

Lawrence Berkeley National Laboratory

Recent Work

Title

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT - OCTOBER, NOVEMBER, DECEMBER, 1953

Permalink

<https://escholarship.org/uc/item/6tn0k6zx>

Author

Lawrence Berkeley National Laboratory

Publication Date

1954-04-01

UCRL 2553
C.2
UNCLASSIFIED

UNIVERSITY OF
CALIFORNIA

Radiation Laboratory

TWO-WEEK LOAN COPY

*This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545*

BERKELEY, CALIFORNIA

C.2

UCRL-2553

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

UNIVERSITY OF CALIFORNIA
Radiation Laboratory
Contract No. W-7405-eng-48

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT
October, November, December, 1953
April, 1954

Berkeley, California

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

October, November, December, 1953

Table of Contents

BIOLOGICAL STUDIES OF RADIATION EFFECTS

Ultracentrifugal Studies of the Macromolecular Constituents of Dividing and Nondividing Yeast	3
--	---

THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

TRACER STUDIES

Lanthanide and Heavy Elements	24
Astatine Studies: Distribution	33
Astatine Studies: Histopathology	35

RADIATION CHEMISTRY

Acetic Acid Studies	50
Effects of Dissolved Oxygen in Irradiation of Aqueous Solutions of Acetic Acid	55
Glycine	55
Separation of Certain Organic Acids by Partition Chromatography.	57

HEALTH CHEMISTRY

Progress Report	59
---------------------------	----

HEALTH PHYSICS

Statistical Summary of Monitoring Program	62
---	----

* Previous Quarterly Reports: UCRL-2418, UCRL-2345

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

October, November, December, 1953

Radiation Laboratory, Department of Physics
University of California, Berkeley, California

April, 1954

BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence, M.D., in charge

ULTRACENTRIFUGAL STUDIES OF THE MACROMOLECULAR
CONSTITUENTS OF DIVIDING AND NONDIVIDING YEAST

Raymond G. Wolfe

Recent cytochemical studies have associated the cytological particles of the cell with certain of the biochemical functions. The relatively large mitochondria have been shown to be carriers of the enzyme systems involved in terminal oxidation of both fat and carbohydrate. The enzymes that appear to be freely soluble in the cytoplasm are responsible for glycolysis. Only recently has evidence been presented^{7, 8} that the microsomes, relatively much smaller than the mitochondria, have a possible role in protein synthesis.

The process of cell division has been studied largely in relation to nuclear division; the process of replication of the cytoplasmic particles seems to have received little attention. This report is concerned with preliminary studies of the influence of cell division on the appearance of macromolecular species in the sedimentation patterns of the Spinco analytical ultracentrifuge. It is felt that such studies are valuable for what they may contribute to knowledge of the basic process of cell division as a fundamental part of biological process related to growth. Greater understanding of the abnormal growth and dedifferentiation associated with neoplastic development may depend on the knowledge of the basic normal process of cell division. The feasibility of such a study on microorganisma was indicated by the recent publication of Schachman et al;⁴ in which it was shown that cell-free extracts of various microorganisms could be prepared and studied in the ultracentrifuge. Furthermore, it was established that the macromolecular components observed in these extracts were observed after

breaking the cells up by a variety of methods. This seems to indicate that the macromolecular components present in the cell extracts are not artifacts of the extraction method so far as the method of cell breakage is concerned.

In the interest of clarity this report is broken up into sections.

Section I is an account of the study of the stability of cell-free extracts. Section II covers the study of the development of certain new macromolecular species in yeast cells as they are induced to divide. Section III covers a study of commercial baker's yeast to ascertain whether it also produces the dividing-cell macromolecular pattern. This study also revealed the change in sedimentation pattern accompanying transformation from exponentially growing to senescent cells. Section IV covers a brief study of the influence of extraction conditions, pH, and ionic strength on the presence of dividing-cell components in cell-free extracts. Section V presents an account of the influence of x-ray irradiation on the presence of microsomes in cell extracts. Section VI presents a study of the influence of x-ray irradiation on the ability of cells to produce the dividing-cell components after cell division is induced.

General Methods

Haploid yeast (S. cerevisiae strain S. C. 7) was used in the experiments reported here except for the commercial yeast (Fleischman's cake baker's yeast) used in section III. Cells were grown in two ways. In some of the earlier experiments cells were grown in YED (1/2 % yeast extract, 1% dextrose) agar and harvested by suspending them in dextrose buffer. In later experiments cells were grown in liquid YED through which sterile air was passed for the purpose of aeration. Cells were nitrogen-starved by harvesting 48-hour cultures of cells, washing twice with glucose buffer (4% glucose M/15 KH_2PO_4), and aerating them in the same medium. During the nitrogen starvation the glucose buffer medium was changed every 12 hours. Approximately 4 grams of damp yeast is suspended in 150 ml of the medium placed in a 250 ml centrifuge bottle equipped with a glass tube for aeration. Division of starved cells was induced by adding yeast extract in the amount to make the concentration 1% with respect to this ingredient.

Starved cells were irradiated after drying down a suspension spread on 2% agar petri plates which had been poured two days previously. Cells were irradiated with 50 kv x-rays at 25 milliamperes at a dose rate of 250 rep per second. The actual dose given is stated in the discussion of each individual experiment.

Harvested cells were extracted by a modification of the method of Chao and Schachman.² The modification of this technique was necessary to increase the concentration of the extracts for the purpose of ultracentrifugation of the extract without preliminary concentration in the preparative ultracentrifuge. The cells were extracted in a cold room at 2°C, and the extracts were kept under refrigeration until the analytical ultracentrifugation could be made. One part by weight of the damp cells was ground in a mortar with a pestle, using six parts of 100-mesh carborundum by a standard technique. The same weight of cells was always extracted in the same size mortar with the same amount of grinding. The ground preparation was then extracted with two parts of water or other appropriate solvent, depending on the individual experiment. After being mixed with filter aid (Hyflow Supercell) the preparation was filtered through a thin filter-aid pad with vacuum. The ground, once-extracted preparation was extracted again with one part of the solvent and filtered again. The combined opalescent filtrates were stored in the refrigerator until centrifuged.

Several of the earlier analytical ultracentrifugations were made at 24,680 rpm for the purpose of studying the forepeak. All later experiments were made at 52,640 rpm and the sedimentation pattern was photographed at two-minute intervals. In the photographs presented the boundaries are moving under the centrifugal field from left to right. Photographs presented in this report were chosen at the time believed to show best the various boundaries present in the preparation. This time is usually 6 to 8 minutes after the machine reaches speed. All photographs comparing preparations made under different conditions are taken at the equal times after the machine is up to speed.

Section I

Study of the Storage Decomposition of the Yeast Extracts

During the study of the ultracentrifuge was not always immediately available. When the sedimentation patterns⁵ of stored samples were examined, appearance of a new peak was observed.

Method

In order to obtain more information on this phenomenon, a new cell-free extract was prepared and aliquots were centrifuged 1, 5, 10, 14, and 21 days after preparation. The extract was stored at approximately -2°C during the study period. Figures 1 through 5 show photographs of these sedimentations after 36 minutes of centrifugation at 24,640 rpm.

Results

It can be observed that under the conditions of storage the microsome peak decreases in size, gradually disappearing with time. Concomitantly a second peak, sedimenting more slowly, appears, but it decreases in size again with time of storage. It was also observed that the extracts of the starved cells have a heterogeneous forepeak sedimenting more rapidly than the microsome fraction. This is believed to represent a polymerized decomposition product of the microsomes (see Fig. 6). Furthermore, this forepeak particle group disappears from the sedimentation pattern very strikingly as cell division is induced by the addition of utilizable nitrogen. This forepeak fraction sediments and spreads so rapidly that a peak is visible only during the time the ultracentrifuge is accelerating up to speed (52,640 rpm), or only shortly after at the lower-speed centrifugations. The time relationship for the appearance of the new peak and the disappearance of the microsome peak suggests that the former may be derived from the latter as a result of decomposition.

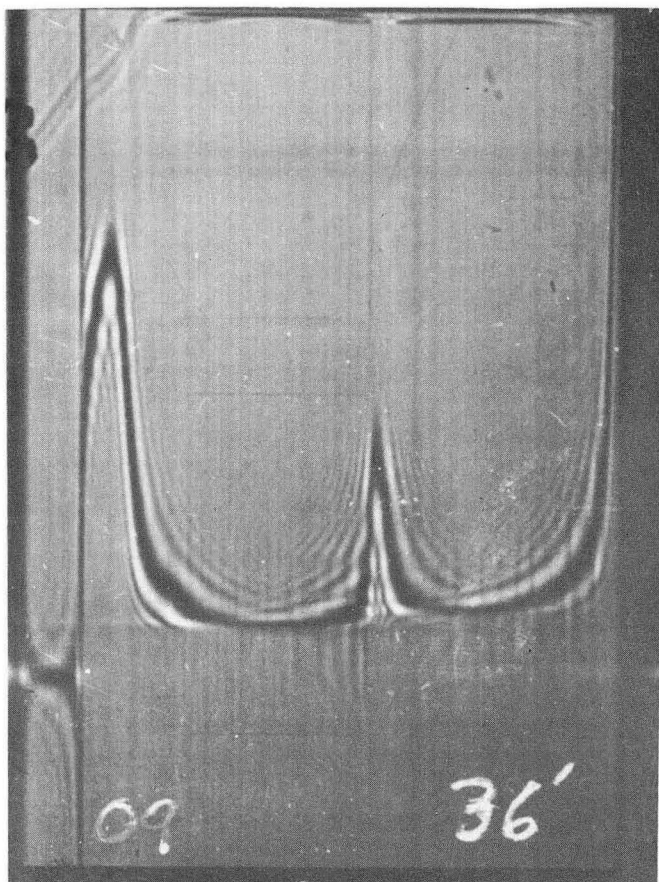


Fig. 1
After 1 day's storage at
 -2°C

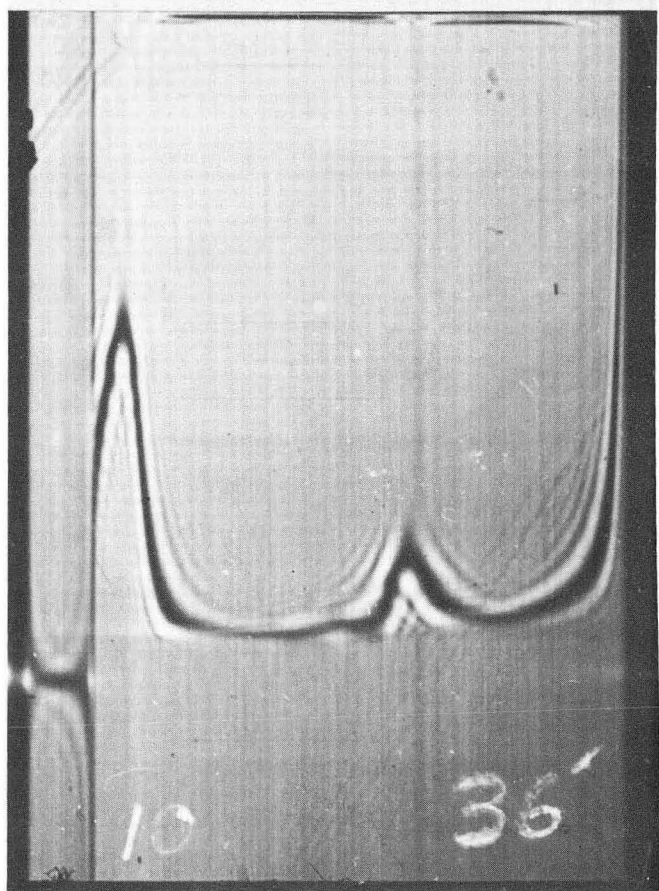


Fig. 2
After 5 days' storage at
 -2°C

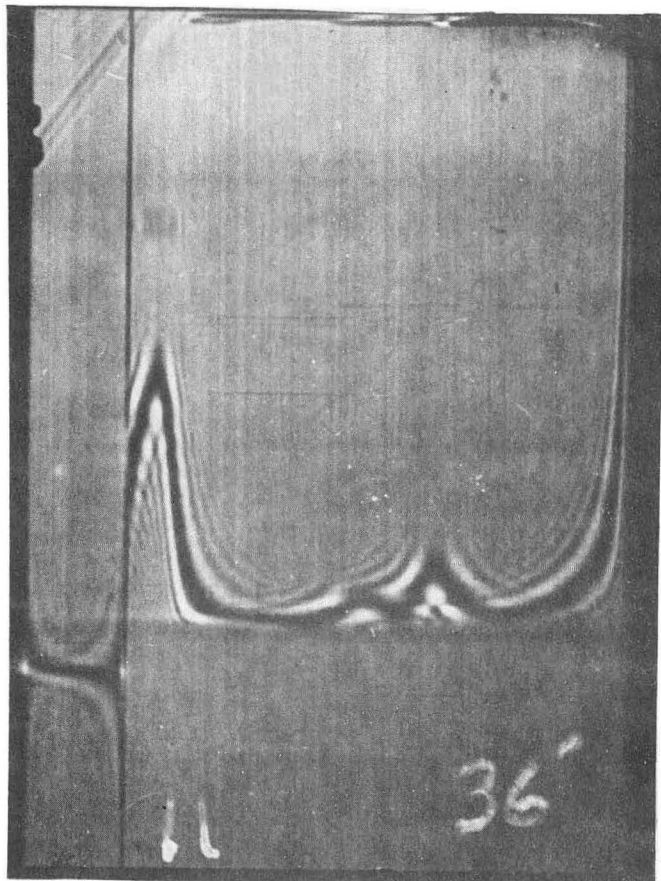


Fig. 3
After 10 days' storage at
 -2°C

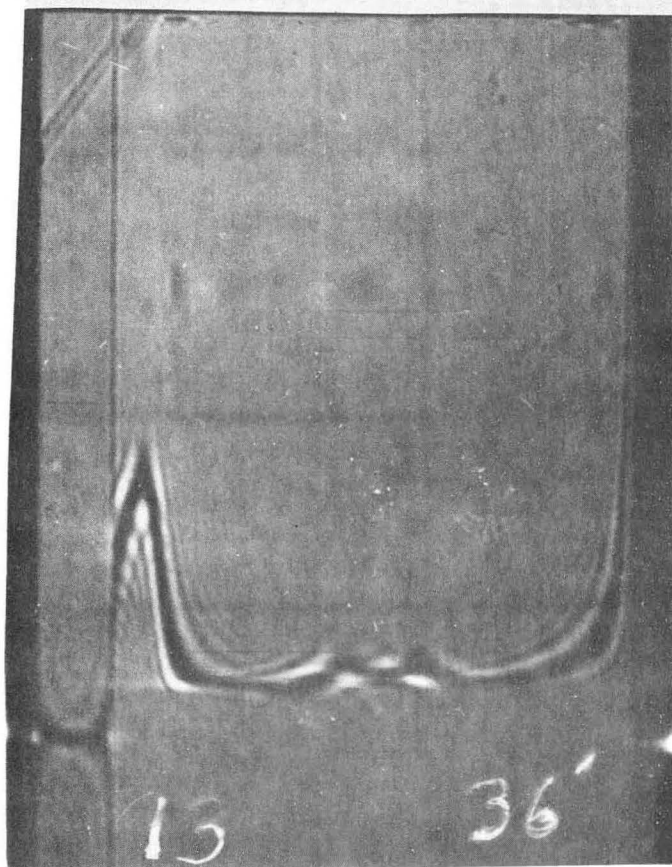


Fig. 4
After 14 days' storage at
 -2°C

ZN-948

Centrifuge sedimentation patterns of cell-free yeast preparations after 36 minutes centrifuging at 24,630 rpm. Figures 1-5 represent various times of storage in the refrigerator at -2°C .

