another. At the same time they become darker and more clearly delimited under the phase microscope. With higher doses of irradiation the bodies may coalesce into five to ten large masses. The doses at which such a marked effect is produced are finally lethal to the animals, but they may survive some hours after almost complete coalescence has occurred. In view of reports of other investigators on the effects of radiation upon nucleoli, it is tempting to think of these bodies in the macronucleus of *Paramecium* as nucleoli although nucleolar material has not been clearly demonstrated so far in this organism. A more pronounced effect appears to be produced by 2804 A than by 2650 A when equal doses of the two wave lengths are given.

*Induction of biochemical mutants in Astasia.* (Schoenborn) An attempt is being made to obtain a series of biochemical mutants of *Astasia longa*, an autotrophic protozoan flagellate, which will permit studies dealing with the process of carbon dioxide fixation. It was first necessary to obtain a complete medium which would support good growth of the parent strain. That work is completed. A number of other experiments were then carried out to establish a suitable method for obtaining an X-ray killing curve and for the isolation of single-rayed cells. Agar plates were found unsatisfactory for two reasons: first, because the organisms move over the surface of the agar; and second, the organisms do not divide to form colonies. Hence, it has been necessary to establish clones by the isolation of single cells into liquid medium. To date approximately 1200 cells have been subjected to various X-ray dosages and isolated into complete medium. When sufficient time has elapsed for the development of macroscopically visible clones, these experiments will provide information for the construction of an X-ray killing curve. Also, the clones that do develop will be tested to determine the mutation rate at the various X-ray dosages employed. This information will then be used to ascertain the dosage for future irradiations.

#### EFFECTS OF RADIATION ON THE RATE OF MITOSIS

J. Gordon Carlson\* (Leader)  
Mary E. Gaulden\*  
M. G. Whittinghill (Visiting investigator)

Nyra Harrington  
Rachel McMaster

*Effects of gamma rays at low intensity upon the rate of mitosis in neuroblast cells of Chortophaga viridifasciata.* (Harrington) Studies on effects of gamma rays upon the rate of mitosis of neuroblast cells are being continued.

Preliminary experiments show that different results are obtained when the embryos are submerged in water during treatment than when they are merely kept moist. Film tests were run and showed that the amount of irradiation from the gamma source was the same through water or air. Experiments were then made on the growth rate of the embryos; those submerged in water were greatly retarded in their development, while embryos not submerged developed normally.

The experiment now under way consists of irradiating embryos with a dosage of 192 r; the dose being given over a period of forty-eight hours. The rate of mitosis of the irradiated embryos is then compared to the controls. The experiment is as yet too new for calculations of statistical data.

*The relative effectiveness of different wave lengths of ultraviolet radiation.* (McMaster) Previous experiments to determine the relative effectiveness of different wave lengths of ultraviolet radiation in producing a given effect upon the nucleolus, and upon the spindle of grasshopper neuroblasts were done without controlled visible light conditions. To investigate the possibility of photoreactivation, two more series of experiments have been done using the same technique as the earlier experiments, but with visible light controlled. In the first series the embryos were treated with ultraviolet in a dark room, and filters which transmitted only yellow and longer wave lengths of light were used in lamps for microscopic observation. The second group were irradiated in a light room, were transferred from quartz to glass cover slips in white light, a process requiring from three to five minutes, and then were placed on the microscope stage under the brightest white light obtainable from a Bausch and Lomb research lamp. The whole embryo was illuminated during the period before and while the effect was observed, except at intervals of fifteen minutes or longer, when observations were made.

Under these conditions it was found that the percentage of cells showing abnormal nucleoli after irradiation with ultraviolet of wave length 2650 A and longer is reduced by exposure to visible light. Any effect of visible light on nucleolar abnormalities produced by shorter wave lengths is not detectable by the methods used. Formation of a hyaline globule, used as the criterion of the action of ultraviolet upon the spindle, is not affected by visible light when the effect is produced by wave lengths 2650 A and above. Studies of the shorter wave lengths have not been completed.

*Micromanipulation of grasshopper neuroblast cell content.* (Carlson). In order to understand better the physical properties of the cell contents

and their changes during division, so that the meaning of certain effects induced by ionizing and ultraviolet radiations may be better interpreted, the interior of the dividing grasshopper neuroblast was investigated with a micro-needle. This study seems to justify the following conclusions:

1. The mitotic spindle is a semisolid body situated in a fluid cytoplasm. It is lightly attached to the cell membrane by fibers running from the pole to the adjacent region of the cell membrane.

2. During prometaphase, metaphase, and anaphase the chromosomes are securely attached to the spindle at their centromeres, distal to which they lie free in the cytoplasm.

3. As anaphase progresses the spindle appears to undergo liquification between the two groups of separating daughter chromosomes. Mitochondria in rapid Brownian movement migrate into this fluid interzonal region which is traversed by fine fibers connecting the distal ends of sister chromosomes.

4. Anaphase separation of chromatids is not simultaneous for the whole length of the chromosomes, but begins proximally and gradually proceeds to the distal ends of the chromosomes.

5. Polarization of the neuroblast, which is manifest in unequal division, is independent of the spindle and chromosomes. It is apparently determined by the cell cytoplasm, the plasma membrane, the position of surrounding cells, or a combination of these.

6. Formation of the cleavage furrow is independent of the spindle and chromosomes but its position is, to a limited extent, determined by the position of the spindle and chromosomes.

Progress has been made in assembling the data and writing up the results of Miss McMaster's action spectrum studies on spheration of the nucleolus and breakdown of the spindle as a result of ultraviolet irradiation.

*Effects of gamma rays upon recombinations and the process of crossing over in Drosophila melanogaster.* (Whittinghill) Two experiments involving some 5,000 and 15,000 offspring respectively in 15 to 30 families were performed to investigate the effect of gamma radiation upon crossing over and recombination in the third chromosome of *Drosophila melanogaster* heterozygous for the eight "rucuca" genes. Large increases were found, especially when total doses of 4,000 r, as contrasted with 2,000 r, were used. In the 4,000 r families there seemed to be no particular differences between treatment at slow and fast rates. No special differences were found following treatment during three different eight-hour periods divided at approximately 8:30 A.M., 4:30 P.M. and 12:30 A.M.

