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PROGRESS REPORT 6

UTILIZATION OF THE GROSS FISSION PRODUCTS
(Unclassified)

By

FACULTY MEMBERS

L. E. BROWNELL, Supervisor
L. C. ANDERSON
H. J. GOMBERG
L. L. KEMPE
J. J. MARTIN
J. V. NEHEMIAS
G. T. VAN WYLEN
E. T. VINCENT

and

RESEARCH ASSOCIATES AND RESEARCH ASSISTANTS

R. CARSTENS
R. J. BASHORE
R. C. DENNIS
C. E. ECKFIELD
H. O. FRANCE
H. A. HARLIN
D. E. HARMER
J. P. HOLMES
R. L. KINNEY
R. F. KLEMM
J. G. LEWIS
S. PEDERSEN

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PREFACE

This report presents the results of research performed during the period June 1, 1953, to January 1, 1954, on Project M943 of the Engineering Research Institute, University of Michigan, under AEC Contract No. AT(11-1)-162.

Results of other research studies in which the irradiation facilities of the Fission Products Laboratory have been used are also reported because it is believed that these studies may provide additional data on the use of waste fission products. For this reason some of the studies supported by Michigan Memorial-Phoenix projects 41, 43, 54, 73, and 83 and some of the academic studies of graduate students are included in this report. It is to be noted that there is no relationship between these studies and Project M943 except that the personnel have a common interest in the effects of radiation and the possible uses of radioactive materials, and they use the same source of gamma radiation. The research of the Michigan Memorial-Phoenix Project is supported by funds of the University of Michigan obtained through contributions by individuals and corporations and is in no way supported by, or connected with, the Atomic Energy Commission. The results of the studies reported by the Michigan Memorial-Phoenix Project in most instances will appear in the scientific literature at some future date. To protect the authorship of research personnel of the Michigan Memorial-Phoenix Project, no part of their results is to be reproduced without permission of the respective authors.

ABSTRACT

Performance tests were made with a diesel engine using 1000 curies of palladium-109 in the combustion chamber. No significant improvement in performance was observed.

Ethylene was polymerized under gamma radiation at pressures of about 1000 psi and room temperature. Radiation dosages of 5 to 7 megarep produced a hard tough polymer having a tensile strength up to 2300 psi with 79 percent elongation upon rupture, and a molecular weight of 37,300. Lesser dosages of radiation produced a soft, brittle, waxy polymer. Toluene was chlorinated under gamma radiation and produced the addition compound. This reaction is considered to be unique in that it is believed to be promoted only by gamma radiation. The product toluene "hexachloride" is being evaluated as an insecticide.

In organoleptic studies on irradiated food the problem of flavor in irradiated food, the design of experiments for flavor evaluation, and the use of statistics in experimental studies are discussed. Experimental results are reported from a taste panel in which the triangle tests and incomplete block ranking tests were used. Pasteurization and sterilization doses of gamma radiation were used in the studies on fresh fruits. The pasteurization and storage life of meat at refrigerator temperature were investigated. Flavor and texture changes were slight in irradiated fresh peaches, dark sweet cherries, and cooked applesauce. Taste panel tests showed that the shelf-life of pasteurized ground pork stored at 40°F could be lengthened to 10 days and possibly longer; ground beef to 8 days. Taste panel tests on irradiated cooked meat indicated that by the combination of heat and gamma radiation it may be possible to sterilize canned meat without undesirable flavor changes.

A new method of wholesaling meat is proposed, based on the prepackaging of meat at the packing house followed by pasteurizing with gamma radiation. Advantages of this process to the retailer, wholesaler, and consumer are discussed. A design for a commercial radiation facility is presented. Separated cesium-137 is selected as a gamma source over 3, 6, 12, and 24 month old fission products on the basis of radiation characteristics and cost. By use of multipasses and by interception of a greater percentage of the radiation flux the efficiency of utilizing radiation was increased fivefold over that of a previous design.

Research reported by the Michigan Memorial Phoenix Project includes the animal feeding experiments using irradiated food, Tetrahymena for the evaluation of the effects of gamma radiation on essential nutrilites, gamma ray sterilization of canned meat, aerobic growth of microorganisms in raw beef exposed to sublethal ("pasteurizing") dosages of radiation, and gamma ray sterilization of tissue culture media.

In the pilot studies with animals fed irradiated food no acute toxicity was observed in animals fed food receiving a 20 megarep radiation dose. However, a marked vitamin deficiency was observed when the complete diet was irradiated with this dosage. This deficiency was removed by supplementing the diet with the water soluable vitamins. Long term feeding and breeding experiments are underway using a colony of 124 initial animals (albino rats) and a diet receiving 4 megarep.

A completely synthetic medium was prepared consisting of the essential amino acids and vitamins required by Tetrahymena. The individual constituents were irradiated in solution and tested for their ability to support growth of the protozoa. These tests showed that thiamine, riboflavin, pantothenate, pyridoxine, folic acid, and thioctic acid were destroyed by irradiation in dilute solution by less than 1 x 10^6 rep, while 2 x 10^6 rep was required to inactivate niacin. Most amino acids proved to be relatively radiation-resistant.

Clostridium botulinum 62A and Putrefactive anaerobe NCA 3679 were used on the tests with canned meat. The sterility dosage for canned meat was found to increase from 2.5 to 4 million rep as the concentration of Clostridium botulinum spores is increased from 4 to 40,000 per gram.

Raw beef exposed to 40, 000 to 160,000 rep was stored for 13 days at 40°F without the development of the off odor of putrid meat; but such an off odor and a slimy growth developed in samples receiving a dose of only about 20,000 rep and stored under similar conditions. Bacterial counts made in this study showed a time lag in the growth of microorganisms in the irradiated meat indicating a decrease in population.

A radiation dosage of 10^6 rep destroyed nonspore-forming organisms in tissue culture medium without detriment to the growth-promoting and tissue-sustaining properties of the medium as measured over a short time.

Studies undertaken cooperatively with industry included the gamma irradiation of glass fibers, of beer, and of cut flowers. The evaluation of results in each case was made by the industrial laboratory concerned.

Glass fibers exposed to gamma radiation had a lower tensile strength than the controls for reasons undetermined. The Young's modulus of glass is not appreciably affected by large amounts of gamma irradiation.

Gamma radiation does not appear promising as a substitute for heat for the pasteurization of beer because dosages sufficient to destroy the brewery organisms also change the odor, color, taste and clarity of the beer.

Roses packaged in polyethylene and irradiated with doses up to 10^5 rep kept twice as long at room temperature as the controls and were perfectly preserved after 30 days storage at refrigerator temperature.

Routine operation of the laboratory has continued according to plan. Wooden equipment in the radiation cave failed under normal load as a result of long-term radiation damage. The design of the ion exchange equipment used to treat the water in the cave well is described.

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PROGRESS REPORT 6

UTILIZATION OF THE GROSS FISSION PRODUCTS

PART I. SUBPROJECT M943-3, THE EFFECT OF IONIZING RADIATION ON INTERNAL-COMBUSTION ENGINE PERFORMANCE

Personnel:

Subproject Supervisors: E. T. Vincent, Professor of Mechanical Engineering and Chairman of Department; G. J. Van Wylen, Assistant Professor of Mechanical Engineering.

A. INTRODUCTION

The study of the effects of beta radiation on the performance of internal-combustion engines, which has been under way for some time, has been completed. This constitutes a final report. Previous progress reports have presented the earlier work and outlined the proposed research which is described in this report. The tests were conducted during the period from August 27 to October 1, 1953, and are described below.

In view of the fact that financial support for Project M943-3 ceased on June 30, 1953, the work was continued with a grant from the Michigan Memorial-Phoenix Project.

B. DESCRIPTION OF DIESEL ENGINE TEST

The engine used on these tests was a single-cylinder CFR diesel test engine, in which the combustion chamber was enclosed with palladium. Two pieces

were used, a cylinder and a disc as shown in Fig. 1, fitted into the combustion chamber of the CFR diesel engine (which was separate from the main cylinder in this engine) in such a way that the combustion-chamber walls were almost entirely made up of palladium.

Several features of this engine permitted constant operating conditions. An electric heater heated the incoming air to 150°F, and the condensing-type

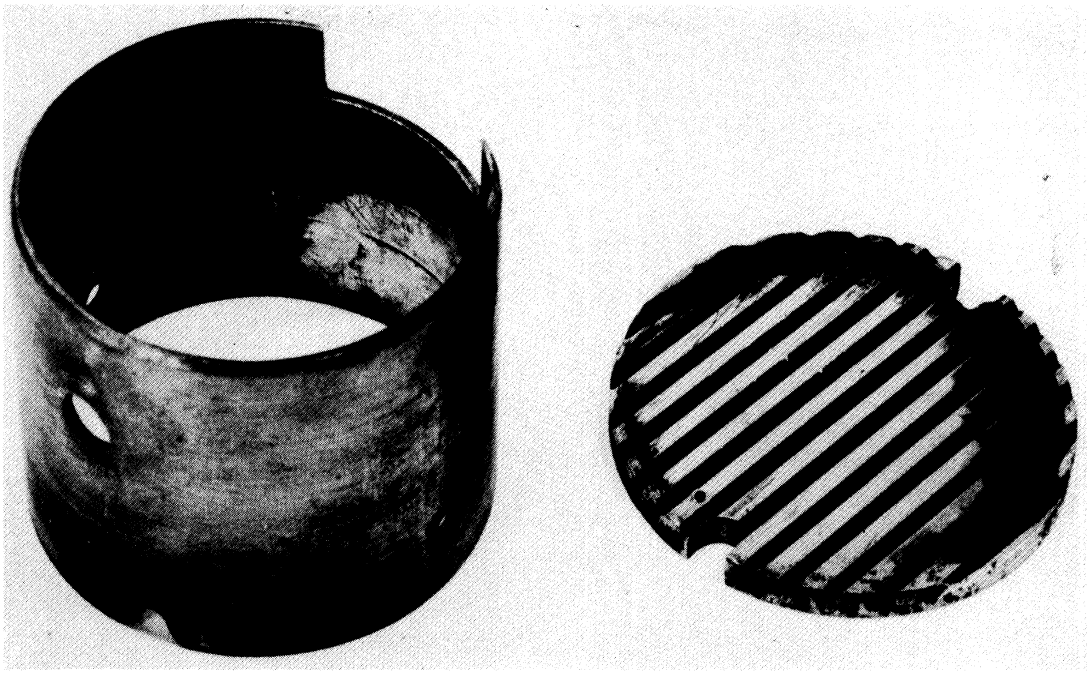

Fig. 1. Palladium Disk and Cylinder for Diesel Engine Experiments.

cooling system maintained the cooling water at a constant temperature of approximately 209°F. An exhaust filter system was used which consisted of a spray washer and a filter.

The proposed test procedure was to use two identical sets of palladium pieces, one of them irradiated. Two runs were to be conducted successively, using inactive palladium in the combustion chamber for the first run and the irradiated palladium for the second run. The effects of beta radiation could then be observed by comparing the two runs under identical operating conditions.

The active palladium was irradiated in the Materials Testing Reactor at Idaho Falls. It was removed from the reactor on August 25 and transported

to Ann Arbor by a special airlift provided by the United States Air Force. The first run was conducted on the afternoon of August 27. Just prior to the arrival of the active palladium, the cold test was conducted, and the test with radiation was begun immediately thereafter. At the time of this first test with radiation, the activity of the palladium was 1,000 curies. The palladium was installed in the engine without difficulty, the area carefully monitored and the time to be spent at various locations limited by radiation safety considerations.

In conducting these tests two parameters were selected as a basis for comparison; namely, specific fuel consumption and pressure-time diagrams. It was felt that these factors would most readily show any effects of beta radiation. Fuel consumption was measured by observing the time required to use 15 cc of fuel, and the output was measured with an electric dynamometer. Speed was measured with an electric tachometer which registered total rpm and elapsed time. A catenary diaphragm-type pickup was used to obtain pressure-time relations, which were recorded by photographing the trace on an oscilloscope. The relative magnitudes of the pressures were obtained from a 40-mv calibrating signal (see Figs. 2 and 3).

In each run four different operating conditions, designated as tests 1, 2, 3 and 4 respectively, were obtained by varying the times at which fuel was injected and the amount of fuel injected. These adjustments were made by adjusting a micrometer screw on the engine. In each run, the following positions were used on the injection and timing micrometer.

	Test 1	Test 2	Test 3	Test 4
Injection Micrometer	0.4	0.4	0.6	0.6
Timing Micrometer	0.9	0.7	0.7	0.9

Increasing the setting on the injection micrometer corresponds to injecting less fuel; while increasing the setting on the timing micrometer results in later injection.

The speed was held constant at 1000 rpm during all runs.

C. RESULTS OF DIESEL ENGINE TESTS

The specific fuel consumption results are presented in the two tables below. Table 1 is for tests with the source in the engine (hereafter referred to as a hot run) and Table 2 for tests with the nonirradiated palladium in the engine (referred to as a cold run).

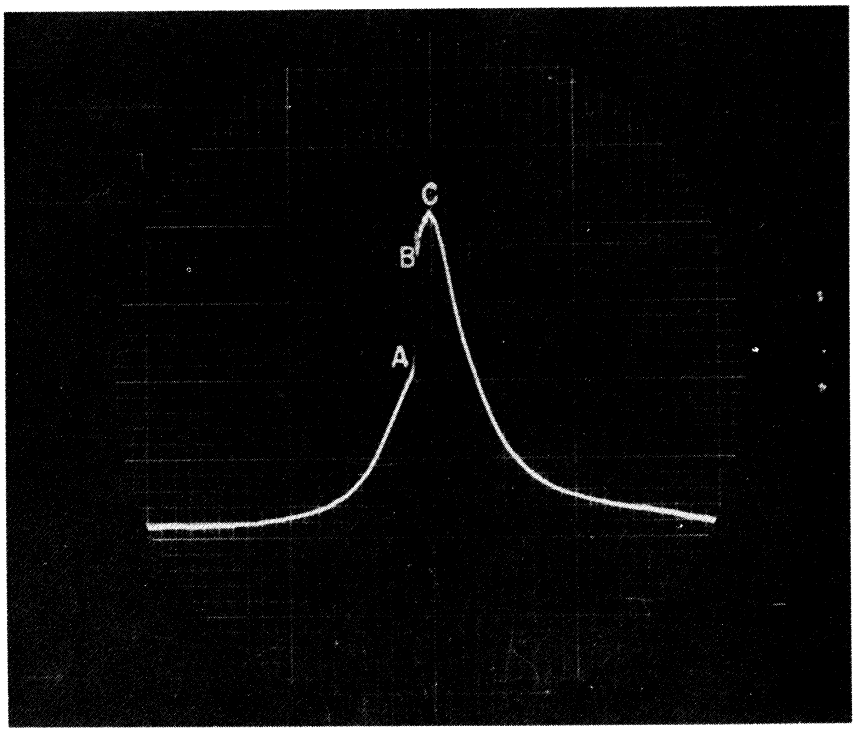

Fig. 2. Pressure-Time Curve:
A. Beginning of Combustion
B. End of Initial Phase of Combustion
C. Maximum Pressure

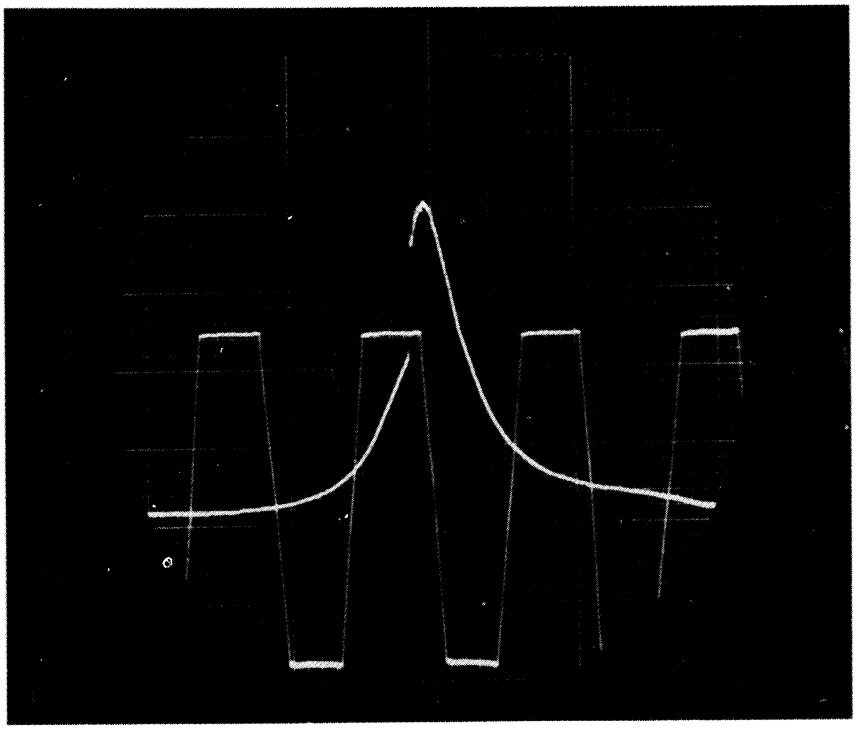

Fig. 3. Pressure-Time Signal Showing Superimposed 40-mv Calibration Signal

TABLE 1

SUMMARY OF SPECIFIC FUEL CONSUMPTION RESULTS ON DIESEL ENGINE TEST

Runs with Radiation

Run No.	Date	Bar., in. Hg	Exh. Pr. Average, in. Water	WB, °F	DB, °F	Activity, curies	Test 1		Test 2		Test 3		Test 4	
							HP	SFC≠	HP	SFC	HP	SFC	HP	SFC
1	9-27-53	29.38	-1-3/4	70	94	1000	2.607	.982	2.653	1.006	.758	2.253	.557	1.735
2	9-29-53	29.32	-1-1/2	70.3	90.8	265	2.647	.965	2.677	.926	.466	2.609	.664	1.797
3	9-31-53	29.33	-1-1/2	73.5	85.4	140	2.626	.967	2.662	.949	.378	3.010	.554	2.129
4	10-2-53	29.23	-1	72.0	87.2	105	2.509	.979	2.514	.975	.246	4.497	.359	3.047
5	10-5-53	29.25	-1	60	73.6	82	2.293	1.014	2.337	1.036	.215	4.871	.330	3.605
6	10-8-53	29.50	-1-3/4	58.0	73.5	65	2.589	.933	2.815	.862	.286	3.81	.397	2.70
6A		29.47	-1-1/4	60.0	77.9	65			2.906	.848				
7	10-10-53	29.43	-1-1/2	60.4	80.6	51	2.932	.834	2.703	.862	*.211	6.04	/.425	2.98
8	10-18-53	29.25	-1	61.5	74.2	28	2.895	.802	2.580	.903	*.479	2.90	*.743	1.77
9	10-24-53	29.42	-1	57.5	72.3	22	2.076	1.123	-	-	-	-	*.735	1.886
10	11-1-53	29.57	-1	58	81.5	20	2.623	.843	2.433	.939	*.548	2.34	*.859	1.556

Micrometer settings for the above tests

	Injection Micrometer	Timing Micrometer
Test 1	0.4	0.7
Test 2	0.4	0.9
Test 3	0.6	0.9
Test 4	0.6	0.7

* Injection micrometer 0.550, timing unchanged.
/ Injection micrometer 0.575, timing unchanged.
≠ SFC units, lbs fuel/BHP-hr.

TABLE 2

SUMMARY OF SPECIFIC FUEL CONSUMPTION RESULTS
ON DIESEL ENGINE TEST

Runs without Radiation

Run No.	Date	Bar, in. Hg	Exh. Pr. Average, in. Water	WB, °F	DB, °F	Same Atm. Conditions as Hot Run No.	Test 1		Test 2		Test 3		Test 4	
							HP	SFC*	HP	SFC*	HP	SFC*	HP	SFC*
1	9-27-53	29.38	----	70	94	1	2.265	1.147	2.427	1.122	.389	3.412	.575	2.247
2	10- 8-53	29.47	-1-1/4	60	77.9	6A	----	----	2.664	.915	----	----	----	----
3	10-10-53	29.43	-1-3/4	60.4	80.6	7	2.685	.881	2.653	.934	.318	3.84	.445	2.77
4	10-18-53	29.25	-1	61.5	74.2	8	2.559	.913	----	----	----	----	----	----
5	10-24-53	29.42	----	57.5	72.3	9	1.477	1.571	----	----	----	----	.212+	6.26

Micrometer settings for the above tests

	Injection Micrometer	Timing Micrometer
Test 1	0.4	0.7
Test 2	0.4	0.9
Test 3	0.6	0.9
Test 4	0.9	0.7

*SFC units, lbs fuel/BHP-hr
+Injection micrometer set at 0.550

It will be observed on comparing the first hot run with the first cold run that a marked improvement in specific fuel consumption occurred in the former.

In view of this marked decrease in specific fuel consumption on the first hot run, it was decided to leave the irradiated palladium in the engine and conduct additional runs in order to determine whether specific fuel consumption would increase as the intensity of radiation decreased. These runs (runs 2 through 6 with radiation) showed no definite trend in specific fuel consumption, and in some cases the specific fuel consumption was higher than on the first cold run. On the theory that variations in specific fuel consumption were at least partially due to other variables, such as relative humidity, it was decided to use the comparison method on additional tests, i.e., to run a hot run and cold run simultaneously.

Therefore on September 8, after run 6 with irradiation was completed, the inactive palladium was installed in the engine. During the next run, the second cold run, a marked change in the tone of noise coming from the engine and decrease in the engine load was noticed after test 2 was completed. When this condition persisted, the palladium was removed from the engine. On removal, the palladium cylinder was found to be bent inward around the hole which leads to the pressure pickup. The reason for this was not apparent; the cylinder was straightened and replaced in the engine, and cold run 3 conducted. No further difficulties were encountered in this run. Immediately following this cold run, the radioactive palladium was placed in the engine and run 7 with radiation was conducted. Comparing cold run 3 with hot run 7 the specific fuel consumption was somewhat lower with radiation present, especially on test 1 and 2. By this time the activity was down to 51 curies.

It was decided to repeat these comparison tests on September 18, run 8 with radiation was first conducted, and then run 4 without radiation was attempted. However, after test 1 of this run was completed, it soon became evident that the palladium was bent again, and this was confirmed by inspection. On test 1 the specific fuel consumption was again less with radiation.

The palladium was again straightened and a comparison test begun on September 24. Cold run 5 was attempted. The palladium soon bent again. Further attempts to use this palladium seemed rather futile because the repeated bending and straightening resulted in a gradual fracture of the material. Two more tests were run with the irradiated palladium in the engine.

Before evaluating these data on specific fuel consumption the results of the pressure time measurements should be considered. The comparison of the first cold and hot runs is presented in Table 3. The pressure at the beginning of combustion, compared in the first two columns, indicates that within the experimental error ignition takes place at the same point in the cycle both with

and without radiation. At the end of the initial combustion period, in tests 1 and 3 the pressure is greater with radiation and in tests 2 and 4 the pressure is greater without radiation. This would imply that the variations are due to factors other than the radiation. The maximum pressure was greater with radiation only on the first test.

TABLE 3

RESULTS AND COMPARISON OF PRESSURE MEASUREMENTS ON RUN NO. 1 WITHOUT RADIATION

WITH RUN NO. 1
WITH RADIATION

Test	Pressure at the Beginning of Combustion		Pressure at the End of Combustion		Maximum Pressure	
	Without Radiation	With Radiation	Without Radiation	With Radiation	Without Radiation	With Radiation
1	.476*	.476	.812	.904	.904	.952
2	.487	.476	.778	.718	.808	.769
3	.465	.463	.731	.764	.817	.802
4	.445	.457	.885	.808	.927	.887

*The pressures given above are averages from three pressure-time photographs for each run. The number given for pressure is an arbitrary unit obtained by measuring the height of the pressure-time curve and comparing it to the height of a 40-mv calibration signal which was photographed at the same time.

D. EVALUATION OF RESULTS ON DIESEL ENGINE TEST

The initial evaluation of the results after the first cold and hot runs was that there was a decrease in specific fuel consumption as a result of the radiation. However, three factors indicate that this difference in specific fuel consumption must be due to causes other than the radiation. First, no

definite increase in specific fuel consumption as the amount of radiation decreases was evident. Second, in the limited number of comparison runs made, the specific fuel consumption was always lower with the radiation source present. This difference did not decrease as the amount of radiation decreased. Finally, the effects of radiation were not detected on the pressure-time diagrams.

If it is concluded that the difference in the specific fuel consumption on the hot and cold runs is not due to radiation, two questions arise. What caused the difference? What caused the nonirradiated palladium cylinder to be dented in? The answers are not clear. The two palladium cylinders and discs were made as nearly identical as possible, and no appreciable difference between the two was evident when checked by measurement or by weighing. In spite of this, however, the most likely answer appears to lie in differences in the physical size and shape of the pieces. It appears that the reason the nonirradiated cylinder deformed was that fuel burned in the small volume which connects the combustion cylinder to the pressure pickup (which fits into a spark-plug hole). The injector is located in the end of the cylinder, very close to one wall. It is possible that slight differences in the thickness of the palladium at this point would influence the spray pattern and permit combustible mixture to accumulate in the passage to the pressure pickup. Had this been anticipated, a comparison test would have been made before irradiation. This was not done because a deposit of carbon collects on the palladium and it was deemed best to have the palladium which was to be irradiated as free from contamination as possible. It should also be pointed out that the nonirradiated palladium was used in the engine a number of times before the test with satisfactory results, and only during the test was the difficulty described above encountered.

E. DESCRIPTION OF SPARK IGNITION ENGINE TEST

The technique used in testing the spark ignition engine was described in Progress Report 5. A palladium tube was inserted in the engine, the palladium tube being secured in a fixture which fits into a spark-plug hole. This tube was then between the inlet and exhaust valves of the engine. This setup was checked a number of times before the hot run was attempted. The only difficulty encountered in these preliminary tests was that the engine, which was fitted with a fuel pump and mixing evaporator tank rather than a carborator, did not give very stable operation. The palladium tube, however, withstood the temperature satisfactorily and did not interfere with the operation of the valves. However, on the hot run on August 27, it was impossible to operate the engine satisfactorily after the hot palladium was inserted in the engine. On removing the palladium tube from the engine it was found that the palladium tube had somehow gotten under one of the valves and was badly bent near the end. This, of course, accounts for the faulty operation of the engine because the valves

were not operating properly. In view of the fact that it would have been extremely hazardous to open up the engine and inspect the damage to the valve because of the small pieces of palladium which had broken off and because it was impossible to straighten out the tube, it was deemed inadvisable to continue the experiment.

F. CONCLUSIONS

The following conclusions summarize the work done on the effect of beta radiation on internal-combustion engines.

(1) The effects of beta radiation on the combustion of fuel in a diesel engine are, if any, very small, and were not detectable with the apparatus used.

(2) Analysis of the material filtered out of the exhaust gases showed an appreciable amount of radiation. This implies the necessity for a good filtering system on the exhaust, which in turn raises the problem of increased exhaust pressure which would tend to decrease the engine efficiency.

(3) Since the performance of an internal-combustion engine is subject to many variations, it would seem best to carry out future experiments in a manner which permits closer control of the combustion process.

G. ACKNOWLEDGEMENTS

In view of the fact that financial support for this project ceased on June 30, 1953, the Michigan Memorial-Phoenix Project provided funds for the completion of the work, which support is hereby acknowledged.

The United States Air Force, 10th Air Force, 2242nd Reserve Combat Training Center, provided the airlift to transport the irradiated material from Idaho Falls to Willow Run, which assistance is also appreciated.

H. REFERENCES

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PART II. SUBPROJECT M943-4, THE EFFECT OF RADIATION ON CHEMICAL REACTIONS

Personnel:

Subproject Supervisors: Joseph J. Martin, Associate Professor of Chemical and Metallurgical Engineering; and Leigh C. Anderson, Professor and Chairman of the Department of Chemistry.

Senior Research Assistants: David E. Harmer and John G. Lewis.

Assistants in Research: F. Bashore, R. Carstens, C. Eckfield, J. Holmes, R. Kinney, R. Klemm.

A. INTRODUCTION

Since the last report the efforts of this group have been concentrated on studying the effects of gamma radiation on two types of chemical reactions: (1) the polymerization of ethylene and other gases at high pressures; and (2) the chlorination of aromatic hydrocarbons. In the case of the ethylene polymerization, considerable amounts of ethylene polymer were produced as a powder and then formed, by heating under pressure, into sheets that could be subjected to physical testing. In the case of chlorination, major interest centered on producing and analyzing the addition product of chlorine and toluene. A large amount of this material was made and some of it was sent to the Department of Agriculture for testing as a possible insecticide. The following sections of the report discuss the details of these reactions, both of which are of sufficient importance to be considered seriously for patenting.

B. POLYMERIZATION OF ETHYLENE BY MEANS OF GAMMA RADIATION

The erratic nature of the results observed when attempting to polymerize ethylene by exposure to gamma radiation has been mentioned previously (see Progress Report 5.[2] In the earlier work some attempt was made to correlate the observed rates of polymerization with oxygen content in the monomeric ethylene

and with the order of the run made. Neither of these approaches yielded satisfactory results.