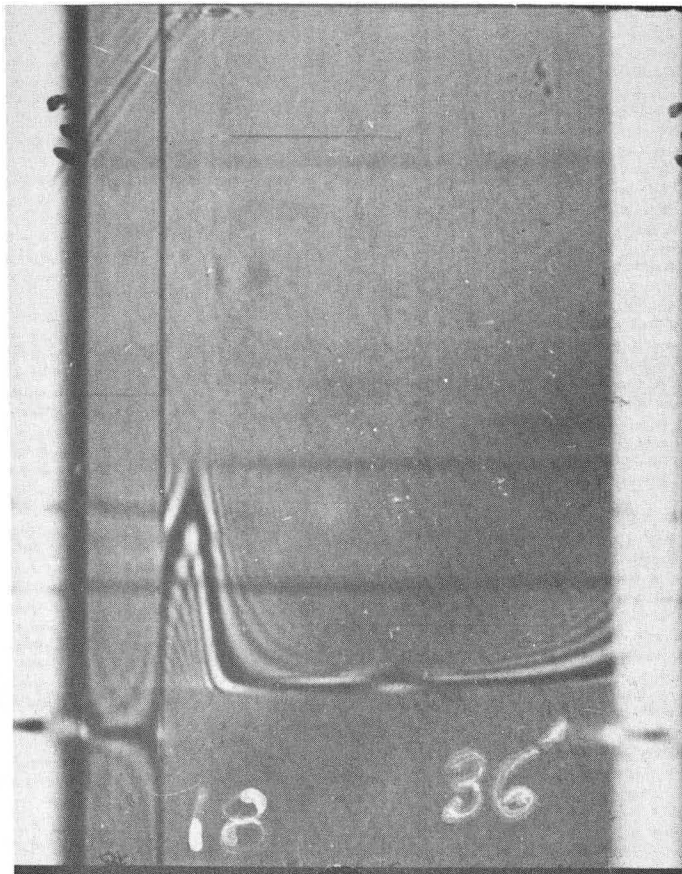


Fig. 5

After 21 days' storage at
-2° C

36 minutes centrifuging

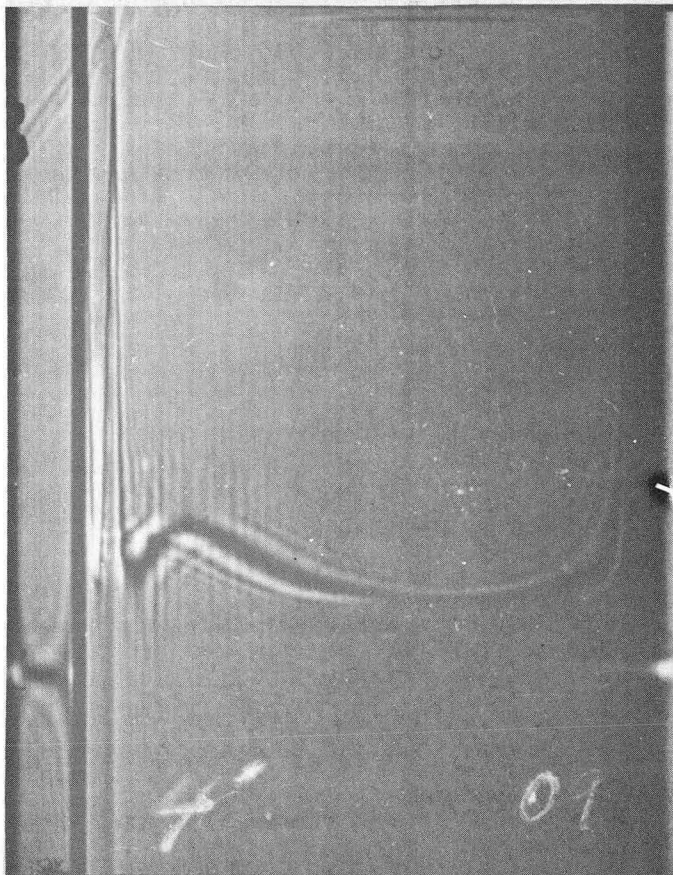


Fig. 6

Photograph of heterogeneous appearing forepeak which sediments before S78 fractions in preparations from starved cells only. The sharp peak about 1/8 in. below the meniscus is the S78 fraction. The peak below the arrow spreads rapidly with further centrifugation.

4 minutes centrifuging

ZN-947

Section II

Studies of the Macromolecular Composition of Dividing Yeast CulturesMethod

Nitrogen-starved yeast suspensions prepared as previously described were induced to divide by the addition of yeast extract. Cells so treated were harvested and cell-free extracts prepared 1.75, 3.5, and 5.0 hours after the addition of the yeast extract. A control harvest was made of the starved cells. Parallel observations of the cell division (budding) was carried out.

Results

The sedimentation patterns of the above preparations are presented in Figs. 7-10. It was observed that two or possibly three new components appeared in the dividing-cell extracts. At 1.75 hours after the addition of the yeast extract, when less than 1% of the cells have formed visible buds, very little dividing-cell components are present in the ultracentrifuge sedimentation pattern. After 3.5 hours, however, when approximately 30% of the cells are budding, the new components are present in the extracts. The extraction technique is standardized and roughly quantitative, excluding such possibilities as changing extractability with stage of cell division. Examination of the area of the microsome peaks in the above figures indicates that the area of the microsome peak increases as the cells commence dividing. Furthermore, this increment in the area of the peak does not occur before the appearance of the dividing-cell components. The apparent increase in the quantity of the microsomes present in dividing-cell preparations is in agreement with the observations of Jeener,³ who has demonstrated that the RNA concentration of the cells is proportional to the rate of cell division. The yeast microsome fraction has been reported to have 80% of the cell RNA and to be 44% RNA by composition.²

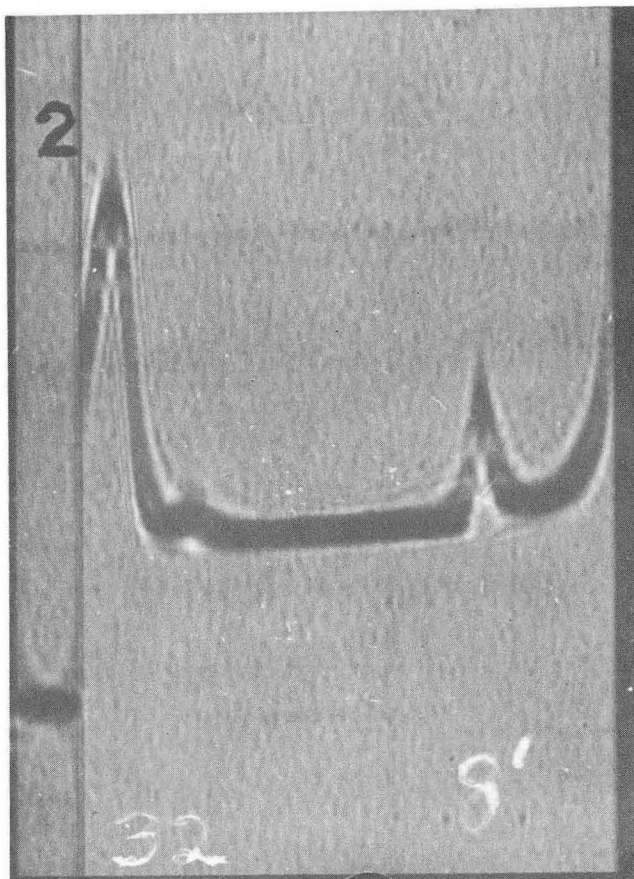


Fig. 7

Control starved-cell
preparation

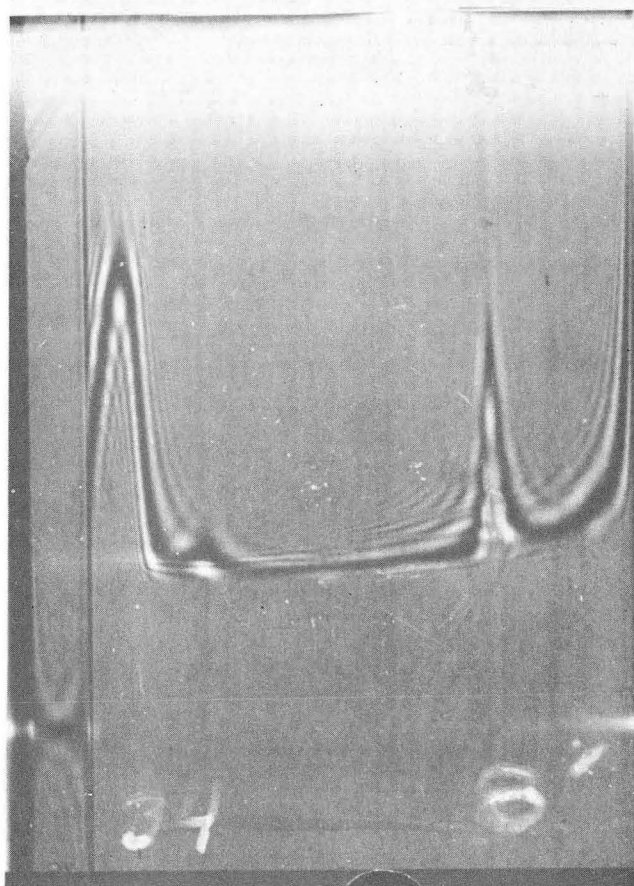


Fig. 8

Preparation of starved
cells 1.75 hours after
feeding utilizable nitrogen

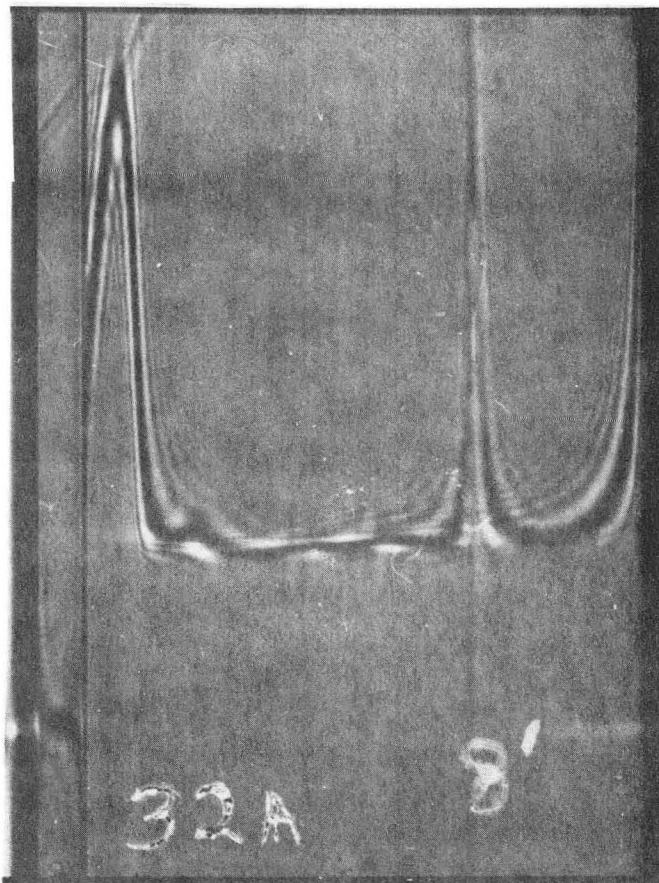


Fig. 9

Preparation of starved cells
3.5 hours after the addition of
utilizable nitrogen

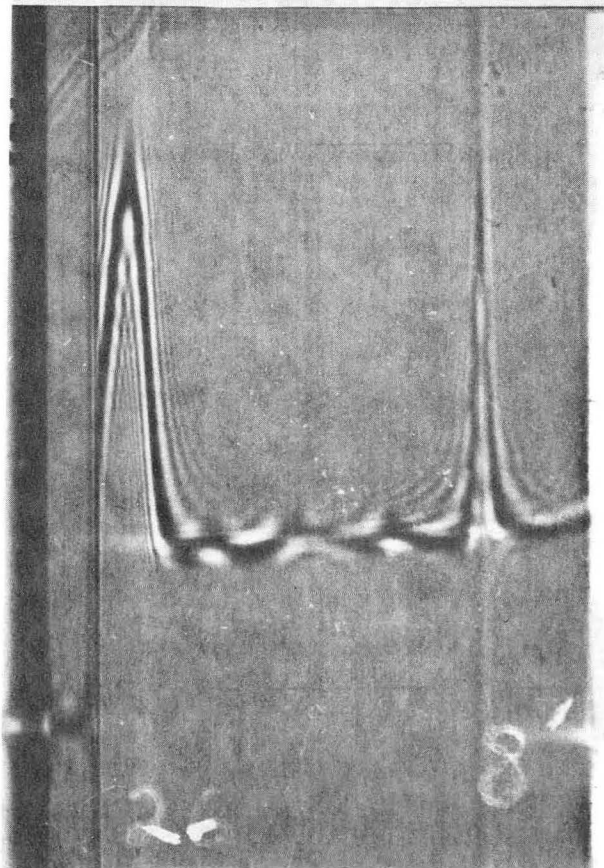


Fig. 10

Preparation of starved cells 5.0
hours after the addition of
utilizable nitrogen

ZN-945

All patterns on this page were photographed after 8 minutes' centrifugation, timed from "up to speed". The preparations were all centrifuged at 52,640 rpm.

The microsome particles appear to decrease quantitatively (as judged by the decrease in area under the peak in the sedimentation pattern) with time of starvation in the glucose buffer solution. Compare Fig. 10, which is of dividing cells, with Fig. 7, which has been starved 48 hours, and also with Fig. 14, which was of a yeast preparation starved 205 hours.

In the interest of clarity throughout the following discussion the various peaks observed in the sedimentation pattern have been designated alpha, beta, and gamma in the pattern from a preparation showing decomposition during storage of the cell free extract. The peaks appearing in the sedimentation pattern from dividing-cell preparations have been designated 1, 2, and 3 (see Fig. 11).

After it was observed that the preparations were unstable on storage in the refrigerator, care was taken to centrifuge all preparations within 18 hours. Stored preparations had no apparent decomposition in this time period.

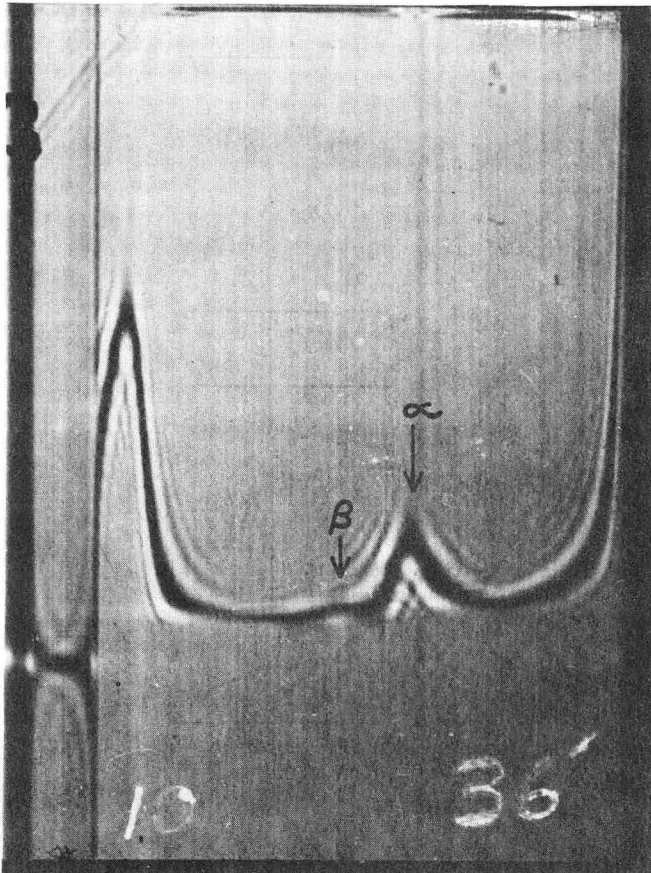
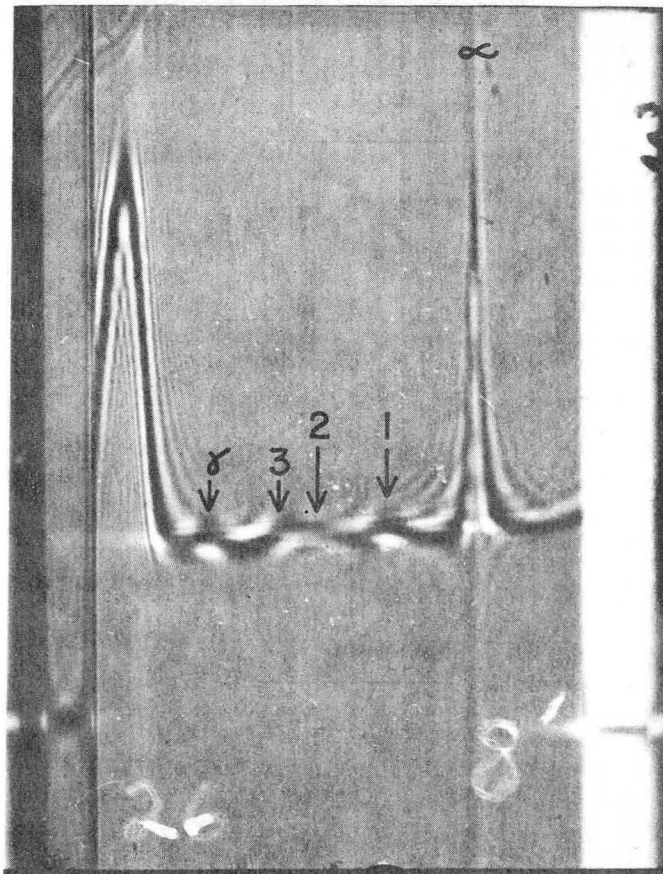


Fig. 11

Sedimentation pattern showing designation of macromolecular components present in decomposing extracts of starved yeast (see text, page 13).