Among all treated families together, increases in the percent of recovered crossovers were greatest at the spindle attachment region and progressively less toward the free ends of the chromosome, where decreases in the recombinations were demonstrated. This fact is entirely consistent with the idea that the gamma rays induced crossing over in oögonial cells. A study of coincidence, the occurrence of double crossovers as compared with their expectation on the usual hypothesis of meiotic crossing over, further indicated that a large amount, if not all, of the induced change is gonially produced. A further gamma-ray experiment will seek to determine whether all of the induced crossovers are gonial.

### EFFECTS OF RADIATION ON *DROSOPHILA* GENETICS

W. K. Baker\* (Leader)

Elizabeth Sgourakis

*Altering the X-ray sensitivity of Drosophila by means of respiratory inhibitors.* (Baker and Sgourakis) Previous preliminary studies indicate that there is a two- to threefold decrease in the number of chromosome translocations induced in *Drosophila* when the flies are irradiated by X rays in an atmosphere of nitrogen rather than air. On the basis of these results a program was initiated about the middle of June to determine if there is any alteration in the X-ray-induced mutation rate when the respiration of the flies is inhibited.

In this study, males of *Drosophila melanogaster* (Oregon-R stock) are kept in a continuous flow of either nitrogen or oxygen during irradiation. An apparatus has been fabricated which allows simultaneous exposure in the X-ray field of the flies in these two gases at one of two temperatures. Three different dosages (1000, 3000 and 5000 r) have been used at each of the two temperatures (2° and 27° C.) By mating these wild-type males to Muller-5 females, the number of induced sex-linked lethal mutations can be determined in the F<sub>2</sub> generation. The results of only five of the twenty-four experiments in this program have been received at this date. These initial results indicate that there is about a twofold decrease in the number of sex-linked lethal mutations in the series treated with nitrogen. Not enough data are on hand to make any statement about a temperature effect.

In the above study it has been observed that the P<sub>1</sub> crosses of the flies exposed to oxygen do not go as readily as the nitrogen series. An investigation

\*Consultant

has just been initiated to determine if there is any difference in survival, willingness to mate, or sperm motility between the irradiated males exposed to oxygen and those exposed to nitrogen.

Preliminary investigations are under way to study the effects of respiratory enzyme inhibitors on X-ray sensitivity. An apparatus has been devised in which a known amount of hydrogen cyanide or hydrogen sulfide gas can be generated and passed over the flies to be irradiated. The concentration of these gases which will "knock out" the flies but still allow recovery after X-ray exposure has been determined. Four experiments using hydrogen cyanide gas and two experiments using hydrogen sulfide gas are under way and the results should be available shortly.

### EFFECTS OF RADIATIONS ON MUTATION OF FUNGI

Alexander Hollaender (Leader)  
G. E. Stapleton                      Frances L. Martin

*Lethal and mutagenic action of ionizing radiations on Aspergillus terreus.*  
(Stapleton and F. L. Martin) Previous quarterly reports have indicated that not only are heavily ionizing particles, such as alpha particles and protons, more efficient in producing lethal effects in these cells but also the order of the reaction changes, first-order killing being produced by the heavy particles, whereas second- or third-order killing results from X irradiation. It has been suggested, upon analysis of the X-ray survival curves, that the lethal effect results from simultaneous occurrence of mixed first- and second-order killing. It is probable that any first-order effect is produced by the tails of electron tracks, where the linear ion density approximates that found along the tracks of the heavy particles.

To test this fundamental point, two pathways present themselves:

1. A study of the effect, using slow electrons, where the entire track is similar to the tails of faster electrons.
2. A study of the effect, using hard gamma rays, where the probability of "tails" occurring in the cells is very much reduced.

Either method should give a clear-cut demonstration.

Because of the difficulty in using the first system, the second was adopted, and small samples of spores are now being exposed to a high-intensity  $\text{Co}^{60}$  gamma-

ray source. Although the results are meager, the general trend is toward a curve of higher order than that obtained using X rays.

# MAMMALIAN GENETICS

## GENETIC EFFECTS OF RADIATION ON MICE

W. L. Russell (Leader)	Josephine S. Gower
J. C. Kife	Mary Henderson
Liane B. Russell	Gloria Jones
Jane Crowell	Louis Wickham

*X-ray induced developmental abnormalities.* (L. B. Russell and W. L. Russell) At the writing of the last report, collection of material for this project, as well as the study of external characters of newborns irradiated during prenatal development had been almost completed. Since then, an extensive study of the ossified skeleton at birth has been carried out. Since, among the known mutants in the mouse, there is a large number that act on the skeleton, a study of skeletal abnormalities is a desirable beginning for the determination of points of reference between changes induced by a general environmental agent and those produced by gene action. Moreover, processes affecting the skeleton, directly or indirectly, occur during a large part of development, while several other systems are critically determined only during certain definite phases. Skeletal abnormalities may be indicative of, or secondary to, changes in other processes, particularly those involving the nervous system and the musculature.

The entire gestation period has now been surveyed and future work will deal with special aspects. The following is a summary of the completed part of the project:

1. Hard ionizing radiation is useful as an injurious agent in mammalian development since its action is largely general and the selective response of structures may be used for the interpretation of intrinsic patterns of sensitivity in the organism. The moment of interference may, moreover, be accurately timed and correlations with the characters produced lead to the discovery of sensitive periods in certain developmental processes.

2. Four hundred and twenty animals, studied externally and skeletally as newborns, had been irradiated in intra-uterine life at stages of development differing by twenty-four hour intervals and ranging from half a day to thirteen and a half days after fertilization. The fetal population, which was a C57 Black  $\times$  NB hybrid, came exclusively from the second litters of C57 Black females, each of which was given one single acute exposure to 250 kvp X rays at a chosen stage in pregnancy timed by the vaginal plug method. The dosages em-

ployed were 200 r (or 100 r in a few cases) for a general survey of all stages; 300 r and 400 r for dosage comparisons at the last five stages.

3. Irradiation with 200 r before implantation ( $\frac{1}{2}$  -  $4\frac{1}{2}$  days), and with 300 r at  $9\frac{1}{2}$  days increases prenatal mortality in early and late stages respectively. The peak incidence of natal death follows irradiation on days  $9\frac{1}{2}$  and  $10\frac{1}{2}$ .