Other ideas were therefore advanced in order to account for the erratic polymerization rates observed. It was suggested that some polyethylene might have been present in the storage cylinders and might have been introduced during charging of the reactant to the pressure reactor; however, the conditions usually required for the polymerization of ethylene were unlikely to have prevailed in the storage cylinder.

Another possibility was that some unknown inhibitor or some unknown promotor was present sporadically. The substances most likely to fall into these categories are impurities in the ethylene, gases from the air, materials used in cleaning the reaction equipment, and the reaction equipment itself. The last possibility was tested tentatively by allowing polymer to accumulate on the walls of the reactor and then checking the rate of reaction in a subsequent run; no influence on the rate of reaction was noted. The influence of various solvents and other materials thought possibly to have been present accidentally in the successful runs was checked by adding the following materials successively to separate batches of the reactant ethylene: acetone, acetaldehyde, air and acetone, air and water, carbon dioxide, sulfur dioxide, and aluminum chloride. Sulfur dioxide and aluminum chloride were the only additives producing detectable effects, and the latter material produced a tar instead of the white powder sought. The addition of sulfur dioxide resulted in the production of a white powder at relatively high rates of reaction (see Fig. 4); however, this powder proved to have a sulfur content rather close to that of the equimolar addition product of sulfur dioxide and ethylene. Matthew and Elder[10] and Snow and Frey[12] have reported similar reactions between sulfur dioxide and olefins under ultraviolet light.

Next, the composition of the reactant gases was examined in some detail. The ethylene was analyzed (see Tables 4 and 5) immediately on removal from the storage cylinders, after charging to the reactor but before irradiation, and on removal from the reactor after irradiation. Components determined were "soluble in bromine," carbon dioxide, oxygen, carbon monoxide, paraffin hydrocarbons, and nitrogen. Higher olefins and acetylenic compounds were not detected separately by the methods used.

From the results of the above experiments it was concluded that an inhibitor could have been present and responsible for the erratic yields observed, but the inhibitor could not be identified. However, the answer was finally found when the reactor was then left in the radiation field for periods longer than those previously used. Larger yields of polyethylene were obtained as a result, and it was shown that after a certain minimum induction period the yield increased to a nearly constant and reproducible value.

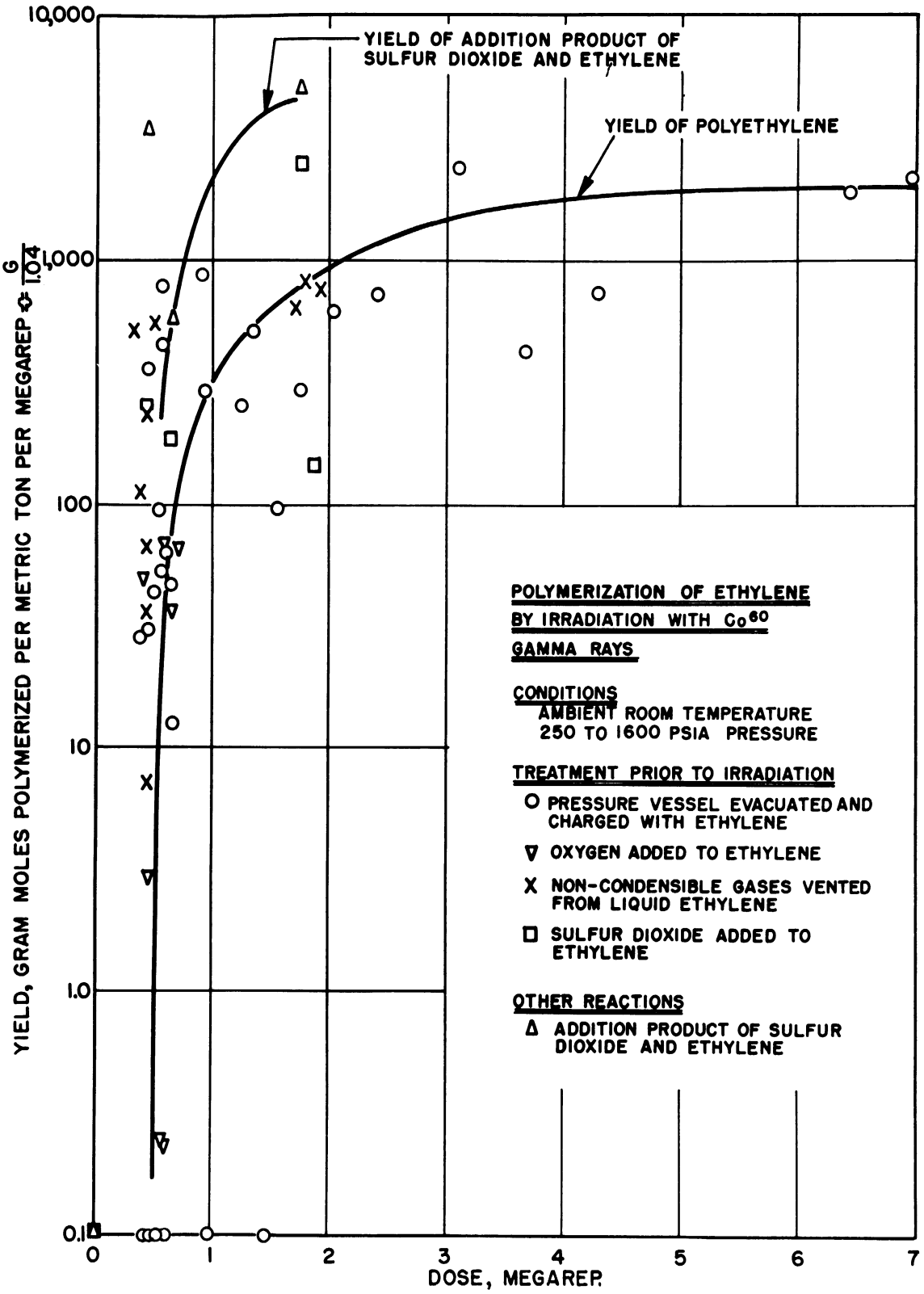

Fig. 4. Radiation Yield as Function of Dose of Radiation in Polymerization of Ethylene.

TABLE 4

ANALYSES OF ETHYLENE FROM STORAGE CYLINDERS

Material No.	Mfg.	%CO_2	%O_2	%CO	%N_2	% Combustible as marked		Number of Determinations
1	Math FF737	0.06	0.02	0.02	0.15	0.1	propane?	duplicate
2	Math 5772	0.06	0.02+	0.00	0.07	0.29	propane	duplicate
3	OC G28087	0.10±	0.02±	0.00+	0.47	total	ethane?	single sample
4	C and C JK370331	0.37	0.02	0.05	1.7	0.8	pentane	triplicate
5	USI IC-1065	0.08	0.05	0.005(?)	0.21	0.45	methane	duplicate

TABLE 5

ANALYSES OF ETHYLENE FROM REACTOR

Material No.	Dose	G	Page No.	%CO_2*	%O_2*	%CO*	%N_2	%Combustible as marked		Number of Determinations
4	3.09	2380	132363	0.37 0.62	0.02 0.07+	0.05 0.07	0.00	3.8	ethane	duplicate; first sample of 1.1 or more
2	2.40	730	132366	0.06 0.05	0.02 0.008	0.00 0.002	0.12	0.14	ethane(?)	duplicate
2	0.45	0	132369	0.06 0.02	0.02 0.05	0.00 0.01	0.22	total	-?-	duplicate
4	0.57	790	132370	0.37 0.34	0.02 0.01	0.05 0.01+	2.49	total	-?-	duplicate
2	0.91	298	132372	0.06 0.05+	0.02 0.005	0.00 0.00	0.23	total	-?-	duplicate; acetaldehyde added
2	6.97	2200	132373	0.06 0.05	0.02 ≤0.01	0.00 0.00	0.09	0.18	methane	single
3	6.43	1915	132375	0.10 0.11	0.02 0.02	0.00 0.009	0.16	0.32	ethane(?)	single
5	4.29	745	132376	0.08 0.07	0.05 0.009	0.005(?) 0.00	0.10	0.63	methane	single

* Top, before irradiation (no removal by distillation and no addition to ethylene unless noted)
Bottom, after irradiation

1. Experimental Procedure. In all of this work ethylene was irradiated with cobalt-60 gamma radiation at room temperature and at pressures of 250 to 1600 psi. Some tests were made in which ethylene was reacted alone and some in which the ethylene was used with other reactants. A stainless-steel bomb (Figs. 5 and 6) was used as the reaction vessel. The bomb was evacuated to pressures of less than 1 mm of mercury absolute and ethylene added from a cylinder. Pressures of about 1100 psi and room temperature were employed in most of the tests. The bomb was then placed in either the 1-kilocurie source or the 10-kilocurie source until the proper dose had been accumulated. After irradiation, the bomb was removed from the source, the unreacted ethylene was vented and analyzed by an Orsat analyzer, and the accumulated polymer was removed mechanically.

In order to remove possible oxygen or other volatile gases from the ethylene, the bomb was evacuated, ethylene was charged under cylinder pressure, and then the ethylene was condensed by immersing the bomb in a flask containing dry ice. The bomb was then vented until the pressure had dropped to a predetermined value or until a given volume of gas had been released. The ethylene was then vaporized and the bomb and contents irradiated as before.

2. Results of Polymerization of Ethylene. In order to conduct a quantitative study of the effect of gamma radiation on the polymerization of ethylene, it was necessary to adopt some criterion of gamma radiation effectiveness. For this purpose it seemed desirable to calculate the radiation yield in terms of the G value, i.e., the number of molecules of ethylene undergoing polymerization per 100 electron-volts of energy absorbed from the radiation. The roentgen equivalent physical, or rep, was adopted as the unit of measure of the absorption of gamma radiation by ethylene. The rep was assumed to correspond to the absorption of 93 ergs per gram of absorber. The dose rate in rep per hour in the absorber was determined by a combination of methods. Chemical dosimetry was conducted, using the method of Weiss[14] and the results were correlated and extended by the methods of Lewis, Nehemias, Harmer and Martin.[8] The dose rates determined in this way were applied to the calculation of the radiation yields.

The degree of polymerization of the ethylene was determined by terminating the reaction at a stage such that the conditions during reaction had been reasonably constant, venting the unreacted ethylene, and then removing the polymer mechanically and weighing it. The conditions prevailing during reaction were averaged and the average values were assumed to have prevailed throughout the course of the reaction. Instead of G, the quantity A was sometimes calculated in this work. A was defined as the gram moles reacted per metric ton subjected to one megarep. By a comparison of units the following relation can be established:

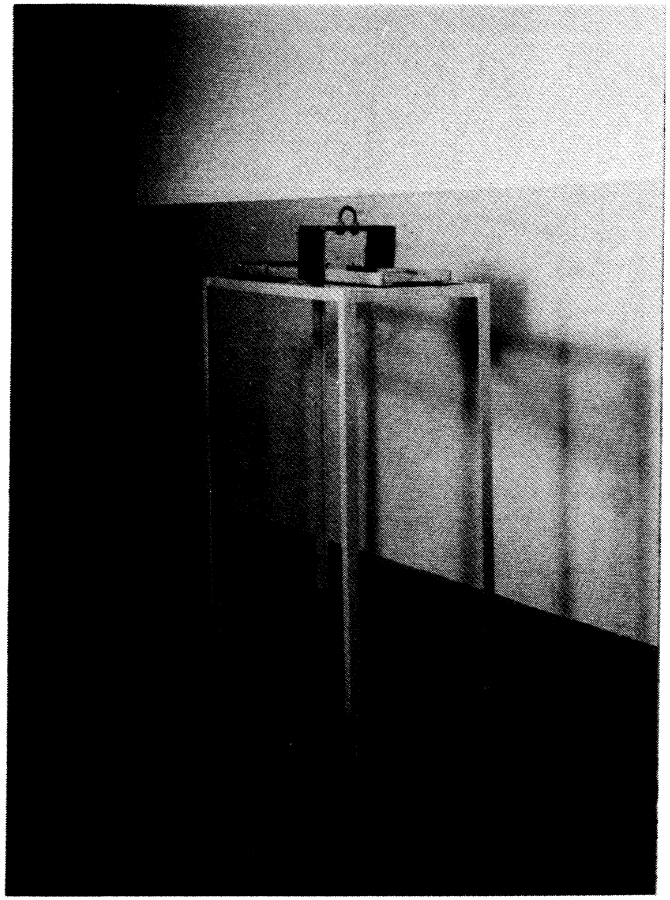

Fig. 5. Rack and Sling for Pressure Reactor.

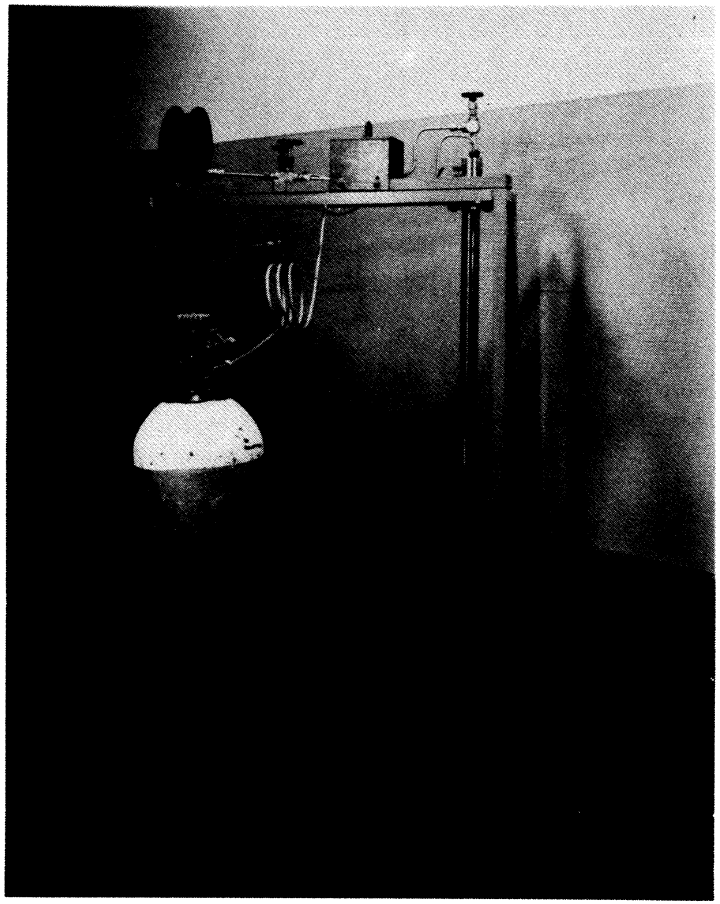

Fig. 6. Pressure Reactor: Tubing Assembly and Gas Cylinder.

$$A, \frac{\text{gram moles reacted}}{(\text{metric ton})(\text{megarep})} = \frac{G}{1.04}, \frac{\text{molecules reacted}}{100 \text{ electron volts}}$$

Consequently, A is almost equal numerically to G. With some simplification the following relation was developed:

$$A, \frac{\text{gram moles reacted}}{(\text{metric ton})(\text{megarep})} = \frac{\text{weight fraction ethylene reacted} \times 10^6}{(\text{molecular weight of ethylene})(\text{dose, megarep})}.$$

This simplified relation was used in calculating A values in the following work.

A white, solid polyethylene resulted from the irradiation of ethylene with cobalt-60 gamma rays. See Table 6 for the experimental results. The yield of polymer was found to be quite small until the system had received a dose of about 1/2 megarep. The yield increased rapidly to a value of about 2500 gram moles reacted/(metric ton)(megarep) at about 3 megarep, and remained nearly constant up to doses of 7 megarep, the highest dose studied (see Fig. 4). About one-third of the monomer was polymerized in three days in the center of the 10-kilocurie source. The amount of ethylene charged to the bomb was calculated from the observed temperature, pressure, and volume and the thermodynamic properties of ethylene taken from the work of York and White.[15]

3. Discussion of Polymerization of Ethylene. From Fig. 4 it can be seen that the yield of polymer per unit of energy absorbed from the radiation is a function of the total dose of radiation. This relation is evidently due to the presence of an induction period for the reaction. No correlation could be observed between contents of the following gases in the monomer and the yield as a function of dose: carbon dioxide, oxygen, carbon monoxide, hydrogen, paraffin hydrocarbons, nitrogen, and sulfur dioxide. It appeared, however, that the venting of noncondensable gases from the liquid ethylene did increase the initial rate of reaction a little. The data for the analyses of gases before and after irradiation are given in Tables 4 and 5.

In view of the evidence just mentioned, it cannot yet be stated what effect chemical additives have on the rate of polymerization of ethylene by gamma radiation. It is possible, however, that the induction period is caused by the presence of chemical compounds other than ethylene.

Average values of dose rates used in these studies varied from about 30 kilorep/hour to about 90 kilorep/hour. It should be noted, however, that errors exist in the method of calculating the dose rates used in estimating the G or A values. A Victoreen ratemeter was used to measure the dose rates on the axis of the bomb. This instrument would detect the secondary photons produced by scatter from the wall of the bomb, but probably would not detect

TABLE 6

IRRADIATION OF ETHYLENE

Order No.	Page No.	Starting Date	Psig Initial	Psig Final	Avg. Psia	Avg. Temp., °F	Grams Polymer	Hours Irrad.	Grams Polymer per Hour	A = G-Moles Reacted / (Metric Ton)(Megarep)	Averaged over Reactor: Dose Rate Kilorep/Hr	Averaged over Reactor: Total Dose Megarep	Other Reactants	Source	Position of Reactor	Mfr.	Cylinder No.	Remarks
2	132113	19 Dec 52	830	785	822	69	1.6+	69.7	0.02+	608.	29.	2.02	1 Ml acetone	1 kc	--	Matheson	FF 737	--
3	132118	12 Jan 53	820	795	822	70	0.666	61.	0.011	291.	28.9	1.76	--	1 kc	--	"	"	--
4	132147	3 Mar 53	1430	1330	1395	230	0.292	17.2	0.017	485.	29.5	0.507	--	10 kc	8" NE 9" up	"	"	--
5	132149-II	7 Mar 53	820 435	700 424	775 445	50	0.004	0.9 15.	0.0003	12.7	42.	0.666	--	10 kc	8" NE 9" up	"	"	--
1	132159	26 Nov 52	790	780	800	68	0.394	42.	0.009	258	29.5	1.24	1 Ml acetone	1 kc	--	"	"	--
6	132250	10 Mar 53	810	715	777	50	0.167	16.2	0.010	94.5	96.	1.55	--	10 kc	℄ on ℄ Base at Base	"	"	--
7	132252	11 Mar 53	1430	1330	1395	200	1.04	16.1	0.06	513.	82.5	1.33	--	10 kc	Do.	"	"	--
8	132253	12 Mar 53	698	692	710	45	1.48	87.7	0.017	423.	42.0	3.69	--	10 kc	8" NE 9" up	"	"	--
9	132254	17 Mar 53	635	655	660	45	--	23.3	--	--	42.0	0.979	650 psi N_2 and 15 psi air vent, chg. C_2H_4	10 kc	8" NE 9" up	"	"	--
10	132255	18 Mar 53	800	710	770	45	0.088	21.5	0.004	865.	42.0	0.904	--	10 kc	8" NE 9" up	"	"	--
11	132256	19 Mar 53	840	760	815	420	0.381	15.3	0.025	1800.	29.3	0.448	--	10 kc	8" NE 9" up	"	"	--
12	132258	23 Mar 53	810	70	455	45	0.010	39.	0.0003	12.5	41.9	1.63	10 Ml acetone	10 kc	--	"	"	Leak
13	132259	26 Mar 53	788	183	485	61	--	15.3	--	--	95.5	1.46	--	10 kc	℄ on ℄ Base at Base	"	"	--
14	132263-I	4 May 53	1600	1425	1528	72	--	21.9	--	--	27.7	0.608	--	1 kc	--	"	"	--
15	132263-II	5 May 53	620	595	623	72	0.022	23.	0.001	45.8	27.7	0.638	--	1 kc	--	"	"	--
16	132264	7 May 53	615	600	622	69	<0.0001	20.7	<0.000005	<0.25	27.6	0.572	100 Ml O_2	1 kc	--	"	"	--
17	132265	8 May 53	620	608	631	67	<0.0001	21.4	<0.000005	<0.24	27.6	0.591	25 Ml O_2	1 kc	--	"	"	--
18	132266	9 May 53	594	577	601	72	0.034	25.6	0.0013	67.5	27.5	0.703	5.0 Ml O_2	1 kc	--	"	"	--
19	132267	10 May 53	600	575	603	75	~0.001	17.0	~0.00005	~ 3.1	27.5	0.468	2.0 Ml O_2	1 kc	--	"	"	--
20	132268	11 May 53	617	597	622	70	0.017	22.3	0.0008	36.6	27.5	0.613	10.0 Ml O_2	1 kc	--	"	"	--
21	132269	12 May 53	605	590	612	71	0.030	21.1	0.0014	71.	27.5	0.581	10.0 Ml O_2	1 kc	--	"	"	--
22	132270	13 May 53	637	629	638	66	0.016	14.8	0.0011	50.	27.5	0.407	5.0 Ml O_2	1 kc	--	"	"	--
23	132275	4 Jun 53	550	525	553	70	0.031	16.8	0.002	94.	31.3	0.526	--	10 kc	8" SW 13-7/8" up	"	"	--
24	132276	7 Jul 53	490	490	505	76	0.021	22.1	0.001	63.	27.0	0.597	23 mm air 1 Ml acetone	1 kc	--	"	"	--
25	132277	8 Jul 53	460	460	475	78	--	20.1	--	0.0	27.0	0.543	95 Ml air 1 Ml water	1 kc	--	"	"	--
26	132278	9 Jul 53	460	455	473	73	0.011	18.3	0.0006	43.1	27.0	0.494	215 mm CO_2	1 kc	--	"	"	--
27	132279	10 Jul 53	445	440	458	~75	0 136	69.8	0.002	147.	27.0	1.88	33 mm SO_2	1 kc	--	"	"	--
28	132280	13 Jul 53	426	395	425	87	Lost, sev< al grams ta-	16.5	--	--	27.0	0.446	4.598 gm $AlCl_3$	1 kc	--	"	"	--
29	132281	14 Jul 53	430	420	440	81	0.013	20.0	0.001	52.2	27.0	0.540	--	1 kc	--	"	"	--
30	132282	15 Jul 53	420	410	430	79	0.657	23.2	0.028	184.	27.0	0.627	1 atm SO_2	1 kc	--	"	"	A_{SO_2} = 575
31	132284	16 Jul 53	420	385	417	83	2.692	16.1	0.167	241.	27.0	0.435	55 psia SO_2	1 kc	--	"	"	A_{SO_2} = 3380
32	132285	17 Jul 53	393	70	246	83	30.9	64.0	0.483	2450.	27.0	1.73	23.98 gm SO_2	1 kc	--	"	"	A_{SO_2} = 4800
33	132287	20 Jul 53	400	390	410	--	--	--	--	--	0	0	27.9 gm SO_2	--	--	"	"	--
34	132293	3 Aug 53	810	790	815	80	0.208	17.0	0.012	357.	26.8	0.456	--	1 kc	--	Ohio Chem.	G28087	--
35	132294	4 Aug 53	930	910	935	76	0.472	21.8	0.022	443.	26.8	0.584	--	1 kc	--	"	"	--
36	132295	5 Aug 53	615	610	628	76	0.010	16.0	0.0006	30.	26.8	0.428	Alk. pyrogallol	1 kc	--	Matheson	FF 737	Powdery, soft lumps, white
37	132297	6 Aug 53	955	950	968	75	4.45	71.5	0.0623	750.	26.8	1.91	--	1 kc	--	"	"	--
38	132350	10 Aug 53	320	320	335	75	0.005	15.7	0.0003	35.6	26.8	0.421	--	1 kc	--	"	"	--
39	132351	11 Aug 53	985	965	990	80	0.084	16.3	0.005	67.	26.8	0.437	--	1 kc	--	"	"	--
40	132353	12 Aug 53	945	930	950	75	0.085	14.6	0.006	112.	26.8	0.392	--	1 kc	--	"	"	--
41	132354	13 Aug 53	860	840	865	80	0.150	16.3	0.009	234.	26.8	0.437	--	1 kc	--	"	"	--
42	132355	14 Aug 53	870	815	857	74	1.70	63.6	0.0268	638.	26.8	1.70	--	1 kc	--	"	"	--
43	132356	17 Aug 53	935	900	932	70	0.309	11.8	0.0262	509.	26.7	0.315	--	1 kc	--	Ohio Chem.	G28087	--
44	132357	18 Aug 53	875	870	888	72	0.428	18.5	0.0231	551.	26.7	0.494	--	1 kc	--	"	"	--
45	132359	20 Aug 53	1015	970	1002	75	~0.005	17.4	~0.0002	7.3	26.7	0.465	--	1 kc	--	Matheson	5772	--
46	132360	21 Aug 53	920	865	907	74	2.6	66.8	0.040	802.	26.7	1.78	--	1 kc	--	"	"	--
47	132361	24 Aug 53	825	785	820	79	--	15.7	--	0.0	26.5	0.416	--	1 kc	--	"	"	--
48	132362	25 Aug 53	975	946	975	79	0.029	14.0	0.0021	28.0	26.5	0.372	--	1 kc	--	"	"	--
49	132363	26 Aug 53	1200	1000	1115	90	21.5	116.5	0.185	2380.	26.5	3.09	--	1 kc	--	Carbide and Carbon	JK 370331	In small to medium lumps, slightly off white, moderately coherent
50	132366	5 Sept 53	1010	924	980	77	4.9	90.5	0.0541	730.	26.5	2.40	--	1 kc	--	Matheson	5772	Powdery, in small, soft lumps, white
51	132369	9 Sept 53	1005	925	980	73	--	17.0	--	0.0	26.5	0.451	--	1 kc	--	"	"	--
52	132370	10 Sept 53	1000	970	1000	70	1.5	21.3	0.0705	790.	26.5	0.565	--	1 kc	--	Carbide and Carbon	JK 370331	--
53	132372	11 Sept 53	513	475	510	60	0.16	34.5	0.00464	298.	26.5	0.912	Acetaldehyde	1 kc	--	Matheson	5772	--
54	132373	14 Sept 53	1500	960	1245	~70	51.3	262.7	0.195	2200.	26.5	6.97	--	1 kc	--	"	"	Had to be cut out of bomb. Some fines, all coherent, white
55	132375	25 Sept 53	1570	1080	1315	~65	43.6	71.8	0.607	1915. avg	89.6	6.43	--	10 kc	On ℄ --	"	"	Had to be cut out of bomb. Some fines, all coherent, white
56	132376	30 Sept 53	1780	1110	1450	79	12.5	17.0 23.7 28.8	0.18 avg	745. avg	29.5 89.5 58.0	0.50 2.12 1.67	-- -- --	1 kc 10 kc 10 kc	On ℄ 6-5/8 out	U.S. Ind. Chem.	I 1065	White, soft powder, very small lumps

the scattered electrons. These scattered electrons would be quite effective in producing chemical reaction because nearly all their energy would be imparted to the chemical system. Consequently it can be seen that more ionization probably occurred than was taken into account by the calculations, in which the effect of the bomb wall was neglected. The effect of this error is that the G values given are too high.

On the other hand, as a calculation device the primary beam was assumed to undergo no appreciable absorption within the ethylene in the bomb. Rather the beam was assumed to maintain within the bomb a value which would be attained on the axis if the bomb were full of air. It was recognized, of course, that absorption within the ethylene was assumed to be causing the reaction. If account were taken of absorption of primaries within the ethylene, then somewhat greater credit for initiating reaction would have to be given to each primary photon, and this would increase the G values given.

Thus, neglect of nonequilibrium secondaries and neglect of the absorption gradient of primary gamma intensity within the ethylene compensate each other to some extent. The importance of accounting for the above errors in dosimetry is recognized. However, the complexity of the measurement problems would seem to indicate the desirability of pursuing this work further in future studies. Therefore, the values given for G in Fig. 4 should be regarded as relative rather than absolute, since all determinations were made in the same equipment and using similar procedures.

No consistent effect of pressure on the G value was noted.

Elevated temperatures were investigated only briefly, but preliminary results indicated that increased rates of polymerization would result in irradiated systems at temperatures of 200-400°F as compared with those obtained at room temperature.

4. Evaluation of the Polyethylene Product. a. General: The polyethylene obtained as a result of gamma irradiation of ethylene was subjected to a brief program of evaluation. The properties considered most basic to an understanding of the material were investigated. Most experimental work was concerned with determinations of solution viscosity, melt viscosity, density, and tensile strength. Melting points of some samples were also determined. Molecular weights were estimated from the determinations of viscosities of solutions and of melts. Crystallinity was estimated from determinations of density. The other measurements were made by conventional means.