Sedimentation pattern showing designation of components present in freshly prepared extracts of dividing yeast cells (see text, page 13).

Section III

Study of Fleischman's Commerical Baker's Yeast

It seemed desirable to determine whether the dividing-cell components observed in section II were strain specific. Consequently, the study was extended to include Fleischman's yeast.

Method

Strain S.C. 7 yeast, starved and dividing, were compared with commercial yeast cake, as purchased and in the dividing condition. The Fleischman's cake was induced to divide by suspending it in 1% YED through which sterile air was bubbled. The dividing yeast was harvested after 5 hours. A parallel preparation of S.C. 7 was made. Cell-free extracts were prepared as described under general methods.

Results

The dividing commercial yeast was observed to have the same components 1, 2, and 3 that are observed in the dividing S.C. 7 cells (Fig. 12). Extracts of the same commercial yeast purchased, however, demonstrated a different sedimentation pattern. Component 1 was absent and either component 2 or 3 was observed to be present (Fig. 13). It was observed that S.C. 7 demonstrated the same sedimentation pattern as untreated commercial yeast as they changed from the log phase to the senescent phase of the growth cycle.

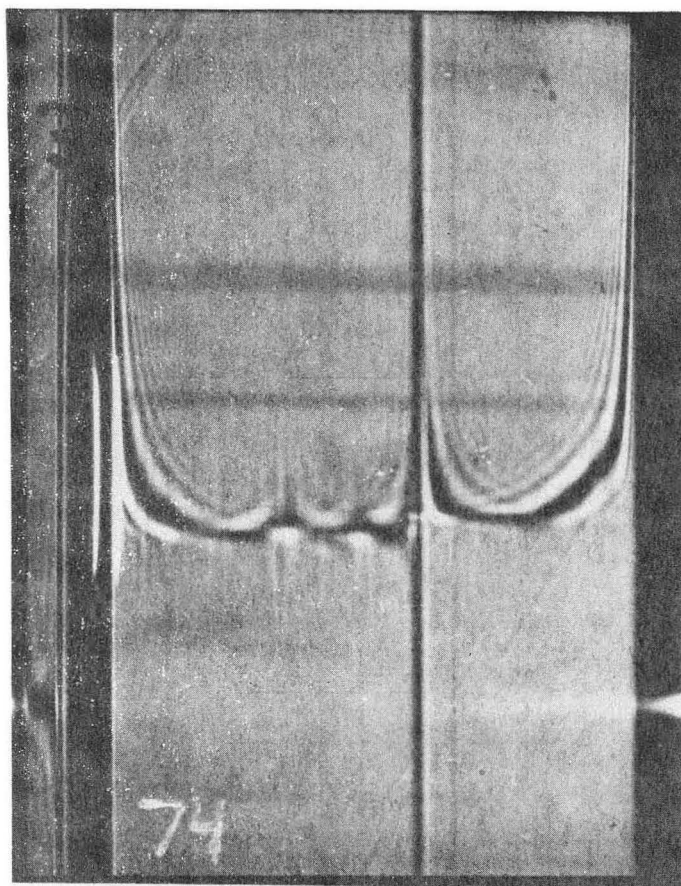


Fig. 12

Sedimentation pattern of cell extract of dividing commercial yeast.

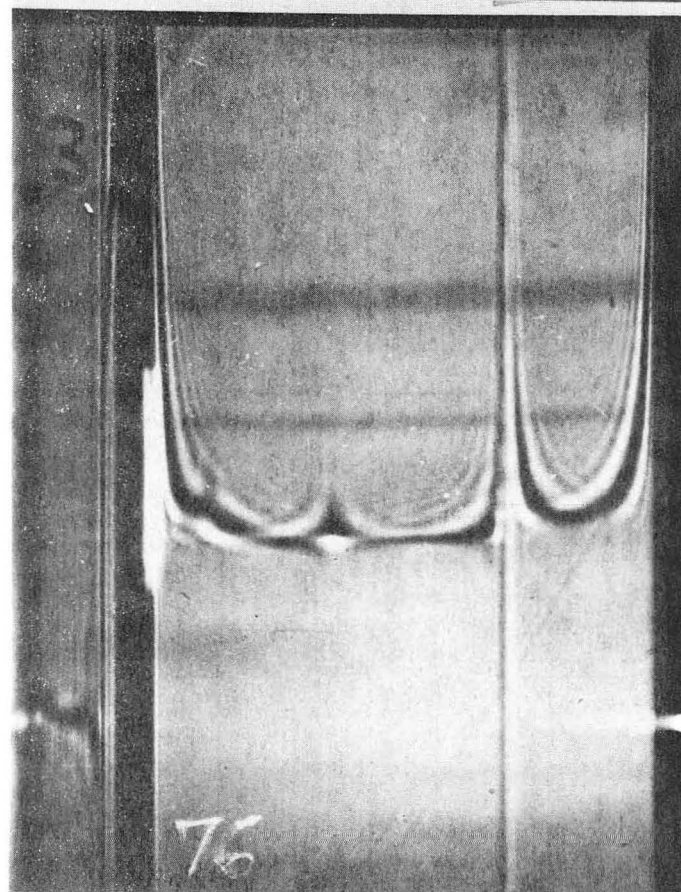


Fig. 13

Sedimentation pattern of cell extracts prepared from commercial yeast as purchased. The sedimentation pattern of the extracts from haploid (SC7) cells harvested in the early senescent stage of the growth cycle are the same.

Section IV

Studies of Various Conditions of Extraction of the
Yeast Macromolecular ComponentsMethod

Dividing S.C. 7 cells were extracted, after being ground in the way previously described, with glycine or phosphate buffers of 0.1 M concentration and of pH values ranging from pH 4.2 to 9.7. In addition, the influence of ionic strength on the solvent was investigated at pH 7.0 by making extractions with this buffer at 0.1, 0.05, and 0.01 M salt concentration. Chao and Schachman² report an improved solvent for the extraction of the microsome (component alpha) fraction. The stability of the dividing-cell components, as well as of the components present in starving cells, was investigated by extraction in Chao and Schachman's solvent and in a 10 times concentrated solution of this salt mixture.

Results

The dividing-cell components appear to be stable at the pH 6-7 range and from 0.1 to 0.01 M concentration at pH 7.0. The microsomes (component alpha) of the dividing-cell preparations appear to be more stable than the microsomes of starved cells, as there is apparent polymerization in extracts of starved cells at pH 7.0, 0.01 M, or even at 0.1 M solvent concentration. The degree of polymerization of the starved-cell microsomes is much greater at 0.1 than it is with 0.01 M solvent. The C-and-S solvent is therefore able to improve the stability of the microsomes. Dividing cells likewise showed no polymerization when extracted with either concentration of the C-and-S solvent. Extracts of starved cells, however, showed considerable polymerization with either concentration of the C-and-S solvent. Dividing-cell components 2 and 3 are more easily destroyed than is component 1; the latter is present in extracts ranging in pH from 4.2 to 9.7. Components 2 and 3 of the dividing cells are present only in the pH 6-7 range.

The preparations in this study were examined only once, within 18 hours after preparing the extracts, and the influence of time of storage on the patterns observed is unknown.

Section V

Study of the Influence of x-ray Irradiation
on the Presence of Yeast Microsomes

Billen and Volkin¹ reported that x-radiation of microorganisms (*E. coli*), followed by bubbling air through a glucose buffer suspension of the cells, resulted in the loss of microsomes from the sedimentation pattern of cell extracts. The study reported here was initiated to make a similar study in yeast cell preparations.

Method

The haploid S. C. 7 yeast were grown in a thin layer on YED medium in petri plates for 48 hours at 30°C. On half the plates the yeast was irradiated with 40,000 rep under the conditions described in general methods. This dose is 99.99% lethal, if colony formation is used as a criterion of survival. After irradiation, the cells were harvested from both the control and the irradiated plates by suspending them in 4% glucose M/15 KH_2PO_4 . Air was bubbled through the suspension as previously described for the purpose of aeration. The cell-free extracts were prepared in the usual manner. Since the above authors had published only an abstract at the time this was written the conditions of their experiment were not precisely known. Therefore, the experiment was repeated three times. The cells were bubbled 48 hours in the first, 80 hours in the second, and 205 hours in the third experiment.

Results

Irradiated cells were observed to have no change in their sedimentation pattern under any of the conditions listed above. Figure 14 gives a comparative example of the normal starved and irradiated cell extracts.

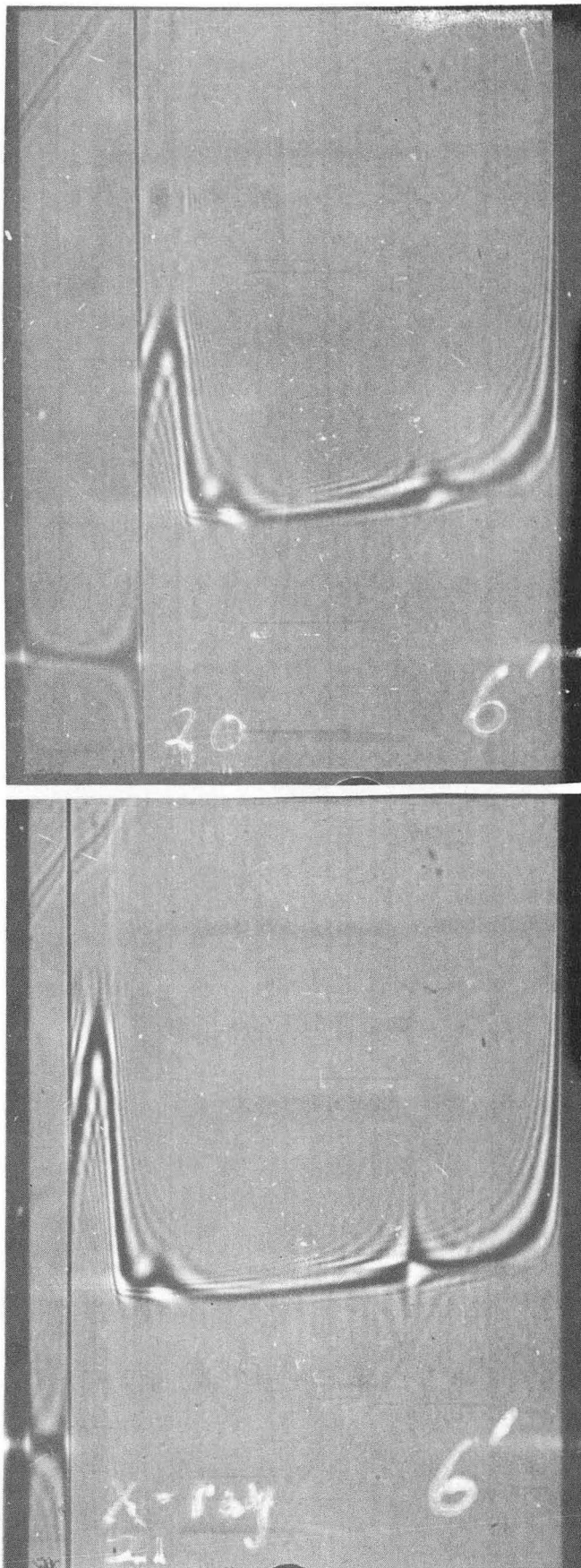


Fig. 14

Sedimentation pattern photographs of normal (at left) as compared to X-irradiated cells (right). All cells were given the same treatment except that the irradiated cells were given 40 kr of 50 kv x-rays at 25 ma. After irradiation the cells were subjected to nitrogen starvation for a considerable period of time before the cell-free preparations were made and studied in the ultracentrifuge. The above photographs were made six minutes after the ultracentrifuge (Spinco) was up to speed at 52,640 rpm.

Section VI

The Study of the Influence of X-ray Irradiation on the Ability
of Irradiated Cells to Produce Dividing Cell Components
Observed in Normal Dividing Yeast Cell Extracts

Since extremely high doses of irradiation are necessary to influence metabolism, whereas relatively low doses produce lethal effects in the same organism, it was believed of interest to study the influence of radiation on a possible synthetic ability of cells after they are induced to divide.

Method

S. C. 7 cells were prestarved in the usual glucose buffer solution for 48 hours. A concentrated suspension of the cells was spread out on a 2% agar plate and, after the water had soaked into the agar or evaporated, the surfaces of the plates were irradiated with 40 krep of x-rays under the usual conditions previously described. This dose was 99.99% lethal under the conditions used. A control suspension was treated identically except for the irradiation. After irradiation the cells were aerated in glucose buffer for 24 hours. Cell division was then induced by adding fresh 1% YED medium and the cells were harvested 5 hours later and extracted in the usual way.

Results

The usual dividing-cell components were observed in the control and in the irradiated cells. The yeast cell is apparently not influenced by a 99.99% lethal dose of x-rays in its ability to produce the dividing-cell components.

Discussion

The possibility that the new macromolecular components observed in the dividing yeast cells may be artifacts of the method must be considered. There are repeated studies showing an absolute correlation between the condition of the division in the culture and the ability to demonstrate new components (1, 2, and 3) in cell extracts. In no case has it been possible to demonstrate any of these components in starved yeast cells, except possibly one (component 1) as a result of decomposition of the extract upon storage. The appearance of

these new components, as cells are transformed from the resting to the dividing condition, has been demonstrated in section II of this report. The loss of two of the dividing-cell components (1 and either 2 or 3) has been demonstrated in section III of this report. The variation in macromolecular composition with the change in physiological conditions, using the same extraction technique, would lend support to the argument that the new components are not artifacts of the extraction technique. A study has been made in which the extraction solvent was varied in its pH and ionic strength (section IV). It was observed that the dividing-cell components (1, 2, and 3) were ~~more~~ stable under a variety of conditions than are the microsomes (component alpha). Rigorous proof that biological components are not artifacts is difficult to establish. The preferred method is the demonstration of the unimpaired biological function of the particle. This course is not open in the present study because the biological functions of the particles in question are unknown, and they have yet to be isolated. Components quite similar to the dividing-yeast components have been observed in extracts of other microorganisms by Schachman et al (4), although no correlation with the condition of cell division was noted. The physiological condition of the organisms studied was reported by the above authors to be late log phase or early senescence, but it is probable that the stability of the dividing-cell components is quite dependent on the physiological condition of the cell. Also stability of these cellular components ~~may~~ vary with the species considered. Either of the last two possibilities could explain differences between yeast and other species so far as the sedimentation patterns reported by the above authors are concerned. It appears certain that the appearance of the dividing-cell components is quite dependent on the physiological condition of the yeast studied. That the extraction procedure may produce an artifact dependent, in some way, on the dividing condition of the cells cannot be conclusively excluded as a possibility, at the present time.

It is interesting the Lindquist⁵ makes no mention of any component similar to component alpha (microsomes) in his recent study of high-molecular-weight compounds from bottom and top brewer's yeast.

The similarity of component 1 in dividing cells to component beta in decomposing-cell extracts is apparent from the photographs. Beta seems to be derived by the slow decomposition of component alpha. Direct proof of the identity of beta and 1 is, however, lacking. Speculation regarding a reversible precursor-product relationship between beta and alpha and 1 and alpha is, therefore, unsupported by experimental evidence.

The presence of the dividing-cell components in Fleischman's cake yeast during division is evidence that these components are not confined to one particular strain of yeast. Studies of other species of organisms to determine the general occurrence of macromolecular species in dividing cells would be of considerable interest from the general biological viewpoint.

It is a distinct possibility that the new macromolecular particles observed and reported here are of some fundamental importance in the process of reproduction of the cytoplasmic particles during cell division. Further study, possibly by isolation and chemical characterization, may give some insight into the poorly understood process of cell division, and yield some information regarding the chemical and physical properties of these particles. Attempts to develop a method of isolation of the new dividing-cell components are now being made.

Under the conditions of the above-described experiments no influence of x-irradiation could be observed, either on the stability of microsomes in yeast cells or on the ability of yeast cells to produce the dividing-cell components.

Summary:

Cell-free extracts of dividing yeast cells were observed to exhibit several new macromolecular components not present in resting yeast cells. The appearance of the new macromolecular components was shown to be related to the condition of cell division by following the appearance of the new components as cells in a starved culture were induced to divide by the addition of an ingredient containing utilizable nitrogen. Preliminary studies of the stability of the macromolecular components under various conditions of extraction were made. Irradiation with x-rays was found to have no influence on the stability of the microsomes or on the ability of cells to produce the new components when induced to divide.

BIBLIOGRAPHY

1. D. Billen and E. Volkin, Radiation Research Society, Iowa City Meeting, Abstract No. 5.*
2. F. Chao and H. K. Schachman, personal communication.
3. R. Jeener, Arch. Biochem. and Biophysics 43, 381 (1953).
4. H. K. Schachman, A. B. Pardee and R. Y. Stanier, Archives of Biochem. and Biophys. 38, 245 (1952).
5. W. Lindquist, Biochimica et Biophysica Acta 11, 90 (1953).
6. C. A. Beam et al., Arch. Biochem., in press; also University of California Radiation Laboratory Report No. UCRL-2345.
7. P. Sickevitz, J. Biol. Chem. 195, 549 (1952).
8. N. D. Lee and R. H. Williams, J. Biol. Chem. 204, 477 (1953).