4. Considerable depression of birth weight occurs in consequence of irradiation between days  $8\frac{1}{2}$  and  $13\frac{1}{2}$ . Birth weight curves are, in general, parallel for different doses and their minima lie between days  $10\frac{1}{2}$  and  $11\frac{1}{2}$ .

5. Following irradiation on days  $\frac{1}{2}$  to  $5\frac{1}{2}$ , inclusive, 98 per cent of the animals surviving to term were indistinguishable from the controls.

6. Irradiation at other stages produces, in general, a high incidence of characteristic abnormalities, making possible the mapping of critical periods. A few of these are as follows:

- a)  $7\frac{1}{2}$  days—intersegmental jumping of thoracic and cervical vertebrae and changes in ribs and sternum, showing general, if not direct, correlation.
- b)  $6\frac{1}{2}$  to  $8\frac{1}{2}$  days—degrees of median approach and fusion of the incisor-bearing portions of the premaxillae, culminating in loss of incisors and formation of single median bone.
- c)  $7\frac{1}{2}$  to  $9\frac{1}{2}$  days—microphthalmia and coloboma (peak at  $9\frac{1}{2}$  days).
- d)  $7\frac{1}{2}$  to  $9\frac{1}{2}$  days—posterior shift of thoraco-lumbar border, i.e., increase in rib number by one; the peak is at  $8\frac{1}{2}$  days when the number of sternbrae and of costal cartilages articulating with the sternum is also increased by one.
- e)  $9\frac{1}{2}$  days—domed forehead and shortened floor of the cranium, possibly indicative of hydrocephalus of the type recognizable at birth.
- f)  $9\frac{1}{2}$  and  $10\frac{1}{2}$  days—virtual absence of the roof of the cranium.
- g)  $9\frac{1}{2}$  and  $10\frac{1}{2}$  days—open eyelids, traceable to excessive eye bulging caused by various skull abnormalities.
- h)  $9\frac{1}{2}$  and  $10\frac{1}{2}$  days—broad fusions of sternbrae; irregular meshwork formation of costal cartilages.
- j)  $9\frac{1}{2}$  days—characteristic lumbo-sacral changes often producing externally visible spina bifida.
- k)  $9\frac{1}{2}$  to  $10\frac{1}{2}$  days—small or imperforate anus; changes in ureters and kidneys.
- l)  $8\frac{1}{2}$  to  $13\frac{1}{2}$  days—shortening of the tail and abnormalities in tail

shape; there are probably two distinct phases with peak sensitivities at  $9\frac{1}{2}$  days and between  $11\frac{1}{2}$  and  $12\frac{1}{2}$  days respectively.

m)  $8\frac{1}{2}$  to  $10\frac{1}{2}$  days—overgrowth in forefeet (rarely) and/or hind feet, resulting in excessive length of digit no. 1, or doubling of digits no. 1 or no. 2.

n)  $10\frac{1}{2}$  to  $13\frac{1}{2}$  days—digital reductions in forefeet (days  $10\frac{1}{2}$  to  $12\frac{1}{2}$ ) and hind feet (days  $11\frac{1}{2}$  to  $13\frac{1}{2}$ ).

p)  $9\frac{1}{2}$  to  $11\frac{1}{2}$  days—drastic changes in long bones and girdles: tibia—only one case—and scapula (day  $9\frac{1}{2}$ ); scapula, clavicle, all arm bones, ilium, femur, fibula (day  $10\frac{1}{2}$ ); ischium and pubis (day  $11\frac{1}{2}$ ).

q)  $10\frac{1}{2}$  to  $12\frac{1}{2}$  days—arch-centrum fusions in the vertebral column and an angulation syndrome in the ribs, both giving the appearance of lateral compression.

r)  $10\frac{1}{2}$  to  $12\frac{1}{2}$  days—cleft palate, without harelip; also occasionally found in other stage groups.

s)  $11\frac{1}{2}$  to  $13\frac{1}{2}$  days—various signs of lateral compression in skulls not otherwise very abnormal.

7. Some abnormalities occurred only in the members of a litter of two irradiated at  $6\frac{1}{2}$  days. They were situs inversus of stomach and kidneys exhibited by one, and possession, by the other, of a shallow cloacal opening for rectum and urethra.

8. Certain developmental interpretations may be made from dosage relationships. Some of these are:

a) Animals of a particular stage-group are continuously distributed with regard to their sensitivities;

b) most frequently, primordia are capable of graded response, but some can apparently give only one response;

c) the sensitivities of certain processes are low and show little scatter;

d) chronological patterns of susceptibility may be traced and reveal that the decrease in sensitivity from a peak period is usually more gradual in time than is the increase.

9. Using the evidence of critical periods, some of the induced abnormalities may be linked with certain phases, or physical events, in the chain of processes known to lead to the formation of affected characters.

10. Many similarities exist between abnormalities produced by X-ray action

on the one hand and gene action on the other. Although it is not legitimate to make assumptions as to the time and form of *primary* gene action, the similarities indicate that some of the reactions in the chain between gene and character occur at the critical time indicated by the radiation effect.

11. The differences between gene and X-ray induced abnormalities are more striking than the parallelisms. No one phenotype shows a one-to-one relationship with X-ray results for a particular stage, the latter usually involving more abnormalities. On the other hand, certain abnormalities frequently produced by physiological agents and by gene action have not occurred as a result of irradiation.

# M I C R O B I O L O G Y

## TRACER STUDIES ON METABOLISM

S. F. Carson (Leader)

D. S. Anthony

M. Kuna

L. A. Nutting

E. F. Phares

W. J. Jefferson

Mary V. Long

*Separation and degradation of acetic and propionic acids.* (Phares, Long, Anthony) Separation of acetic and propionic acids from fermentation by use of the Celite column described in a previous report proved quite satisfactory. However, in order to eliminate overlapping of bands, other column materials were tried. Of these, Polycel, a wood fiber, showed most promise.

The standard method for acetate degradation has been the dry distillation of calcium salt. This method has the serious drawback of giving uncertain and disproportionate yields of the two end-products, acetone and calcium carbonate. Two other reactions, the bromination of the silver salt and the Schmidt reaction, were studied and gave the desired results.

The action of bromine on silver acetate yields  $\text{CO}_2$  from the carboxyl group and methyl bromide from the methyl group. The activity of  $\text{CO}_2$  can be easily determined and by difference one can also get the activity in the methyl group. To date it has not been possible to carry out direct determinations of the amount of activity in the methyl bromide from the above reactions.