These measurements and derived quantities probably need no further explanation with the exception of the concept of the crystallinity of a polymer. The degree of crystallinity of a polymer is measured by the degree to which the

molecules of polymer are arranged parallel to each other. An arrangement of parallel molecules results in a repetitive structural pattern such as that found among the molecules of a crystal. A random orientation of molecules similar to a pile of jackstraws might be expected to be less dense than a parallel arrangement such as that just described, and it has been found that percentage crystallinity may be correlated with the density of polyethylene (see Kirk-Othmer).[7]

From the data obtained in experiments on the polymer, molecular weight and crystallinity are presented as functions of dose and all the properties of the polyethylene are presented as functions of the radiation yield of the polymerization reaction because of the following considerations. The radiation yield of the polymerization of ethylene may be expressed as the G value. Lind[9] has shown that in many gaseous systems, approximately one molecule reacts per ion pair formed in the system. In the irradiation of ethylene a variable number of molecules, usually much greater than one, react for each ion pair formed. The polymerization of ethylene is therefore evidently a chain reaction. For this calculation it is assumed that one chain is initiated for every ion pair formed, that all chains are of equal length, and further that the formation of each ion pair requires 32.5 electron-volts of energy, a value approximately correct for gases at one atmosphere. The densities of ethylene under the conditions of reaction were greater than at one atmosphere, however, and therefore the energy required per ion pair may be quite different from the value given. The G value may therefore be divided by three to give the approximate number of molecules reacted for each ion pair formed, and this result may then be multiplied by the molecular weight of the monomer in order to arrive at the molecular weight of the polymer.

Consequently, the G value is directly proportional to the molecular weight which would be expected of the polymer if the above assumptions held. Furthermore, the properties of a polymer are frequently found to be functions of its molecular weight. It therefore seems advantageous to consider the properties of the polyethylene as functions of the G value.

The results of most determinations could be correlated against the G value, or radiation yield, somewhat better than they could against dose, although the G value has been shown to be a function of dose. In Fig. 4, for example, the G value was about 0.1 to 1.0 until about 0.5 megarep had been received. The G value then increased rapidly with increasing dose until it reached a nearly constant value of about 2000 molecules per 100 electron-volts for doses of about 3 to 7 megarep.

b. Experimental: All the samples of polyethylene were white. Some were fluffy powders and others were tough, coherent masses.

Portions of each of the samples of polyethylene which were obtained in yields of 4 grams or more were molded into sheets as an operation preliminary to further examinations. A two-compartment mold was used, one compartment at a time. Samples were placed between aluminum foil in the mold, preheated to 300°F, pressed at 1000 psi, and cooled to about 125°F under pressure. The resulting sheets were 2.5 by 4 by 0.025 inch. All such sheets proved to have the characteristic milky, translucent appearance of polyethylene. The sheets molded from the powders were brittle, while those from the tough reaction products were also tough.

Molecular weights were estimated from viscosities of solutions, measured as follows: Solutions of some samples were prepared in concentrations of 0.01 percent and of 0.125 percent by weight in tetralin. Viscosities of these solutions and of the tetralin were measured in modified Ostwald pipettes at 212°F. Specific viscosities were calculated and divided by the respective concentrations. The resulting ratios were plotted as a function of the concentration of polymer, and the plots were extrapolated to zero concentration to give intrinsic viscosity. Intrinsic viscosity was assumed to be directly proportional to molecular weight. The concentration was computed in units of gram moles of monomeric ethylene per liter of solution. The constant of proportionality was computed by the author to cause the observed value for the molecular weight of Bakelite DYNH to agree with the value of 20,000 for the weight-average molecular weight given by Dienes and Klemm.[4] The value of the constant was computed in this way to be 0.42×10^{-4} liter per gram. (See also the work of Tani[13] on intrinsic viscosities of polyethylene in tetralin.)

The method of Dienes and Klemm[4] was used to estimate molecular weights from melt viscosities. Viscosities were measured in a parallel-plate plastometer with an attached dial gauge reading to 0.01 millimeter. The entire assembly was placed in an oven. Temperatures of 248°F and 266°F were used. The samples were placed between sheets of aluminum foil about 1-1/2 mils thick. The thickness of the sheets of foil was measured in the plastometer before each determination.

Crystallinity was estimated by correlation with density (see Kirk-Othmer).[7] Densities were determined by the use of Archimedes' principle. Weighings were made directly in water, after the sample had first been degassed by use of reduced pressure while it was immersed in water.

Tensile properties of the polyethylenes were examined by the following procedure. Specimens for testing were cut from the molded sheets by means of a die. The resulting specimens were 0.079 by 0.025 inch in the smallest cross section. The narrowest section was 1-1/2 inches long. Tension was applied in a Gardner-Parks testing machine shown in Fig. 266 of Gardner.[5] This machine had a capacity of 2.5 kilograms.

Melting points were determined on a melting-point bar of the type used by Dennis[3] (see Fig. 8).

c. Results. Results of evaluation of the properties of the radiation-polymerized polyethylene are summarized in Table 7. The molecular weight is plotted as a function of radiation yield in Fig. 7, and the molecular weights and crystallinities are plotted as functions of dose in Fig. 9.

The significance of the determination of molecular weights by means of solution viscosity is not clear. The values obtained were assumed to be weight-average molecular weights, based on the weight-average molecular weight of Bakelite DYNH of 20,000 (see Table 7). However, differences in crystallinity and cross-linking, mentioned above, may invalidate the comparison of the thermally polymerized sample with the radiation-polymerized samples.

Determinations of molecular weight by melt viscosity may be subject to similar criticism. As shown in Figs. 7 and 9, the molecular weights determined by solution viscosity do not agree well with those determined by melt viscosity. Neither do the molecular weights from solution viscosity appear to display any regular variations with dose or with G value, in contrast to the regular behavior of molecular weights from melt viscosities. The reasons for these discrepancies are not clear.

Values of crystallinity are plotted as a function of radiation yield in Fig. 10. The crystallinities varied from about 77 percent for samples of low radiation yield to about 71 percent for samples of high radiation yield. All these samples were of considerably higher crystallinity than was the Bakelite DYNH, which had a crystallinity of about 61 percent. It is possible that the radiation-polymerized samples were of higher crystallinity than the thermally polymerized sample of DYNH because the temperature of polymerization was lower for the radiation-polymerized samples. The samples of low radiation yield would be expected to be more highly crystalline than those of high radiation yield. Since radiation yield has been shown to increase with dose (Fig. 4), cross-linking and branching would probably also increase with dose, and increases in either cross-linking or branching would cause decreased crystallinity.

Tensile properties are reported in terms of stress as a function of strain in Fig. 11. The irradiated samples all have properties similar to those of a brittle material, and the samples subjected to higher doses have higher tensile strengths and are more ductile than those subjected to lower doses of radiation. Such behavior would be likely if the irradiation increased cross-linking and branching. The Bakelite DYNH shows the characteristic elongation of several hundred percent before rupture (see Kirk-Othmer[7] page 942). Ultimate tensile stress as a function of radiation yield is plotted in Fig. 10. The ultimate tensile stress increased markedly with radiation yield and consequently

TABLE 7

PROPERTIES OF POLYETHYLENE PRODUCED

Page Number	Dose, Megarep	Radiation Yield, A	Melting Point, °F Lower/Upper	Density g/cm^3	Ultimate Tensile, psi	Elongation, Percent at rupture	Crystallinity Percent	Molecular Weight by Melt Viscosity	Molecular Weight by Solution Viscosity
132250	1.55	95	219/226						
132268	0.61	37	216/225						
132269	0.58	71	205/217						
132276	0.60	63	207/214						
132281	0.54	52	196/205						
132297	1.91	750	241/244	0.951	450	4	77	26,300	insol.
132362	0.37	28	234/235						
132363	3.09	2400	248/689	0.941	2200	42	71	34,400	insol.
132366	2.40	730	210/248	0.951	770	2	77	28,100	4200
132369	0.45	0.1							
132370	0.57	790	203/252						8800
132372	0.91	298	199/207						insol.
132373	6.97	2200	234/720	0.943	2100	29	72	40,500	insol.
132375	6.43	1900	241/610	0.941	2300	79	71	37,300	insol.
132376	4.29	745	241/244	0.951	630	3	77	11,900	3700
Bakelite DYNH				0.921	1500	550	61	21,800	20,000 assumed

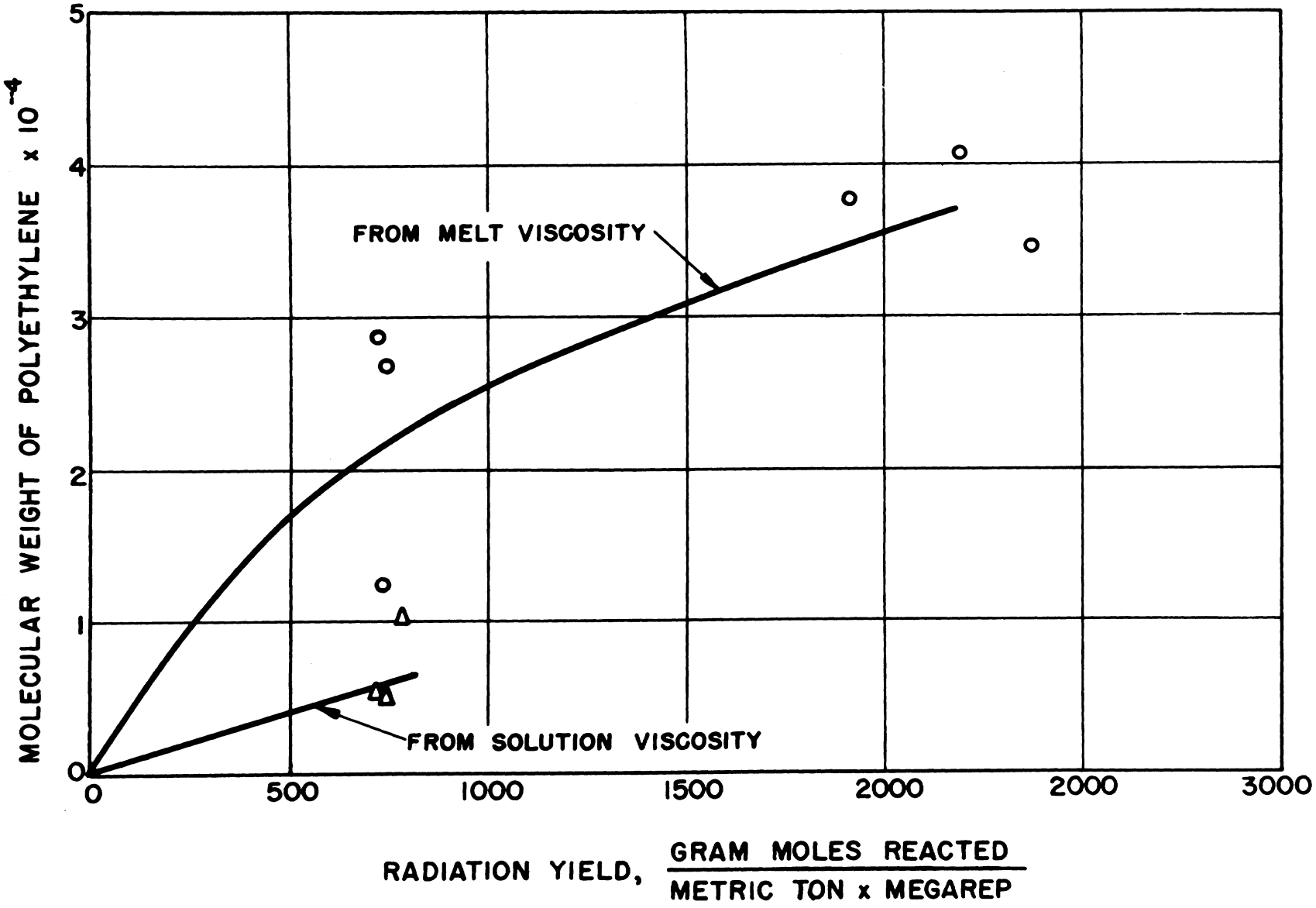

Fig. 7. Molecular Weight as Function of Radiation Yield of Polyethylene.

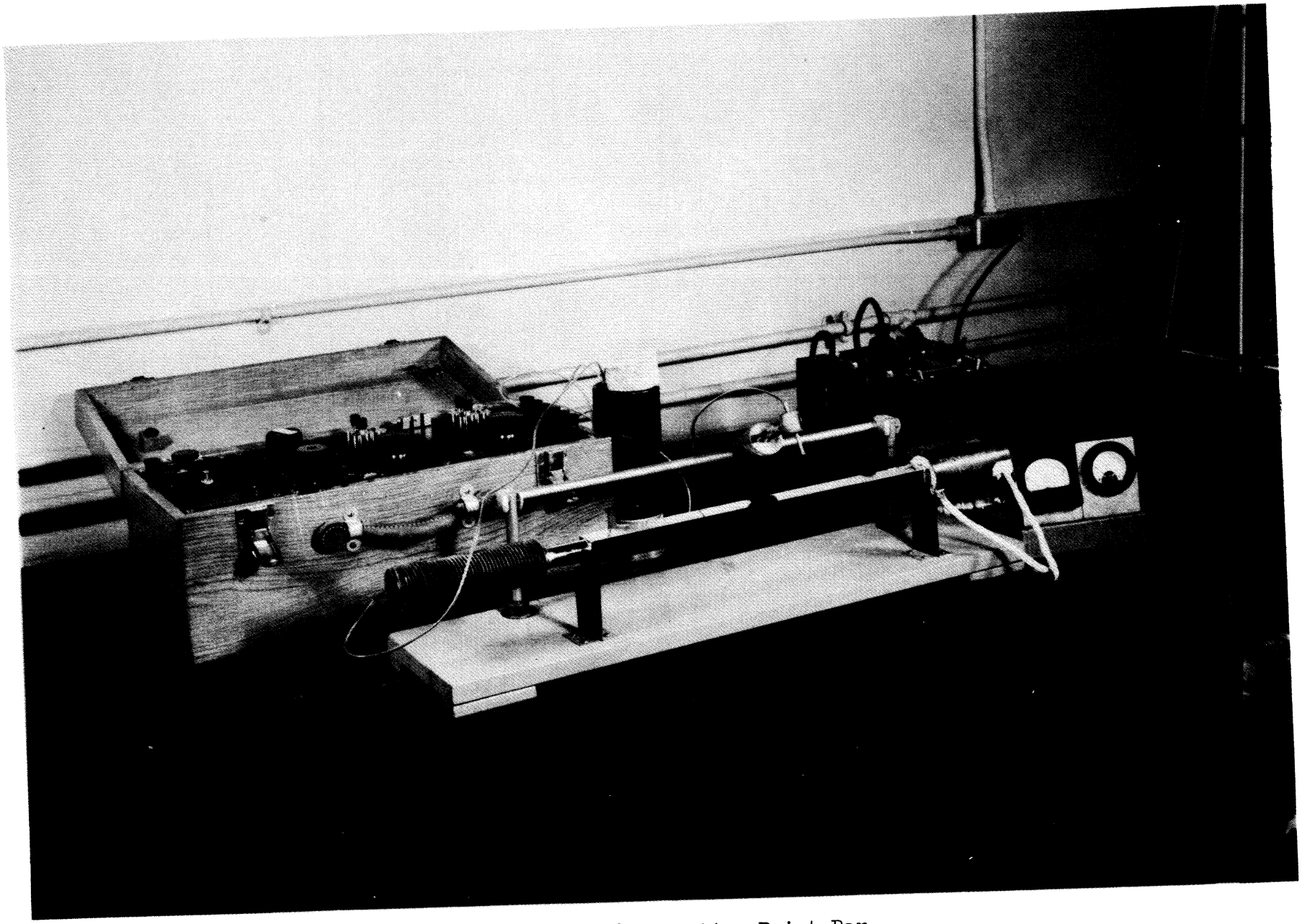

Fig. 8. Melting-Point Bar.

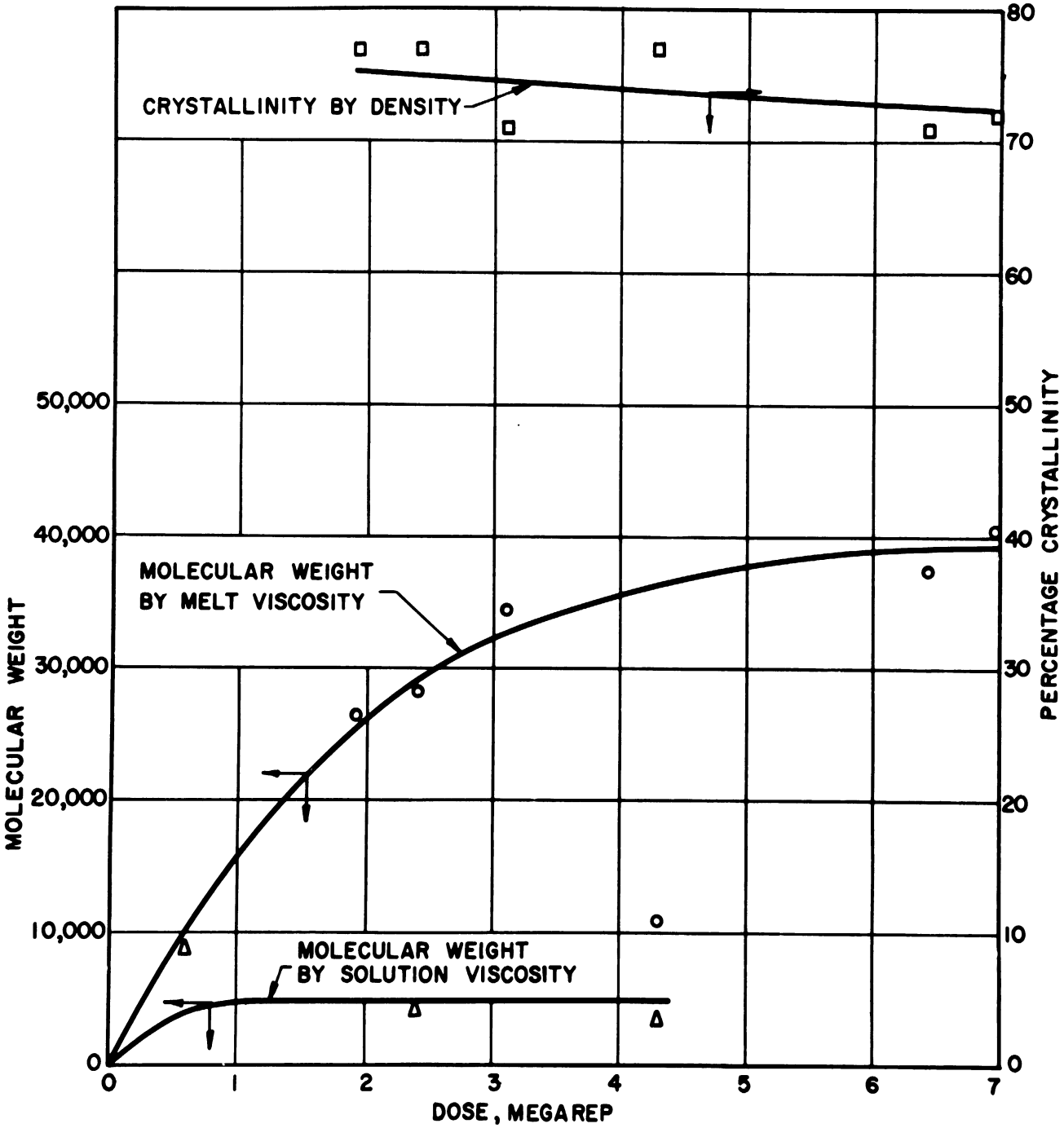

Fig. 9. Molecular Weight and Crystallinity as Functions of Radiation Dose for Polymerization.

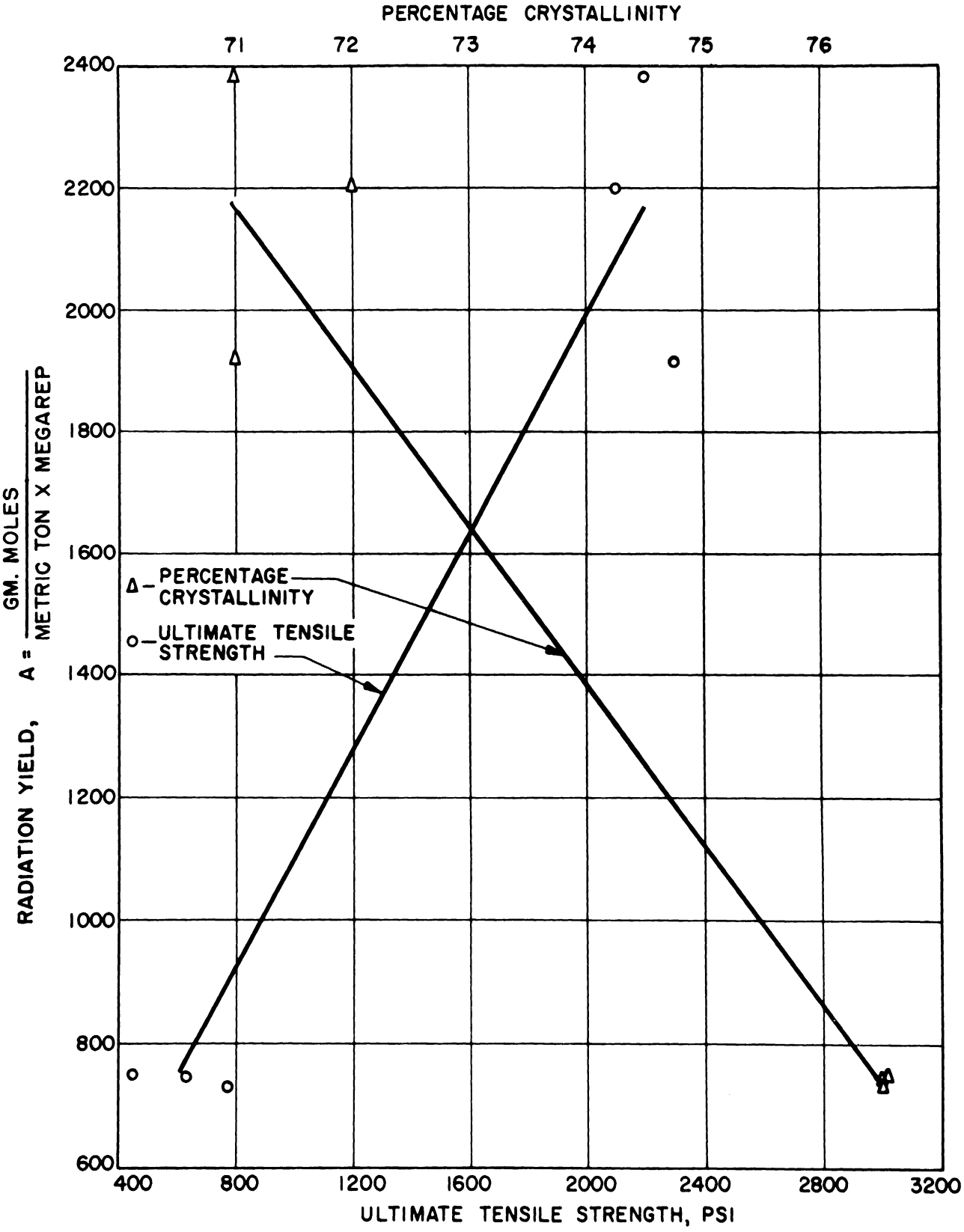

Fig. 10. Crystallinity and Tensile Strength as Functions of Radiation Yield for Polyethylene.

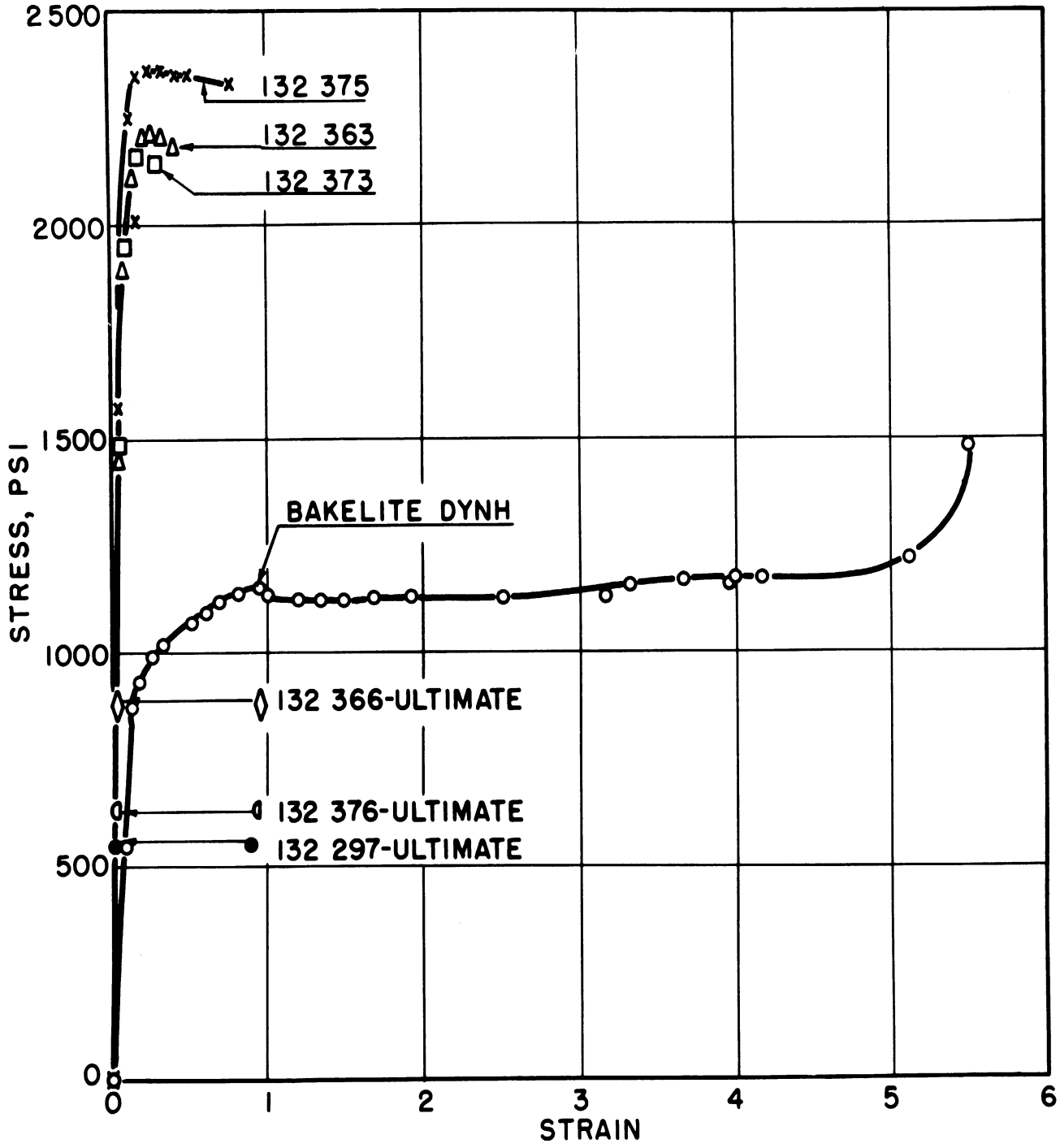

Fig. 11. Stress-Strain Plots for Test Specimens of Polyethylene.

with dose. A set of structural properties such as those just described for the radiation-polymerized polyethylene might be desirable for certain applications, but the properties differ from those of most polyethylene currently marketed.

Melting points are plotted as a function of radiation yield in Fig. 12. Curves are given for both the upper and the lower ends of the melting-point range. The results show that there is a small increase in the temperature of initial softening with increase in radiation yield, and that there is a large increase in the temperature of complete melting. The higher melting points indicate higher degrees of cross-linking as a result of the higher doses of radiation, and are thus in conformity with the results of the other determinations.

C. CHLORINATION OF AROMATIC COMPOUNDS

The reactions of chlorine with various aromatic hydrocarbons have been reported by this laboratory previously.[2] During this period covered by this report, the study of toluene reactions has been continued and monochlorobenzene has also been reacted with chlorine.

1. Description of Apparatus. In order to utilize the 10-kc cobalt-60 gamma source for reactions employing large volumes of toluene, new equipment was designed and constructed. In this equipment, up to 1.5 liters of toluene may be reacted with chlorine gas. The chlorine was fed from a cylinder located in the second floor chemical laboratory of the Fission Products Laboratory. Gases returning from the reacting system passed through bubbler bottles or solutions for absorption and were vented to the outdoors. This part of the system was the same as that illustrated in Progress Report 4 (1, pages 26 and 29) in the system used with the 1-kc source.