* D. Billen and E. Volkin, J. Bact. 67, 191 (1951), The Effect of X-rays on the Macromolecular Organization of Escherichia coli.

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

October, November, December, 1953

THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

Joseph G. Hamilton, M. D.

TRACER STUDIES

LANTHANIDE AND HEAVY ELEMENTS

Joseph G. Hamilton, Patricia Durbin, Marshall Parrott, Marilyn Hemenway,
Margaret Gee, and Ruth Newman

This work is being continued actively, using the procedures described in previous quarterly reports. Rare earths of very high specific activity are being used, and are injected via the intramuscular route using adult Sprague-Dawley female rats as the laboratory animal. Each rare earth is complexed with sodium citrate, and injected as an isotonic solution at about pH 8.

Cerium-144

The 64- and 256-day cerium studies, which had previously been set up, are continuing, and will be reported on when the 256-day study is complete.

Thulium

In a similar manner these tracer studies are being carried through 256 days. A complete report on the metabolism of this element will be made when the 256-day study is completed.

Europium-152, 154

Data from the 1- and 4-day tracer experiment are now available and appear in Table I. Results for the more extended intervals, namely the 64-day and 256-day studies, will appear in a subsequent report.

A mixture of europium-152, 154 was obtained from Oak Ridge as solid $\text{Eu}_2(\text{C}_2\text{O}_4)_3$. The europium oxalate was dissolved in 4N H_2SO_4 , and a fraction of this was further diluted in a sodium citrate solution (30 $\mu\text{g}/\text{ml}$) at a pH of 8 for animal injection by the intramuscular route. The general aspects of the techniques used were the same as those described in the previous report given

on cerium-144, and five rats were used for each time interval. In order to minimize the amount of stable europium given, the animals received only about 2 microcuries of europium-152, 154. To facilitate the counting of such a small amount of activity, the ashed animal samples were dissolved in a suitable amount of 4N HNO₃ and aliquots of the acid solutions were transferred to gold plates for counting on a thin-window Geiger counter at the best possible geometry. Appropriate corrections were made for self-absorption.

It may be noted that the liver uptake is approximately one-half that of the cerium-144 at the 1- and 4-day time intervals, and there appears to be a significant increase of deposition and retention by the skeleton. There may be seen here a transition point between radiocerium and the heavier group of which the thulium-170 studies given in the last report serve as an excellent example. The same effect in the case of thulium-170 may also be seen with the 6-day lutetium-177, which is the last of the rare earth group and -- owing to the lanthanide contraction -- possesses the most acidic properties of the group. The lutetium-177 data are shown in Table II. Here the liver uptake is about one-twentieth that of cerium-144 and the deposition in the skeleton nearly three times greater.

Terbium-160

Detailed studies employing the 76-day terbium¹⁶⁰ should be highly interesting, and will cover the usual time intervals up to at least 64 days and possibly 256 days, depending upon whether sufficient specific activity for satisfactory completion of the 256-day time interval is possible.

Attention should be drawn to the fact that these rare earths have been made available to us through the cooperation and continuing interest of Professor Seaborg and his colleagues. Needless to say, we at Crocker Laboratory are most grateful for the opportunities made available to us by his group.

Radium-223

An attempt was made to secure satisfactory 64-day data, but owing to the high degree of radiotoxicity of the alpha-active isotope of radium and the energy released successively from its alpha-active daughter products, it was not feasible to adequately do a 64-day experiment. The radiotoxicity of this radioelement has been demonstrated and is presented later in this quarterly report.

Table I

EUROPIUM-152, -154

The Deposition of Europium-152, -154 Complexed with Sodium Citrate in the Rat 1 and 4 Days after Intramuscular Injection. Values are Corrected for 100 Percent Recovery and Expressed in Per Cent of Absorbed Dose. Each Rat Received 1.75 Microcuries of Europium-152, -154, 5.2 Micrograms of Europium, and 5.7 Milligrams of Sodium Citrate

<u>Tissue</u>	<u>1 day</u>		<u>4 days</u>	
	% per organ	% per gram	% per organ	% per gram
Spleen	0.15	0.20	0.13	0.21
Blood	0.10	0.01	0.06	<0.01
*Liver	33.3	4.94	25.0	2.69
Kidney	3.36	1.86	2.39	1.23
Gastro-intestinal tract	1.17	0.14	0.92	0.12
Gastro-intestinal content	4.11	-	2.02	-
Muscle	3.09	0.03	3.17	0.03
**Skeleton	35.3	1.40	35.6	1.40
Balance	4.71	-	2.65	-
Skin	3.03	0.08	2.43	0.06
Urine	11.0	-	16.6	-
Feces	0.68	-	9.08	-
Left leg	15.9		14.1	
Actual Recovery	106.5		109.4	
*Mean Std. Error	±1.6	±0.35	±1.3	±0.10
**Mean Std. Error	±1.9	±0.09	±1.8	±0.11

Table II

The Deposition of Lutetium-177 Complexed with Sodium Citrate in the Rat 1, 4, and 16 Days Following Intramuscular Injection. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Absorbed Dose. Each Rat of the 1-, 4-, and 16-Days Groups Received 7.3, 5.7, and 29.2 Microcuries Lutetium-177 Respectively; 0.47, 1.4, 1.9 Micrograms Lutetium Respectively; 4.8, 5.4, and 7.2 Milligrams Sodium Citrate. The Standard Error of the Mean for the Liver and Skeleton is Shown at the Bottom of the Table.

<u>Tissue</u>	<u>1 day</u>		<u>4 day</u>		<u>16 day</u>	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Spleen	0.14	0.29	0.13	0.25	0.13	0.26
Blood	0.11	0.01	0.05	≤0.01	≤0.01	-
* Liver	3.67	0.59	2.74	0.35	1.35	0.18
Kidney	1.33	0.96	0.63	0.39	0.44	0.28
Gastro-intestinal tract	1.92	-	0.86	0.13	0.46	0.06
Gastro-intestinal content	4.52	-	0.35	-	0.12	-
**Skeleton	65.9	3.88	67.6	3.57	65.5	3.41
Muscle	2.07	0.02	2.36	0.03	1.38	0.01
Balance	3.98	-	1.81	-	1.10	-
Skin	2.16	0.07	1.04	0.03	1.08	0.04
Urine	13.0	-	15.6	-	16.4	-
Feces	1.23	-	6.91	-	12.1	-
Left leg	7.35	-	6.50	-	4.69	-
Actual Recovery	115.7		121.2		109.4	
* Mean Std. Error	±0.13	±0.04	±0.12	±0.03	±0.29	±0.04
**Mean Std. Error	±0.90	±0.13	±0.37	±0.12	±0.65	±0.06

Thorium-227

Data are now available for thorium-227, which was complexed with sodium citrate and given by the intramuscular route to adult Sprague-Dawley female rats. The animals were sacrificed at intervals of 1, 8, 15 and 32 days after injection. Since the decay of thorium by alpha emission (half life 18.6 days) to radium-223 (half life 11.2 days) results in only a transient equilibrium, it was found necessary to separate the thorium-227 from its radium-223 daughter and from the tissue ash by chemical means. The separation was carried out by employing a modification of the TTA extraction method developed by Scott and Hamilton¹ for the separation of plutonium from animal ash. The method is as follows: The samples were dissolved in 4N HNO₃ and a suitable aliquot was placed in a centrifuge cone. To the sample were added 6 mg of La(NO₃)₃ and 10 ml of 12N HF, with stirring. After centrifuging, the supernatant was discarded, 10 ml of 1.5N HF was added, and the precipitate was resuspended. After a second centrifugation the supernatant was again discarded. The precipitate was dissolved by the addition of 25 ml of saturated Al(NO₃)₃, the pH of which had been adjusted to 1.5 with the addition of NH₄OH. After the precipitate was dissolved the liquid was transferred to a separatory funnel and extracted with 10 ml of 0.25 M TTA in benzene. The aqueous layer was discarded and the organic layer washed twice with distilled water and then back-extracted with 2N HNO₃. An aliquot of the nitric acid layer was then pipetted onto a gold plate for alpha counting. Samples thus prepared were counted immediately so that the growth of the radium-223 daughter would not interfere with the thorium count. Each time samples were counted an aliquot of the dose was also chemically extracted, so that a direct measurement of the thorium-227 was possible. The decay of the thorium-227 -radium-223 mixture was measured, as well as the decay of the thorium-227 samples extracted from this mixture, both as a check on the purity of the preparation and as a check on the accuracy of the chemical separation.

¹ K. G. Scott and J. G. Hamilton, Proc. Soc. Exptl. Biol. and Med. 83, 301-305, (1953).

The results of the thorium-227 data together with the standard deviation appear in Table III. The low level, on both a per-gram and a per-organ basis, in the liver and spleen indicate that little if any of the thorium-227 existed in the body in a colloidal state. The fact that absorption was almost complete from the site of injection indicated the value of using sodium citrate as a complexing agent. Note should be taken of the high skeletal values, which remain essentially unchanged throughout the time intervals from 1 to 32 days. There appeared to be a little increase of excretion, but its effect was small, and up to the 32-day interval, although some loss of thorium-227 from the associated tissues was to be expected, the effect was not large except in the blood, kidney and gastro-intestinal tract.

Iron-59

The Fe⁵⁹ studies have been completed. The purpose of this experiment is to set up the procedures for studying the damage to the blood-forming tissues in the acute and long-term changes of iron metabolism as a function of dose of internal irradiation. The irradiation may be from such sources as the beta-active lanthanide series, radium-223, and members of the actinide group, including actinium-227 and possibly astatine-211. Iron-59 was given intravenously to adult Sprague-Dawley female rats as the ascorbic acid complex prepared according to the method described by Wintrobe et al.² Because the iron-59 uptake in the red cells is to be used as an index of the damage to the blood-forming tissues in the chronic radiation studies, in which other radioelements will be present in the samples, the 7-day samples were subjected to a chemical separation as follows: The samples were dissolved in 8N HCl to which was added 5.4 mg of FeCl₃. The samples were then extracted twice with an equal volume of diisopropyl ether. The ether was evaporated to dryness in porcelain counting dishes to which had been added 4 ml of 2N HCl. This technique appears to be satisfactory. The recovery values are occasionally erratic, but fairly reliable in view of the type of chemical separation employed.

² Wintrobe, M.M., Greenberg, G.R., Humphreys, S.R., Ashenbrucker, H., Worth, W. and Kramer, R.J., Clin, Investigation 26, 103 (1947).

Table III

The Deposition of Carrier-Free Thorium-227 Complexed with Sodium Citrate in the Rat 1, 8, 15 and 32 Days After Intramuscular Injection. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Absorbed Dose. Each Rat of the 1- and 4-Day Groups Received 0.77 Microcuries of Thorium-227 and 1.5 Milligrams of Sodium Citrate; Each Rat of the 15- and 32-Day Groups Received 1.54 Microcuries of Thorium-227 and 3.0 Milligrams of Sodium Citrate.

Tissue	1 day		8 days		15 days		32 days	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Spleen	0.36	0.53	0.30	0.52	0.40	0.70	0.32	0.48
Blood	0.27	0.03	0.06	<0.01	0.02	<0.01	<0.01	-
*Liver	4.70	0.72	4.09	0.45	5.20	0.56	4.66	0.53
Kidney	4.44	2.66	3.26	2.01	2.25	1.32	1.73	1.03
Gastro-intestinal tract	2.13	0.30	1.74	0.24	1.68	0.22	1.36	0.19
Gastro-intestinal content	1.17	-	0.38	-	0.23	-	0.14	-
**Skeleton	64.9	4.00	66.3	3.69	65.7	3.46	68.3	3.50
Muscle	5.16	0.06	3.41	0.03	3.91	0.04	3.96	0.04
Balance	4.48	-	3.86	-	3.61	-	2.56	-
Skin	3.73	0.14	2.84	0.10	2.90	0.10	2.50	0.09
Urine	7.00	-	8.67	-	10.9	-	11.1	-
Feces	1.51	-	5.11	-	3.23	-	3.32	-
Left leg	6.26	-	8.12	-	6.09	-	5.71	-
Actual Recovery	100.0	-	98.6	-	95.6	-	91.5	-
*Mean Std. Error	±0.15	±0.04	±0.35	±0.03	±0.57	±0.08	±0.48	±0.06
**Mean Std. Error	±0.8	±0.10	±1.2	±0.04	±1.1	±0.12	±0.6	±0.07

The rapid accumulation in the erythrocytes at the 3-day interval agrees quite closely with the data given by Furchner and Storer.³ The high liver-uptake values observed are thought to have been due in part to the blood present in this organ; similarly, a rather high value, in terms of concentration per gram of the administered dose, may be noted in the spleen. It is difficult to determine which fraction represents radioiron accumulation held up in these organs and how much may be radioiron broken down from previously tagged erythrocytes. The data for the 3-, 7-, 14-, and 28-day time intervals appear in Table IV.

During the past quarterly interval a series of adult Sprague-Dawley females weighing approximately 250 grams each have been given actinium chloride complexed with sodium citrate at a pH of approximately 8 by intramuscular injection. The actinium-227 had been previously freed from its radioactive descendents. The animals are to be sacrificed in groups of five at 1, 4, 16 and 256 days. The use of sodium citrate in this experiment is to secure rapid absorption from the site of injection and thus obtain a clearer picture of the behavior of this particular radioelement as far as its metabolic characteristics are concerned.

³ Los Alamos Report, LA-1544, March, 1953.

TABLE IV

The Deposition of Iron-59 Complexed with Ascorbic Acid in the Rat 3, 7, 14, and 28 Days Following Intravenous Injection. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Administered Dose. Each Rat Received 3.0 Microcuries Iron-59, 14.5 Micrograms Iron and 0.5 Milligrams of Ascorbic Acid at pH⁸.

Tissue	3 days		7 days		14 days		28 days	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Spleen	0.95	1.44	0.99	1.87	0.95	1.34	1.28	2.18
*Red Blood Count	52.5	10.9	45.7	8.18	38.2	7.26	47.1	8.72
**Liver	20.0	2.45	19.0	2.12	22.9	2.68	25.7	2.69
Kidney	2.10	1.19	2.71	1.37	1.90	1.03	2.32	1.19
Gastro-intestinal tract	3.47	-	2.63	-	1.62	-	1.81	-
Skeleton	8.36	0.41	9.64	0.36	8.54	0.45	9.13	0.37
Muscle	7.67	0.07	11.7	0.09	8.09	0.07	12.1	0.10
Skin	4.22	0.11	5.69	0.12	4.62	0.12	4.47	0.11
Urine	1.45	-	2.15	-	2.80	-	2.43	-
Feces	4.94	-	10.0	-	15.0	-	11.2	-
Actual Recovery	115.9		87.6		89.1		89.7	
*Mean Std. Error	±1.0	±0.3	±3.4	±0.50	±3.0	±0.56	±2.0	±0.37
**Mean Std. Error	±0.9	±0.19	±0.7	±0.09	±1.4	±0.16	±1.4	±0.14

ASTATINE STUDIES: DISTRIBUTION

An experiment was set up to determine the oral absorption of astatine in the rat following intragastric administration. The purpose of doing this experiment was to determine an approximation of the feasibility of giving astatine by mouth in tracer amounts to human subjects suffering from different types of thyroid diseases. Five plateaued Sprague-Dawley female rats were used. Each rat received 62.5 microcuries of astatine, and the data are summarized in Table V. The point of importance was to ascertain whether there was a marked decrease in the accumulation of astatine by the thyroid gland. From the table it may be noted that the accumulation by the thyroid gland averaged 1.06 percent after suitable correction had been made for recovery which in itself was quite good. This value is slightly less than the uptake following intravenous administration. An interesting observation was the high level of astatine in the gastrointestinal tract and its contents. When astatine was given intravenously the amount present at 19 hours was approximately 22 percent, whereas in this experiment the amount present was found to be 56.6 percent at 24 hours. How much of this represents unabsorbed material, and how much recycling, is not clear. Inasmuch as the animals were fasted 24 hours prior to the administration of astatine, the question of absorption in the food does not appear to be a significant factor. Finally, it must be remembered that this was a pilot experiment to determine the feasibility of oral administration of astatine in small amounts in human beings.

TABLE V

The Distribution of Astatine in the Rat 24 Hours after Intragastric Administration. Values Presented Are Expressed in Percent of Administered Dose and Are Corrected for Deviation of Recovery to 100 Percent. Each Animal Received 62.5 Microcuries of Astatine in Isotonic Saline.