Using the Schmidt reaction, with general conditions as described by Scheurch and Huntress (J. Am. Chem. Soc., 71:2233, 1949) but with only 0.5-millimole quantities of acetate, both carbons were recovered in usable form, according to the reaction:  $\text{RCO}_2\text{H} + \text{HN}_3 \longrightarrow \text{RNH}_2 + \text{CO}_2 + \text{N}_2$ . The acetate was dissolved in 8- to 10-molar amounts of concentrated sulfuric acid, and approximately two equivalent amounts of sodium azide were added over a period of one-half hour. The mixture was kept at a temperature of  $60^\circ \text{C}$  and was stirred magnetically. The  $\text{CO}_2$  was collected in an alkali trap. It was weighed and counted as barium carbonate. The yields were 80 - 90 per cent of the theoretical but there was some evidence of slight contamination from the methyl carbon.

After two hours, the reaction material was diluted with water and made alkaline. The methyl-amine was distilled into an acid trap, and a wet oxidation was carried out. Yields of  $\text{CO}_2$  indicated recovery of 40 - 60 per cent of the

methyl carbon uncontaminated with carboxyl carbon.

*Synthesis of substrates containing C<sup>14</sup>.* (Kuna, Anthony) (1)  $\beta$ -labeled propionic acid: Carbonyl-labeled sodium pyruvate, prepared according to the method described in the previous report was reduced in a micro Craig apparatus with hydrogen and Raney nickel catalyst. The purified lactic acid was taken up in hydrogen iodide and reduced in a bomb at 140° (Lauteman, Ann. der chem., 113:218, 1860). Iodine was removed by filtration and by use of mercury. Iodide was removed with silver sulfate. After steam distillation, 100 ml of approximately N/10 acid were obtained with a specific activity of ~3  $\mu$ c/ml.

(2) Carboxyl-labeled propionic acid: From 3 millimoles of active BaCO<sub>3</sub>, with 32 millimoles of added BaCO<sub>3</sub>, 250 ml of N/10 carboxyl-labeled propionic acid, activity ~1.5  $\mu$ c/mg, were obtained by carbonation of ethyl Grignard reagent.

*Fermentation studies - coupled reactions involving acetate.* (Carson, Jefferson) Nontracer studies were carried out using the Warburg technique with resting cells of *P. pentosaceum* in order to obtain a better understanding of the reactions involving acetate. Tracer experiments are to be designed from the results of these studies.

The following substrates were used both separately and in combination with acetate: acetate (alone), pyruvate, glycerol, fumarate,  $\alpha$ -keto glutarate, citrate, succinate, malate and maleate.

On the basis of about ten such experiments there is a clear indication that a coupled reaction occurs between acetate and fumarate, and between acetate and malate.

*Interchange of methyl and carboxyl carbon of acetate in the propionic acid fermentation.* (Carson, Anthony, Phares, Long and Jefferson) Large-scale fermentations were run using pyruvate as substrate, with methyl-labeled acetate as tracer in one case, and carboxyl-labeled acetate in another. The fermentations were carried out for six hours, after which the residual pyruvate was separated from the end-products. Propionate and acetate were separated on the column and the acetate degraded by new and much superior methods, as set forth in subsequent paragraphs.

The following table summarizes the main results of two such experiments:

INITIAL C*	PER CENT OF INITIAL C*	
	C*H <sub>3</sub> COOH 100 PER CENT	CH <sub>3</sub> C*OOH 100 PER CENT
CO <sub>2</sub>	0.8	9.0
Cells	13.0	17.0
Supernatant	87.0	72.0
Nonvolatile	30.0	21.5
Volatile	54.5	42.0
Propionate	5.5	9.0
Acetate	49.0	33.0
Acetate	49.0	33.0
-CH <sub>3</sub>	1.0	29.0
-COOH	48.0	4.0

It is indicated that there is considerable decarboxylation of acetate in the initial stages of the fermentation. During later stages, considerable amounts of propionate are synthesized, as well as acetate itself. The final acetate apparently contains the labeling in the end opposite the starting point; which indicates not only a considerable turnover of acetate, but also its importance in both the fermentation cycle and in cellular syntheses.

Using pyruvate as substrate and C<sup>14</sup>O<sub>2</sub> as tracer, the incorporation of CO<sub>2</sub> into propionate and acetate was followed. Results of two such experiments are summarized below, one of which was run for one hour, and the other for twenty-two hours.

INITIAL C*	PER CENT C*	
	ONE HOUR	TWENTY-TWO HOURS
	100	100
C*O <sub>2</sub>		
Final CO <sub>2</sub>	92.5	88.2
Supernatant	6.1	14.6
Nonvolatile	3.1	3.2
Volatile	3.0	11.4
Propionate	2.9	11.2
Acetate	0.1	0.2
Ratio total propionate/acetate	30/70	38/62

The above results, as well as those of the preceding experiments, indicate that both CO<sub>2</sub> and acetate (independently) are precursors of propionate, but that CO<sub>2</sub> plays little, if any, part in acetate synthesis.

## RADIATION BACTERIOLOGY

E. H. Anderson (Leader)

R. W. Whittle                      B. B. Hill

*Reactivation of ultraviolet-irradiated bacteria.* (Anderson, Whittle and Hill) During the past quarter the radiation bacteriology group has been investigating the reactivation of ultraviolet-irradiated cells of *Escherichia coli*. Microorganisms exposed to ultraviolet light become inactivated or non-viable in that they are incapable of dividing and forming visible colonies when plated on a suitable solid medium. Under certain conditions of treatment after irradiation so-called inactivated cells may recover their ability to divide and form colonies when plated on a solid medium. A study of conditions necessary for recovery of bacterial cells might yield information on the nature of radiation damage and the mechanisms involved. Strains B and B/r of *Escherichia coli* have been used in these studies. Strain B/r is a radiation-resistant mutant of strain B and shows a multiple-hit survival curve to ultraviolet while strain B shows a single-hit type of curve.

It was found that both strains B and B/r respond to reactivation with visible light. Indications are that the wave lengths responsible for this type of photoreactivation lie in the near ultraviolet region of the spectrum.