The apparatus located in the source room consisted of a water-cooled reactor section, a storage section, and inlet and outlet tubes for gases leading to the chemistry laboratory. The apparatus is shown in Fig. 13, and details of individual parts of this apparatus appear in Fig. 14. Referring to Fig. 15, the gases entered the apparatus through tube (1), and passed through the jet injector assembly (2). The gas then bubbled through the outer jacket of the reactor section (3), passed through the connecting tube (4) and bulb (5), and emerged from tube (6). In passing through the jet injector (see Figs. 16 and 17 for detail), and in rising through the vertical reactor section, the gases caused the reacting liquids to circulate through the tubes of the system. After being carried across the upper connecting tube (4), the liquid returned to the storage bulb (7) until it was recirculated through the lower connecting tube (8). The upper storage bulb (5) provided space for increase in the volume of the

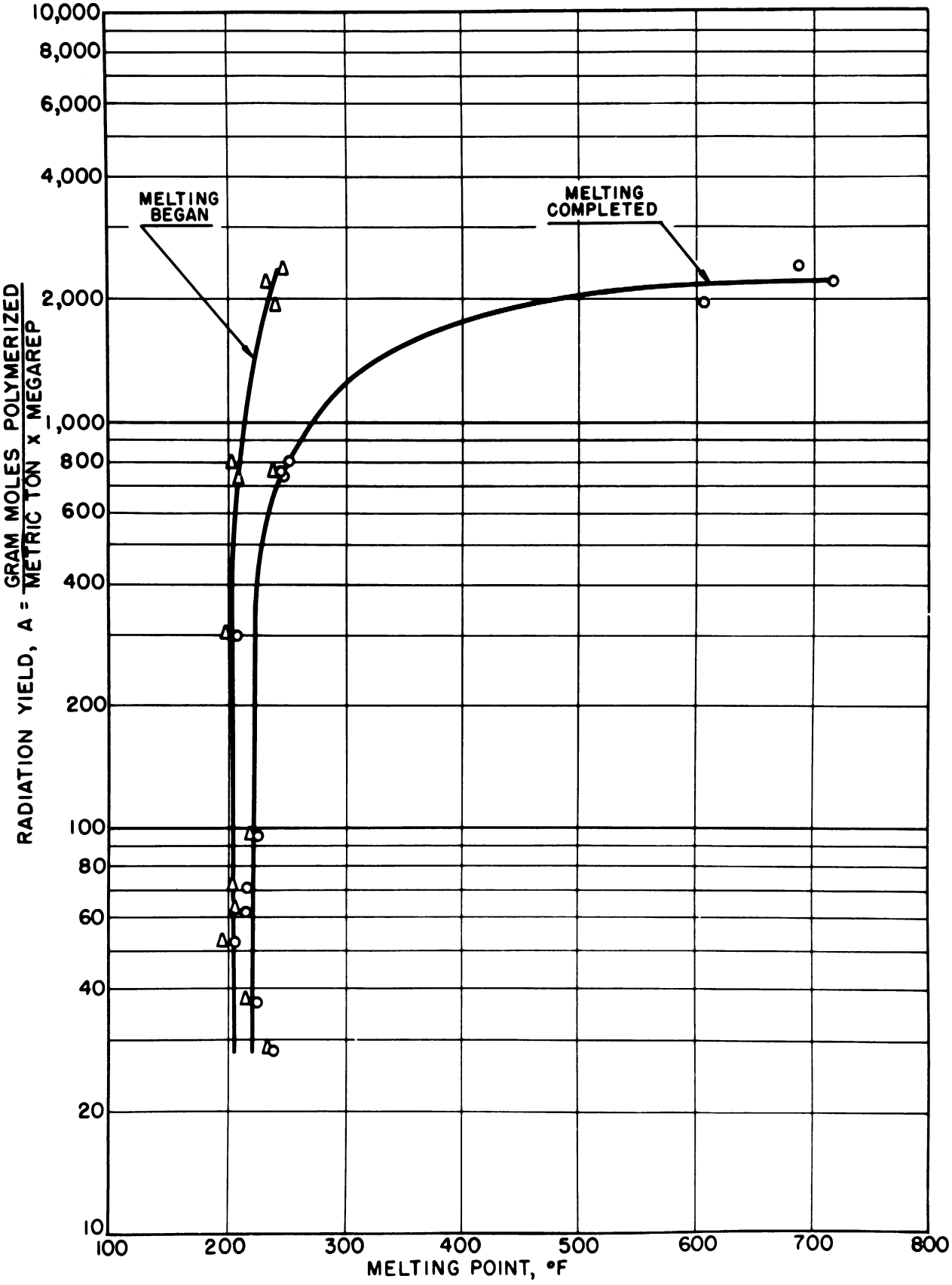

Fig. 12. Melting Points as Functions of Radiation Yield of Polyethylene.

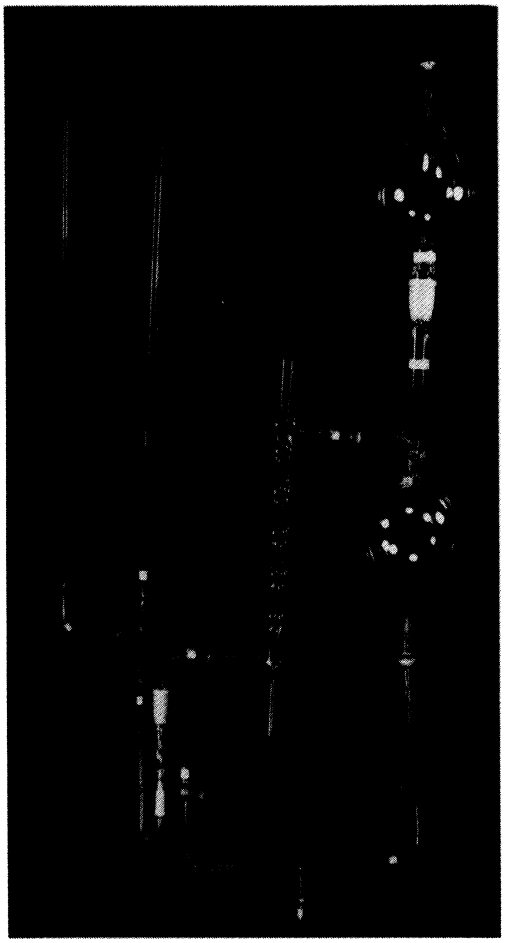

Fig. 13. Apparatus for Reaction of Liquids with Chlorine Gas in the 10 kc Cobalt-60 Gamma Source.

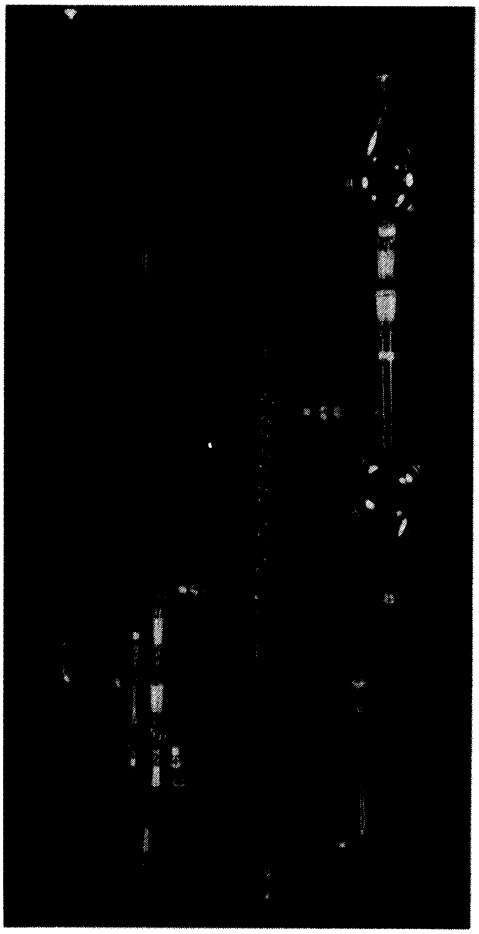

Fig. 14. Individual Parts of the Apparatus for Reaction of Liquids with Chlorine Gas.

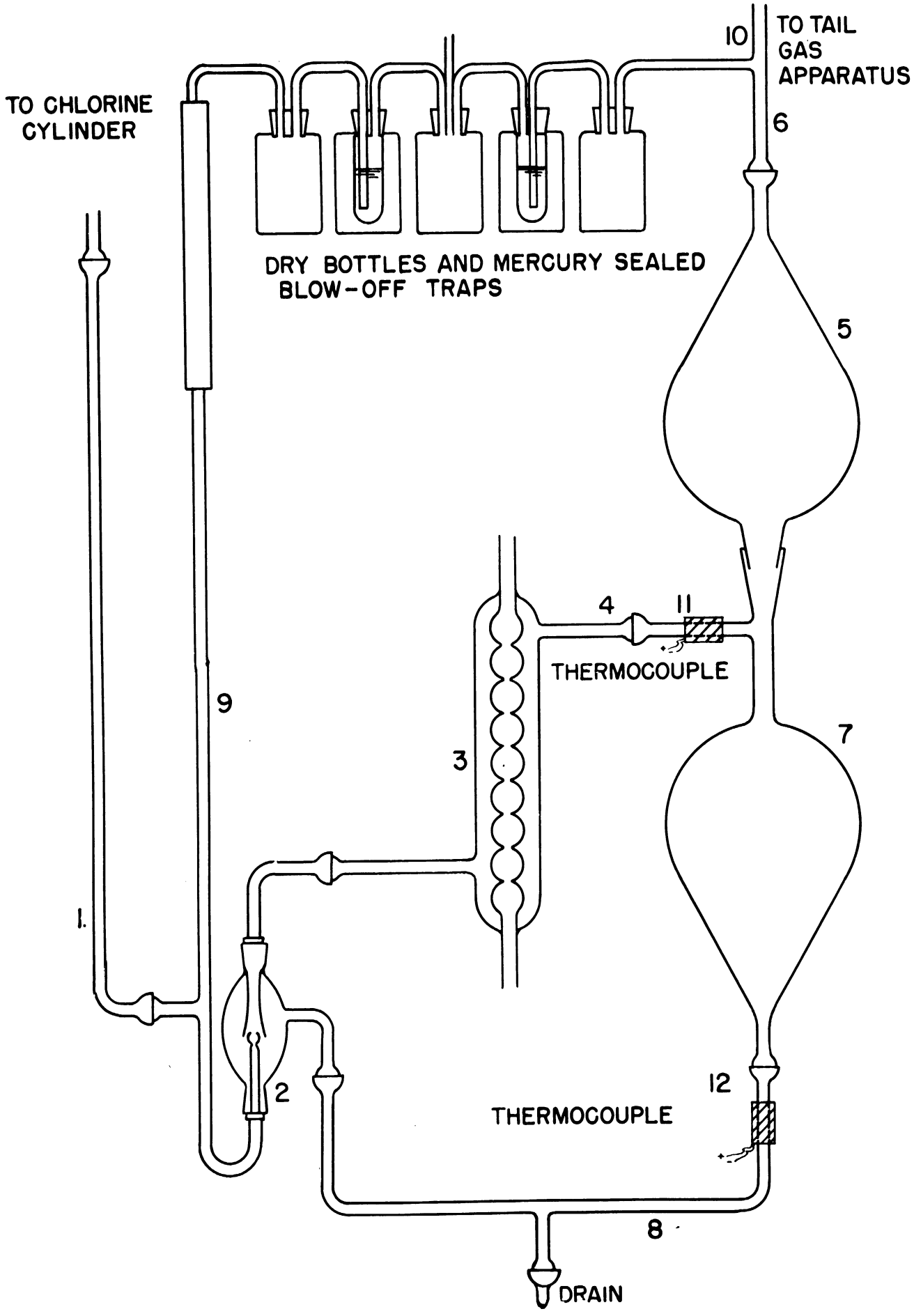

Fig. 15. Drawing of Apparatus for Reaction of Liquids with Chlorine Gas.

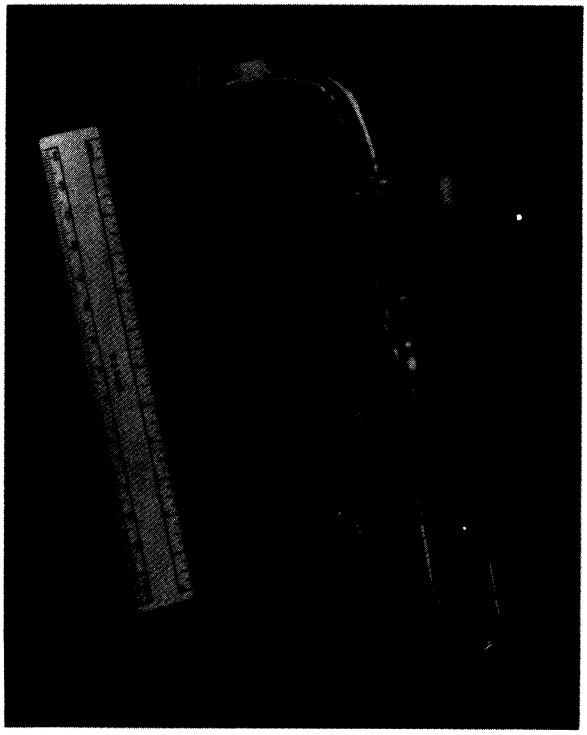

Fig. 16. Photograph Showing Detail of the Jet Injector for Gases used on the Apparatus for Liquid-Gas Injections in the 10 kc-Source.

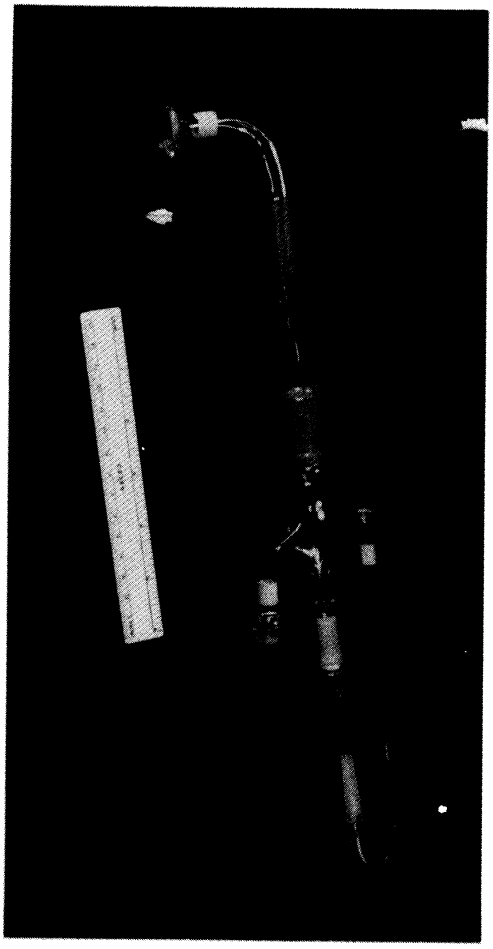

Fig. 17. Component Parts of the Glass Jet Injector, Disassembled.

reacting mixture, since the apparatus was initially filled to the level of the upper connecting tube (4). It also served to separate the exit gases from spray and foam at the surface of the liquid.

Vent lines were included at the inlet (9), and exit (10) of the apparatus. These lines were connected to dry bottles and thence to mercury-sealed traps so that safety was assured in case of rapid reaction or buildup of gas pressure due to plugged lines.

For the recording and controlling of temperature, thermocouples were fastened to the outside of the glass at points (11) and (12). The temperatures of the thermocouples were recorded on chart recorders located in the chemical laboratory. The thermocouple on the upper connecting tube (4) also activated a temperature controller, so that the temperature of the coolant circulating through the inner jacket of the reactor section (3) was controlled by the temperature of the liquid as it left the reacting section.

The coolant was recirculated through a modification of the cooling system described in Progress Report 4 (1, pages 28 and 30). Figure 18 is a schematic drawing of this apparatus. By means of a pump (1) the coolant was recirculated through the cooling jacket of the reaction apparatus, and returned to the mixing can (3). A knife heater (6) in this mixing can was activated by the recorder-controller to which the thermocouple was attached and was wired to a recirculating pump (2) through a double-throw switch. When this pump was activated by the controller, water was pumped into an upper cooling can (4), from which it overflowed into the mixing can (3) again. In the cooling can (4) was a smaller can (5) containing a mixture of dry ice, carbon tetrachloride, and chloroform which cooled the recirculation liquid as it was pumped around it. When only moderate cooling was required, water was used as the recirculating coolant, in which case a layer of ice was kept frozen around the inner part of the cooling can arrangement. For low-temperature runs, methanol can be used as the recirculating coolant.

When ready for use, the glass reactor equipment was assembled on a portable support frame, and placed in a portable exhaust hood adjacent to the 10-kc gamma source (Figs. 19 and 20). In use, the reaction section could be centered as close as 2 inches from the gamma source. A plastic shield was placed over the open side of the hood to prevent the corrosive liquids from splashing out into the source room in case the glass reaction equipment should break.

After the apparatus had been set in place in the source room, it was connected to the gas control rack in the chemical laboratory by means of glass lines connected by ball-and-socket ground joints. The installation of these feed lines required some special consideration. As shown in Progress Report 5

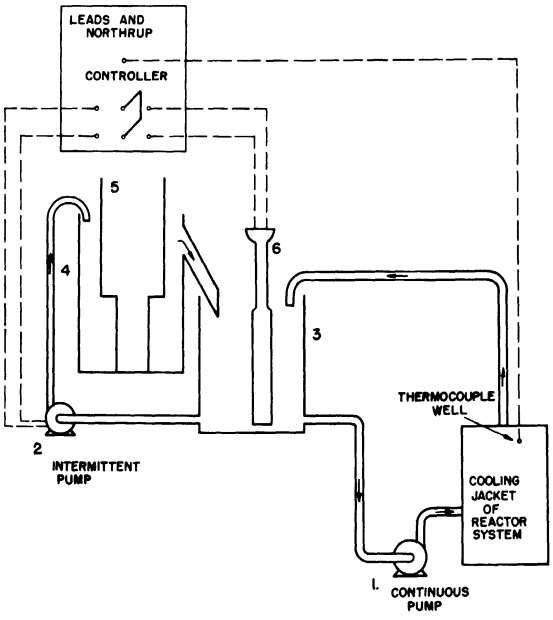

Fig. 18. Schematic Drawing of Apparatus for Temperature Control of Reactions in the 1 and 10 kc Cobalt-60 Gamma Source.

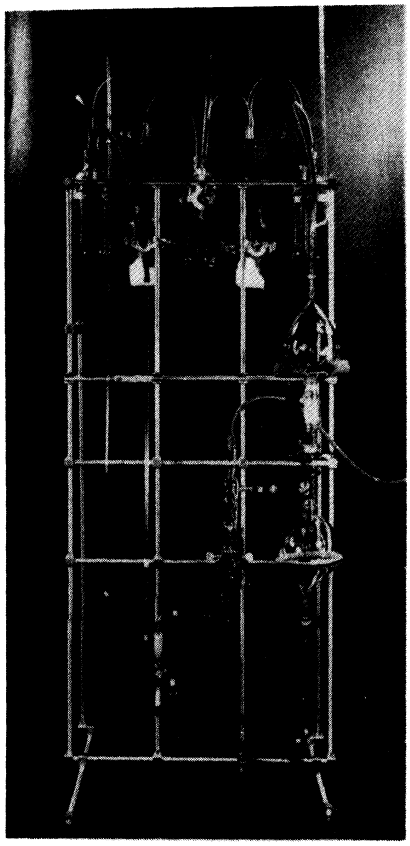

Fig. 19. Apparatus for Reaction of Liquids with Chlorine Gas, Assembled to go into the Portable Hood next to the 10 kc Gamma Source.

Fig. 20. Portable Hood and Glass Apparatus in use for Reactions in the 10 kc Source.

(page 66)[2] access to the source room from the chemical laboratory was gained through a small chute with a right angle through the shielding. In this chute were the leads for thermocouples and electrical cable. The problem of making a gas-tight connection of glass tubing at the right angle of the chute was solved in the following manner. A wooden support board was constructed which was of the same width and somewhat longer than the horizontal portion of the access chute (see Fig. 21). The glass tubes, which were fitted with ball-and-socket joints, were affixed to this board by means of small spring clamps made from spring clothespin clips. One end of each tube had a short right-angle bend in it. These were rotated to lie flat against the supporting board so that the entire assembly could be pushed into the access tube. Wooden strips along the sides and one one end of the board acted as runners and allowed the glass tubing to hang from the bottom of the board, while cables and aluminum tubing lay on top of the support board in the chute. Once in place, the glass tubing was rotated so that the right-angle bends lead downward placing the ground glass ball in position to receive the next (vertical) section of tubing with its ground-glass socket. Since clips are necessary to assure a gas-tight seal at the ball-and-socket ground joint, a special tool was devised to affix a clip to the joint located at the right angle of the chute. This tool (Fig. 22) was inserted through the vertical section of the chute from below in the source room, and could be manipulated to attach or remove the ball-joint clips. A diagram of the entire setup is shown in Fig. 23.

2. Experimental Procedure and Results. A number of runs using toluene and chlorine were made in the apparatus described above. For these runs, about 1.5 liters of toluene were placed in the reactor. Nitrogen was run through the system for 10 to 20 minutes before each run to displace all air and the solutions were saturated with chlorine before the source was raised at the start of the reaction. During the entire period of chlorination to minimize photochlorination, only red light was used whenever it was necessary to observe the equipment. It should be noted that when the source is raised in the dark, a faint blue glow can be seen in the toluene solution, probably caused by Cerenkov radiation. At periodic intervals, the source was lowered to allow personnel to add more dry ice to the temperature control system. When desirable, however, it is possible to place part of the cooling system at a remote position so that uninterrupted runs can be carried out.

In most runs, the storage-bulb section of the glass apparatus (7, Fig. 15) was shielded by lead bricks from the direct radiation of the source. In one run, however, no shielding was employed and the reaction became so violent after about 15 seconds of irradiation that the chlorine and hydrogen chloride escaping from the hot toluene solution blew open a glass joint. In another run, the shielding bricks were removed after the run had continued for some time, in order to attempt to compensate for an apparently decreasing reaction rate as the reaction proceeded; the rate of reaction increased, but the temperature could still be controlled.

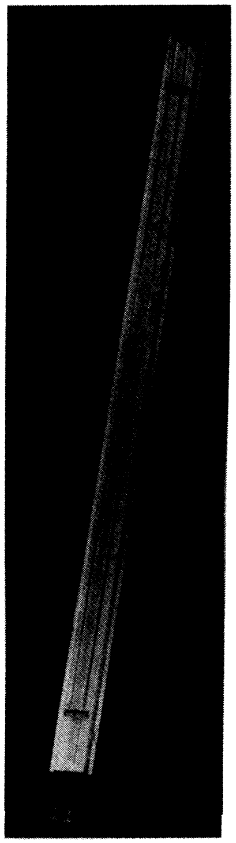

Fig. 21. Wooden Support Board for Glass Tubing to be put through the Chute Providing Access to the 10 kc Source Room. In Use, the Side Shown is Placed Down, when the Assembly is Put into the Chute.

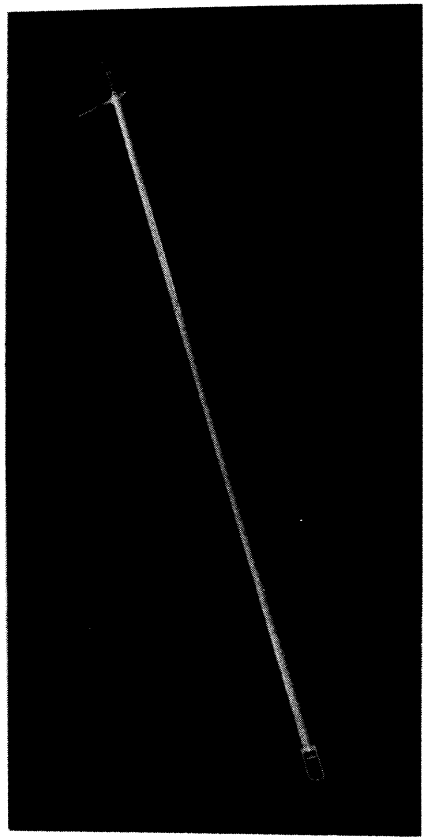

Fig. 22. Special Tool for Attaching and Removing Clips from Joints Located at Right Angle of the Access Chute to 10 kc Gamma Source.

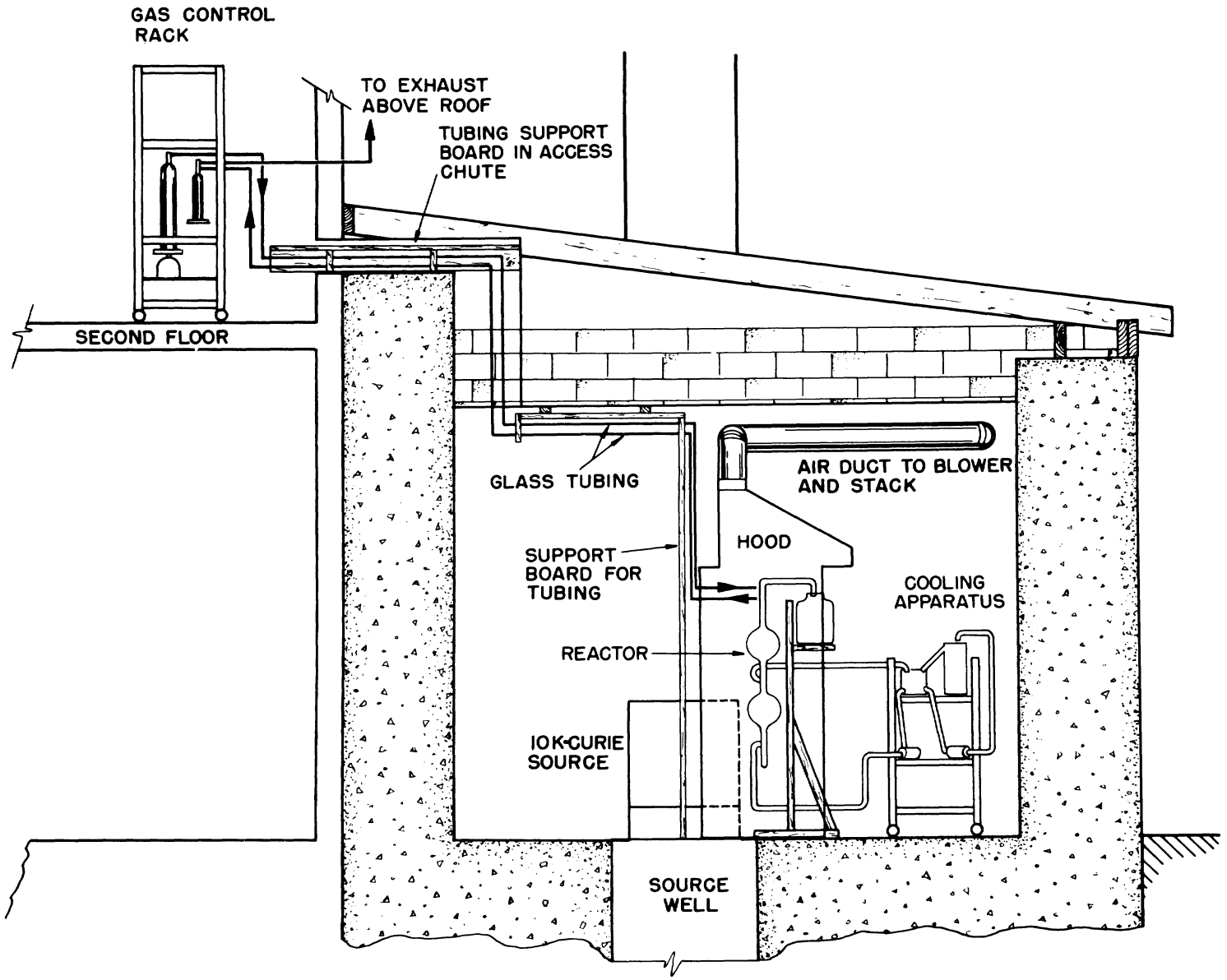

Fig. 23. Diagram of Complete Set-up for Reactions of Liquids with Chlorine Gas in the 10 kc Source.

The reaction mixture from one of these runs was distilled under vacuum, and high-boiling fractions were vacuum-distilled again. Table 8 summarizes the information on these fractions. Overlapping of temperature ranges was probably due to variations in pressure as receivers were changed. The theoretical chlorine content for a hexachloro addition product of toluene is 69.78% Cl.

TABLE 8

HIGH BOILING FRACTIONS OBTAINED FROM REDISTILLATION OF PRODUCTS OF REACTION BETWEEN TOLUENE AND CHLORINE

Fraction Number	Volume, ml	Temperature Range, °C	Pressure, Microns Hg	% Cl
VII-A	6.6	85-102	100-200	
VII-B	29.4	98-111	100-200	68.6
VII-VIII	4.0	110-115	100-200	
VIII-A	18.4	111-115	100-200	69.6
VIII-B	18.0	112-120	100-200	70.5
VIII-IX	3.1	120-125	100-200	
IX-A	10.0	125-135	100-200	70.7

The individual fractions were syrupy, crystal-clear liquids. After several weeks of standing, part of which was under refrigeration, some of the liquids became cloudy and crystals appeared to be precipitating out slowly. This production of liquids is perhaps not unusual, for if the product is 1,2, 3,4,5,6-hexachloro-1-methylcyclohexane, a great number of stereoisomers may be expected from total addition of chlorine to toluene. It is possible to write twenty such different stereoisomers, and it seems probable that at least half of these should be important constituents of the mixture of reaction products. Thus it is not surprising that the combined effects of many mixed isomers and high viscosity prevented appreciable crystallization of the products.