Tissue	% per organ	% per gram
Spleen	0.37	1.07
Liver	1.65	0.17
Gastro-intestinal tract	56.6	-
Carcass	18.6	-
Skin	14.2	0.53
Thyroid	1.06	-
Urine	6.59	-
Feces	0.93	-
Actual Recovery	98.8%	-

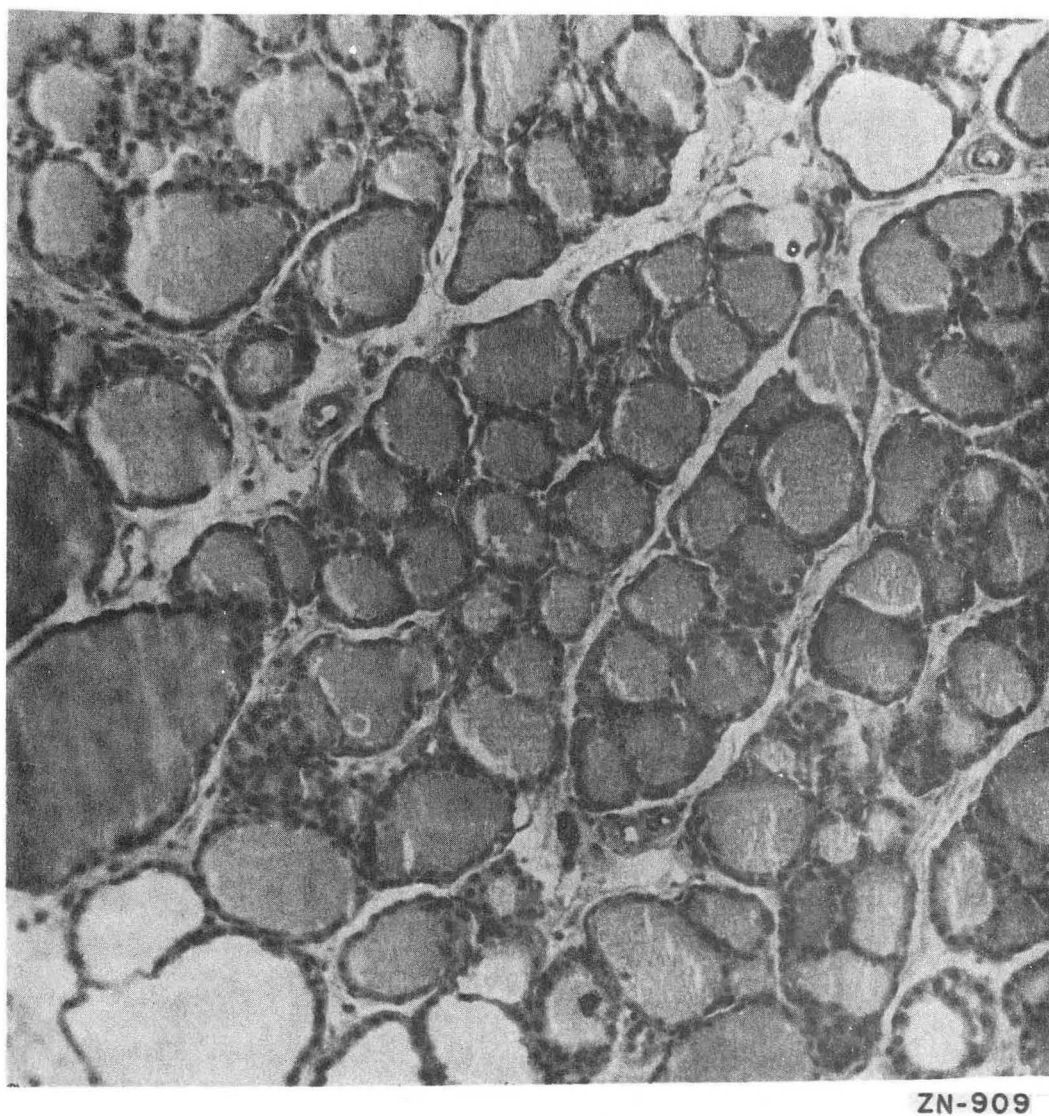
ASTATINE: HISTOPATHOLOGY

Patricia Durbin, Muriel Johnson, Marshall Parrott
Nylan Jeung and Joseph G. Hamilton

Histopathological preparations associated with different experiments currently under way have been made in increasing numbers. A good deal of additional information has been accumulated in the astatine studies. The experiments, described in this report and presented with a series of photomicrographs, were done with astatine preparations of which an estimated 20 percent of the astatine was in the colloidal state. It has been shown that accumulation of astatine by the thyroid gland is not significantly influenced when some of the astatine is in a colloidal state. Thus, the photomicrographs show changes of the thyroid gland under these conditions that may be considered essentially the same as from administration of the more recent clean preparations in which no colloid was present. In the lymph nodes and spleen the greater accumulation of astatine that is partially colloidal may be anticipated to produce a greater effect, since the astatine uptake under these conditions is somewhat greater in these organs.

Figure 1 shows a photomicrograph of thyroid tissue a rat that received by intravenous injection a total of 130 microcuries of astatine (which was equivalent to 0.75 microcuries per gram) was sacrificed at the end of two days. The thyroid gland showed no evidence of change from the normal rat thyroid gland. Figure 2 presents a photomicrograph of tissue thyroid gland of a rat that received the same amount of astatine and was sacrificed at the same time. Here may be seen some apparent changes in the colloid which would suggest early damage.

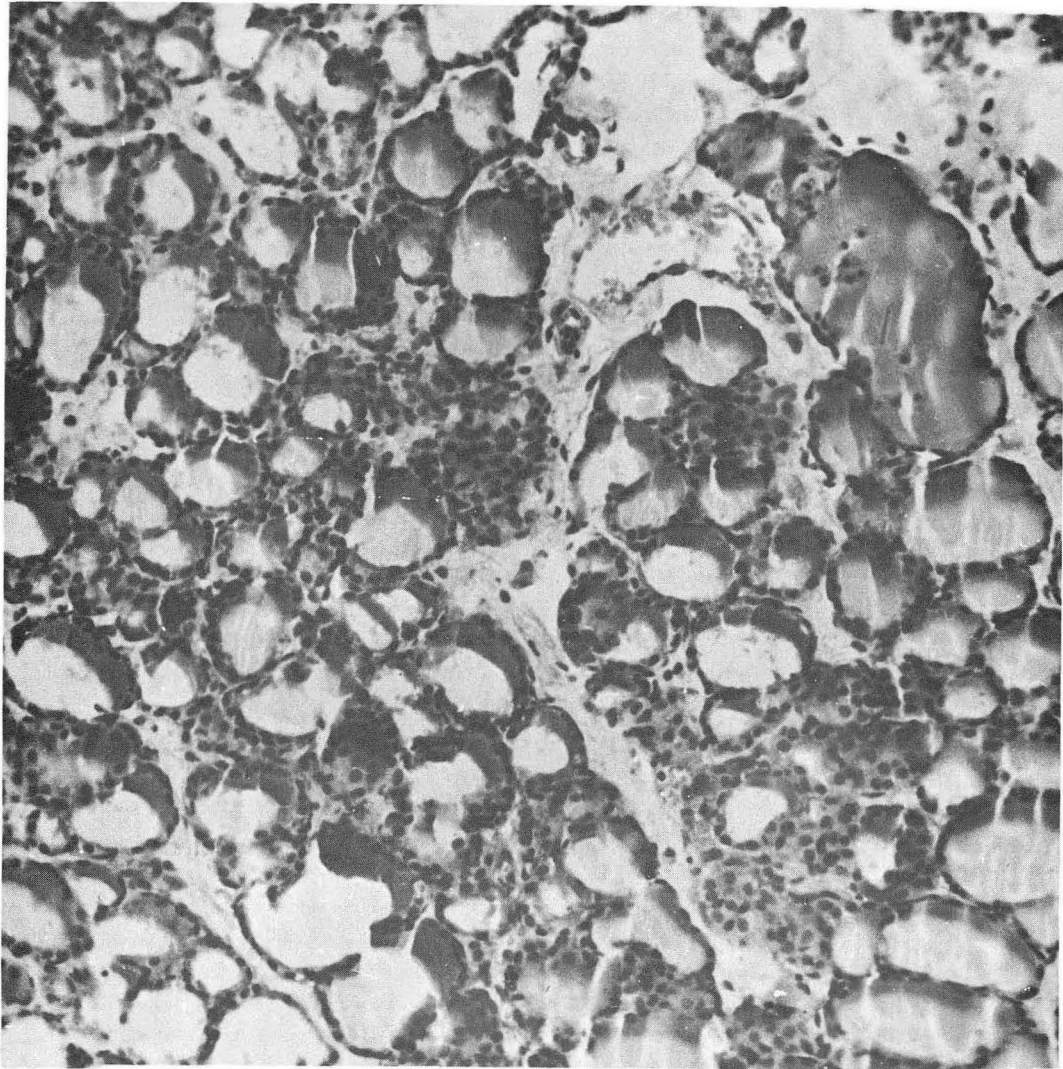
An extreme degree of injury to the thyroid gland of the rat can be seen in Fig. 3. In this instance, the animal received 175 microcuries of astatine by intravenous injection, or 1.8 microcuries per gram, and was sacrificed at the end of five days. A few follicles may be seen, which appear to be devoid of colloid, and the follicular cells have lost their normal structure. There was evidence (not shown in the photomicrograph) of some hemorrhage and infiltration by polymorphonuclear cells. This type of injury was seen to the same degree in other animals receiving this dose of astatine and also sacrificed at the end of five days.



ZN-909

Fig. 1

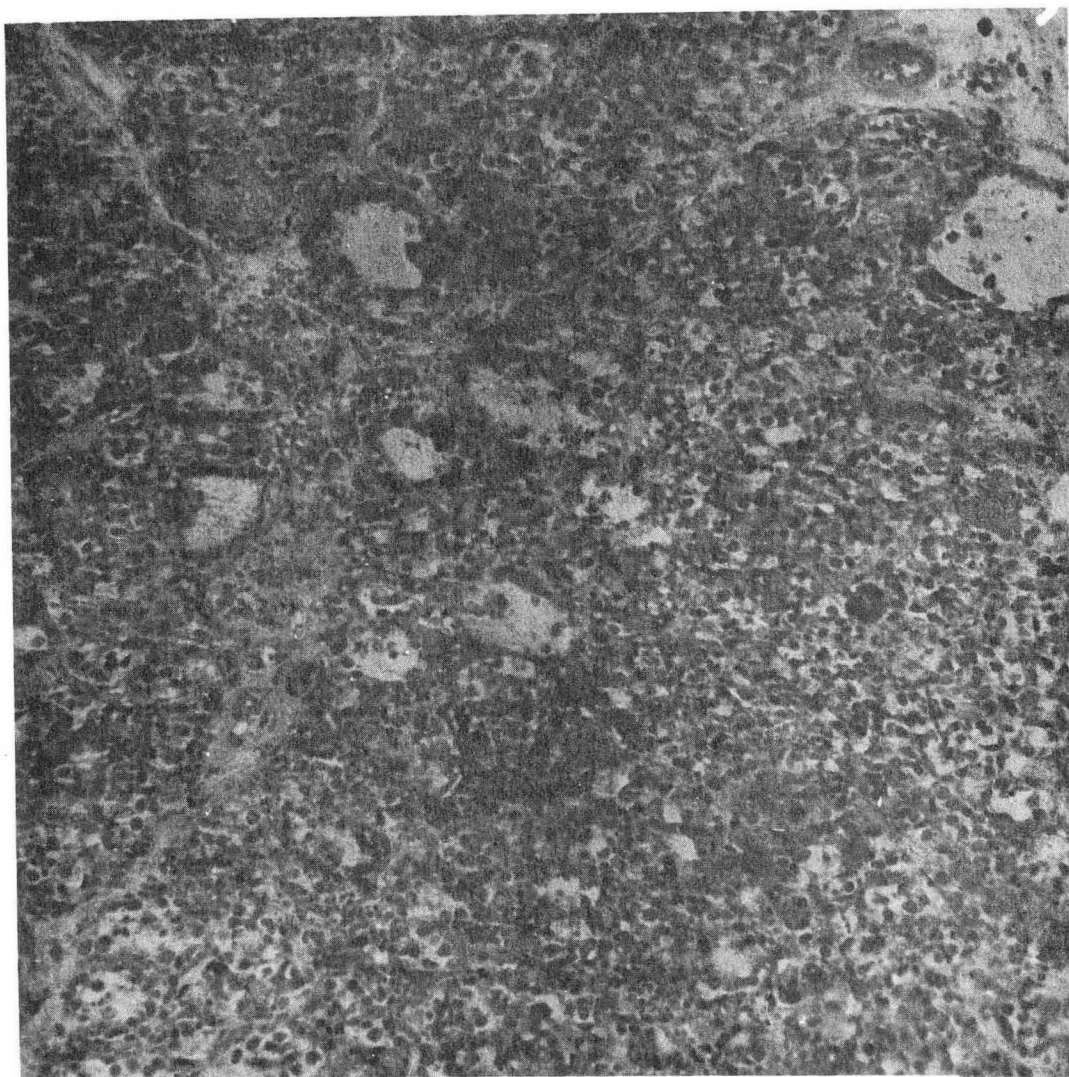
Thyroid gland of the rat which received 130 microcuries of astatine (0.75 microcuries per gram) by intravenous injection and was sacrificed two days later. Magnification x 100.



ZN-910

Fig. 2

Thyroid gland of the rat, indicating some colloidal changes following administration of 130 microcuries of astatine-211, (0.75 microcuries per gram) by intravenous injection, and sacrifice of the rat two days later.



ZN-911

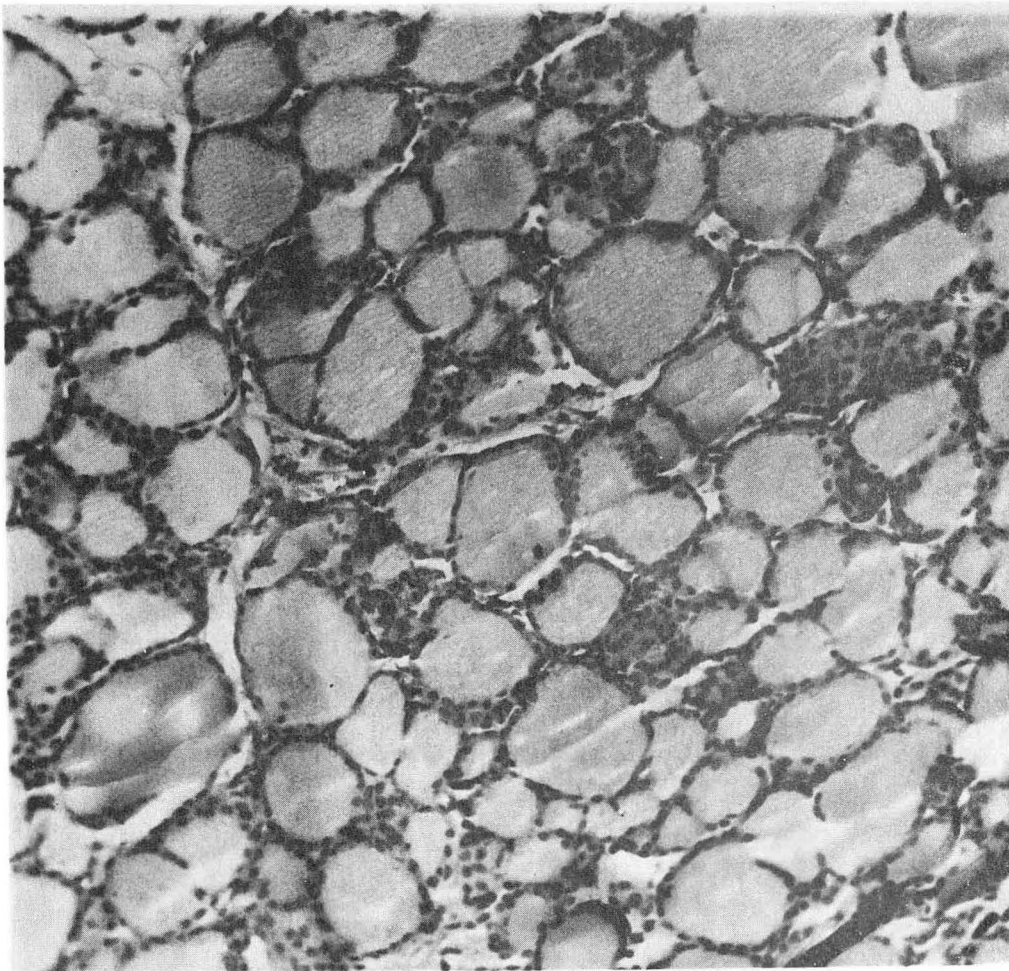
Fig. 3

Thyroid gland from the rat which had received 175 microcuries of astatine (1.8 microcuries per gram) by intravenous injection and was sacrificed at the end of five days. Here may be seen a very marked degree of injury to the gland. x 100.