In studying the effect of temperature on photoreactivation it was found that the B strain, unlike the B/r strain, can be reactivated by heat alone. Experiments to determine rates of heat reactivation of strain B have been carried out, in which aliquots of the irradiated suspension have been held at various temperatures. Assays of samples removed at intervals up to four hours in length showed an increase in survival ratios with time for each holding temperature, the most rapid increase in each case occurring during the first two hours.

Data have been obtained which indicate that heat reactivation and photoreactivation may be independent factors. This is further substantiated by the observation that strain B/r reacts to photoreactivation but not to heat reactivation. Survival curves of the B strain carried out under carefully con-

trolled conditions, with respect to light and temperature, do not show a change in slope at about one per cent survival as do the published curves of Witkin.

# BIOCHEMISTRY

## BIOCHEMISTRY OF NUCLEOPROTEINS, AMINO ACIDS AND ENZYMES

C. E. Carter (Leader)

D. G. Doherty

M. L. Morse

E. Volkin

Ann R. Webster

P. B. Lowrance (Visiting investigator)\*

W. E. Cohn

J. X. Khym

F. Vaslow

M. Helen Jones

P. G. Williams\*

*Chemical and enzymatic degradation of ribonucleic acid.* (Carter, Cohn and Khym) The products of the enzymatic separation of yeast ribonucleic acid by crystalline ribonuclease have been analyzed by ion exchange and paper chromatography. The mononucleotide product is almost exclusively uridylic and cytidylic acids in approximately equal amount. The small quantity of adenylic and guanylic acid appearing as mononucleotides seem to arise from higher molecular weight compounds during the analytical separation on the ion exchange resin. Several discrete fractions, which were bound more strongly to the anion exchanger than are mononucleotides, were analyzed and each was found to have a different polynucleotide composition. These latter fractions may be artifacts resulting from the analytical procedure of polynucleotide subgroups arising from the action of nuclease on nucleic acid or degradation products of such polynucleotides.

The preparation of uridylic and cytidylic acids from an acid hydrolysate of yeast ribonucleic acid employing the anion exchanger Amberlite IRA-400 and elution with formic and hydrochloric acids has been accomplished. From 100 grams of nucleic acid, 4.9 grams of crystalline diammonium uridylate were obtained in a preparation involving formic acid elution and conversion of uridylic acid to the diammonium salt. In another preparation, starting with 110 grams of yeast nucleic acid and employing a hydrochloric acid elution, 17.8 grams of a barium uridylate fraction were obtained which, by spectrophotometric assay, contained 52 per cent uridylic acid (theory 70 per cent). A method for conversion of barium uridylate to free uridylic acid employing a cation exchanger is described. Cytidylic acid was readily crystallized from fractions eluted from the column by either dilute formic or hydrochloric acid. The yields were 7.5 grams and 6.9 grams respectively.

*Chemical synthesis and identification of new nucleic acids.* (Doherty) Dibenzylchlorophosphonate was prepared and used to phosphorylate benzylidene adenosine and benzylidene guanosine yielding 3<sup>5</sup> benzylidene adenosine 2' dibenzylphosphate and 3<sup>5</sup> benzylidene guanosine 2' phosphate. Hydrogenation of the

\*Left the laboratory before end of quarter.

adenosine compound to remove the protecting groups was attempted with inconclusive results, little adenosine 2'phosphate being formed. Conditions for removal of the benzyl groups are under investigation at this time.

Adenylic acids "A" and "B" were purified and their physical properties determined. Adenylic acid "B" has been positively identified as adenosine 3'phosphate through its rotation, melting point, acridine salt, dibrucine salt, and adenine has been isolated from it and characterized as the picrate. Similar work with adenylic "A" has so far yielded adenine picrate. Further chemical work has confirmed the differences between adenylic "A" and adenosine 2'phosphate found on the ion exchange column and paper chromatograms.

*Investigation of the equilibria involved in the formation of enzyme substrate complexes.* (Vaslow and Doherty) Radioactive parabromoaniline of high specific activity has been prepared using bromine obtained from the pile neutron bombardment of  $\text{BeBr}_2$ . Purification of isomers and other impurities has been effected with an ion exchange column using  $1/2 N$  hydrochloric acid to elute the material.

Complexes of parabromoaniline with papain and with chymotrypsin have been demonstrated using the dialysis equilibrium technique and measurements of specific radioactivity. Special apparatus has been designed and built for this work.

Preliminary and apparent dissociation constants at  $5^\circ \text{C}$  for unimolar complexes are  $1 \times 10^{-2}$  and  $1.8 \times 10^{-4}$  for papain and chymotrypsin respectively.

*Biochemistry of nucleic acids.* (Volkin) Purified ribonucleic acids from calf spleen, pancreas, thymus and liver, and from yeast have been prepared by the method involving their precipitation by guanidine salts as described previously (cf. Biology Division Quarterly Report, ORNL-244). The molecular homogeneity of the nucleic acids, as well as their sedimentation rates have been estimated with the use of the analytical ultracentrifuge.

The analytical composition of the purified nucleic acids is now being resolved using methods of ion exchange and paper chromatography.

The *in vivo* incorporation of  $\text{P}^{32}$  into the nucleotides of the ribonucleic acids from rabbit and rat liver has been investigated. The liver nucleic acids were prepared and purified by the method indicated above. Resolution of the nucleotides was effected by the method of ion exchange. The enzymatic degradation of  $\text{P}^{32}$  containing nucleic acid is also being investigated.

## RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)  
W. T. Burnett, Jr.  
Maryann Huddleston

Leon Wish  
W. R. Martin  
Gertrude Beyl

During the last quarter several reports (listed in the Introduction) describing work which was done during Dr. Tompkins' tenure have been completed.

*Mineral metabolism.* (Burnett) The distribution of manganese-56 in various tissues of rats injected intravenously with 65  $\mu\text{c}$  of the radioisotope in solution is shown in table 1. The high uptake by the pancreas is of particular interest since other investigators have obtained similar results with the dog. Elimination of the element through the bile is in agreement with earlier observations by Greenberg et al.