In an effort to study the behavior of the reaction during its course, a run was made in which the chlorine-gas cylinder was weighed continually, and in which samples of the exit gas were taken for analysis. Figure 24 summarizes the resulting data. The analytical methods were not precise in this run, so data on the material balance of the chlorine can be regarded as only roughly indicative of the true reaction rates.

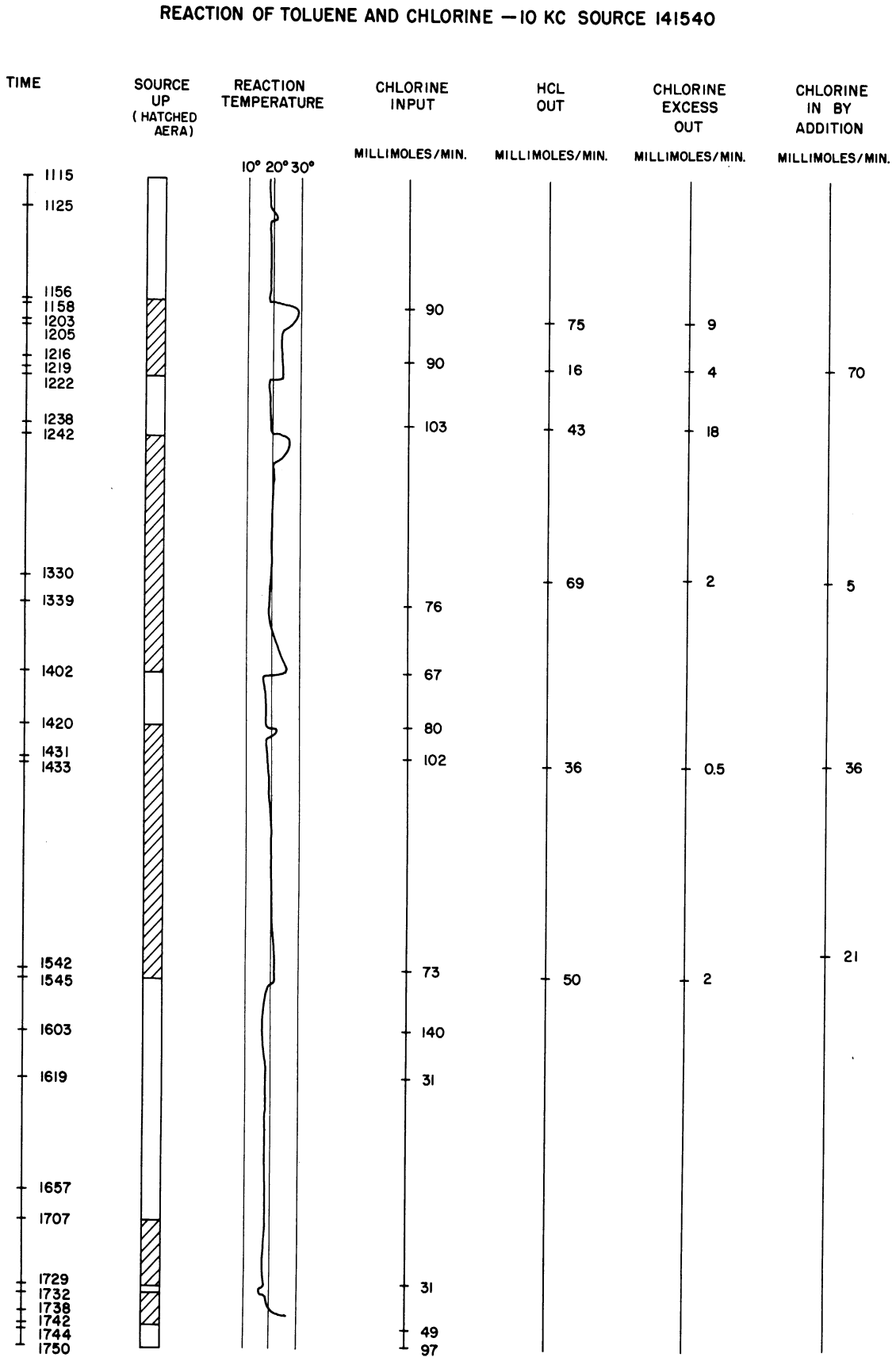

Fig. 24. Chart and Graph Showing Radiation, Temperature, and Material Balance at Various Times during a Reaction of Chlorine and Toluene in an Average Radiation Flux of 22 k Rep/Hour.

The behavior of the temperature curve is a striking example of the dependence of the reaction on the radiation field. Because of the nature of the temperature control system, a short time for readjustment was always required after a sudden change in heat production of the system. Thus, it can be seen that each time the source was raised into position for irradiation the temperature displayed a sharp increase, while each time the source was lowered the temperature dropped off. Furthermore, this effect is more pronounced at the start of the reaction than at a later time, when the rate had apparently decreased.

A summary of information on the runs made with toluene in the 10-kc source is presented in Table 9. The estimated average gamma flux rate was based on flux measurements in air at the centers of each of the two components (reaction and storage) of the glass apparatus. The overall yield was based on moles of supposed hexachloro addition products (high-boiling fractions with chlorine content in the 69-71% range) obtained from a given number of moles of toluene originally placed in the apparatus. Calculation of radiation yields in moles per liter per kilorep and in terms of "G" (molecules reacted per 100 electron-volts of gamma energy) was made using the density which was determined from a reactor solution used in one of the runs saturated with chlorine gas.

Inspection of the G values (Table 9) for the addition reaction in these compounds reveals that they are all in the neighborhood of 17,000.

The high values for G found in all these cases are somewhat unusual in radiation chemistry at the present time, and indicate a long reaction chain. Assuming about three primary ionization events per 100 electron-volts,[11] the chain lengths must be of the order of 5×10^3 or greater. This figure also assumes that each primary ionization event produces a chain of molecules which react completely and solely to give the hexachloro addition product. The fact that appreciable substitution takes place indicates that the chain length for the addition process itself might be much larger. It is very interesting that the products obtained by gamma activation differ markedly from those usually obtained through actinic radiation activation (mostly sidechain substitution in this case). In view of the long reaction chain lengths the product isolated contained negligible amounts of those molecules originally activated in the primary process. Thus the reaction chains in the gamma-activated process would seem to be propagated by a different mechanism than those of the ultraviolet-activated process. This difference is being investigated further.

From one of the reaction runs of toluene and chlorine, 120 grams of those fractions which distilled over at 90-156°C (90-240 microns Hg pressure) were sent to be tested for entymological poison activity, since the supposed compound is the methyl-substituted derivative of commercial benzene hexachloride, which is currently used extensively as a pesticide.

TABLE 9

SUMMARY OF THREE REACTION RUNS OF TOLUENE AND CHLORINE IN THE 10 KC 60 GAMMA SOURCE

Run Number	Volume of Sample ml	Control Temp., °C	Total up Time, min	Estimated Average Gamma Flux Rate, kilorep/hr	Total Dose Received, kilorep in air	Weight of Hexachloro Addition Product Produced, grams	Moles of Product Produced	Overall Yield, %	Average Moles per (liter) (kilorep)	Average Value of G for Addition
141500	1440	20	71	24	28	185	0.61	4.5	0.014	14,000
141534	1450	20	121	22	44	371	1.2	8.8	0.017	18,000
141540	1500	20	226	22	82	778	2.5	18	0.018	19,000

After consideration of the rapid addition of chlorine to benzene and to toluene, and the nonreactivity of benyl, benzal, and benzo trichlorides under the same conditions[2] (page 29), a run was carried out in which chlorobenzene was treated with chlorine gas in the 1-kc cobalt-60 gamma source. In this case, the reaction proceeded at a more rapid rate than that for toluene. Table 10 summarizes the information obtained in this run.

The yield in Table 10 is based on the weight of crude product obtained by steam-distilling off the unreacted chlorobenzene and drying the solid residue; this procedure may yield values that are high. A sample of recrystallized material gave a chloride analysis of 76.0%, as compared to the calculated value of 76.3% for heptachlorocyclohexane, which would result from addition of six chlorine atoms to chlorobenzene. A run has been made in darkness with other conditions the same as those used during the gamma-induced reaction. Analysis of reaction products gave a value for chlorine content which was so close to the calculated value for unreacted chlorobenzene that virtually no reaction was indicated.

D. SUGGESTIONS FOR FUTURE WORK

It would be of interest to investigate further the polymerization of ethylene under gamma radiation with the objective of determining the cause for the induction period observed for the polymerization. It seems possible that small concentrations of impurities are responsible for the induction period. However, it is also possible that such an induction period might be characteristic of the gamma-induced polymerization and independent of chemical parameters.

If the ethylene could be removed shortly after polymerizing, then a continuous process for the polymerization might be developed. Such a procedure would permit closer control of those properties of the polymer which are dependent on total dose of radiation.

As mentioned earlier, under "Discussion of the Polymerization of Ethylene," elevated temperatures in conjunction with irradiation caused greater rates of polymerization of ethylene than did irradiation alone. Investigation of the influence of both elevated temperatures and irradiation of the polymerization of ethylene is certainly in order. Some suggestions for such a program were advanced in Progress Report 5,[2] based on some of the foregoing studies of radiation chemistry and on the work of Kennard.[6]

The electrical properties of the polyethylene made by gamma irradiation might well be studied.

The reaction between ethylene and sulfur dioxide proceeds rapidly enough under gamma radiation that it should be possible to secure much

TABLE 10

REACTION OF CHLOROBENZENE WITH CHLORINE UNDER GAMMA IRRADIATION

Sample Volume, ml	Control Temp., °C	Total Reaction Time, min	Gamma Flux Rate in air, kilorep/hr	Total dose, kilorep in air	Weight of Hexachloro Addition Product, Grams	Moles of Product Produced	Overall Yield, %	Moles per (liter) (kilorep)	G for Addition
100	20	30	52	26	38	0.12	12	0.042	43,000

information concerning the behavior of this reaction. The physical properties of this polymer molded into massive form should be investigated.

The work on chlorination of aromatic compounds should be pursued with the object of attempting to find and correlate those reactions which differ from reactions promoted by chemical catalysis or ultra-violet radiation. This work would involve further study of reaction rates for those reactions already partially investigated, and the study of the reaction of other aromatic compounds with chlorine. Such studies might provide insight into the manner in which gamma irradiation promotes reactions which have large G values, and establish unique uses for the gamma radiation of fission products.

E. SUMMARY

1. Polymerization of Ethylene. (a) Ethylene has been polymerized by exposure to gamma irradiation from cobalt-60. The rates of reaction were sufficiently large that further work on this reaction appears to be promising.

(b) Polyethylene formed by gamma irradiation has been subjected to a preliminary evaluation. The polymer was found to be denser, less ductile, and of a higher ultimate strength than Bakelite DYNH polyethylene. Molecular weights of the radiation-polymerized materials increased with radiation dose to a value of about 40,000 when estimated from melt viscosities. Most samples were insoluble in tetralin, but estimates of molecular weights from solution viscosities were not conclusive. Crystallinities estimated from densities varied from 71 to 77 percent.

2. Chlorination of Aromatic Compounds. (a) Toluene and chlorine were reacted in the presence of gamma irradiation using up to 1.5 l. of toluene in one run. Fractional vacuum distillation of the reaction products yielded high boiling materials whose chlorine content is close to that for a hexa-chloro addition product of toluene and chlorine in addition to products of substitution reactions. For some runs of varying total gamma dosage, the G value for addition was in the neighborhood of 17,000. Preliminary rate data for one run have been taken.

(b) Chlorobenzene and chlorine were reacted under gamma irradiation. It was found that the addition reaction proceeded with a G value of about 43,000.

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PART III. GAMMA IRRADIATION OF FOOD
SUBPROJECT M943-5

A. ORGANOLEPTIC STUDIES

Personnel:

Subproject Supervisor: L. E. Brownell, Associate Professor of Chemical Engineering and Director of Fission Products Laboratory.

Advisor: L. L. Kempe, Assistant Professor of Bacteriology and Assistant Professor of Chemical Engineering.

Research Associates: S. Pedersen, Biochemist, H. O. France, Biochemist; J. V. Nehemias, Health Physicist.

Research Assistants: Miss H. A. Harlin, Food Chemist; R. C. Dennis, Statistician.

Laboratory Assistant: E. Ambo.

1. INTRODUCTION

a. Review of Previous Progress Reports

The literature on the effects of radiation on microorganisms, enzymes, and food was reviewed in Progress Report 1. The initial experimentation on food preservation by means of irradiation was initiated at the University of Michigan and covered a general exploration of the effects of x-ray and gamma irradiation on a variety of foods. This work was continued and was reported in Progress Reports 2 and 3. It was found that systems of microorganisms such as molds, yeasts, bacteria, etc. could be destroyed by gamma radiation and that food could be sterilized by gamma radiation. Some exploratory work on enzyme systems was performed.

In Progress Report 2 some studies were reported on the effects of gamma radiation on foods in the frozen state, evacuated or packaged under nitrogen, carbon dioxide, or other gas atmospheres, using different pretreatments before irradiation such as blanching or soaking in dilute ascorbic acid solution. In

Progress Report 3, it was reported that green asparagus, fresh spinach, green peas, green beans, green broccoli, carrots, bacon and ham could be irradiated with only relatively slight flavor changes, and that some protection of the flavor of fresh meat could be obtained by using small amounts of protective chemicals such as thiourea and ascorbic acid.

Since cured meats such as bacon and ham contain sodium nitrate and sodium nitrite, it was thought that these compounds might be responsible for the protective effect on flavor in treated meat. In Progress Report 4 it was reported that sodium nitrite in a concentration of 100 ppm gave some protection to the flavor of irradiated meat; but variable results were obtained with different batches of meat. Some preliminary experiments on the peroxide values of irradiated animal fats were also reported in Progress Report 4. With pork and beef fats there was a definite increase in peroxide oxygen, but organoleptic tests did not prove that they were objectionable.

Little work was reported on the irradiation of foods in Progress Report 5, since research during that period was supported only by the Michigan Memorial-Phoenix project 41 and a major portion of the funds of that project were allocated to an extensive animal-feeding experiment. During this period the 10-kilocurie cobalt-60 source was received and put into operation and some preliminary tests were made on the irradiation of intact foods in larger volumes than could be irradiated in the small cobalt-60 source.

After the completion of the work reported in Progress Report 5, a statistician and a food chemist have been added to the laboratory personnel and taste-panel experiments have been initiated.

b. Literature Review of Biochemical Changes in Irradiated Foods.

Biochemical systems in general are more complex than inorganic systems and chemical changes in living and/or dead biochemical systems are consequently much more difficult to detect, follow, and identify despite the use of all available equipment and procedures.

Changes resulting from gamma-ray irradiation are not well understood, but an attempt will be made to review what is known and what has been postulated. Gamma rays are electromagnetic in nature and more penetrating than either alpha or beta rays; consequently, the absorption is correspondingly less. Small-scale studies are possible using cobalt-60 gamma sources in lieu of actual fission products which are not yet generally available and adaptable for experimental procedures. In the first preliminary report from this laboratory some applications of gamma-ray irradiation in the food industry were outlined.[1]

Changes induced by gamma rays may be grouped into those involving the solvent and those involving the solute.

(1) Changes in Solvent. Considering for brevity only the universal solvent water, gamma rays at a high energy level may give up some of their energy with a resulting ionization of water and the formation of highly active free radicals as follows:

H_2O	$H_2O^+ + e^-$	(1)
H_2O^+	$H^+ + OH$	(2)
$e^- + H_2O$	H_2O^-	(3)
H_2O^-	$OH^- + H$	(4)
$e + H^+$	H free atom	(5)
OH^-	$OH^- + e^-$	(6)

Free hydrogen and hydroxyl radicals formed from the solvent water react freely with other oxidizing and reducing substances dissolved in the water, especially when present in dilute solutions.[2] Further, reactive substances may be added to water, thereby exerting a protective action on the solute by competing for and reacting with the free radicals formed from the ionization of water.

Foods contain varying amounts of water, ranging from 55 to 75 percent in meats, except pork products, up to more than 90 percent in certain fresh vegetables. The very penetrating nature of the gamma rays permits easy piercing of the cell walls, both animal and vegetable, and promotes action within the cells. As protoplasm may be considered to be in part an aqueous solution, it can be perceived that numerous reactions may take place within the cells. In view of the complexity of the protoplasma with its content of proteins, fats, carbohydrates, and inorganic salts, the possibilities of reactions are very numerous. Many of the reactions known to take place in vitro, especially those of reduction and oxidation, may be assumed to proceed in the protoplasm, as some ionization of the intracellular water undoubtedly takes place. That flavor and odor changes do take place in foods during gamma irradiation is an established and proven fact.

(2) Changes in Solute. Proctor et al.[2] have demonstrated that the enzymatic activity of dilute aqueous solutions of pepsin decreased when exposed to cathode radiation and that this decrease in enzymatic activity could be prevented by the addition of sodium D-isoascorbate to the system. This protective action was considered to be due to the competition for free radicals from the ionized water which otherwise would have reacted with definite groupings in the pepsin molecule.

Dainton,[3] after a review of the literature, formed the opinion that the free radicals may be assumed to account for the action with appropriate grouping

in the solute molecules. Alden and Eyring[4] have formulated a mechanism for the decomposition of solute in dilute aqueous solution on the basis of the relationship between ionic yield and solute concentrations.

(a) Carbohydrates: Irradiation of dilute aqueous solutions of glucose in preliminary exploratory experiments has resulted in an increase in acidity beyond that observed when water itself is irradiated.[5] While no oxidation products have been identified so far, oxidation of either one or both the terminal carbons could yield carboxylic acids. A breakdown into two trioses and subsequent oxidation is also possible.

Irradiation of "nonreducing" sucrose and subsequent treatment with Fehling's solution indicates the formation of reducing sugars, possibly hexoss.[4] Proctor[2] has shown that irradiation by cathode rays of an aqueous solution of crystalline pepsin results in a decrease in the activity of the enzyme and that this effect can be counteracted by sodium D-isoascorbate. However, it is pointed out that it is not simply a case of the additive protecting the enzyme by preventing its oxidation, since niacin, which is relatively stable toward oxidation, also exerts a protective action.

(b) Fats: Fats are not stable in the presence of oxygen and by autoxidation may break down, yielding aldehydes, ketones, peroxides, etc. Investigations of this breakdown in the past have been based on the estimation of the amount of peroxides formed. Unfortunately, results obtained by chemical methods for the determination of peroxides, considered to be a measure of fat deterioration, do not always correspond with results obtained by an experienced taste panel on products with high rancidity. In the case of bacon, for example, chemical methods might show low peroxide values, and members of the taste panel still consider the product inedible.[6] In gamma irradiation of fats and fatty materials it is possible that an oxidation in the presence of air also takes place. In this case there might be little or no ionization of water into free radicals. However, the degree of deterioration in fats and fatty products resulting from gamma radiation would be expected to be greatly influenced by the amount of naturally occurring antioxidants in the processed products.

Autoxidation of foods has long been known, especially in those foods rich in fats where the chief manifestation of rancidity could easily be detected. The extent of autoxidation taking place depends not only on the ratio of so called prooxygenic and antioxygenic compounds found in the food, but also on temperature, light, and the presence of certain metals in trace quantities. It is generally assumed that autoxidation begins at the double-bond carbon-carbon linkage with the formation of peroxides or perhaps free radicals. Antioxidants, approved by the Food and Drug Administration, are available for use in the inhibition of food losses through spoilage by autoxidation.

In fresh vegetables the liquids are enclosed by the cell walls and in intact citrus fruits the normal high acidity and antioxidants inhibit oxidation. However, in the presence of air, fruit juices and peeled fruits are subject to oxidation and loss of vitamin C.

Rancidity in fats may be of several types. The most common type is the oxidative, which occurs at the double bonds in the presence of oxygen. Hydrolytic rancidity is due to the liberation of free fatty acids. Such rancidity may easily be detected by odor alone if low molecular-weight fatty acids are liberated, such as butyric acid in rancid butter; but it may not be so detected if only high-molecular-weight nonvolatile fatty acids are liberated. Both, however, can be detected by titration. Fungi such as Aspergillus niger and others may act on vegetable oils to liberate aldehydes and/or ketones; this is called ketonic rancidity.

Experiments conducted by this laboratory have clearly indicated that gamma radiation of animal fats results in definite structural changes of the fat molecule, as shown by peroxide values before and after radiation.[7]

(c) Proteins: Proteins, in general, may be described as being high-molecular-weight compounds with a very complex composition. Simple proteins are made up from amino acids, whereas conjugated proteins contain other compounds in addition, as indicated by names such as glucoproteins, lipoproteins, and flavoproteins.

The chemistry of simple proteins is essentially that of the constituent amino acids, while for conjugated proteins it is more complex. In aqueous solution amino acids yield hydroxy or keto acids by oxidation of the amino groups. Proctor[2] has shown experimentally that dilute aqueous solutions of histidine hydrochloride are protected against the actions of cathode rays by the addition of D-isoascorbic acid. A reaction with the thiol group (SH) should also be possible.[8]

In the complex protein molecule, similar action can be expected with free amino, thiol, and carboxyl groups in the molecule. In food products such as meats, especially fat meats, the reactions undoubtedly proceed at different rates. Nickerson[9] treated mackerel tissues with ionizing radiation and concluded that the proteolytic enzyme action remained essentially the same after 1,500,000-rep irradiation.

Proctor and Bhatia[10] have studied the effect of high-voltage cathode rays on amino acids in haddock muscle at 900,000, 2,700,000, and 5,700,000 rep. Ten amino acids were determined by a microbiological method from which it was concluded that there was no significant destruction of the amino acids. From

organoleptic tests it was deduced that there may be some persons who could not detect any unfavorable flavor changes in haddock fillets broiled subsequent to irradiation.

Gamma irradiation of meat in doses up to 2,400,000 rep was found to cause color changes on the meat surface; but the mechanism of these changes were not identified, although indications pointed toward oxidation.[11] Definite changes in the more common proteolytic enzymes, themselves protein in nature, also took place when they were irradiated in acid solution, as indicated by a decrease of enzymatic activity on a standard medium.[12] Elliott and Gross[13] outlined definite studies involving the protozoa Tetrahymena pyriformis, including growth responses on an irradiated complete diet and on a nonirradiated control diet. In another section of this report[14] Elliott and Gross have reported their results in detail; but a few of their findings will be mentioned here. Dilute aqueous solutions of individual amino acids underwent definite changes when exposed to massive dosages of gamma rays. While their decomposition products were not isolated, certain organoleptic changes were clearly discernible; this was especially true for tryptophan and methionine in which detectable odors became evident. It was shown that amino acids irradiated with high dosages in dilute aqueous solutions and later incorporated into the basal medium did not promote normal growth of the Tetrahymena, whereas the growth of this protozoa was normal when these amino acids were irradiated in a dry, solid state and incorporated into the medium afterwards.

(d) Enzymes: Oxidation and reduction reactions have long been associated with activation and inactivation of certain enzymes. Such systems would involve those in which the sulfhydryl group, either reduced or oxidized, would influence or govern the state of activity. Gamma radiation could certainly affect such systems, as cathode rays have been shown to inactivate pepsin in aqueous solutions with or without acetate buffers,[2] but in foods the action would be greatly minimized. Enzyme studies of this laboratory have been reported previously in Progress Reports.[12,15]

(e) Vitamins: Any vitamins which are easily destroyed by oxidation are also subject to deterioration and destruction by gamma rays. Actual destruction has been observed in the case of aqueous solutions of vitamins; but the destruction in food products would be conditioned by the nature of the product and except in fruit juices might be assumed to be of relatively low magnitude, especially in view of the fact that gamma-ray sterilization would be "cold" sterilization. The effects of gamma irradiation of vitamins will be reported elsewhere in this report in the discussion of animal-feeding experiments.[16]

(f) Pasteurization and Sterilization: Gamma radiation has been used for these purposes in fruits as well as in meats on a small scale.[17] The

industrial importance of using this new tool for pasteurization and sterilization of food products has not yet been fully realized, and the practical application in industry has therefore not yet been explored. As a public health measure, gamma radiation of pork is a suitable new approach to the control of trichinosis.[18]

Gamma-radiation treatment of operational rations for the Armed Forces is a promising method for lengthening the shelf-life of these rations, insuring a greater palatability and better utilization of these rations as well as financial savings.

Trump and Van de Graff[19] have shown that there is a 2°C rise in temperature for each 1,000,000 rep, which leads to calling sterilization by ionizing radiation "cold" sterilization.

(3) Formation of Toxic Products. Before gamma-ray radiation can be accepted as a means of pasteurization and sterilization of food products at ordinary room temperatures, the process must be approved by the Food and Drug Administration.

Animal experiments are now in progress in this laboratory in an attempt to ascertain if gamma-irradiated food has any deleterious effects on rats, resulting in delayed body growth and/or pathological changes in the body organs at the time of autopsy. Results to date are not far enough advanced to warrant any definite conclusion but they appear very promising in view of present knowledge. Detailed information will be found elsewhere in this report.[16]

Summarizing this section, it may be stated that although gamma radiation may and does cause definite chemical changes in simple inorganic and organic systems, the biochemical changes in complex systems, exemplified by food products, may be expected to occur on a much smaller scale for the same dosage because of the mutually protective action of the naturally occurring and widely different compounds present within the food itself as well as the compounds which are added to the food during industrial processing.

In extended storage experiments, it should be kept in mind that if nonsterilizing dosages are used, changes may occur in foods due to bacterial and/or enzymatic action which, though not toxic in themselves, may influence the food intake and, consequently, the growth curve of laboratory animals. It is also possible that such changes may seriously affect the findings of the taste panel since its members could confuse flavor changes due to irradiation itself with flavor changes ascribable to products formed as a result of bacterial and enzymatic activity.

c. Importance of Food Acceptance by the General Consumer.

Acceptance or nonacceptance of a food is affected by the food habits of the individual consumer. These food habits are determined by such factors

as age, sex, national-social grouping, economic status, geographical location, and availability of the food as influenced by processing and transportation. Sometimes foods are rated low in acceptability because of emotion prejudices, physiological reactions to the food, poor cooking methods, flavor dislikes, unfamiliarity, or other factors.

Consumers have accepted over a period of time the flavors of food processed for preservation by means of canning, freezing and refrigeration, drying and dehydration, smoking, salting, etc. Gamma irradiation may be another means of sterilizing and thus preserving food, but irradiated food may have a slightly different flavor from that to which the consumer is accustomed. Through research, undesirable flavor changes in some foods, particularly foods high in protein and fat, may be overcome by the addition of various chemicals and antioxidants, but it still may be necessary to educate the consumer to the flavor of food treated with gamma radiation.

Any new food product must meet the requirements of the Food and Drug Administration. It must be clean and free from contamination with extraneous materials, and contain no harmful or deleterious ingredients. It must be wholesome, free from spoilage or harmful bacterial organisms, and safe to eat. In addition, new foods must contain no inadequately tested ingredients that might conceivably be harmful, or the use of which might result in the lowering of the nutritional value of the food. Also, the food must be processed in such a manner as to assure the retention of the expected nutritional value of the food. Last, but not least, the food must be attractive in appearance and sufficiently economical that it can be sold at a reasonable price.

In line with the Food and Drug Administration requirements for foods, it is known that microorganisms can be destroyed by irradiation, In addition to sterilization and preservation of irradiated food, other factors are being considered, such as food value, loss in vitamins, and the possibility of producing toxic compounds. Animal-feeding experiments are being conducted to establish food values and to determine whether or not toxic compounds are formed in irradiated food.

Changes in odor, consistency or texture, and color have also been encountered in the irradiation of certain foods. For example, dark sweet cherries became much softer in texture at the higher levels of treatment (1 megarep or greater) and the natural red color is bleached from irradiated cherries in proportion to the dosage level. Changes in odor are particularly noticeable in protein foods such as meat. Usually these undesirable odor molecules are partially driven off when the meat is cooked.

d. The Importance of Food Acceptance to the Armed Forces.

The enormous increase in the global military commitments of the United States has resulted in a concomitant increase in the number of widely scattered personnel on active duty in peace time. Although transportation methods have been tremendously improved and now include shipment of food supplies by air (in some particular locations that is the only method which can be used) the shipping lanes and the time in transit of food supplies have been greatly lengthened. This is not so important for the more stable components in the present B ration and in the operational rations available; but it is very important if the more desirable fresh items in the A ration are to be made available to the individual Army mess hall or Navy ship on the high seas.

Even with the modern industrial methods and refrigeration equipment, certain types of foods occasionally reach the end of the line in a state which cannot be considered palatable. The adequacy of a serviceman's food intake is determined by what he actually consumes, not by what is placed before him, and the amount he consumes is greatly influenced by the palatability of the food served to him.

Maximum efficiency of the serviceman presupposes a continuous supply of adequate amounts of food received in good condition. The studies in gamma-ray irradiation of foods so far completed and those now in progress, may thus provide a valuable supplementary method of food preservation for the military services.