There may be seen in Fig. 4 the interesting effect of daily pretreatment of the rat by the intraperitoneal injection of potassium iodide at the dose level of 10 milligrams per kilo for one week preceding the intravenous administration of 270 microcuries of astatine or 1.8 microcuries per gram by weight, the animal being sacrificed at the end of 10 days. The complete absence of apparent injury to the thyroid glands is notable. This protective effect upon the thyroid gland, however, did not apply to the animal as a whole. It is becoming increasingly apparent that pretreatment with stable potassium iodide at these high dose levels enhances the radiotoxicity not only of astatine, but also of x-rays. This effect upon the thyroid gland was not unexpected, inasmuch as it had been known that pretreatment of rats with stable potassium iodide decreases the accumulation of astatine in the thyroid gland.

An attempt has been made to correlate the histopathological changes in the livers, lymph nodes, and spleens of rats that have received astatine at the lethal range with and without pretreatment with stable potassium iodide at the dose levels indicated above. Figure 5 shows the changes observed in the liver of a rat that had received 1.8 microcuries of astatine per gram and was sacrificed at 14 days, having had no pretreatment with potassium iodide. Figure 6 presents a photomicrograph of a liver from another animal, which had received the same amount of astatine and was sacrificed at 10 days, but had received pretreatment with stable iodide for six days. There appears to be a difference in the histopathological changes. Obviously, much effort must be devoted to determine whether these differences, which are quite obvious here, are of statistical significance. There would seem to be a greater degree of hepatic injury to those animals which were not pretreated with stable iodide than pretreated animals.

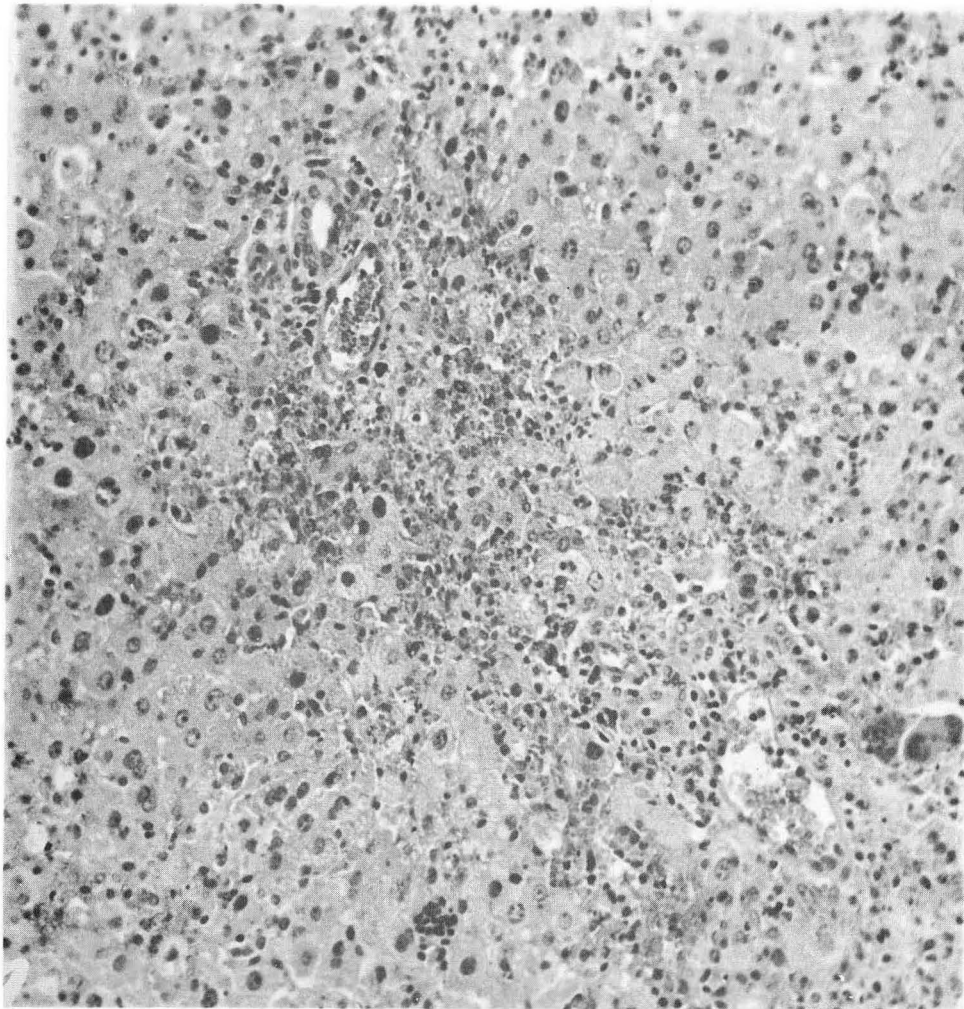
Figures 7 and 8 are representative photomicrographs of mesenteric lymph nodes of the rat with and without pretreatment with potassium iodide, respectively. The dose level of astatine was the same, namely 1.8 microcuries per gram for each animal. The photomicrograph shown in Fig. 7 was obtained from an animal sacrificed at 11 days, and that in Fig. 8 from one sacrificed in 7 days. The variations in the date of sacrifice arise from the fact that we are attempting to obtain at the same time reasonably adequate



ZN-912

Fig. 4

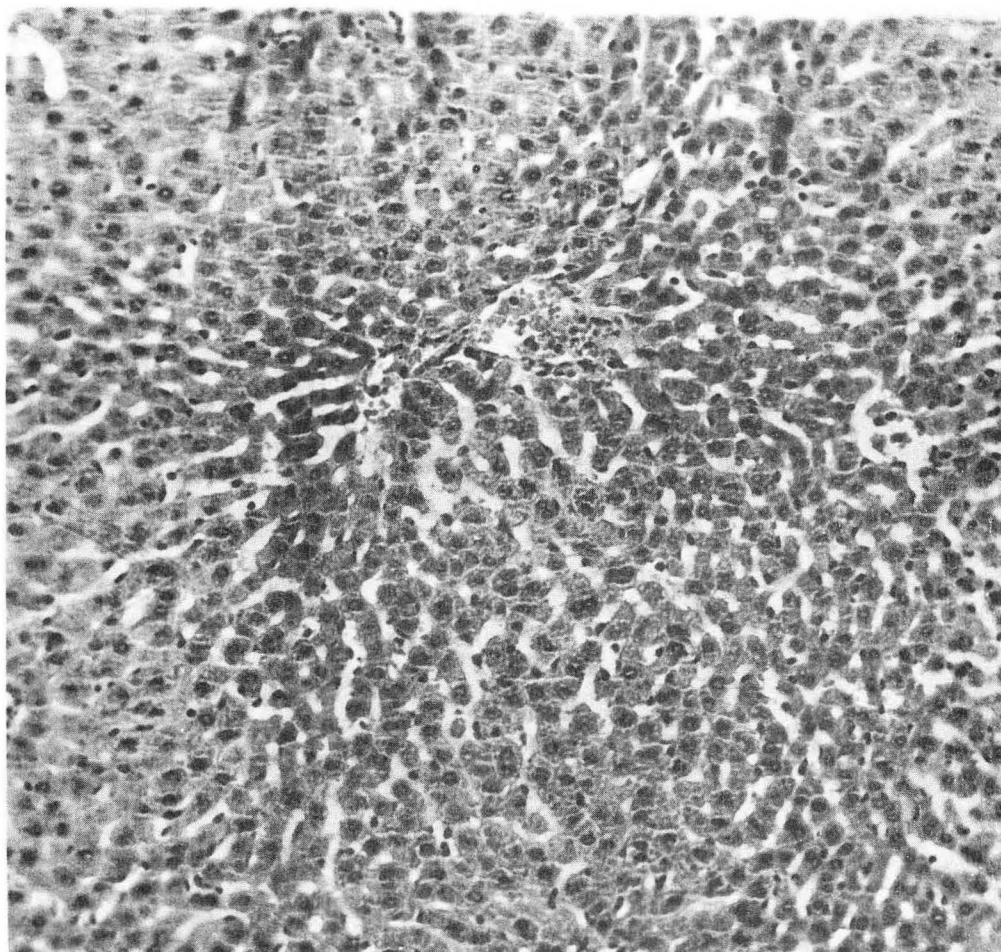
The effect of pretreatment with potassium iodide upon the thyroid gland of a rat subsequently given 270 microcuries of astatine (1.8 microcuries per gram) by intravenous injection and sacrificed ten days later. x 100.



ZN-913

Fig. 5

A section from the liver of a rat which had received no pretreatment with stable iodine but had been given 1.8 microcuries of astatine per gram by intravenous injection and sacrificed at 14 days. The distortion of the normal architecture and the hemorrhage are noteworthy. x 100.



ZN-914

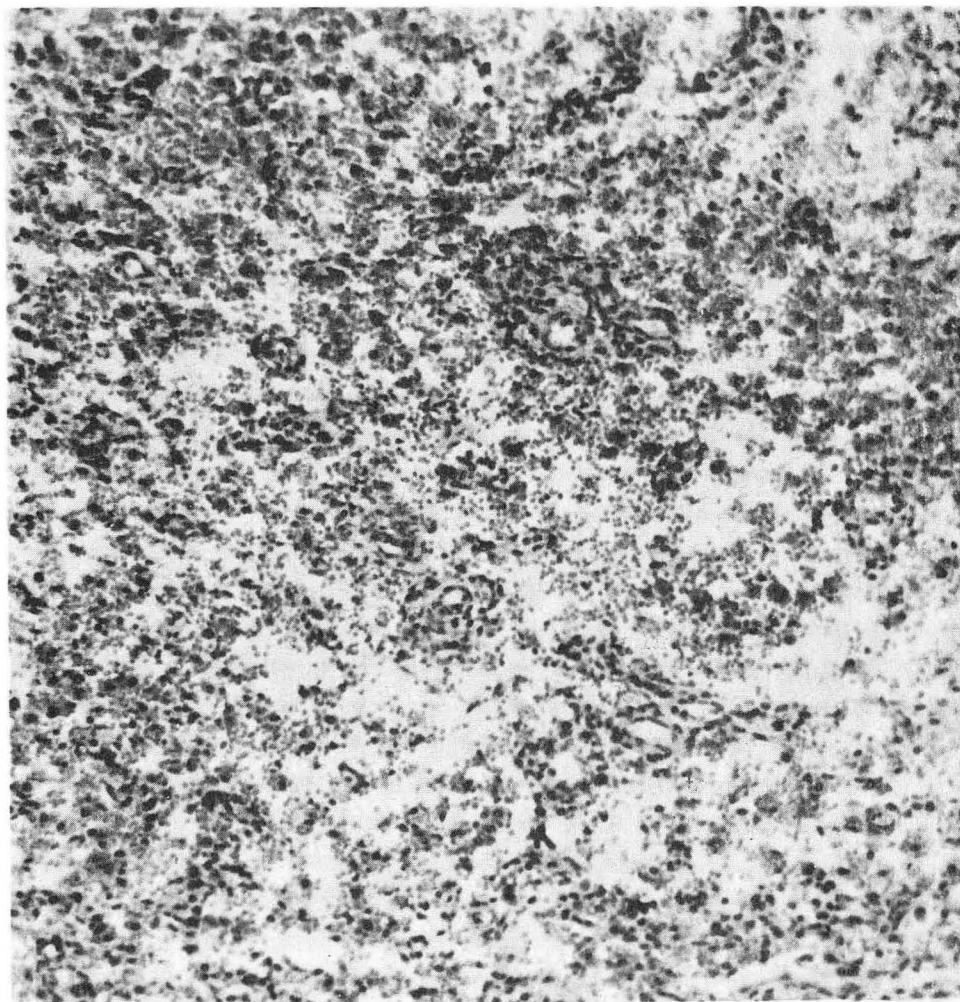
Fig. 6

A section from the liver of a rat which had received six days' pretreatment with stable potassium iodide and then was given 1.8 microcuries of astatine by intravenous injection and sacrificed at 10 days. Dilatation of the sinusoids and loss of nuclear structure are the principal observations to be made in this particular section. x 100.

lethality data; when the animal appears to be the point of death it is sacrificed. Because it is not feasible to maintain a 24-hour observation of the animals, and the groups are small, these variations in the sacrifice dates are inevitable. The impression gained to date, for which these two photomicrographs are representative evidence, is that pretreatment results in greater injury to the cervical and mesenteric lymph nodes. In the spleen, the picture is as yet ill defined and must await the accumulation of more data. It should be pointed out that these animals received astatine preparations that were free of colloidal particles such as were observed and described in this and a previous quarterly report.

Figure 9 presents a photograph of a rat which had received 1.8 microcuries of astatine-211 40 days previously and was 95 days of age at the time the photograph was taken. On the right is shown a control animal 89 days of age. The difference in length of the two animals is apparent. Figure 10 presents a similar picture, but in this case radioiodide was given rather than astatine. The control rat on the right was 89 days of age when the photograph was taken and the experimental animal on the left was 87 days of age. This rat received 70 microcuries of radioiodide per gram and the photograph was taken 35 days later.

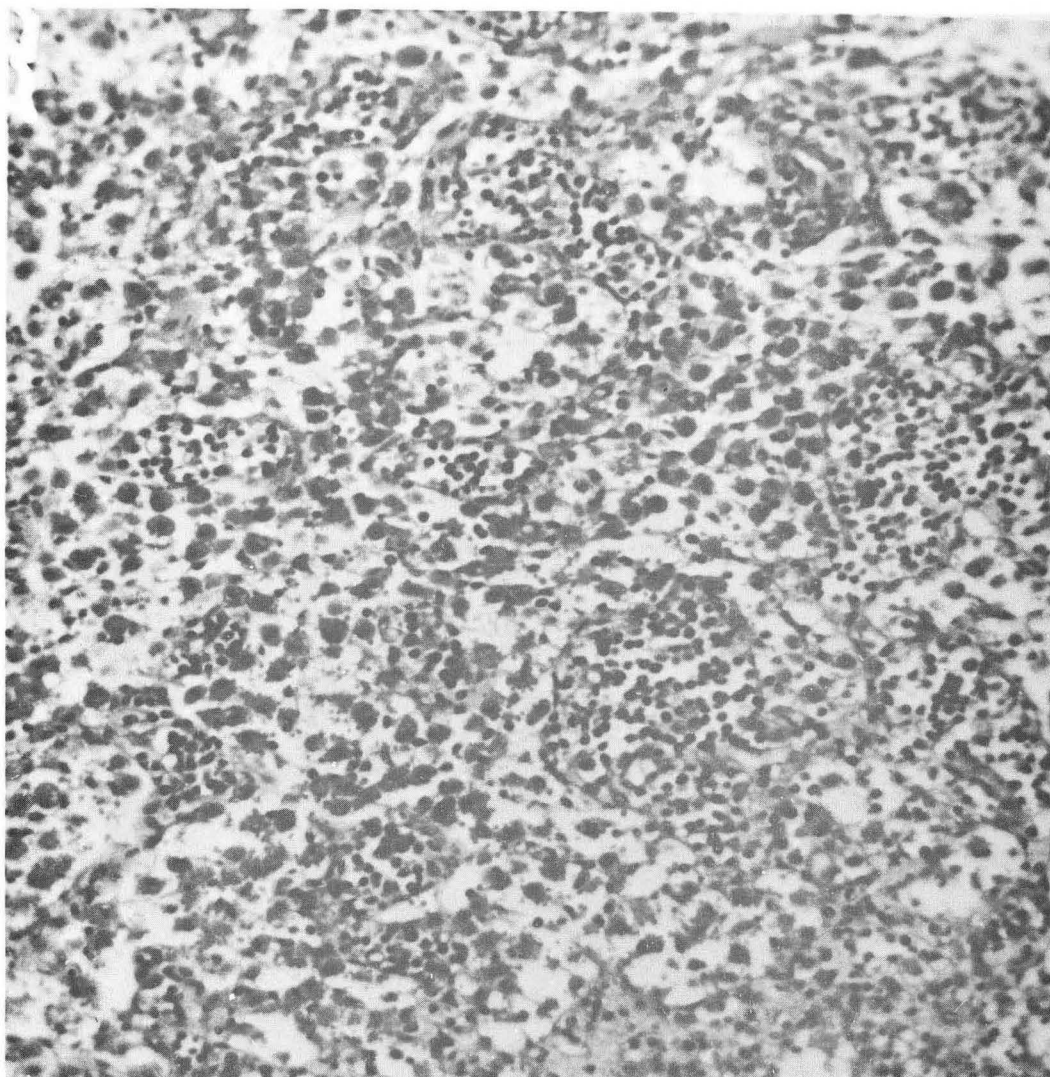
The presence of colloidal particles in the astatine preparations may be significant, both in the mortality aspects of high dose levels of this radioelement and in the histopathological changes. In the preceding quarterly report, photomicrographs of colloidal particles containing astatine in the liver were shown. When this effect was recognized, subsequent experiments were performed with material known to contain colloidal particles upon which presumably some of the astatine was adsorbed. The presence of these presumed colloidal particles containing astatine was demonstrated by the use of NTB stripping film emulsion. The liver experiments were repeated in more detail; also, the spleen and bone marrow were examined by this radioautographic technique 18 hours after the intravenous administration of 50 microcuries of astatine to each rat. Further controlled experiments were also done, in which the astatine was filtered through a fritted glass disk and centrifugation was done at 100,000 G for 30 minutes. Whenever preparations were used that had not been filtered and subjected to ultracentrifugation, stars were seen in the stripping film emulsion, indicating the