TABLE 1

TISSUE	PERCENT OF INJECTED DOSE PER GRAM OF TISSUE			
	ANIMAL NO.			
	1	2	3	4
	TIME AFTER INJECTION (HOURS)			
	1.4	2.5	4.2	5.8
Large Intestine	0.39*	12.50	10.39	13.76
Pancreas	1.08	6.11	3.34	3.82
Small Intestine	0.71	5.13	2.86	1.45
Liver	1.76	1.11	0.71	0.67
Heart	1.25	0.48	0.19	0.15
Kidneys	1.76	1.87	1.02	1.21
Spleen	0.59	0.36	0.14	1.04
Testes	-	0.22	0.11	0.12
Muscle	0.02	0.05	0.05	0.37

\* All values corrected for decay back to time of injection.

In a cooperative project with the Pathology and Physiology group, a series of mice have been given, interperitoneally, solutions of iodine-131.

Preliminary hot animal work has been in progress on a small scale in the semihot laboratory to serve as a pilot plant for the later hot animal farm. A convenient, inexpensive glass cage which permits a very high recovery of radioactive material from small animals has been developed. Other devices recently developed for handling radioactive material include a remote control injection device and other special equipment.

*Blood volume.* (Wish) The red blood cells of the mouse have been successfully tagged with radioactive phosphorus *in vitro*. In cooperation with the Pathology and Physiology Section of this division, these tagged red cells have been used for blood-volume determinations with apparently good results.

A method has been developed for the tagging of gelatin with  $I^{131}$ . The material is now being tested as a method of determining the plasma volume of mice. *In vitro* experiments with dog's blood showed that at least 99 per cent of the  $I^{131}$  remained in the plasma when a portion of the tagged solution was shaken with the blood for 30 minutes or less.

*Erythrocyte respirations.* (Beyl) Harris (1941) and Danowski (1941) have presented evidence that the reentry of potassium into erythrocytes after storage at 5° C may be a metabolic process. Their reports of increased oxygen uptake prior to hemolysis made it conceivable that the redistribution of cellular potassium would have associated with it similar changes. Therefore, oxygen consumption of washed human red blood cells, suspended in buffered saline-dextrose medium, was measured by the direct Warburg technique both before and after cold storage, as well as in the presence of methylene blue. Potassium leakage from the cells during storage was established with the flame photometer. Small increases in oxygen consumption were noted but the results were not impressive. The methylene blue respirations showed consistent rates of 10-11  $\mu\text{c}/10^9$  cells/hr regardless of previous storage. The methylene blue system was sensitive to saponin hemolysis but was not affected by cyanide or fluoride.

*Cation exchange in erythrocytes.* (Sheppard, Martin, Huddleston) A series of potassium exchange rates were established on human blood at different temperatures, ranging from 6 to 41° C. Points for the first few hours only were taken. These data were sufficient to show the variations in potassium exchange rate with temperature. The log of the exchange rates was found to vary linearly with the reciprocal of the temperature, giving a  $Q_{10}$  of about 2.5.

Experiments on sodium exchange in canine, human and bovine blood at physiological temperature have been started.

## BIOLOGICAL SYNTHESIS

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

*Isolation and separation of C<sup>14</sup>-labeled carbohydrate.* (Noggle, Bolomey)  
The problem of the isolation and separation of C<sup>14</sup>-labeled carbohydrates has been approached from several new angles during recent months. The flowing chromatogram on Magnesol for separating glucose and fructose has been relatively successful on pure mixtures but has given considerable difficulty when dealing with mixtures from plant extracts. The plant material, after exposure to C<sup>14</sup>O<sub>2</sub>, is killed in boiling alcohol and then extracted eight hours with alcohol. The extract is freed of alcohol, taken up in water and then extracted with ether to remove the pigments and lipids. The water extract is then passed through Duolite C<sub>3</sub> cation and A<sub>3</sub> anion exchange resins to remove salts and acids. The resulting neutral solution contains the sugars. Following inversion with invertase, the sugar solution is concentrated for passage through the Magnesol column. Ethanol has been used as an eluant. In order to obtain good separations it is essential to operate the columns at a rate of about one drop every ten or more seconds, as faster flow rates result in considerable cross contamination. In practice it is advisable to add the glucose-fructose mixture on the column in as small a volume of 95-per-cent ethanol as is possible, to develop to chromatogram with 95-per-cent ethanol until most of the glucose has been eluted, then to continue the elution with 80-per-cent ethanol. The lower ethanol concentration speeds up the elution of fructose so that good recoveries may be obtained in a fairly small volume of eluate, i.e., two-column volumes instead of six or seven. Representative C<sup>14</sup> experiments in small photosynthesis chambers are as follows:

Sugar beets - Wanzleben variety

Fresh weight, 11.0 g

890  $\mu$ c C<sup>14</sup> introduced as BaC<sup>14</sup>O<sub>3</sub>

14.6  $\mu$ c C<sup>14</sup> found in chamber after 24 hours photosynthesis

263 mg of total sugar in alcohol soluble fraction

7 mg of sugar from starch fraction

30 mg of sugar from acid digestion of cellular residues

Green beans - Burpee's Stringless variety

Fresh weight, 7.0 g

822  $\mu\text{c}$   $\text{C}^{14}$  introduced as  $\text{BaC}^{14}\text{O}_3$   
5.2  $\mu\text{c}$   $\text{C}^{14}$  found in chamber after 24 hours photosynthesis.  
85 mg of total sugar from two alcohol extracts  
Starch sample not completed

The sugar sample was assayed for radioactivity and found to contain approximately 24,000 d/s per mg sugar.

A careful study of different methods of analyzing glucose and fructose mixtures has been made. The Van der Plank fructose method (hypoiodate destruction of glucose) had given consistently good results when used with the Somogyi sugar reagents.

To aid in the identification of the sugars found in plant extracts several new technics have been used. Paper chromatography methods have given good results. A solvent mixture of n-butanol, ethanol and water has been most successful. One per cent ammonia is added to the aqueous phase. Both descending and ascending chromatograms have been tried. The sugars have been identified by spraying with either ammoniacal silver nitrate or naphthoresorcinol in alcoholic hydrochloric acid. Xylose, raffinose, rhamnose, ribose, sucrose, glucose, fructose, lyxose, galactose, arabinose, 5 phosphoxylose and 5 phosphoribose have been run on the chromatograms.

Radioautographs of paper chromatograms of  $\text{C}^{14}$ -labeled sugars have been made. An exposure of 48 hours to the X-ray film (Eastman Industrial Type K) has given successful radioautographs. This method has indicated that the principal sugars of the green bean extract are sucrose, glucose and fructose.