2. THE PROBLEM OF FLAVOR IN IRRADIATED FOODS

a. Flavor.

Kirchner[20] has commented that "the popular conception of flavor is synonymous with taste, perhaps because there is no separate verb to describe the act of determining the flavor of a substance. When we taste we are actually judging the flavor, for flavor is an elusive blend of sensations. It includes not only taste, but also the sense of smell and touch." According to E. C. Crocker[21] "flavor is that quality of food which makes it register favorably or otherwise on the senses." Also Crocker[21] has stated that "no less than three distinct senses may be engaged in the perception of any particular flavor, the sense of tongue taste, the sense of smell, and the sense of feeling." Examples of tongue taste are the flavors of salt, citric acid, sugar, and quinine. Some flavors which depend more on odor than on tongue taste are the flavors of fruits, coffee, and butter. The sense of feeling in flavor perception must be considered in order to account for the burning sensation caused by mustard, cloves, and pepper and for the coolness of peppermint.

Biologically, the flavor senses constitute a complex situated at the beginning of the gastrointestinal tract and are, of course, intimately related

to nutrition. Considering the flavor senses as a unit is more than a matter of convenience, for confusion among these senses has introduced great difficulties in scientific analysis. These difficulties are not confined to the failure to distinguish one sense from another. For example, one difficulty shared with the other senses is known to introspective psychology as the "stimulus error." This "error" consists in reacting not only to the sensory impressions received but also to the object and its associations. In technical terms, therefore, we react on the basis of our perceptions rather than our sensations, which is to say that our behavior is influenced by much that is not immediately present. When we look at a sizzling, juicy T-bone steak, we are stimulated by a certain pattern of light rays. We cannot see all that we associate with this food; but what we see has associated reactions and sensations that are all tied together in one perceptual process. When one of its constituents is sensed, we behave as though all were sensed and attribute the immediate source of the sensation to some thing apart from ourselves, as when we say that we smell hot bread, rather than a certain odor. In this case, we have no term for the odor itself.

Such perceptual processes are learned through experience and, although we can learn again to react independently to the single sensory quality, training is required.

From the scientific point of view, it may be important to distinguish objectively individual sensory impressions. For practical purposes, however, we can, and do, take advantage of the perceptual processes learned through long experience. This is the basis for the connoisseur's sensitivity to differences in flavor too minute to be noticed by the average untrained (in this sense) individual. The tobacco connoisseur, tea and coffee tasters, and the wine connoisseur all have this quality in common--the knowledge, based on experience, of what to look for and how to interpret what their senses convey to them.

Because an individual may not be generally aware of his past learning, and generally does not analyze his reactions, his statements about the basis of such reactions are untrustworthy, even in regard to whether a particular taste or smell is perceived. They are even more unreliable when the question is directed toward his reasons for any preference. Roses may be disliked because they were the favorite flower of someone who is disliked, or because a bee stung the person when he picked them as a child.

Whatever the motivation may be, it is often not so important to know how an individual may have acquired a preference or what sensory process is involved as it is to know how large a proportion of the population has this preference or how many in a given group can distinguish between two flavors. Scientific procedures should be designed to guard against uncritical acceptance of assertions which, even if true, could be complicated beyond usefulness in a practical situation. For instance, it is quite possible that the use of "pure"

stimuli, in choosing food testers, as has been done by a number of investigators (for example, in the use of quinine sulphate to represent bitterness, cane sugar to represent sweetness, sodium chloride solutions for saltiness, etc.), is a refinement that has neither scientific validity nor practical value.

b. The Receptors Participating in Flavor.

Four groups of receptors are recognized as participating in the sensation of flavor. These are the kinesthetic, cutaneous, gustatory and olfactory receptors.

(1) Kinesthetic sensations are the foundation of our knowledge of the positions and movements of parts of the body. Distinctions between kinesthetic sensations are made on the basis of location rather than quality. Muscles, tendons, and joints have receptors from which these sensations arise, those important for flavor coming largely from muscles of the tongue and muscles and joints of the jaw. Toughness of meats, softness of candy creams, crispness of lettuce, and chewiness of caramels are examples of judgments based on kinesthetic sensations.

(2) Cutaneous receptors are the same as those found generally in the skin. While the receptor organs are not known definitely, the sensory qualities themselves are touch, cold, warmth, and pain. Cutaneous receptors are located in the mucous membranes lining the mouth cavity, nasal passages, and digestive tract; little is known about their occurrence in the stomach and intestines. Textures of foods, such as smoothness, creaminess, or oiliness, probably involve touch receptors; the tang of carbonated beverages is a "pain" sensation; and many "warm" and "cool" factors enter into flavor, as well as modify other sensations.

(3) Gustatory receptors ("taste buds") mediating our tastes are largely on the tongue, grouped with other kinds of cells into structural units called papillae. Some papillae are also located in the pharynx and larynx.

(4) Olfactory (smell) receptor cells are located high in the nasal passages, out of the most direct path of inhaled air. Sniffing is of importance because it creates air currents directly over the receptors, although strong odors will diffuse to them in perceptible amounts without strong inhalation.

Since there is an anatomical separation of the receptors for taste and smell, attempts have been made in the course of investigation to stimulate each type of receptor separately, although it is difficult to prevent diffusion or jaw movements which waft odors from substances on the tongue into the olfactory passages or to avoid the penetration of olfactory stimuli into the pharyngeal and laryngeal regions containing taste buds. The sweet "smell" of chloroform and the sour "smell" of vinegar probably represent taste sensations. Pieces of onion, apple, and potato are confused by most individuals when these stimuli are simply

laid on the tongue while the nose is held. The cutaneous receptors are the most difficult to avoid in these investigations, giving us "warm" odors (such as alcohol and heliotrope) and "cool" odors (such as ammonia and mustard) whose sharpness is probably a function of pain receptors.

c. Types of Flavor.

Four components of taste are recognized: sweet, sour, salty, and bitter. The bitter and sweet tastes are usually nonionic, whereas the sour and salty tastes are ionic.

(1) Sour. The sour taste has been known since antiquity and was included as one of the nine (sweet, salty, sour, bitter, astringent, dry, pungent, vinous, and oily) listed by the Greeks. One good example of the sour taste is vinegar. In the early days of chemistry, acids, in general, were observed to be sour; but the dissociation theory was required to explain the nature of sour taste on the basis of the presence of hydrogen ion and to establish the relation between the concentration of hydrogen ions and the degree of sourness. Certain acids may taste bitter as well as sour, as for an example picric acid, or there may be a sweet taste as in citric acid. Kirchner[20] expressed the opinion that the anion must also play a part, since very dilute solutions of acetic acid are more sour than would be expected on the basis of hydrogen ion and total acid concentration. Most citrus fruits are sour because of the presence of malic, citric, or tartaric acids. Some sour and astringent herbs such as rhubarb, Swiss chard, and spinach contain oxalic and other acids together with some calcium oxalate.

(2) Sweet. Sweet-tasting foods were in great demand in order to provide variety to the rather unpalatable fish and meat diet of the ancients. A good example of a sweet-tasting food would be honey, which was also used for the preparation of the alcoholic beverage mead. Generally, foods having a sweet taste can be classified as carbohydrates, although certain salts such as some of those of lead and beryllium also possess a slightly sweet taste. While the sour taste has been associated definitely with the hydrogen ion, the sweet taste in carbohydrates has not been established to depend on ionization or any specific ion.

In general, the compounds which impart a sweet taste have little in common. Examples given by Kirchner[20] include the polyhydroxy sugars, saccharin, and amino acids. Compounds of the same type show variations in sweetness; for example, when maltose is formed from two molecules of glucose there is a decrease in sweetness, but when fructose and glucose are joined to form sucrose, sweetness is increased. Nearly all fruits are sweet, and sweetness due to sugars is conspicuous in sweet potatoes, yams, Jerusalem artichokes, parsnips, carrots and beets. Onions and garlic are also very sweet; but their sweetness is hidden by their strong aromatic content.

(3) Salty. The typical salty taste is exemplified by common table salt, sodium chloride. Centuries ago when food habits were simpler than today, salt was at a premium because of its seasoning and flavoring effects on meats and was used for barter and even in lieu of currency. Many other salts were found to have a salty taste, especially the chlorides of magnesium, potassium, lithium, rubidium, and ammonium; but some of the other halides have in addition a slightly bitter taste such as that found in potassium bromide. Potassium iodide has a very pronounced bitter taste. The salty anions include all the halides, the lower members of the acetic acid series, the carbonates, nitrates, and sulfates. All starchy and protein foods would be uninteresting without the presence of salt. Salt is just noticeable when it constitutes between 1/2 and 1 per cent of the food, and it is not desirable to exceed this amount in most foods.

(4) Bitter. A bitter taste may be found in a variety of compounds, especially in the alkaloids; but it also is found in the glycosides, the tannins, and in certain inorganic salts such as magnesium sulfate (Epsom salt). The bitter taste may also be noticeable in lower-grade table salt (sodium chloride) containing impurities such as magnesium salts. Organic acids such as picric acid also have a bitter taste, presumably due to the presence of the three nitro groups in the molecule. Examples given by Kirchner[20] were for the alkaloids, quinine and brucine; for the glycosides, hespiridin in oranges and naringin in grapefruit; for the tannins, the astringent taste of unripe apples and persimmons, and the bitterness of tea that has been steeped too long. The flavor of meat is mild; but among animal products the gall has a strong bitter flavor and liver frequently has some bitterness. Oysters are more or less bitter and astringent in addition to sweet, but this is due to an appreciable copper content.

d. Odors.

Distinctive taste and smell are frequently found in foods, a fact which undoubtedly was of great value prior to the present-day protective measures through the enforcement of regulations instituted by the various federal agencies. Associated with deterioration and decomposition of certain foods are definite changes in odor which warn of possible danger to health if that particular food is eaten. In the animal world the sense of smell is undoubtedly the factor which causes certain animals to avoid eating poisonous berries and fruits. Only a relatively few persons, on the other hand, can distinguish between edible and inedible mushrooms, although complete classification and description are available. With the changes in the habits of man from a nomadic existence to present-day life, the sense of smell may be assumed to have decreased, perhaps because of less usage.

The relation between taste and smell can be further demonstrated in man by the temporary lack of taste sensations in persons suffering from a respiratory infection such as the common cold. A more or less similar manifestation can be observed when the nostrils are clamped close together. When there is an

absolute lack of smell, anosmia exists. If the sensation of taste is greatly impaired in man, a former voracious appetite for food may change into a very sluggish appetite, resulting in a marked loss in body weight. It can be seen, therefore, that smell as well as taste affect the food intake.

In flavor perception the olfactory apparatus plays a very important part. Coffee without aroma would be repulsive and kola drinks without aromatics would not be popular. It is possible to detect odor not only by sniffing through the nose but also from within the mouth to the smelling area in the head by way of the throat. According to Crocker[21] the odor detection area is located high in the nasal cavity immediately below the eyes. It is not known how certain chemicals are able to stimulate the tiny olfactory hairs in this region so that their presence is accurately recognized.

Many attempts have been made to classify and identify odors; the A. D. Little System[21] is the most common one. Using the four fundamental odor sensations, fragrant, acid, burnt, and caprylic, it is possible to express the individuality of an odor with quantitative values for each of the four sensation elements by assigning to each component intensity digits of 0 to 8 for the nine recognizable degrees of sensation intensity. Each of the four sensation elements in most odors are evaluated in intensity by comparison with graduated values in an external set of standard chemicals.

It is important to mention that most foods in themselves are lacking in flavor. For example, the normal flavors of milk, cream, and most meats are low. Cooking is an art, and herbs, spices, or flavoring can transform a lowly dish to a dramatic one. Epicures are noted for their distinction in the seasoning and flavoring of food, and this is an art which may be learned by almost any individual if he or she really enjoys food and is willing to experiment and taste.

e. The Relation of Chemical Groupings to Taste and Flavor.

In organic chemistry, particularly in relation to the chemistry of dyes, it is known that certain groups impart definite colors to compounds. The groupings responsible for taste and flavor are not known as well. The development, control, and possible prevention of "off flavor" in foods subsequent to gamma-ray radiation, constitutes a relatively new, unexplored field.

Another topic which requires elucidation is the determination of the threshold dosage of gamma rays at which the "off flavor" first becomes noticeable.

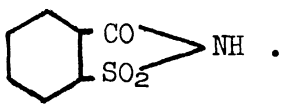
The structure of the "off flavor" compound or complex is not known. It is known that the "off flavor" either is not tasted or is less noticeable after irradiated meat is cooked, a fact which indicates a compound volatile at less than 100°C. It could possibly be a low-molecular-weight fatty derivative; but this is just a conjecture at this time.

The "off-flavor" taste incurred by large dosages of gamma rays may be markedly decreased by irradiating the food item in the frozen state, in vacuo, in atmospheres other than air, and by the use of free-radical acceptors. This indicates that the formation of the "off-flavor" complex is favored by the presence of oxygen and that the complex might be the oxidized form or decomposition product of a naturally occurring food constituent normally possessing a good taste. Further, the increased peroxide numbers in a fatty food item with an "off flavor" might indicate development of unsaturation in the food itself and/or the "off-flavor" complex. If in oxidative rancidity of fats the attack is primarily at the site of the double bonds, it can be postulated that fat-containing foods yield shorter-carbon-chain compounds on gamma radiation in air, possibly in the form of alcohols, ketones, or aldehydes which possess properties that could alter the normal taste.

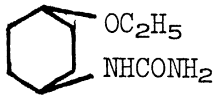
In fruits and vegetables certain lower alcohols, acids, and esters are found occurring naturally in varying proportions, with the relative amounts or predominance of certain compounds accounting for the total composite flavor of the individual fruit or vegetable. The paucity of data relating chemical structure to flavor may be ascribed to the facts that flavor compounds frequently are volatile and unstable and that this phase of food chemistry has been neglected.

The relation of chemical constitution to dyeing properties was explained by Witt[22] on the basis of the presence of chromophoric groups with double bonds and auxochromic groups reacting to give a colored compound; but it was not until several years later that speculations on constitution and taste appeared. Many German investigators worked in this latter field. Among them were Cohn, who found that hydroxyl groups and amino groups frequently occurred in pairs in sweet compounds. He called these groups sapophores. From studies of members in a homologous series he observed that the lower ones seemed to have a sweet taste, while the higher members had a more bitter taste. Neff associated the formulae $(CH_2O)_n$ with sweetness, and in his work on amino acids Emil Fischer found several with a sweet taste.

While the sweetness of a compound may have a relation to the hydroxyl groups present, as exemplified by sugars, there is no direct relation of the number of hydroxyl groups to the degree of sweetness, as can be shown by arranging the sugars in their order of sweetness. Furthermore, a much sweeter compound known as saccharin, discovered by Ira Remsen in 1879, with a sweetness approximately seven hundred times that of sugar, has the formula



Another synthetic compound, less sweet than saccharin, bears the appropriate name of dulcin and has the formula



Neither of these two compounds possesses hydroxyl groups. Perhaps the sweetest compound known today is a substituted phenylpropyl ether claimed to be 3300 times sweeter than a 1% solution of sucrose.[23]

In 1919 the American investigators Oertley and Myers[24] published a new theory relating constitution to taste and introduced two new terms: Glucophore, a group of atoms which has the power to form sweet compounds by uniting with a number of otherwise tasteless atoms or radicals, and auxogluc, an atom or radical which combined with any of the glucophores yields a sweet compound. On the basis of a careful review the authors listed six glucophores and nine auxoglucs, simple examples of which are:

Glucophore	Auxogluc	Compound
CH_2OH-CHOH-	H-	CH_2OHCH_2OH (glycol)
CH_2OH-CHOH-	CH_2OH-	$CH_2OHCHOHCH_2OH$ (glycerol)
HOOC-$CHNH_2$-	H-	$HOOCCH_2NH_2$ (glycine)

This article represented a great step forward; but there was no explanation for the behavior of saccharin and dulcin. Ten years later Druse[25] published a short note pointing out that constitution and taste had not been correlated factually and that little attention had been paid to this field. Still later Finzi and Colonna[26] stated, after a careful review, that it was not possible to develop any clear-cut evidence on the relation between taste and aromatic compounds.

On the subject of constitution and taste, it should not be forgotten that a compound may be exceedingly sweet and its isomer not, as in the case of saccharin and pseudo-saccharin. Stereoisomers also may have different tastes; thus D. valine and D.L. valine are both sweet, while L. valine is tasteless. Therefore taste is dependent not only on chemical structure, but also on space configuration. In the case of alpha amino acids, where the amino group and the carboxyl group are in close proximity, there is frequently a sweet taste, whereas ordinarily compounds with the amino group in the beta or gamma position or even further away are not sweet. For example, alanine, alpha amino propionic acid, is sweet; but beta amino propionic acid is not. Glycine is sweet, but phenylglycine is not; this shows the influence of the phenyl group. The effect of the amino group is shown very clearly in propionic acid which is sour itself, although alpha amino propionic acid (alanine) is sweet. Acetic acid is distinctly sour, but amino acetic acid (glycine) is sweet. Many more examples can be cited among compounds occurring naturally in foods. A very good example of a compound

introduced into this country because of its meatlike taste is sodium glutamate, which is used in the Orient as a condiment.

Beet tops fed to cattle are believed to be responsible for the undesirable odor in milk caused by the presence of trimethylamine. Betaines are found in Steffen's waste and also in molasses. The good flavor in butter is considered to be due to biacetyl (diacetyl), which may be formed by oxidation of acetylmethylcarbinol formed by butter starter microorganisms. Amines in fish and sulfur compounds in eggs are probably responsible for off odor and taste in these foods.

It may be concluded on the basis of this review that flavor is very complex, involving not only taste and odor but also the condition of the food (whether it is smooth or coarse, hot or cold, etc.) and other factors. Recent reviews in this field have been written by Moncrieff[27] and by Kirchner.[20]

f. Evaluation of Flavor

As pointed out by Stevens,[28] flavor has only recently come within a reasonable degree of quantitative control. "There are indications recently that many aspects of consumer preference can be anticipated in the laboratory, with a trained small panel, at appreciable saving and with increased reliability as a basis for management decisions."

Chemical tests have so far not been satisfactory for flavor testing. The physical and chemical tests used have had to be supplemented with selected taste-panel tests for there are no satisfactory objective tests to measure flavor, odor, and, most important, individual preference.

Flavor evaluation tests are included in the general class of subjective tests, i.e., tests in which a particular characteristic or property of a material is ranked or scored on the basis of the judgment of an individual or groups of individuals. The principal difficulties involved in this field may be attributed to the limitations and uncertainties of human behavior.

Boggs and Hanson[29] have shown that individual responses are subject to variations for many unknown reasons; therefore, results are expressed in relative terms. A great deal of time and data often are required to obtain valid results and small laboratories sometimes do not have sufficient personnel from which to select satisfactory panels. However, there are many procedures that can be followed to increase the accuracy of taste-panel tests.

3. THE DESIGN OF EXPERIMENTS FOR FLAVOR EVALUATION

a. The Use of Statistics in Experimental Studies

(1) General Considerations. Variability of results during repeated trials is a characteristic of many fields of research. This variability, caused generally by a number of undetermined factors, introduces some question as to the true value of the parameter under study. Thus, it must be considered what the results would be if a large number of repeated trials were performed. Often such a performance would not be feasible or perhaps would be impossible. Also, a large number of repetitions of the experiment may radically alter the conditions of the original experimental design.

A solution to this problem has been developed by the theory of statistics. Basically, the solution consists of a systematic technique of inducing statements concerning large numbers of repetitions on the basis of the results of a small number of trials; statistically speaking, this is induction from the sample to the population.

Obviously, such a solution will not provide exact information. A more realistic goal is to provide a solution and an associated level of confidence, i.e., the probability that the statistical solution is correct. For example, if a statistician states a solution with a confidence level of 95 percent, he says in effect; if he were to make a number of statements of this nature, he would be correct about 19 times out of 20.

One of the fundamental problems of statistical inference is that of testing statistical hypotheses. Generally speaking, the procedure is to design an experiment such that on the assumption that the hypothesis under test is true, all possible outcomes of the experiment and their associated probabilities of occurrence can be predicted.

To cite a familiar example, in order to test the hypothesis that a certain die is true, repeated throws of the die would be made. All possible outcomes and their associated probabilities can be described if it is assumed that the die is true; i.e., the appearance of each of the various faces is equally probable. Next an acceptance level is prescribed usually 0.01 or 0.05. For purposes of illustration, suppose 0.05 were selected. Further suppose a sequence of 100 throws of the die results in the appearance of one particular face 72 times. Now if this die were true, the probability of such a sequence occurring would be extremely small, much less than 0.05. Hence, in this case, the hypothesis would be rejected and it would be concluded that the die was not ture. Note that when a true die is subjected to many such sequences of trials, with such a standard of acceptance 95 percent would result in acceptance and 5 percent in

unjust rejection. Erroneous conclusions of this type, i.e., rejection of the hypothesis under test when it is actually true, are called "errors of Type I." An "error of Type II" is committed when the test hypothesis is accepted although in reality it is false.

Thus, the problem of the statistician is as follows: given certain objectives, select the appropriate hypothesis and design an experiment to test this hypothesis. Generally speaking, it is hoped that the hypothesis can be rejected; hence the term, "null hypothesis."

The measurements derived from an experiment are affected by (1) the treatments under test and (2) other factors which are not the subject of the experiment. Variations due to the latter are termed "experimental errors" (these are not to be confused with mistakes). Experimental errors may be classified either as (a) errors derived from the material to which the various treatments are applied (the judges in the case of flavor evaluation) or (b) errors derived from the manner in which the experiment is conducted.

The terms "precision" and "accuracy" are used interchangeably in most fields of endeavor. However, statisticians attach different meanings to these terms: "precision" refers to the ability to repeat a particular measurement, while "accuracy" signifies the closeness with which a measurement approaches the actual or true value of some parameter. For example, if an ammeter consistently indicates 1.5 amperes when 1 ampere is passing through the line, the meter is precise but certainly not accurate. Thus, in experimental design cognizance of both precision and accuracy is necessary. Generally, if the test statistic is unbiased, measures introduced to improve precision will improve accuracy; however, if the data are biased, greatly improved precision will have little effect on accuracy. In the following discussion the data will be assumed to be unbiased.

A number of types of experimental designs have been developed; these designs may be roughly classified as to the methods employed for increasing accuracy. However, there exist several procedures for increasing accuracy which are utilized to some extent in virtually every design.

(2) Types of Designs. In designing an experiment, the statistician can often anticipate the fact that certain factors will have a decided influence on the measurements; e.g., in flavor evaluation, the choice of judges to be employed will determine, in part, the results obtained. However, usually there are unknown or unrecognized factors present which introduce additional experimental errors. To guard against the possibility of any such factors biasing the data, a device known as "randomization" is employed. For example, if a panel of judges is required to taste a number of differently treated foods, the order in which the judges taste these foods could conceivably influence their judgment.

Therefore, the experimenter would present the samples to the judges in a random order; i.e., so that the probability of a given treatment appearing at a given position in the design is the same as that for any other treatment and is completely independent of the previous order. While the result of any specific randomization may favor some particular treatment, this occurs only to an extent that is allowed for in the calculations used for tests of significance.

(a) The Randomized Block Design: A randomized block design is one in which the experimental material is divided into groups, each of which constitutes a trial or replication. This design is especially useful when a uniform experimental technique cannot be maintained throughout an experiment. An example of such a design might be the testing of three types of fertilizers by applying them to a large field of corn. Since wide variations in natural fertility of the soil, drainage, etc., might reasonably be expected, the field could first be divided into four equal main sections or groups. (See Fig. 25 below). Each of these four sections could in turn be subdivided into four equal plots. In each of the four main sections, the three different fertilizers and a control (no fertilizer) may be assigned to the four subplots in a random manner. No design is more frequently used than randomized blocks. Any number of treatments and replications may be included, the accuracy obtained is greather than that of complete randomization, and the analysis is straighforward.

At this point it is well to observe that the simplest design which provides the accuracy desired is the best design to use. Certain of the more complex designs, however, are particularly well adapted to flavor evaluation studies, as will be shown later.

None	T_2	T_1	T_2	T_3	None	T_3	T_1
T_3	T_1	T_3	None	T_3	T_2	None	T_2

Fig. 25. Typical Randomized Block Design.

(b) The Latin Square Design: Sometimes it is apparent that some restriction on a randomization scheme would be advantageous. For example, if there are four judges and four treatments, the order of presentation could be randomized with the restrictions that each judge be allowed to test all four treatments and that each treatment be tested exactly once as the first, second, third, and fourth of some series (see Fig. 26).

	Order of Presentation of Treatments			
Judge 1	T_1	T_3	T_2	T_4
Judge 2	T_3	T_2	T_4	T_1
Judge 3	T_4	T_1	T_3	T_2
Judge 4	T_2	T_4	T_1	T_3

Fig. 26. A Latin Square Design.

In this case, for example, if the treatment which is tasted first is thereby given some advantage, each treatment obtains this advantage exactly once. Experimental designs such as this, where each treatment appears once and only once in each row and in each column, are known as "Latin squares."

Still another device for increasing the accuracy of an experiment is "replication." Suppose the above experiment were repeated several times, each time with a new block constructed according to the same restricted randomization procedure. Whatever the source of experimental error, replication will decrease the error associated with the "difference" between the "average" results for two treatments at a rate which is predictable from statistical theory. (Roughly speaking, the error will usually vary inversely as the square root of the number of replications).

Refinements in experimental technique, i.e., the physical conduct of the experiment under standardized conditions, will also tend to reduce experimental error.

Finally, the effects of variability of the test data can be reduced by the method of handling the experimental material. This can be accomplished

by careful selection of material, by taking additional measurements, or by skillful grouping of the experimental units so that the units receiving one treatment are closely comparable to those receiving another treatment.

If there is no planned grouping included in the experimental plan the procedure employed is known as "complete randomization"; in such a case the treatments are allotted to the experimental units entirely by chance. For example if the experiment illustrated in Fig. 26 were completely randomized, then the probability of any one treatment occurring at a specific position in the design is the same as for any of the other treatments. While the advantages of such a design are complete flexibility and ease in analysis, there is an objection on the ground of accuracy. Nevertheless, complete randomization is the obvious design to use in many cases, e.g., in physical experiments where several constituents are combined by mixing and then divided into batches. As for ease of analysis, this question is deferred until later, when the topic of basic assumptions underlying the analysis and design of these experiments will be discussed.

The latin square is a logical extension of the randomized block design. In the Latin square design, variations among the groups of experimental units which correspond to the rows and in turn to the columns of the schematic diagram of the design may be separated from the experimental error. Thus in the example illustrated in Fig. 26, any variation introduced by the judges or by the order in which the samples are presented can be eliminated from the experimental errors.

(c) The Incomplete Block Design: In some cases, the number of treatments tested in any one block must be held to a minimum. In flavor tests, for example, unless the number of samples compared by the judge in any one block is small, the phenomena of taste fatigue and adaptation introduce a prohibitive amount of variation in the experimental data. In cases such as this, the general procedure is to employ experimental groups which do not include every treatment under test, thereby keeping the size of the groups small. Such designs bear the general classification "incomplete blocks."

(d) The Split-Plot Design: Certain particular types of incomplete block designs are recognized. Suppose a number of different storage techniques are to be compared for application to corn-fed hog carcasses and also to hogs fattened on an antibiotic-supplemented diet. Flavor comparisons between cooked samples of meat from hogs with the same diet would be more important; hence the various treatments could be grouped in incomplete blocks in such a way that an incomplete block would contain either all the different storage techniques as applied to the corn-fed hog carcasses or all the techniques as applied to the hogs fed as antibiotic-supplemented diet. Thus there would exist pairs of blocks which together would form a complete replication. Such an experimental design is known as a "split-plot" design.

(e) The Factorial Design: In certain cases, the experimenter is interested in investigating the effect of a number of different factors simultaneously. For example, suppose two different strains of albino rats are to be studied as to their susceptibility to two different strains of a certain microorganism which in turn are being cultured in two different media. An experimental design known as a "factorial" design might profitably be employed, especially if the comparisons of immediate interest are those of the differences between the average responses for the two strains of rats, the two strains of microorganisms, and the two culture media. In the factorial design one of each of the various principal factors under test is represented in each incomplete block, every possible combination of factors being represented by its corresponding incomplete block. The factorial design for the experiment cited as an example might be of the type shown in Fig. 27.