ZN-915

Fig. 7

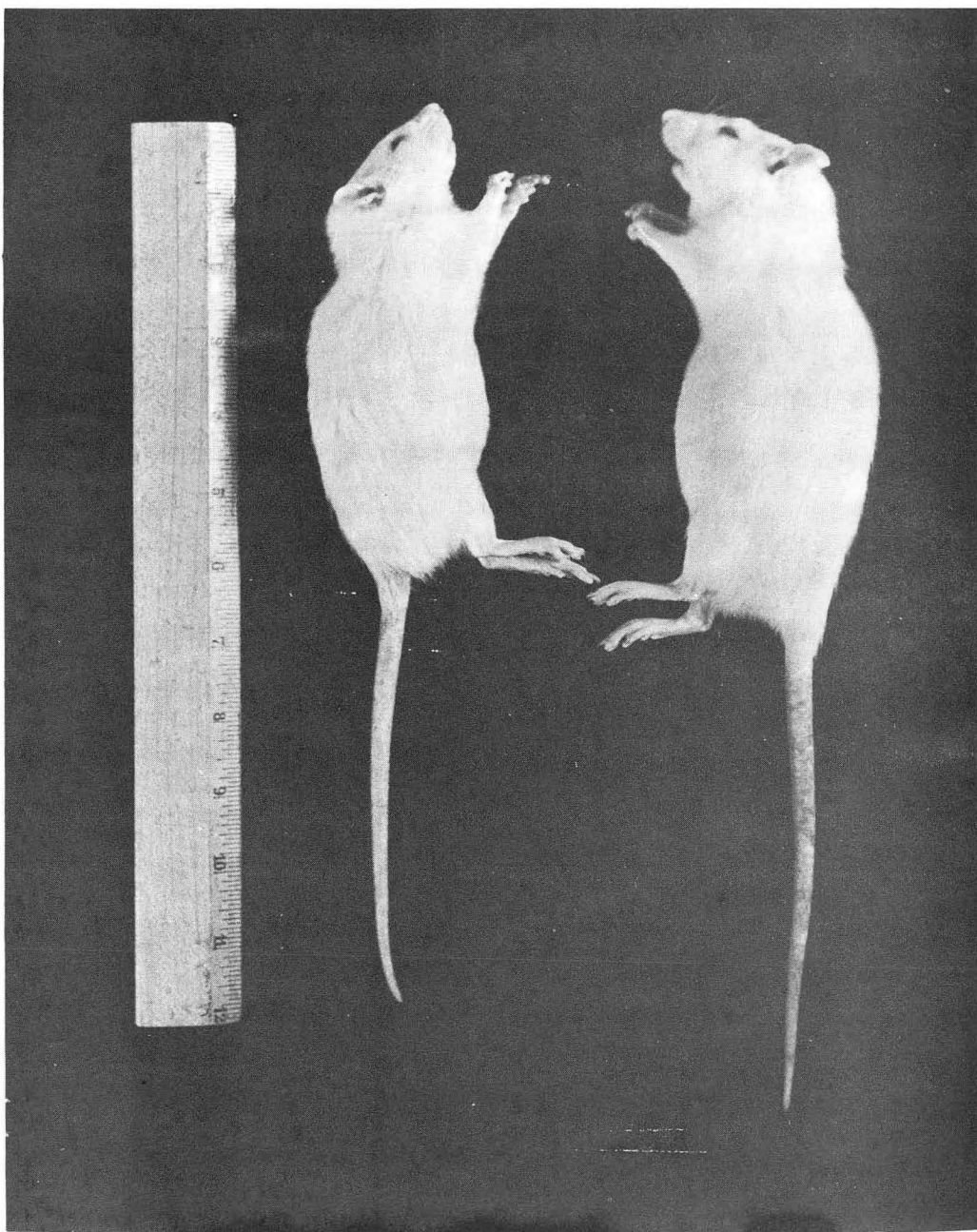
A section from a mesenteric lymph node of a rat which had had no pretreatment with potassium iodide and received 1.8 microcuries per gram of astatine by intravenous injection, and was sacrificed at 11 days. There may be seen evidence of marked hemorrhage, atrophy and loss of the normal structure.



ZN-916

Fig. 8

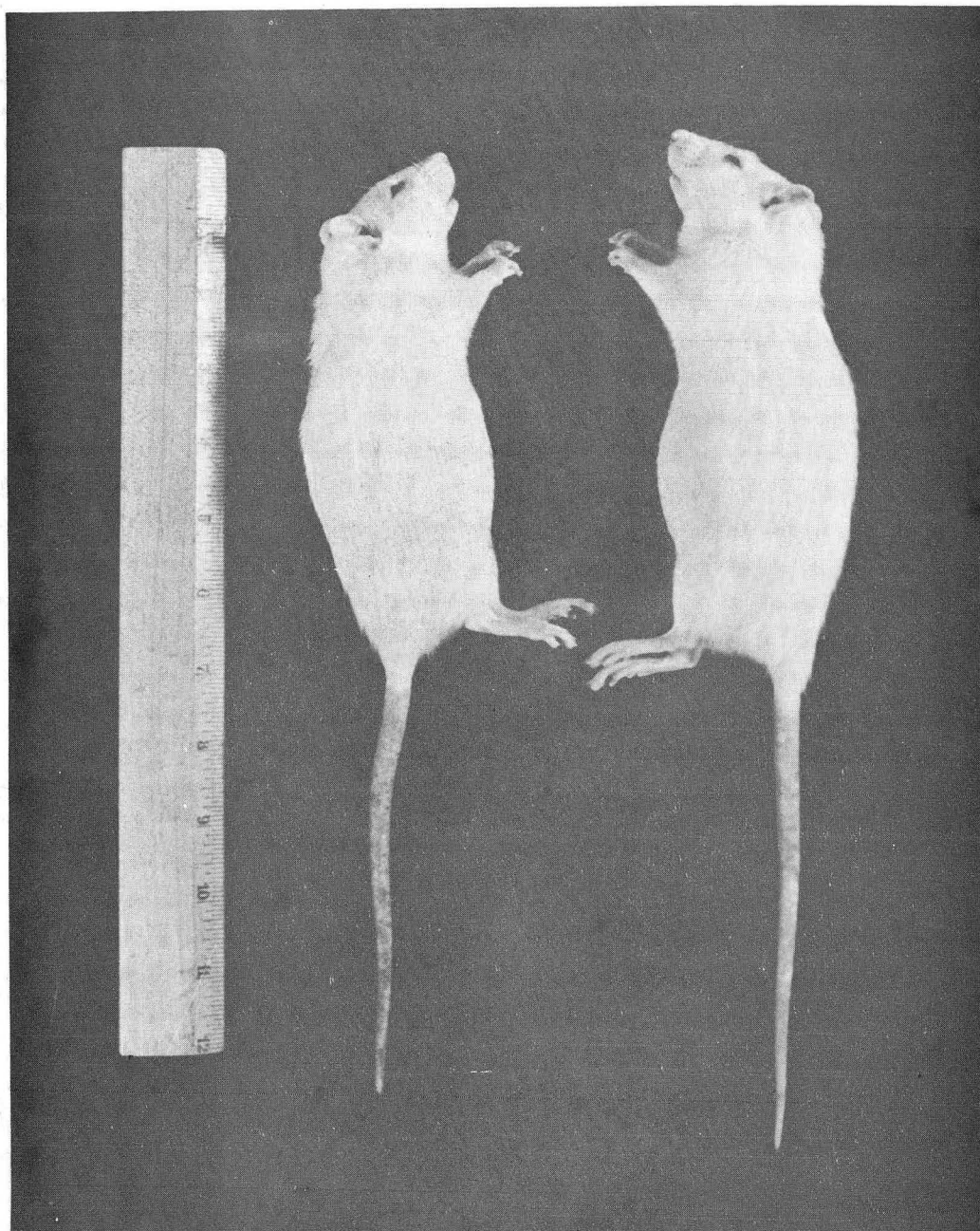
A section from a lymph node of a rat which had been pretreated with potassium iodide for six days and then received 1.8 microcuries of astatine by intravenous injection and was sacrificed at 7 days. The degree of atrophy and hemorrhage appears more extensive than in Fig. 7. x 100.



ZN-917

Fig. 9

Left: a rat that had received 1.8 microcuries per gram of astatine and was 55 days of age at the time of injection. The picture was taken 40 days later. The control animal, on the right, was 89 days of age.



ZN-918

Fig. 10

Left: a rat that had received 70 microcuries per gram of radioiodine at an age of 52 days. The photograph was taken 35 days later. The control on the right was 89 days of age.

presence of aggregates of astatine atoms in liver, spleen, and bone marrow. The treated astatine preparations showed no stars in any of these three tissues. This evidence is corroborated by tracer studies employing astatine that had not been filtered and centrifuged as described earlier. The untreated preparations of astatine gave much higher astatine content in the liver and spleen than the treated preparations of astatine. It is of interest that when an untreated preparation of astatine was administered intraperitoneally, there were fewer stars to be seen in the spleen, liver, and bone marrow than when the astatine was administered intravenously. This would suggest that the peritoneal membrane served to act as a filter.

As a corollary to this work, a careful search of the literature was made for lethality data on iodine-131. We were interested in comparing whatever relative biological effects might exist between astatine-211 and iodine-131 in the rat. The search proved fruitless. Accordingly, it was decided to set up a group of experiments to ascertain approximately the lethality of iodine-131 in Sprague-Dawley female rats approximately 150 grams in weight and 55 days of age. These parameters were selected so that the best possible comparison could be made to the astatine lethality studies. Initially, four groups of ten rats each were used; they received the radioiodide by intraperitoneal injection. The initial dose levels employed were 10, 30, 50 and 70 micro-curies per gram respectively. The percent lethality at 30 days was 10, 33, 33, and 35, respectively, for these four dose levels. The animals that died the first three days after the administration of radioiodide showed grossly very little except for hemorrhage in the thyroid gland; the largest number of early deaths was within this time interval. About the 15th day, the animal receiving the two higher dose levels developed a rather curious respiratory symptom, which could be heard when listening to the animals breathe in a quiet area. The sound might be best described as having a quacking quality, and presumably arose from radiation damage to the larynx and trachea as a result of accumulation of radioiodide by the thyroid gland. Grossly, the lymphatic structures (which included lymph nodes, spleen, and thymus) appeared to be slightly hyperemic; surprisingly these were not markedly reduced in size. The thyroid gland appeared very small, was white, and was firmly attached to the trachea by fibrous tissue. Histopathological preparations

should be made available for the next quarterly report. The estimation of amount of total body irradiation received is somewhat difficult, for several reasons. Space and facilities did not permit the maintenance of 30 animals at one time in separate metabolism cages so that the fraction of iodine excreted could be accurately established; moreover, there was no available facility for determining the total amount of activity in each animal for the first 24 hours. It must be kept in mind that iodine is rapidly excreted, except for that accumulated and retained by the thyroid gland. Another factor was that the animals had to be maintained in groups in the cages, and there was some cross-irradiation, since these animals tend to sleep in a group rather than spread about the cage. Circumstances were such that some three to five animals were maintained in each cage, but the individual cages were separated from one another by adequate lead shielding. However, from making the best approximation possible, it would appear reasonable that groups receiving the values for the 10, 30, 50 and 70 microcuries per gram were 132, 395, 660 and 923 rep respectively. These values would appear to be somewhat out of line in light of the fact that only 35 percent of the high-dose-level group died in the first 30 days. This work is being continued; a more detailed report will be presented and attempts will be made to more accurately evaluate the amount of total body irradiation received by each animal.

In the previous quarterly report it was mentioned that pretreatment with stable potassium iodide apparently enhanced the lethality for rats of both astatine-211 and x-rays. This work is now being very actively pursued, and in the next quarterly report a complete account should be possible, establishing the significance of this effect in young and plateaued Sprague-Dawley female rats treated with 230 KVP x-rays. By this time, we will have accumulated studies on nearly 200 animals, which should substantiate how real this effect may be. The radiotoxicity studies with radium-223 are being continued and a more complete report will be presented.

RADIATION CHEMISTRY

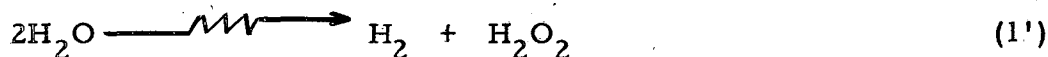
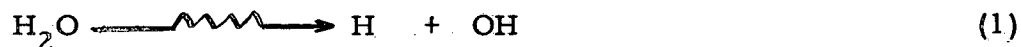
Warren M. Garrison

ACETIC ACID

Winifred Bennett, Sibyl Cole, H. R. Haymond, and Boyd M. Weeks

In previously reported studies of chemical change induced in aquo-organic systems by cyclotron irradiation, it was necessary to use beam intensities $> 0.100 \mu\text{a}$ to insure quantitative measurement with the then existing beam-monitoring equipment. It has been apparent for some time, as discussed in earlier reports, that it would be desirable to obtain data at lower beam intensities, so that results from cyclotron irradiation studies could be more adequately correlated with other current research, much of which is done with radioactive sources and at considerably lower dose levels. Recently, the Crocker Laboratory cyclotron group installed a beam-monitoring circuit which permits radiation chemical studies to be made with existing target assemblies at beam intensities as low as $0.010 \mu\text{a}$. Data have been obtained on the acetic acid system at this dose rate level.

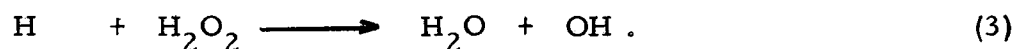
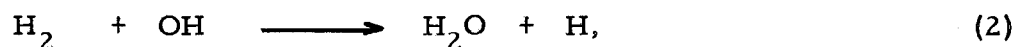
Irradiation of dilute aqueous solutions of acetic acid results in the formation of hydrogen, hydrogen peroxide, and succinic acid as principal products at radiation doses below 1×10^{20} ev/ml. The product yield, G^1 , increases with acetic acid concentration and approaches a limiting value in the range 0.25 - 1.0 M acetic acid. With 35-Mev helium ions at a beam intensity of $0.2 \mu\text{a}$ for a total dose of $0.030 \mu\text{ahr}$ the limiting values are $G_{\text{H}_2} = 1.0$; $G_{\text{H}_2\text{O}_2} = 0.46$; $G_s = 0.36$.² On the basis of recent work³ on the mechanism of water decomposition by ionizing radiation, it would appear that the total number of water molecules dissociated by the primary process



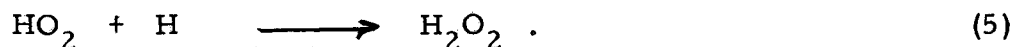
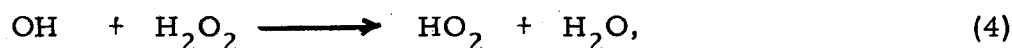
is a constant equal to ~ 3.2 independent of the type of radiation; i. e., ionization density is important only in determining the relative number of water molecules decomposing via Eq. (1) or (1'). Therefore, in the irradiation of dilute acetic

-
1. Radiation yield (G) represents the number of molecules formed or decomposed per 100 ev absorbed energy.
 2. Quarterly report, July, August, September, 1953.
 3. E. J. Hart, ANL-4833.

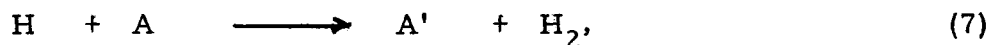
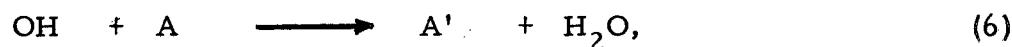
acid with 35-Mev helium ions, the hydrogen yield should approach a value of at least 1.5 - 1.6 unless a net reduction of acetic acid is involved. No such reduction has been detected in amounts that would account for the low hydrogen yield ($G_{H_2} = 1.0$) at 0.200 μ a. Apparently, as discussed in previous reports², the radical-catalyzed recombination⁴ of hydrogen and hydrogen peroxide is involved;



The H and OH radicals are regenerated, and in pure water the chain is terminated by reactions of the type



In acetic acid, competing reactions for OH and H are involved:



Although evidence for Eq. (7) has been obtained², reaction (6) is considered the principal source of A' (CH_2COOH), which dimerizes to form succinic acid by the reaction

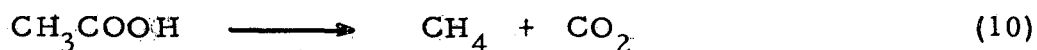


As the H_2 and H_2O_2 concentrations increase, reactions (2) and (3) become more important. This results in a decrease in the experimentally observed radiation yields for H_2 and H_2O_2 . Therefore, to insure that initial yields are being measured, both a low dose rate and low total dose are necessary. We have accordingly utilized the recently available facilities for monitoring low beam currents on the Crocker Laboratory cyclotron to study the effect of beam intensity

4. A. O. Allen, et al., J. Phys. Chem. 56, 575 (1952).

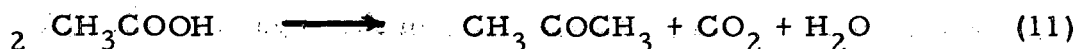
on radiation yields in acetic acid solutions. Some of these results are summarized in Table I. It is apparent by inspection of these data that previous studies of 0.2 μ a do not represent initial yields. At 0.010 μ a, however, the observed G values for H_2 and H_2O_2 indicate that beam intensity effects are becoming negligible. Further studies of dilute acetic acid solutions at low beam intensity are in progress.