The study of the growth and chemical composition of four sugar beet varieties (Wanzleben, Half-Sugar Rose, A U.S.D.A. selection, a Michigan selection) is still in progress. The results have shown that the petioles of the sugar beets are very high in sugar. With this idea in mind a large leaf chamber (45-liter volume) has been constructed and will be used for preparing  $\text{C}^{14}$ -labeled compounds from sugar beet petioles and leaves by short exposures to  $\text{C}^{14}\text{O}_2$ .

# B I O P H Y S I C S

## PHOTOSYNTHESIS

W. A. Arnold (Leader)  
A. S. Holt Imogene A. Brooks

*Effects of ultraviolet radiation on algae.* (Holt, Arnold, Brooks) Further work has been done on the comparative effects of hydrogen cyanide and hydroxylamine on normal and irradiated cells of *Scenedesmus*. The findings support the previous work which indicated that the inactivation does not occur stepwise but results in a complete cessation of the photosynthetic mechanism as a whole.

Comparative experiments on the respective sensitivities of photosynthesis by *Chlorella* and *Scenedesmus* were undertaken. Cultures of the two organisms were grown simultaneously under similar conditions and the absorption at 2537 Å and cell volumes made equal. The results show that *Chlorella* is far more sensitive.

Glucose oxidation by *Chlorella* has been found to be inactivated in a manner suggesting that two separate processes of glucose oxidation are occurring simultaneously.

*Oxidation reduction potential changes during illumination of isolation chloroplasts.* (Holt, Arnold, Brooks) Equipment and preliminary experiments show that a definite change does occur when chloroplasts are illuminated.

## ULTRAVIOLET MICROSCOPY

R. W. Koza

*Assembly and testing of equipment.* (Koza) A number of indicating circuits for use with the photomultiplier tube in direct measurement of the light intensity in the microscope image have been tested and none with sufficient sensitivity, stability, and period was found. To make the measurement photographically a projection densitometer is needed, and preliminary design work on such an instrument has been started.

The Zeiss stand of the microscope was found unsatisfactory in several particulars and the optics were transferred to a suitably modified American Optical Company stand. A calibration of the fine focus control against wave length is being carried out with the 6-mm objective.

Test and calculations have been performed with the monochromator and 6-mm objective which show that the present monochromator does not have sufficient aperture to fill the field of this objective with light of the proper numerical aperture. A beginning has been made in computation of the requirement of a monochromator fitted to the microscope.

The Daniel and Heidt lamps were finally constructed with graded quartz to tungsten seals before leakage of the seals was stopped. Even these lamps have a life of only 30-40 hours under the conditions in which they are used in the monochromator. The H-4 lamp is again being tested under somewhat different conditions from those used last spring.

## SPECIAL PROBLEMS

Seymour Benzer (Leader)

*Ultraviolet irradiation of bacteriophage.* (Benzer, assisted by M. B. Hill and R. W. Whittle) Experiments have been continued along the lines of Luria and Latarjet (J. Bact. 53: 149, 1947) to follow the development of bacteriophage by analysis of survival curves after exposure to ultraviolet irradiation. Specifically, it is sought to determine the effect of multiplicity reactivation as a possible explanation for the peculiarities observed by Luria and Latarjet, who used bacteriophage  $T_2$ .

A series of experiments using phage  $T_7$  reveals marked differences from  $T_2$  which are of particular interest because  $T_7$  does not show multiplicity reactivation.

The effect of multiplicity reactivation upon the curves for  $T_2$  has been studied more directly by infecting washed bacteria in buffer. Under these conditions, absorption of phage occurs, but there is no shift of the survival curve with time. The data obtained for various multiplicities of infection, up to ten particles per bacterium, are in fair agreement with the theory of Luria and Dulbecco (Genetics 34: 93, 1949). Due to the multiplicity reactivation, a relatively small multiplicity of infection is sufficient to produce a large increase in resistance to radiation, even without any growth of the phage. This is believed to account for the discrepancy observed by Luria and Latarjet between experiments using multiplicities around 0.01 and those around 0.3.

Studies of the burst size and latent period show that these are strongly affected by irradiation during phage growth, the effect being to decrease the yield and increase the latent period.

With Dr. M. R. Zelle, of Cornell University, preliminary experiments were done on photoreactivation with visible light (after infection) of phage T<sub>2</sub> inactivated (before infection) by monochromatic ultraviolet light of various wave lengths ranging from 2250 to 3022 Å. Photoreactivation was found in all cases.

Photoreactivation studies on phage inactivated during intracellular growth and then exposed to visible light before plating are being continued. The rate and maximum amount of photoreactivation are found to vary when the ultraviolet is administered at different stages in the latent period.

# PHYSIOLOGY AND PHARMACOLOGY

(Old Program)

H. I. Kohn (Leader)

Nancy Swingley Germaine Click

W.D. Robertson Mary Pigford

John Lane E. Ledford

W.D. Gude

## RADIATION SICKNESS

The activity of the group is being concluded by the writing of reports, to be finished during August and September. The principal objective was to supply more data concerning the acute reaction of the rat to ionizing radiation. Tentative titles of papers in preparation, together with some of the salient findings are listed below.

*Chemical Changes in the Blood of the Normal, Hypophysectomized and Adrenalectomized Rat Following a Single Total-Body Exposure to X Irradiation.* A pattern of reaction, elicited by as little as 125 r, was demonstrated in four strains of rats (Sprague-Dawley, Osborn-Mendel, Holtzman, Tumblebrook Farm Hooded), with some variation from strain to strain. The sequence of changes in the pattern was independent of dosage, but the changes themselves were proportional to dosage. The results were independent of food consumption, and were in no sense "terminal".

The animals being exposed to the LD-15 per cent on day 0, the principal elements of the pattern were as follows. (1) Sugar and NPN were elevated by 45 per cent and 25 per cent respectively for three days. (2) Plasma chloride rose about 10 meq. per liter on day 3, and, depending on the strain, returned to normal in three to ten days. Erythrocyte chloride usually changed in the opposite direction, so that whole blood chloride was relatively constant. (3) Total protein showed its maximum fall of one gram per cent on days 4 - 6. (4) The albumin/globulin ratio (A/G) reached a maximum of three (normal, two) on day 4, and in some cases remained above 2.4 for more than a month. Preliminary ether extraction of the plasma invariably abolished this effect. (5) Cholesterol rose on day 2 or 3, showed a maximal rise of more than 50 per cent on day 4, and returned directly to normal on day 6 (Holtzman), or after several days of subnormality (Sprague-Dawley).