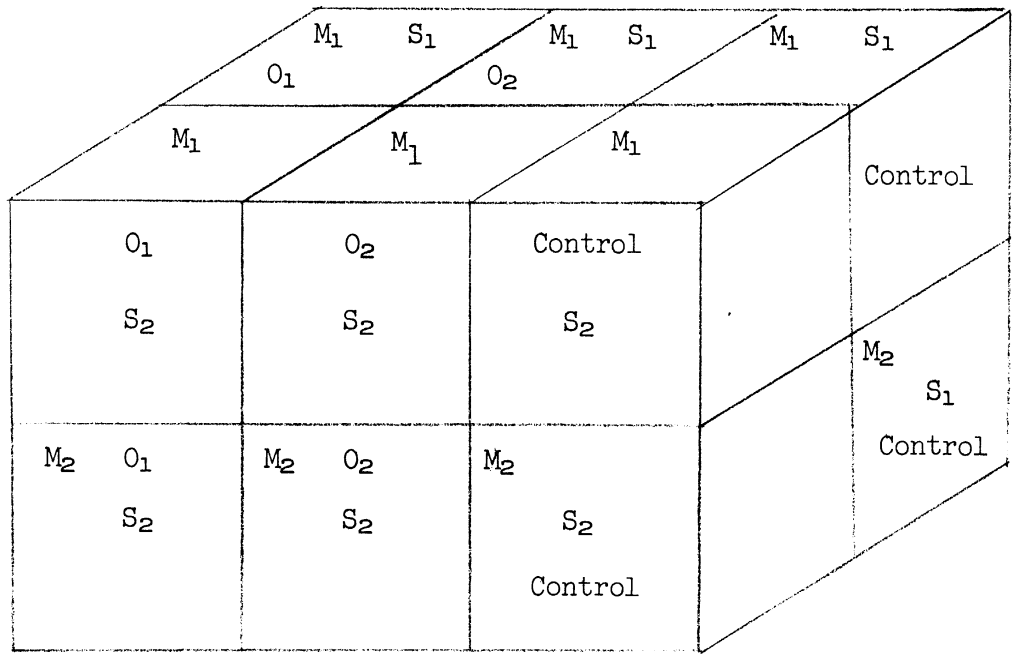


Fig. 27. An Example of a Factorial Experimental Design. Each block is composed of a number of innoculated rats. Here the blocks on the top layer all have organisms which were cultured in medium 1, while those in the bottom layer were cultured in medium 2; the blocks of the back layer have rats of strain 1, the front layer having rats of strain 2; finally, microorganisms 1 and 2, and a control (perhaps a sterile saline solution) are represented in the left, middle, and right layers respectively. Thus all possible combinations of treatments are represented by an incomplete block.

(f) The Balanced Incomplete Block Design: In both the split-plot and the factorial designs certain comparisons are of more importance than others. Now suppose that all comparisons between treatments are of equal importance. If an incomplete block design is utilized, two treatments which occur in the same block are more precisely compared than those which occur in separate blocks. Thus in order that all possible pairs of treatments are afforded the same advantage, a "balanced incomplete block design" must be employed; i.e., a design wherein all possible pairs occur in the same block as often as any other possible pair. As shall be demonstrated later, this class of experimental designs is particularly adaptable to the problem of flavor evaluation.

Those balanced incomplete block designs where the number of treatments under test, n, is an exact square; i.e., $n = k^2$ for some positive integer k, and the number of treatments occurrring in a single block is k, are known as "lattice" designs.

These types constitute the principal designs which have been developed so far. They have been presented in order of increasing complexity. The choice of which particular design to employ for a specific subject will depend on the objectives of the study, the precision desired, and the economic or practical aspects of repetition of the experiment. All these designs have a common method of analysis, the method of least squares. A short discussion of this method follows.

(3) Mathematical Analysis. In any of the experiments described above, each of the observations may be considered as being made up of four components: (i) a general mean; (ii) the effect of the treatment applied; (iii) the effect of the block to which the treatment is applied; and (iv) a residual effect, made up of all other factors which are influencing the observations (this is generally termed the "experimental error"). In symbols this can be expressed as

$$y_{ijk} = \mu + \tau_i + \beta_j + e_{ijk} ,$$

where μ = the general mean,

τ_i = the effect of the treatment,

β_j = the effect of the block, and

e_{ijk} = the experimental error.

Basically, the analysis of these experiments consists of obtaining precise and accurate estimates m, t_i, and b_j of the components μ, τ_i, and β_j. The method of least squares derives these estimates by minimizing the sum of the squares of the residuals; i.e.,

$$\sum e_{ijk}^2 = \sum (y_{ijk} - m - t_i - \beta_j)^2$$

taken over all observations.

When the estimates t_i of the various treatment effects are obtained it is necessary to test certain hypotheses concerning the significance of the differences exhibited between these t_i. The quantity most often used for such a test is Student's t-distribution, the test is commonly referred to as the t-test. In order to perform this test it is necessary to use the residual sum of squares, which quantity could be obtained by calculating for each observation y_{ijk} the value $(m + t_i + b_j)$ predicted by the least squares solution. However, the residual sum of squares can be computed more easily by a method known as the "analysis of variance." Briefly, the basis of this analysis is that under certain assumptions the following relationship holds:

$$\sum y_{ijk}^2 = \sum m^2 + \sum t_i^2 + \sum b_j^2 + \sum (y_{ijk} - m - t_i - b_j)^2 .$$

The assumptions underlying the analysis of variance are reasonable for a wide variety of applications.

Before stating these assumptions, the concept of a "normal distribution" shall be defined. A normal distribution is a theoretical distribution characterized by two parameters, its mean μ and its standard deviation σ. Explicitly, the function

$$\phi(x) = \frac{1}{(2\pi)^{1/2}} e^{-[(x-\mu)^2/2\sigma]}$$

is called the "normal density function"; its integral

$$\Phi(x) = \frac{1}{(2\pi)^{1/2}} \int e^{-[x-\mu)^2/2\sigma]} dy$$

is the "normal distribution function." The graph of $\phi(x)$ is the symmetric, bell-shaped curve shown in Fig. 28. If repeated observations of some parameter are distributed in such a manner that a normal density function approximates the distribution of these observations, it is reasonable to suppose that these observations were drawn from a normally distributed universe of observations. For example, suppose the heights of several hundred randomly selected adult American males are recorded to the nearest 1/2 inch by means of a curve such as that of Fig. 29, where the height of the curve for each of the groups is proportional to

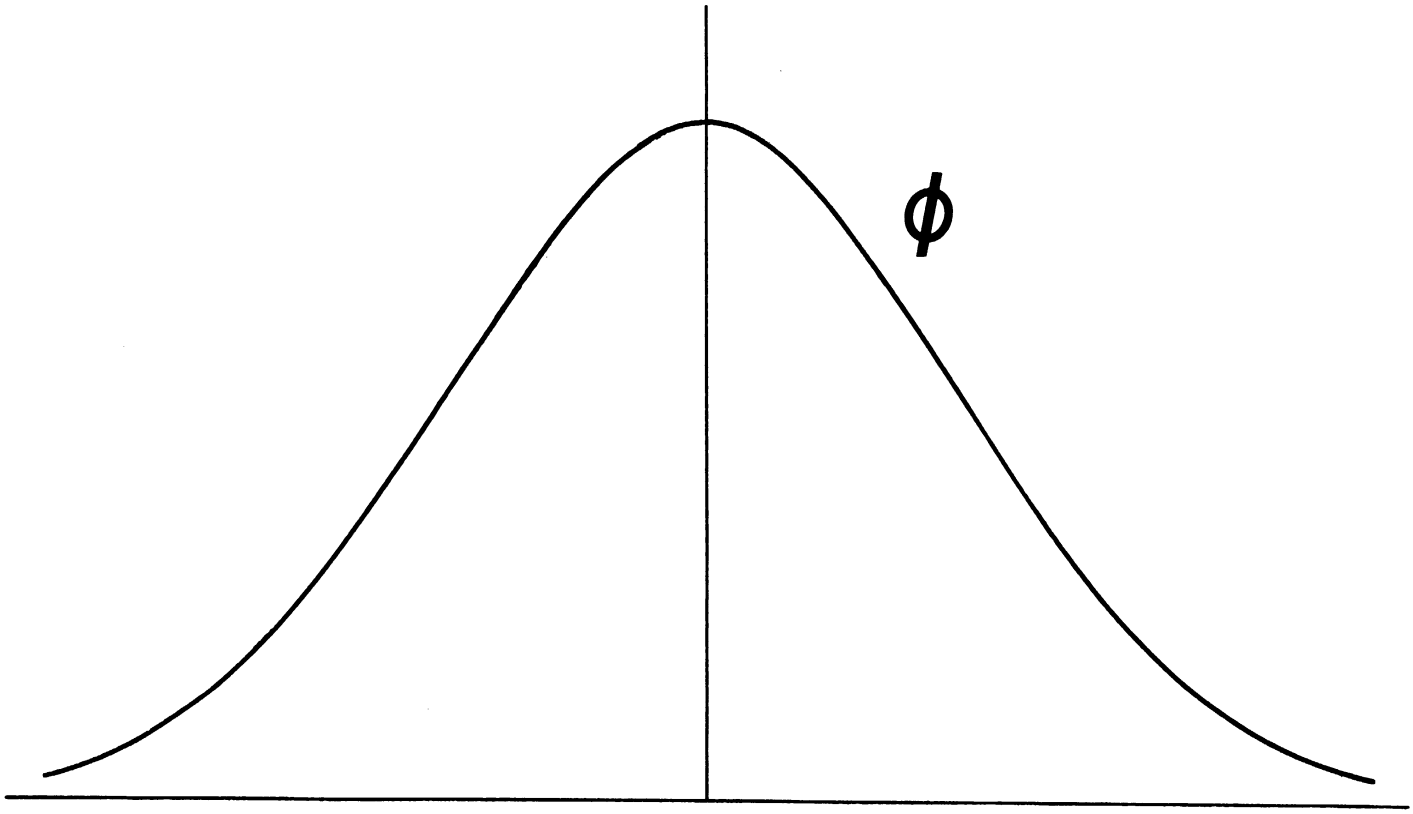


Fig. 28. The Normal Density Function.

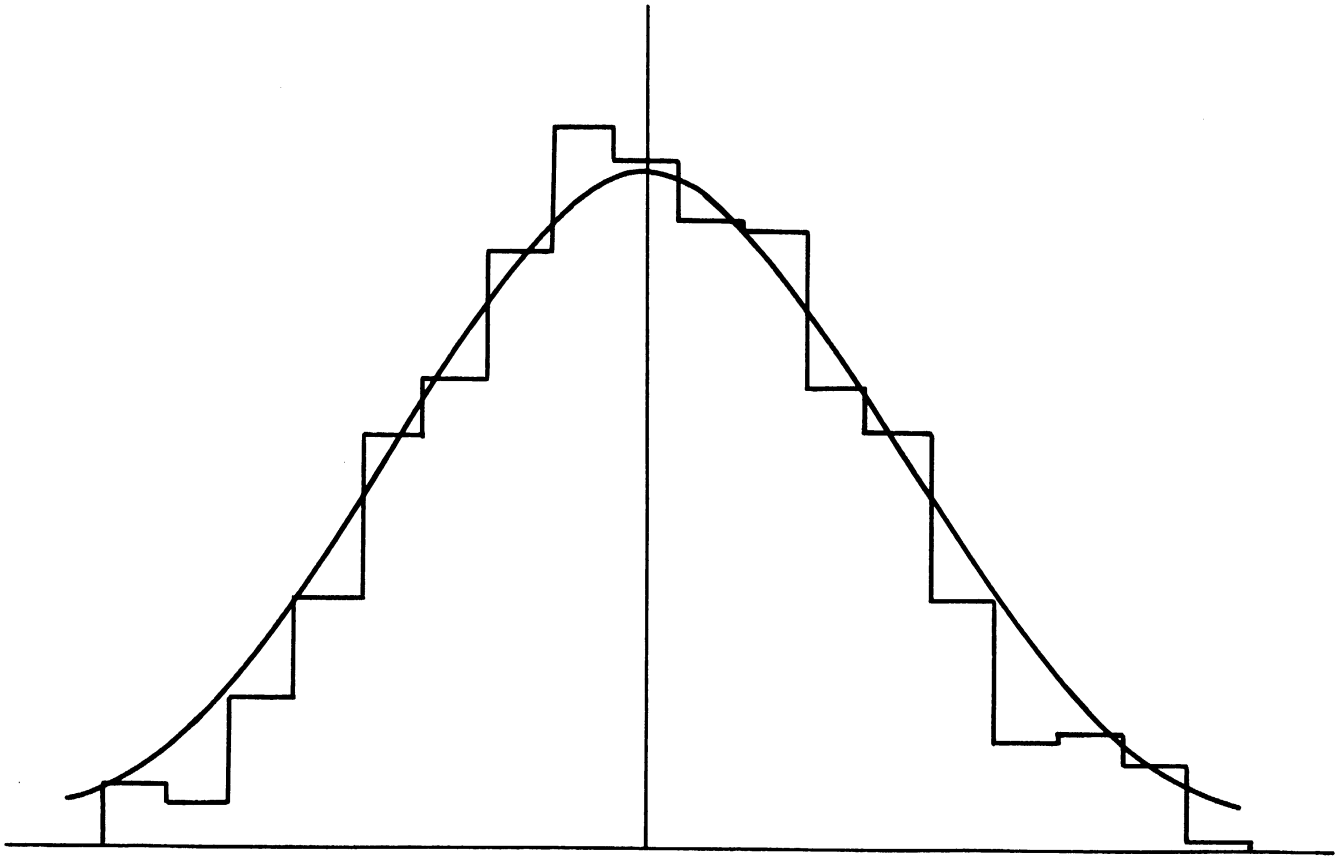


Fig. 29. Comparison of the Distribution of the Heights of Several Hundred American Males and a Normal Density Function.

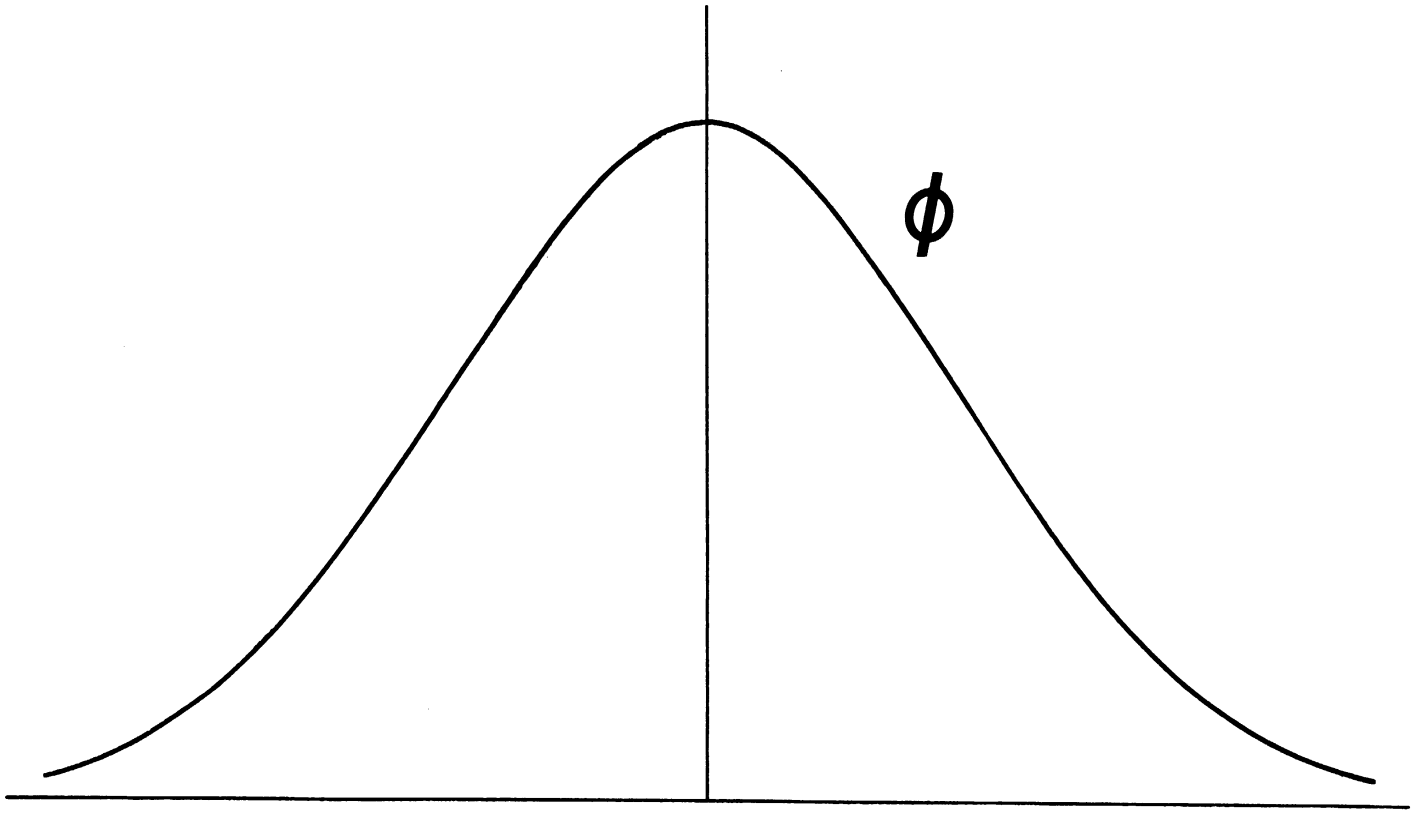


Fig. 28. The Normal Density Function.

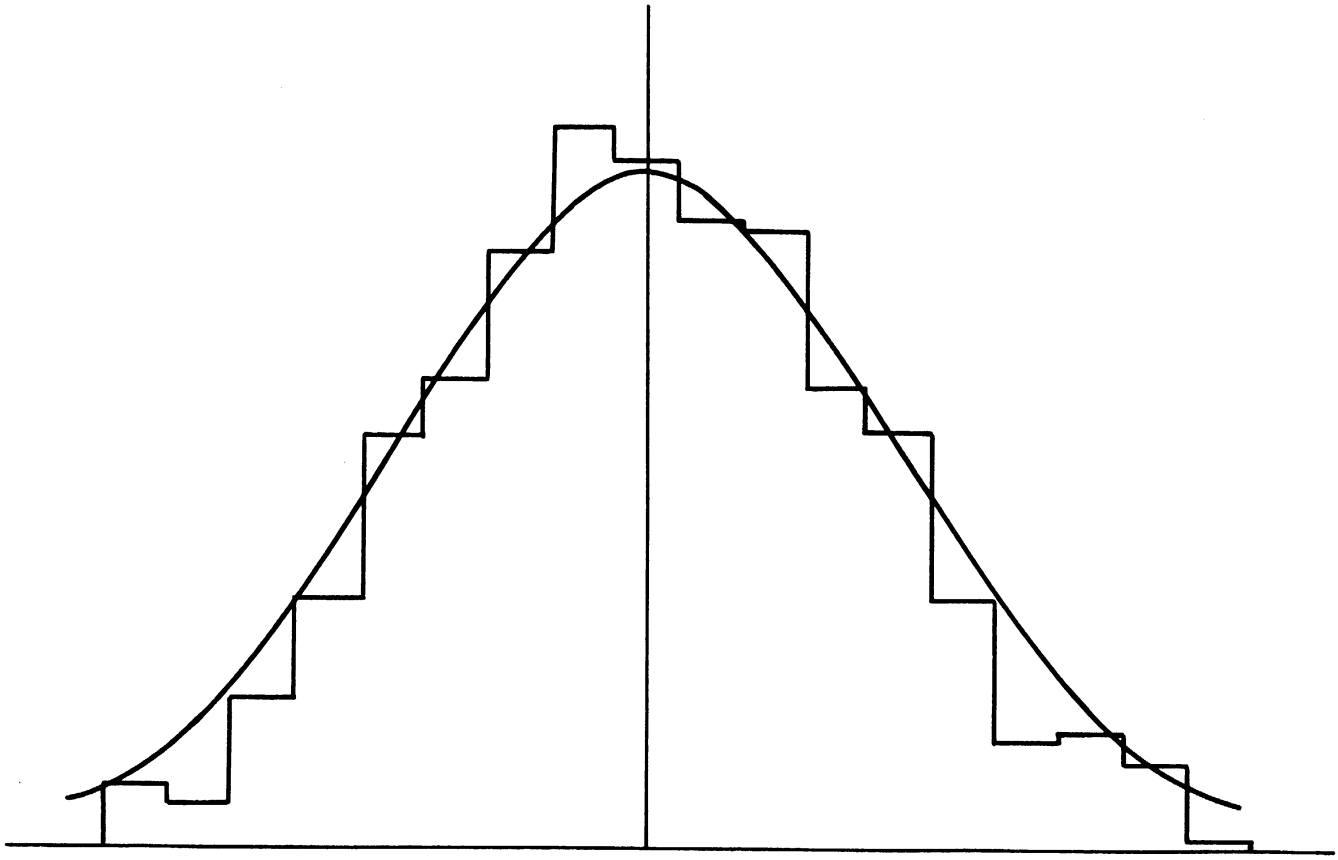


Fig. 29. Comparison of the Distribution of the Heights of Several Hundred American Males and a Normal Density Function.

must be assigned some preset score, and often the very introduction of these standards modifies the scores of those treatments under test due to the phenomemon of psychological "adaptation." The chief advantage of a scoring system as opposed to an ordering or ranking system is that it permits ties, whereas a ranking system forces the judge to rank one treatment above the other even if he cannot detect a difference in the attribute being tested. However, most scoring systems lead to some doubt with regard to the validity of the basic assumptions underlying the analysis of variance. If standards are employed, taste fatigue is necessarily increased; and taste fatigue may invalidate the assumptions that observations are independently distributed and that error variances are homogeneous. If standards are not employed, then error variances are probably not homogeneous. While it is believed that in some cases the analysis of variance may be applied without introducing serious error, in case of any doubt ranking methods should be used.

The simplest ranking techniques are those involving paired comparisons, in which treatments are compared two at a time and the judge's decision is purely qualitative.

A general class of ranking experiments long recognized by psychometricians is that of so-called "triangle tests." In such a test the experimenter asks the panel member to select on the basis of some attribute, the odd sample from a trio of samples of which two are identical. This test provides a means of detecting "true differences."

The mathematical model is based on the "binomial probability distribution." Repeated independent trials are called "Bernoulli trials" if there are only two possible outcomes for each trial and their probabilities remain the same throughout the trials; the most familiar example of Bernoulli trials is afforded by successive tosses of a true (symmetric) coin. As a means of distinguishing between the two possible outcomes, the convention is to term one a success and the other a failure, with probabilities p and q respectively. Disregarding order, n successive Bernoulli trials can result in n + 1 possible outcomes. The probability that exactly k of the n trials will result in success is

$$b\,(k!n,p) = \binom{n}{k} p^k q^{n-k} \quad , \qquad (1)$$

where

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$

Equation 1 is called the "binomial distribution"; the term "binomial" refers to the fact that Equation 1 represents the k^{th} term of the binomial expansion of

$$(p + q)^n \ .$$

The initial taste-panel experiments conducted in this laboratory were based on a modification of the "triangle" test. The procedure employed was as follows:

(1) A taste panel was selected.

(2) Each panel member performed a series of modified triangle tests. In this test the panel member was presented with three samples of a given food, one marked as control or nonirradiated food. He was informed that one of the remaining two samples, identified only by random code numbers, was also control. By tasting first the identified control and then the remaining two samples, the panel member was required to select the odd or treated sample. In order to enable the judges to express ties, they were also asked to rate the difference in flavor according to a six-point scale:

0 no difference
1 slight
2 moderate
3 decided
4 very decided
5 gross

These data were not employed in the test of significance; they served only as a psychological outlet when the judges were forced to rank a pair of treatments which in their opinion did not differ in flavor.

(3) For each treatment-food combination the above triangle was replicated both on individual judges and on the panel as a whole.

The null hypothesis under test is: "There is no true flavor difference between the treated and untreated food." Assuming this statement to be true, the probability, p, that the panel member will make a correct decision is 0.5. Thus, assuming there is not true flavor change, the probability that for n trials r or more of these trials will result in correct decisions can be determined by means of the binomial distribution. For example, if 23 of 36 triangle tests resulted in correct decisions, then under the null hypothesis the probability that this variation occurred by chance alone is about 0.07. It would then be deduced that no "difference" in flavor exists at any usual significance level. The results of the application of this test appear in Table 16, on page 18

In some instances where the judges were successful in identifying the odd sample, some of the panel members expressed a preference for the flavor of the irradiated sample. Thus, the employment of a statistical design which permits not only detection of flavor differences but also assignment of a ranking in terms of preference seemed in order. The rank analysis of incomplete blocks developed by Bradley and Terry[31] provides tests of various hypotheses pertinent to the problems of flavor-preference testing. This method permits tests of hypotheses of a general class and the estimation of treatment ratings or preferences. The mathematical model developed is relatively simple and easy to interpret and apply (statistically speaking). Ranks are used in incomplete blocks of size 2, which permits later generalization to blocks of larger size (such extension of the theory is now under consideration). The method of maximum likelihood is employed, and tests depend on the likelihood ratio statistics. (For a more detailed discussion of the mathematical model, see Appendix A and Reference 30 and 31. Two special tests are featured in Reference 30 the null hypothesis that true treatment ratings are equal. The alternative hypothesis (i) makes no assumptions of equality of treatment ratings and (ii) makes the assumption that there are only two groups of treatments, within which treatments do not differ in ratings, but the two groups themselves may have different ratings. Attention is called to the fact that in application to our problems, the control food will be considered as a treatment, as far as the nomenclature of this design is concerned. These procedures are particularly applicable here, since qualitative measurements alone are reliable. The method of combining data permits an overall test of significance without the usual assumption that members of a panel agree on the nature of the differences to be detected.

The general procedure finally adopted for the taste-panel experiments is as follows:

(1) A competent panel of judges is selected.

(2) As an example, five judges are employed to test three treatments against a control, i.e., a total of four treatments. For each judge and for each repetition twelve containers are coded: three of these containers contain samples of control food, three contain samples of treatment 1, etc. These twelve containers are presented to the judge pairwise so that he compares all possible pairs of treatments.

(3) For each pair the judge tastes each sample and records the value 1 for the sample preferred and 2 for the other sample.

(4) The experimenter collects and decodes the data for each judge and records the results.

The analysis of the data is based on the total score accumulated by each particular treatment and may best be demonstrated by means of an example. Table 11 presents the data of a typical experiment of this type, where a control C and two different treatment levels, T_1 and T_2 are tested. The treatment sums for C, T_1, T_2 are (18, 16, 11) for Judge 1 and (11, 19, 15) for Judge 2; combining the data yields (29, 35, 26).

TABLE 11

DATA FROM A TYPICAL EXPERIMENT WITH THREE TREATMENTS AND TWO JUDGES

Repetition	1			2			3			4			5		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
Pair								Judge 1							
C, T_1	2	1	-	2	1	-	1	2	-	1	2	-	2	1	-
C, T_2	2	-	1	2	-	1	2	-		1	2	1	2	-	1
T_1, T_2	-	1	2	-	2	1	-	2		1	-	1	-	2	1
								Judge 2							
C, T_1	1	2	-	1	2	-	-	2	-	1	2	-	1	2	-
C, T_2	2	-	1	1	-	2	1	-	2	1	-	2	1	-	2
T_1, T_2	-	1	2	-	2	1	-	2	1	-	2	1	-	2	1

From the appropriate table of Bradley and Terry[31] we find

	p_C	p_{T_1}	p_{T_2}	B_1	P
Judge 1	0.06	0.12	0.82	2.767	0.0386
Judge 2	0.81	0.03	0.16	2.274	0.0112

and $B_1^c = 2.767 + 2.274 = 5.034$, $P < 0.005$. The nomenclature is that of Bradley and Terry and should be read as follows:

p_C, p_{T_1}, p_{T_2} = estimates of the true preference ratings of the control and each of the two treatments; note that these have meaning only when P is significant; in such cases the log P's may be composed on a linear scale.

B_1 = the test statistic employed to test the null hypothesis thesis that all three preference ratings equal 0.33 against the hypothesis that each food has its own preference rating with the condition that the sum of the three ratings be equal to 1.

P = the probability that B_1 will not be exceeded if the null hypothesis is true.

B_1^c = a statistic used for a test of agreement between judges (see below).

In this case, it was concluded that differences detectable by these judges did exist at the 1 percent level. If it were decided to combine the data we would have $p_C = 0.34$, $p_{T_1} = 0.14$, $p_{T_2} = 0.52$, $B_1 = 7.752$ and $P = 0.0674$. $B_1 - B_1^c = 7.752 - 5.034 = 2.718$ affords a test of agreement and in this case is indicative of poor agreement of the preferences of the judges; in fact, by use of the large-sample analysis $(B_1 - B_1^c)$ is distributed as $X^2 = 12.51$ with two degrees of freedom, a result significant at the 1.0 percent level.

In summary, the experimenter would conclude on the basis of this test that flavor differences at a level detectable by these judges were present, but that they disagree as to which was the preferred flavor.

c. Selection Of a Taste Panel

The concept of flavor is dependent on the specific members of a particular population. No matter how accurately an experiment measures the flavor of a particular food, the best that can be expected of the result is that the true flavor, as conceived by the population represented by the panel, has been determined. Thus, an attempt must be made to select a panel representing the population of interest. While the factors which influence the flavor conception of a given population are not definitely known, such factors as region, sex, and age are highly suspect.

In the selection of panel members the following questions must therefore be answered:

(1) Does the candidate represent the population under study?

(2) Can he repeat his judgments?

(3) Is his flavor perception sufficiently acute?