At higher acetic acid concentrations the G values for hydrogen, hydrogen peroxide, and succinic acid decrease linearly above 1 M with increasing acetic acid concentration; the G values for carbon dioxide and methane, however, increase linearly with acetic acid concentration from 0.0625 M to 16 M acetic acid. It has been suggested² that this linear dependency of carbon dioxide and methane represents a direct interaction of the effective radiation with acetic acid. That this process cannot be formally represented only by



is indicated by the fact that carbon dioxide and methane are not produced in equal amounts. The ratio G_{CO_2}/G_{CH_4} is ≈ 3 over the entire concentration range.

A reaction in addition to that in Eq. (10) is presumably involved in the "direct effect". Some time ago³ it was found that acetone is produced in the radiolysis of 16 M acetic acid. More recently, we have obtained evidence that total carbonyl, calculated as acetone, increases linearly with concentration, and at 16 M represents a G value of ~ 1.50 . Mass spectral analysis⁵ of the volatile fraction separated from an irradiated 16 M acetic acid solution by fractional distillation showed only acetone and acetaldehyde in appreciable amounts. Acetone accounted for approximately 80 percent of the observed volatile products. These results suggest that a second direct process represented by



is involved. Whether or not reactions (10) and (11) involve radical intermediates is yet to be determined. If radical reactions are involved in (10) and (11) it

5. We are indebted to Dr. Amos Newton for this analysis and for all gas analytical data presented.

TABLE I

Effect of Beam Intensity and Exposure Time on Radiation Yields (G)*
 in Evacuated 0.25 M Acetic Acid Solutions -- Target Vol: 100 ml;
 Radiation: 35-Mev Helium Ions

Intensity (μ a)	Exposure (μ ahr)	$G_{H_2O_2}$	G_{H_2}	G_s
0.010	0.005	0.86	1.40	**
0.010	0.005	0.85	1.44	**
0.010	0.030	0.51	1.19	0.40
0.200	0.005	0.67	1.05	**
	0.005	0.66	1.10	**
	0.030	0.46	1.08	0.36
	0.060	0.40	1.1	0.32
	0.100	0.33	0.98	0.32
1.00	0.050	0.48	1.09	0.34

* Radiation yield (G) represents number of molecules formed per 100 ev absorbed energy.

** To be determined.

would seem logical to assume that these could compete with reactions involving H and OH or CH_2COOH . The observed shape of the product yield curves would indicate that such processes are not occurring. Because of the importance of these phenomena in both radiation chemistry and radiation biology, radiation chemical studies of concentrated solutions are being continued.

THE EFFECTS OF DISSOLVED OXYGEN IN IRRADIATION OF AQUEOUS SOLUTIONS OF ACETIC ACID

H. R. Haymond

Recent work on the problem has been related to (1) studies of the effects of dose rate, total dose, and acetic acid concentration on the yields of the products of irradiation, and (2) definite identification of some products indicated by calorimetric tests. The data are incomplete and a detailed discussion will appear in a future report.

In the presence of oxygen, the yield of succinic acid decreases and the yields of hydrogen peroxide and carbon dioxide increase as compared to amounts in solutions irradiated in vacuo. However, the stoichiometry does not account for complete oxidation of acetic acid to carbon dioxide nor for complete conversion² of oxygen to hydrogen peroxide. One possibility, discussed in a previous report, is that oxygen reacts with the radical CH_2COOH to form other products as yet unidentified. The investigations indicated above should help to clarify how oxygen enters into the reactions occurring during irradiation and what the resultant products are.

GLYCINE

B. M. Weeks

Various aspects of the current study of the radiation chemistry of aqueous glycine solutions have been discussed in previous quarterly reports beginning with October-December 1952. Work in the past quarter has been directed primarily toward the problems involved in the separation and determination of the several nitrogen products as well as the purification of glycine for bombardment.

Chromatographic technique

In addition to the very convenient and sensitive method of C^{14} labeling for the determination of product concentration in the column effluent in the chromatographic fractionation of the nitrogen products, another method had to be developed that could be used simultaneously and independently so that co-chromatographs could be obtained and also so that larger quantities of material could be analyzed. Several different methods were tried, but it was found most

practical to titrate directly with NaOH the hydrochloride salts of the minute amounts of the amino acid and nitrogen base products that come off the ion exchange column. Since the eluents used are 1.5N to 6N HCl, every trace of free acid has to be removed from each sample (or aliquot thereof) before titration. This has been done so far in a large vacuum desiccator at room temperatures. Very satisfactory elution curves have been obtained for several targets and for glycine control.

To facilitate collection of uniform samples of the column effluent, an automatic sample changer has been constructed. This employs a 25-inch-diameter horizontal turntable with 60 equally spaced radial partitions into which beakers or various other collecting vessels of from 5 to 20 ml capacity can be accurately positioned. If some partitions are removed, beakers of larger capacity as well as Geiger counter sample dishes, etc. can be conveniently positioned; the capacity of the turntable is thereby reduced, however, from 60 collecting vessels to 30, 20, 15, etc., depending on their size. The turntable is automatically advanced 6 degrees, or one partition, each time a predetermined number of drops from the column have fallen through an electrical counting device into a collecting vessel on the turntable. The drop falls between two platinum electrodes in an open tube between the delivery tip of the column and the collecting vessel. The momentary contact with the electrodes fires a thyratron tube which in turn activates the count relay of a signal impulse counter, which upon completion of a counting cycle starts the turntable motor. A cam on the motor shaft actuates a microswitch for every 6-degree rotation of the turntable, which automatically shuts off the motor and resets the impulse counter.

A heating and ventilating system is planned that will dry the samples down more conveniently than is now possible with a conventional vacuum desiccator.

Purification of glycine for bombardment

Repeated recrystallization from water of glycine as supplied by the Nutritional Biochemicals Co. seems so far to be fairly satisfactory. However, much glycine is lost in the process, and some other procedure for the purification of glycine labeled with C^{14} is necessary. As discussed in previous quarterly reports, there are objections to chromatographic purification methods. An attempt has therefore been made to develop a method of vacuum sublimation of glycine at temperatures between 150° and $200^{\circ}C$. (Glycine melts with decomposition at about $230^{\circ}C$). First indications are encouraging, and perhaps the method can be used for the preparation of larger quantities of inert glycine as well as the C^{14} -labeled material.

SEPARATION OF FORMIC, GLYCOLIC, GLYOXYLIC AND
OXALIC ACIDS BY METHODS OF PARTITION CHROMA-
TOGRAPHY ON SILICIC ACID COLUMNS

Winifred Bennett and Sybil Cole

Evidence has been obtained² which indicates that glycolic, glyoxylic⁶, and oxalic acids may be formed in the radiolysis of aqueous formic acid solutions and in the radiolysis of oxygen-saturated acetic acid solutions. Methods have been developed for the quantitative separation of these acids in irradiated formic acid solutions. The standard procedure of Marvel and Rand⁷, in which a water-saturated butanol-chloroform solvent sequence is employed, gives a quantitative separation of glycolic and glyoxylic acids. Oxalic acid, however, gives erratic results and is not quantitatively eluted. A quantitative method for the separation of formic, glycolic, glyoxylic and oxalic acid has been developed using a modification of the method of Bulen⁸, et al. The standard column dimensions and general procedures have been previously described² in detail. In the present modification the column length is 1.5 times that of the standard column. The formic and glyoxylic acids are separated by eluting with 10 percent butanol-chloroform saturated with 0.50 N hydrochloric acid. Glycolic and oxalic acids are then separated by 25 percent butanol-chloroform. A typical chromatograph for the separation of glyoxylic acid and oxalic acid is shown in Fig. 1.

6. Prepared from di-n-butyl d-tartrate by lead tetraacetate oxidation (N. E. Tolbert, unpublished procedure).

7. C. S. Marvel and R. D. Rands, Jr., J. Am. Chem. Soc. 72, 2642 (1950)

8. W. A. Bulen, et al., Anal. Chem. 24, 187 (1952).

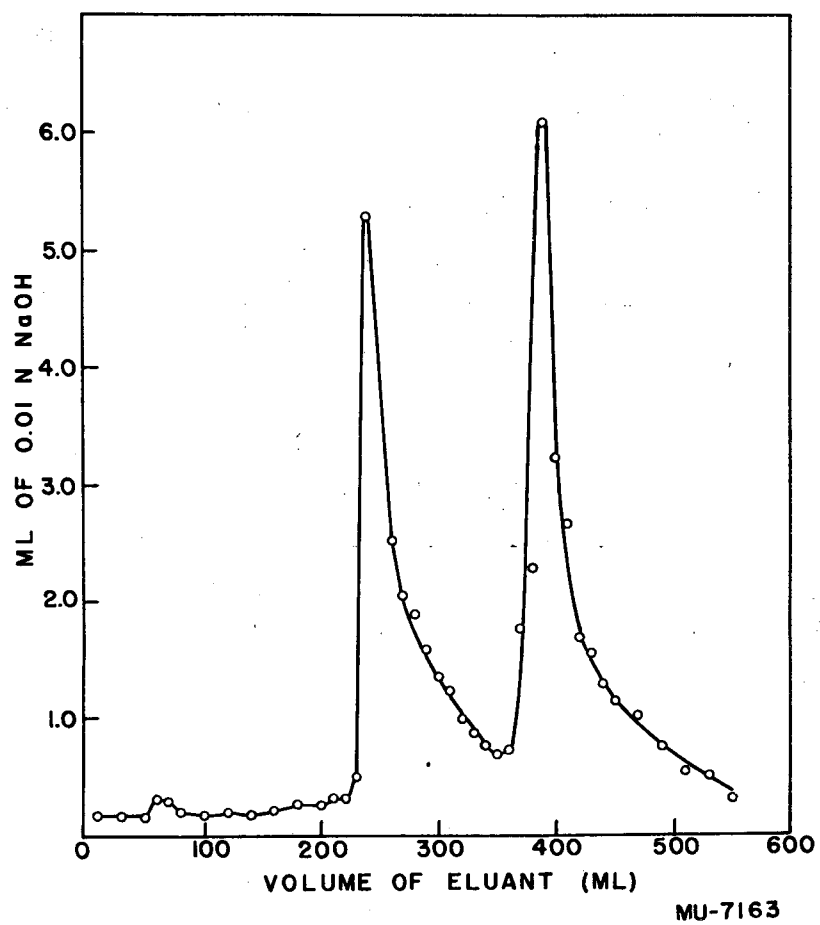


Fig. 1

HEALTH CHEMISTRY

N. B. Garden

PROGRESS REPORT

Previous progress reports have mentioned the large-scale operation that took place in Bldg. 106, Livermore, in which equipment was designed and built by Health Chemistry for processing multicurie quantities of fission products combined with multicurie alpha-emitting substances which had been irradiated in the Materials Testing Reactor at Idaho Falls. As noted before, this operation consumed the greater part of the effort of the Equipment Development and the Airborne Activity Control groups, with assistance from personnel in the other Health Chemistry groups. The material was processed successfully and safely.

The over-all design of the setup involved a completely enclosed system at slightly negative pressure, in which all air within the system (and therefore all air in contact with any phase of the operation) could be completely entrapped within the system, being recirculated after chemical and physical treatment or stored as indicated in expandable accumulators. The system included a chemistry processing box, a materials encapsulation box, transfer boxes, and expandable accumulators, switching boxes, air treating equipment, etc. The chemistry and encapsulation boxes were shielded by straight-type lead cave walls, access and visibility being achieved through ball-socket manipulators and lead glass windows.

The equipment for chemically and physically treating the off-gases and leaks in the closed system consisted of electric furnaces to remove hydrogen in a copper oxide bed, recirculating NaOH scrubbers for acid vapor and removal, dewatering condensers, graded filter beds (glass fiber and CWS No. 6) for particle removal, and silica gel beds for air drying. The control mechanism and electrical equipment employed involved pressure-sensitive regulators, recorders and alarms, refrigerator systems, water pumps and motorized valves, electrical blowers, continuous-flow ionization chambers, scrap samplers, filter-paper samplers, numerous manometers and other pressure measurement devices, thermometers, expandable accumulators. All of the above was connected together and to the processing enclosures by 100 feet of noncorrodable polyethylene piping via more than a hundred lucite valves.

This operation afforded considerable experience in ascertaining equipment requirements for processing quantities of material of this order of magnitude. While the closed system described above was completely successful in keeping the air in the locality free of radioactive contamination, further improvements or even different methods of attack were suggested. It was concluded that for larger-volume multicurie chemistry, metal boxes should receive preferred consideration.

The above-mentioned principles were tried with success in the processing of crud remaining from the Livermore work; this crud processing took place in a two-inch lead cave in Bldg. 5, Berkeley. The operation definitely showed that with the handling of large quantities of highly active alpha emitters, the employment of swiftly flowing air, recycled through scrubbers and condensers, finally to be discharged through filters, is to be looked upon with favor.

The encapsulated material from the Livermore operation, being highly active on the outside surface, was further encased, this job taking place in Health Chemistry equipment in Bldg. 4., with health chemists and chemists performing the task; this work was preliminary to calorimetry operation, in which the absence of contamination was mandatory.

On the termination of the program described above, an equipment disassembly and decontamination period was started. The bulk of the active equipment has been removed and prepared for sea disposal. A few items of less contaminated gear await removal and decontamination for possible reuse. The larger pieces of equipment, such as the chemistry and encapsulation boxes, the switching boxes and air dryers and ducting, were placed in metal boxes in Livermore and transported to Berkeley, where cement was poured into the space between the object and the metal box. These units were then dumped at sea.

The remainder of the stock material from which the above-mentioned samples processed at Livermore were created is being processed in Bldg. 5A annex, and slugs of transuranic materials for irradiation in the Materials Testing Reactor at Idaho Falls are being made.

Research continues on other MTR irradiations and various handling and processing devices have been furnished by the Health Chemistry Equipment Development group. These include a slug cutter for opening irradiated samples encapsulated in quartz capsules of less than four mm diameter. This cutter, based on one used at the MTR, consists of pipes in a telescoping position for breaking the quartz. One irradiation from Idaho Falls was successfully repackaged at the airport in equipment set up on a Health Chemistry truck, and was immediately returned for further irradiation.

A special inventory of materials in the Pit Room in Bldg. 5 was made, and a new indexing system was created, with a goal of having the holders of the materials therein constantly review their holdings and make new decisions whether or not the material should continue in storage. It is hoped that this system will tend to eliminate the accumulation of vast numbers of odds and ends of no value.

HEALTH PHYSICS

Burton J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

1. B- γ Ionization Chamber	62
2. I. D. L. Portable Survey Instruments.	20
3. Cutie Pies.	3
4. Recording γ -Intensity Meters.	22
5. Victoreen Proteximeter	3
6. Fast-Neutron Proportional Counters	8
7. Slow-Neutron Proportional Counters.	15
8. Fast-Neutron Proportional Counter (Portable).	11
9. Slow-Neutron Portable Unit	4
10. Balanced Chamber - Fast Neutron - Portable	3
11. Special Tissue Wall Survey Instrument.	1

Personnel Meters in Use

1. Total Personnel Covered with Film Badges	2,853
2. Total Man-Days Coverage with Pocket Chamber	5,236
3. Total Man-Days Coverage with Pocket Dosimeters.	5,236
4. Total Man-Days Coverage with Pocket Chambers (S.N.). . . .	4,593

Cases of Weekly Exposure above 0.3r

<u>Weekly Film Expos. above</u>	<u>184" Area</u>	<u>60" Area</u>	<u>Lin. Acc.</u>	<u>Chem.</u>	<u>Other</u>	<u>Total</u>
0.3	4	12	8	100	9	133
0.5	1	2	2	61	3	69
1.0	0	0	0	9	1	10
1.5	0	0	0	2	0	2
2.0	0	0	0	1	0	1
2.5	0	0	0	0	0	0