Judged by survival, the immature animals (30 - 40 days old) were more sensitive than the mature. Adrenalectomy greatly increased sensitivity, as compared with a moderate increase by hypophysectomy. Both operations increased the "reactions" of cholesterol, protein and A/G following total-body irradiation, but diminished those of glucose and NPN. Immature hypophysectomized animals showed no chloride reaction (rise on day 3); hypophysectomy in the adult animal did not affect the chloride reaction, but prevented the NPN.

The principal changes in the pattern centered on days 2 - 4. This may be correlated with the finding of Hagen and Simmons (MDDC-1210) that after exposure, three days is the minimum time for death, and that the first wave of deaths occurs during days 4 - 7.

*Some Chemical Changes in the Plasma of the Guinea Pig Following Total Body X Irradiation.* The guinea pig shows changes in sugar, chloride, A/G and cholesterol which resemble those shown by the rat.

*Studies on a Factor in the Plasma of the Irradiated Rat Which Changes the A/G Ratio* (with E. Volkin of the Biochemistry Section). The rise in A/G ratio noted under the first title was determined by precipitation with sodium sulfide. Electrophoretic analysis of serum showed no such rise, while ultracentrifugation did confirm the rise. Material has been extracted from plasma which, when added to normal plasma, elevates the A/G ratio. More of it can be obtained from the plasma of irradiated than of normal animals. It is biuret negative and insoluble in petroleum ether.

*The Effect of Total Body Irradiation Upon the Serological Response of the Rat to Sheep Erythrocytes Injected Before and After Irradiation.* Under our experimental conditions, the maximum titer was reached five to seven days after the injection of antigen. Irradiation of the animal (single total-body exposure) before the injection of antigen inhibited antibody production, as was well known. However, irradiation *after* the injection of antigen also inhibited antibody production, the effect occurring almost, if not immediately, after exposure. This was true even on the fourth day after antigen injection, when a high and growing titer was present in the plasma.

*The Effect of Immunization with Sheep Cells upon the LD-50 per cent of the Rat* (total-body X irradiation, single dose). Injection of sheep cells one week before exposure increased the LD-50 per cent by about 75 r in the Sprague-Dawley strain, but not in the Holtzman strain. The protective action was *not* correlated with the titer. Such immunization changed the pattern of reaction in the plasma following irradiation (see first title) by abolishing the rise in

A/G ratio, i.e., preventing the appearance of the ether-extractable factor. It was also observed that when the lymphocyte count fell 24 - 48 hours after irradiation (or four to eight hours after injection of adrenal cortical extract), no rise in titer occurred.

*Changes in the Mitotic Index of the Cornea Following Local and Total-Body X Irradiation.* The experiments involved exposure to a single dose of 250 KV X rays, delivered in the course of a few minutes or less. In one series of experiments, the changes in the corneal mitotic index were followed during the six hours after exposure. It was found that barbiturate anesthesia during exposure doubled the sensitivity of the corneal mitotic index, and inhibitions were obtained after as little as two r, delivered to the head alone or to the entire body. When the trunk alone was exposed to as much as 600 - 700 r, an inhibition of the mitotic index was observed, but was no greater than could be accounted for by scatter. In another series of experiments, the changes in mitotic index were followed for a week, or more, in animals subjected to as much as the LD-50 per cent. The evidence indicated that the mitotic index was then subject to two distinct inhibitory influences, the direct effect of the radiation upon the cornea, and an indirect effect resulting from the changed physiological status of the animal. The latter was most evident after the second day following exposure.

*On the Inheritance of the Plasma Levels of Cholesterol and Sugar in the Rat.* It was noted that the Sprague-Dawley and Osborn-Mendel strains have a plasma total-cholesterol level twice that of the Tumblebrook Farm Hooded and Holtzman strains. It was also noted that following hypophysectomy, the difference between the Sprague-Dawley and Holtzman strains disappeared, both rising to a new and equal level. In addition, the plasma sugar fell much more rapidly during starvation in the Tumblebrook strain than in the others. Crosses were therefore made to test the genetic behavior of these characters. Crosses between the two high or the two low cholesterol strains resulted only in "high" or "low" offspring, respectively. Crosses between a high and a low strain resulted exclusively in offspring whose cholesterol level was the arithmetic mean of the parents. The same type of result occurred for the sugar levels. The various F-1's have been inbred, and the F-2's will be old enough for analysis during the next three weeks.

## PATHOLOGY AND PHYSIOLOGY

(New Program)

Jacob Furth (Leader)  
John Lane                    R. H. Storey  
Mary Knoohuizen            W. D. Gude  
Jane Beale                   G. Click  
                                 E. Ledford

New work was initiated by this section on July 1 and is still in the stage wherein equipment is being assembled and procedures worked out.

*Blood-volume.* (In conjunction with Dr. Sheppard's Radiobiochemistry Group. Wish, of Radiobiochemistry, Storey, Beale, Lane, Knoohuizen) The work on this problem falls into three divisions -- biochemistry,  $P^{32}$  technique, and animal experimentation. The plans call for determination of factors involved in the maintenance of blood volume, using  $P^{32}$ -tagged erythrocytes and other tagged substances.

*Studies of X-ray induced neoplasma.* (Knoohuizen) These studies, including an estrogen-secreting and a progestin-secreting type, are being carried out by transplantations. The endocrine disturbance leading to the development of these tumors, and the disturbances caused by the tumors, themselves are subjects of study.

*Neutron effects on small mammals (mice and rats).* (Lane, Stapleton --of Cytogenetics -- Gude, and Sheppard and Conger, consultants) The conditions of exposure in the reactor are being explored and the first experiments will soon be under way.

*Effects of administration of  $I^{131}$ .* (In conjunction with Radiobiochemistry Group. Burnett -- of Radiobiochemistry, and Knoohuizen) It appears from the literature that the changes produced by  $I^{131}$  involve organs other than the thyroid. This possibility is being explored.

*Radioautography.* (Gude) The radioautography unit is under construction and assembly. Pathological study will require radioautography of all tissues which may exhibit radioactivity.