(4) Is his motivation sufficient?

For preference testing (3) is not too important as long as (1) is satisfied; however, failure to satisfy (4) will seriously impair any experiment in which the candidate participates.

A taste panel will, in general, fall into one of four categories depending on the objective of the experiments to be conducted.

(1) Taste Panels for the Detection of Differences. Such a panel is generally small; i.e., about three to ten in number. Rather intensive training of panel members is usually undertaken; however, agreement among panel members in regard to preference is generally not necessary. It is necessary that a judge demonstrate precision; i.e., ability to repeat his judgments.

(2) Taste Panels for Quality Control. Taste panels for quality control are usually panels of long standing and of more experience than the first type. Such panels are utilized for the maintenance of fixed standards and must test for lack of differences. As for type (1), quality-control panels are not concerned with preferences.

(3) Taste Panels for Consumer Preferences. Panels of this type are usually large and untrained. Generally, no standards are provided and decisions are based on preferences alone. It is important that the taste panel be representative of the population of interest.

(4) Taste Panels for Quality Evaluation. Taste testing for quality evaluation is usually one phase of a more elaborate evaluation procedure. Composite quality scores consist of weighted averages of a variety of determinations. This kind of taste procedure is used in certain United States Standards for Grades. The tasting is usually done by a very small number of official graders. An attempt is made to conform to a uniform scoring system over long periods of time. Interest is in an absolute taste score and not in comparative scores for several products, as is usually sufficient in the other types of panel testing.

The proper weighting of attribute measurements in quality evaluation is one of the problems in taste-panel studies. Also important are the selection of the panel and the choice of experimental designs and scoring techniques.

In the experimental plan the number of characteristics to be judged by a taste panel should be limited to one or two; in this panel work only one characteristic, "flavor," was evaluated. Cooking procedures were standardized for each food and the best quality of the food obtainable was used, since variability of scores is much greater on low than on high-quality material. As far as possible the raw material used for each repetition was of similar quality, and the samples used for controls were drawn from the same lot of material as the other samples. Replications of an experiment can be reduced if uniform material is selected for samples and the judges employed are accurate and consistent.

In the selecting and training of judges for a panel, the probability of finding persons with superior taste acuity is greater if at the beginning an appreciable number (such as thirty) are tested. Six judges can be selected from such a group with a sufficiently high confidence level. Such a procedure produces a more efficient panel than if members are selected simply because they happen to be available. However, in small laboratories, some compromise usually must be made between these selection methods because the number of persons available is limited. Once judges are selected for the panel, the consistency of each judge has to be checked statistically from time to time during the course of the experiments. The interest of the judges should be maintained by giving them enough information to sustain interest; but not enough to bias their opinions.

Testing environment is a factor to be considered if reliable and accurate data are to be obtained. Disturbing factors such as cooking odors, interruptions and tardiness should be reduced to a minimum. In order to avoid communication among the judges by speech or facial expression, each judge should be tasting a different set of samples at any one time. The utensils used for serving food to the judges should be uniform and have no noticeable odor or flavor. Equal amounts are used for each sample, usually bite-size portions, but more can be allowed for a difficult decision.

The sample temperature should be between 25°C and 35°C, as this is the optimum temperature range for taste perception. In order to lessen fatigue of the sense organs resulting from tasting too many samples at one time, several procedures can be followed. Just before commencing the taste experiments a trial sample is given to the judges to sharpen their senses for the tests to follow. Ejecting the food after tasting into a suitable container instead of swallowing has been shown by numerous taste-panel experts to give better results. Rinsing the mouth with lukewarm water also helps to prevent a taste carry-over from one sample to the next. It is better to schedule judging for the middle of the forenoon or afternon as the senses have then had time to recover from the previous meals. Each judge should be allowed as much time as he needs for each set of samples because individuals differ in their ability to recover from taste sensations of different foods.

d. Description of Taste-Panel Procedure

(1) General Procedure. In the work on irradiated food reported here, most of the suggestions enumerated above for obtaining reliable and accurate data were followed. Panel members were selected from the personnel of the Fission Products Laboratory who were available at the time tests were held, and it was not always possible to keep the same people. At first the taste-panel tests were conducted in the Fission Products Laboratory, where the environment was not wholly satisfactory because of chemical and animal odors from adjoining laboratories. In October permission was obtained from the Food Service Department of the University of Michigan to use their test kitchen for the food preparation and for

conducting the tasting experiments. Figure 30 is a photograph of the taste panel participating in a test in the Food Service Kitchen. All the food used in the irradiation experiments was obtained through Food Service, and was of standard quality. In the taste experiments the judges were asked to eject their food, but some preferred to eat their samples; no strict rules were enforced on this point. Water was used for rinsing the mouth between taste tests.

(2) Types of Tests. A study was made of the different types of taste-panel tests before one was chosen for the work to be conducted on irradiated food.

Paired and triangle difference tests indicate whether there is any difference between samples or a difference in one or two characteristics, such as flavor, tenderness, etc. In the "paired" test two samples are submitted to the judges and the judges may be asked, "Which of the two samples of cake is more tender or which of the two has better flavor?" In the "triangle" tests three samples, two of which are duplicates, are given to the judges, who are asked to identify the identical samples.

The triangle test is often used in the selection of judges for a panel. For the purposes of selection of judges it is important that the controls and the treated sample be of just sufficient difference to permit the selection of persons of superior acuity. For example, if cherries treated with 800,000 rep are used, the difference in flavor between the treated sample and the control should not be of such magnitude that everyone could detect the odd sample.

The test known as the "dilution" test has been used to determine the smallest amount of unknown that can be detected when it is mixed with a standard material. According to Boggs and Hanson[29] dried egg is the only product to which the method has been applied, and fresh egg was the standard used. The method is used only with homogeneous substances but many nonhomogeneous foods can be made homogeneous without deleterious effects on quality.

"Scoring" or numerical "rating" tests have been used more frequently than any of the other methods. The literature describes many scoring forms that have been used by different workers. There is considerable variation in the range of scores used by different investigators; but scales ranging from 1 to 5, 7, or 10 are the most common.

The "ranking" test is another method of evaluating foods in which the judges are asked to rank samples in decreasing or increasing order of some characteristic, such as the rancidity in fat samples.

(3) Selection of Test for Evaluating Irradiated Food. In the evaluation of the flavor of irradiated food a method was desired which would reveal

Fig. 30. Photograph of Taste Panel Personnel Testing Food in Food Service Kitchen.

any flavor differences brought about by irradiation. The first planned taste-panel tests with irradiated foods employed an adaptation of the triangle test. Six judges constituted the panel, and each judge performed a sequence of four tests on food samples given four different dosages of irradiation. In each test each judge was given three samples in glass petri dishes. One sample was marked control and the others, one of which was a control, were marked with random numbers. The judge was asked to select thesample which he or she thought was different, i.e., the irradiated sample, and to write the code number of the treated sample on his or her score card (Table 12). The judge was also asked to check the magnitude of flavor difference using a six-point scale from 0 to 5. By checking 0, No Difference, the judge can show that the ranking was forced. Table 13 shows a page of the taste-panel director's code for one series of tests. It can be observed that the judges are not tasting the same level of irradiation treatment at one time. For each treatment-food combination the above triangle was replicated both on judges and on the panel as a whole.

The early taste-panel studies were conducted using the triangular method with six fresh fruits that were in season. When these tests were completed it was decided that this method was not giving all the information desired. The triangular test method permits the judges to detect flavor differences, but it does not reveal whether the flavors of irradiated foods are preferred to the flavors of nonirradiated food.

The taste-panel experiments were then modified to the "incomplete block" design employing paired comparisons. This method permits both detection of flavor differences and ranking in terms of preference. The panel still consisted of six persons, but only three treatments and the control were used instead of the four treatments used in the triangle test. An example of the taste-panel director's code sheet is given in Table 14. In this test twelve small containers are coded for each judge. These twelve containers represent the six possible combinations of the control and three treatments taken in pairs; i.e., each series consists of a pair of samples, each sample identified by random code numbers. The score card used is shown in Table 15. For each pair of samples the judge is asked to write down the code number of the sample he or she prefers, and whether he or she is being forced to rank by checking no difference, or whether the difference in flavor between the two samples is slight, moderate, or decided. The taste-panel director collects and decodes the data for each judge and records the results. The data are then analyzed by the statistician.

4. RESULTS OF THE TASTE-PANEL EXPERIMENTS

a. Tests with Fresh Fruit

(1) Dark Sweet Cherries: Triangle Test. Whole dark sweet cherries were given 4×10^5, 8×10^5, 1.2×10^6, and 1.6×10^6 rep of gamma radiation.

TABLE 12

SCORE CARD FOR TRIANGLE TEST

Do Not Write in this Space

Judge No. _____
Sitting No. _____
Series No. _____

Code No. of Treated Sample _____
Magnitude of Flavor Difference
(Check one)

No Difference	0
Slight	1
Moderate	2
Decided	3
Very Decided	4
Gross	5

Comments:

TABLE 13

CODE SHEET FOR TRIANGLE TEST

Test No. _____ Sitting _____ Series _____

Judge	Identified Control	Unknown Control	Treatment	Treatment
1	C - 1	25	3	11
2	C - 2	76	4	86
3	C - 3	16	3	44
4	C - 4	92	1	72
5	C - 5	22	2	96
6	C - 6	48	2	52

TABLE 14

CODE SHEET FOR RANKING BY MEANS OF PREFERENCE

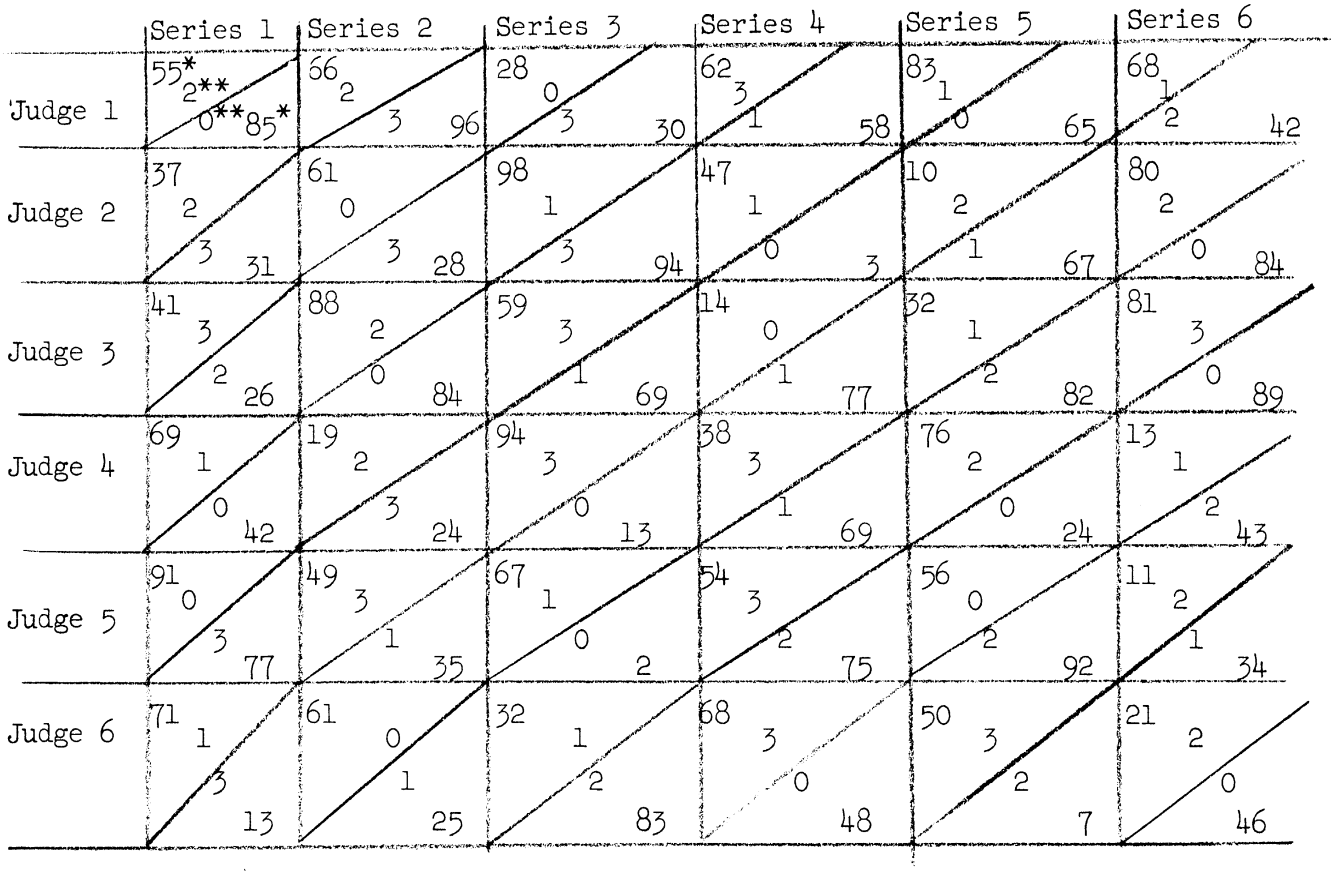


TABLE 15

SCORE CARD FOR RANKING BY MEANS OF PREFERENCE

Judge No. _____
Sitting No. _____
Series No. _____

Which sample do you prefer? _____
How much better is it?
(Check one)

___ No better-I was forced to rank
___ Slightly better
___ Moderately better
___ Decidedly better

Comments:

*Code numbers used by judges to identify samples.
**Numbers used by director to identify irradiation doses.

The cherries used in each experiment were first sorted, discarding all those which were overripe or underripe, and then washed and mixed well. Enough cherries were set aside for controls; these were left at room temperature while the others were irradiated. The judges on the panel had no difficulty selecting the treated cherries, chiefly because of texture changes. With increasing irradiation dosage the cherries were progressively softer, juicier, and brighter red. The cherries given a dosage of 4×10^5 rep were not as distinguishable from the controls as those given the higher treatments.

In an attempt to prevent visual and kinesthetic detection of the treated cherries, a puree was made after the whole cherries were irradiated: the cherries for each dosage and for the controls were pitted and liquified by placing in a Waring blendor for two minutes. In the taste-panel tests using the puree, the judges were given a few cc of each sample to taste. Once again the judges were able to select the irradiated samples by visible appearance rather than by flavor, because the puree prepared from the irradiated cherries tended to gel more and had a smoother external appearance than the puree from the control cherries, particularly at the three higher dosage levels. In some tests an equal amount of water was added to the puree made from the control and the irradiated cherries to prevent gelatinization, but it was found that the puree of treated cherries would not gel if the cherries were irradiated in the puree stage, so this method was used thereafter.

The color of the irradiated cherry puree was somewhat bleached at the higher dosage levels, i.e., 8×10^5, 1.2×10^6, and 1.6×10^6 rep; therefore food coloring was added to all the samples, even to the control, to make the puree colors similar. The amount of red color bleached from the irradiated puree appeared to be approximately proportional to the dose level.

With purees prepared in this manner differences in appearance no longer influenced the judges, and flavor became the criterion for selection. The statistical interpretation of the results is shown in Table 15. To summarize, the statement can be made that in most instances the judges were able to select the irradiated puree, but the flavor was good, and to some judges the irradiated puree had a better flavor than the control puree. Some judges reported that the irradiated puree had a nut-like flavor.

(2) Sliced Peaches in Syrup: Triangle Test. In the first tests with peaches, whole peaches were given irradiation dosages of 4×10^5, 6×10^5, 8×10^5, and 1.0×10^6 rep. When the whole peaches were sliced, however, all the peach slices tended to brown. The peaches given 8×10^5 and 1.0×10^6 rep browned to a greater extent than the controls and those given lesser dosages. Since it was impossible to shorten the time interval between slicing and the taste-panel, test browning was minimized by slicing the peaches into a 40° Brix syrup containing 0.1% ascorbic acid. For each dosage tested a container with the same amount of sugar and ascorbic acid solution was used.

The slices from different peaches were distributed among the containers for the different dosages. Dosages of 4×10^5, 6×10^5, 8×10^5, and 1.0×10^6 rep were used again. Taste-panel tests were held the day following irradiation. Using peach slices in syrup and these irradiation dosages, observations immediately after irradiation showed no marked texture changes in any of the irradiated peach slices, and the color of the irradiated peach slices was similar to that of the controls. The flavor of the irradiated peach slices differed from the flavor of the controls but was not objectionable to the judges. Some of the judges described the flavor of irradiated peach slices as resembling peaches flavored with almond extract. These tests were encouraging and were followed by other tests on peaches.

(3) Stored Whole Peaches. In another experiment using fresh peaches, whole fresh irradiated peaches and controls were packed in saran and polyethylene bags and stored in one of the cold rooms of the Food Service Building held at 40°F. These experiments were an attempt to increase the storage life of fresh whole peaches; the results are discussed in another section of this report. The radiation dosage levels used in this experiment were 5×10^5, 1×10^6, 1.5×10^6, and 2×10^6 rep. These dosages were slightly higher than those used in the previous experiment with sliced peached in syrup; therefore, some additional taste-panel tests were held using peaches at these higher dosages and sliced in 40° Brix syrup and 0.1% ascorbic acid.

The texture and color changes of peaches given 1.5×10^6 and 2.0×10^6 rep were quite marked, the irradiated peaches being softer and browner than the controls. To most of the taste panel members the odor and flavor of peaches given 1.5×10^6 and 2.0×10^6 rep were objectionable. These peaches lacked the almond flavor associated with peaches irradiated at levels between 4.8×10^5 and 8×10^5 rep and were flat and flavorless instead.

After one month of storage, some of the peaches packed in saran and polyethylene bags were removed from the cold room for taste-panel experiments. The peaches were sliced in a 40° Brix syrup and 0.1% ascorbic acid. Flavor changes in peaches stored in saran and given 1.5×10^6 and 2.0×10^6 rep were quite drastic, there being no characteristic flavor of peaches whatsoever. The peaches given 5×10^5 and 1×10^6 rep and stored in saran had an almond-like flavor which was not objectionable, but which was quite different from the flavor of fresh peaches.

After two months of storage at refrigerated temperature, the external and internal appearance and the flavor of the control peaches were superior to those of any of the irradiated peaches and the experiment was discontinued.

Saran seems to be a suitable material for packaging and storing fresh whole peaches. After two months of storage, 50 percent of the control peaches

packaged in saran were still in excellent condition, and 31 percent after three months of storage.

After one month of storage the peaches packaged in polyethylene were very poor in both flavor and appearance. The taste-panel members had no difficulty selecting the peaches given 5×10^5 rep, and actually the color and texture changes were less at this level of radiation than at the higher levels. All the peaches packaged in polyethylene, even the controls, were very unpalatable due to fermentation and overripening; thus the taste testing of peaches packaged in polyethylene was likewise discontinued. After three months of refrigerator storage most of the peaches packaged in polyethylene and given 2.0×10^6 rep were free of mold growth; but they were so very unattractive in appearance, i.e., dark brown and soft, that the peaches were considered to be spoiled.

(4) Stored Sliced Peaches in Syrup. In connection with the storage experiment using whole fresh irradiated peaches and controls packaged in saran and polyethylene, another storage experiment was planned with sliced peaches. The peaches were sliced into Mason jars containing 40° Brix syrup with 0.1% ascorbic acid before irradiation. The dosages or irradiation given were 5×10^5, 1×10^6, 1.5×10^6, and 2.0×10^6 rep. A sufficient quantity of peaches was irradiated to furnish material for taste panels once a month for a period of six months during which they were stored at room temperature in Mason jars.

Owing to the fact that the peaches were packaged in cold Brix syrup without evacuation, the control peaches and the peaches given 5×10^5 and 1×10^6 rep fermented in a short period of time. The peaches given 1×10^6 rep had not fermented as much in four months as those given just 5×10^5 rep, however, and both had fermented less than the control peaches. The peaches given 1.5×10^6 and 2.0×10^6 rep did not ferment at all over a period of four months, and the peaches still had a good color and flavor. The peaches given 1.5×10^6 rep were slightly better in color, texture, and flavor than those given 2.0×10^6 rep.

(5) Plums: Triangle Test. The tests on plums were quite limited because they were first undertaken near the end of the season for plums. Blue Italian plums were sliced in a 40° Brix syrup and given 4×10^5, 6×10^5, 8×10^5, and 1×10^6 rep. When fully ripe plums were used, there were no differences in texture between irradiated plums and the controls. When the plums were not fully ripe, the irradiated plums were found to have a softer texture. However, the taste of irradiated plums, regardless of dosage given, was not well liked by the taste-panel members, as the irradiated plums were lacking in fresh-plum flavor.

(6) Cantaloupe: Triangle Test. In the first tests with cantaloupe the melons were pared, cut into bite-size pieces, and sealed in No. 2 cans.

The controls were left at room temperature while the others were being treated. The taste-panel members reported that some of the control melon pieces had soured; thereafter whole melons were irradiated. In these tests groups of three melons were given 4×10^5, 6×10^5, 8×10^5, and 1×10^6 rep. The melons given 8×10^5 and 1×10^6 rep were softer in texture, but were not so soft as to be objectionable. The flavor of irradiated cantaloupe was described by some panel members as being similar to that of honeydew melon, while others though that the melon simply lost flavor on irradiation. To most of the panel members the flavor was not objectionable; but the difference between the control and irradiated melon was easily detected.

(7) Apples: Triangle Test and Ranking by Means of Paired Comparisons. Apples of the Delicious variety were pared and then sliced into a 40° Brix syrup with 0.1% ascorbic acid. The amounts of irradiation given to the sliced apples were 5×10^5, 1×10^6, 1.5×10^6, and 2×10^6 rep. The treated apples were observed to be softer in texture, with greater texture changes accompanying greater doses. Also, the apple slices browned progressively with increasing dosages of irradiation. The flavor of irradiated raw apples was very different from the controls, and was not liked by taste-panel members. At the lower dosage of 5×10^5 rep, the irradiated apples seemed to have a slight almond flavor; but at the higher dosages the flavor was flat and obnoxious, being somewhat similar to that of rotten apples.

Since the color and texture changes in irradiated apples were visibly apparent to the judges, it was decided to use applesauce rather than whole or sliced apples. First, MacIntosh apples were given dosages of 5×10^5, 1×10^6, 1.5×10^6, and 2×10^6 rep; then applesauce was made with the irradiated apples. The applesauce made from the irradiated apples was increasingly darker in color and more fluid with higher dosages of irradiation. In order to inhibit enzyme action, cooked applesauce was made before irradiation. Although it is probably commercially unfeasible to irradiate cooked applesauce, this test was used because it was the only way to obtain information about the flavor of irradiated applesauce without visible changes influencing the judges.

Both the triangle test and ranking by means of paired comparison were used with the irradiated cooked applesauce; thus this experiment afforded an excellent comparison of difference and preference type taste tests. Results of the former tests are presented in Table 16.

Judging of irradiated applesauce, wherein the judges were merely attempting to detect "true differences" in flavor, indicated that irradiation produced a change in flavor at a level detectable by the panel members. The results of the preference tests appear in Table 16; for purposes of illustration of the methodology andthe format of succeeding tables, let us consider Sitting No. 1 in detail. The doses tested (0, 1.0×10^6, 1.5×10^6, and 2.0×10^6 rep)are shown

TABLE 16

THE APPLICATION OF THE NULL HYPOTHESIS TO
TEST DATA ON FLAVOR DIFFERENCES IN FOOD SAMPLES

Experimental Food	Dose	No. of Correct Decisions	No. of Correct Decisions	p*
Cherry Puree	1	32	4	-
	2	27	8	-
	3	32	5	-
	4	33	3	-
Whole Cherries	1	15	9	.15
	2	20	3	-
	3	23	2	-
	4	20	4	-
Peaches	1	26	11	.01
	2	23	13	.07
	3	22	13	.09
	4	32	4	.09
Plums	1	24	6	-
	2	27	3	-
	3	25	6	-
	4	24	5	-
Cantaloupe	1	24	6	-
	2	24	5	-
	3	26	5	-
	4	24	6	-
Applesauce	1	17	1	-
	2	14	4	-
	3	16	2	-
	4	16	2	-

* "-" indicates a value less than 0.01.

immediately to the right and one line above the sitting number. Immediately below the doses are the cumulative scores (31, 23, 27 and 27) received by each of the respective treatments. The probability P, that the preference ratings of the control and the three irradiated samples are equal, is 0.15; this information is shown in the column second from the right in the table. Since the 5 percent significance level will be employed in the analysis of these experiments, this result is not significant; that is, in this case preference differences do not exist in the opinion of this taste panel. For each case where a significant difference is demonstrated, estimates of the respective preference ratings are provided immediately below the cumulative scores. These preference ratings are scaled so that their sum is 1 and the more preferable the treatment the greater the rating. The results appearing in this table indicate that there is no general agreement among the members of the panel as to whether the irradiated or the nonirradiated flavor is preferable; hence the panel as a whole did not feel that any of the treatments were significantly different with respect to flavor preference. Many of the judges liked irradiated applesauce, even that which was given a very high treatment of gamma radiation. The irradiated applesauce was sweeter, more full-bodied, and more fluid. Data are presented below in Table 17.

TABLE 17

RANKING OF APPLESAUCE BY MEANS OF PAIRED COMPARISONS

Sitting No.	Dose and Total Score for each Dose				P	Significant Difference?
1	0 31	1.0×10^6 23	1.5×10^6 27	2.0×10^6 27	0.15	No
2	0 29	1.0×10^6 29	1.5×10^6 22	2.0×10^6 28	0.13	No
3	0 29	1.0×10^6 26	1.5×10^6 26	2.0×10^6 27	0.87	No
4	0 30	1.0×10^6 25	1.5×10^6 26	2.0×10^6 27	0.58	No

(8) Naval Oranges: Ranking by Means of Paired Comparisons. A bitterness factor in the juice of California navel oranges has limited the use of this juicd. Some correspondence with the Sunkist Growers indicated that there was

reason to believe that low dosages of irradiation might change or destroy the bitterness factor. Some exploratory taste-panel tests were therefore made. Whole oranges (Sunkist, Navel 126's) were given dosages of irradiation from 1.5×10^3 to 1.2×10^5 rep. At least six oranges were used for each sample and additional whole oranges were set aside for controls. After iradiation, and just before the taste-panel tests were held, the oranges were squeezed by hand using a Foley juicer. The juice obtained from the oranges given dosages of irradiation from 1.5×10^4 to 6.0×10^4 rep was very definitely preferred by the taste panel over the juice from the control oranges and those given greater dosages. The oranges given greater dosages produced a juice more bitter than the juice from the controls.

As these initial tests were encouraging, more Navel Sunkist 126 oranges were obtained, and given dosages of irradiation from 3.0×10^4 to 2.0×10^5. After irradiation, juice was prepared from the oranges and they were squeezed using the method described. The oranges used for the second experiment were found to be entirely different from those used previously. With dosages up to 100,000 rep there was no preference for either irradiated or nonirradiated, and none of the orange juice had the bitterness associated with the first lot of Navel oranges. The control orange juice was preferred to juice obtained from oranges given dosages of irradiation higher than 1.0×10^5 rep.

A third experiment was tried with more Sunkist Navel 126 oranges. The same preparation procedures were followed. The dosages of irradiation given were 3.0×10^4, 6.0×10^4 and 1×10^5 rep. This time the taste-panel members seemed to prefer the control juice more often than the irradiated. However, the oranges were like those used in the second experiment and all the juice was again free of the bitterness factor.

It may be stated that navel oranges subjected to gamma-ray doses of the order of magnitude used in these experiments undergo a change in flavor. Further investigation will be necessary, however, in order to determine if there exist optimum levels of radiation which result either in no flavor change or in flavor changes resulting in new flavors preferable to the nonirradiated flavor. The results of these experiments appear in Table 18.

Because of this observed difference in the amount of bitterness from separate lots of oranges, the tests were halted and results were discussed by correspondence with the Sunkist Growers in California. According to Baier[32] Navel oranges eaten out of the hand and freshly extracted orange juice have no bitter taste, even if the oranges are immature and noticeably sour. The bitter taste develops only after the juice stands. Juice from early-season Navel oranges of root stock susceptible to bitterness development may require a storage period at room temperature of one hour or more before bitterness can be detected; but extracted juice from midseason fruit may require storage overnight before

TABLE 18

RANKING OF NAVEL ORANGE JUICE
BY MEANS OF PAIRED COMPARISONS

Sitting No.	*Total Scores For Each Dose				P	Significant Difference?
1	0 35 0.01	1.5×10^3 22 0.48	3.0×10^4 28 0.12	6.0×10^4 23 0.38	<0.01	Yes
2	0 24	8.0×10^4 29	1.0×10^5 25	1.2×10^5 30	0.26	No.
3	0 22	1.5×10^3 30	3.0×10^4 27	6.0×10^4 29	0.10	No
4	0 26 0.24	8.0×10^4 23 0.43	1.0×10^5 34 0.04	1.2×10^5 25 0.29	0.01	Yes
5	0 27	3.0×10^4 24	6.0×10^4 27	1.0×10^5 30	0.39	No
6	0 21 0.62	1.2×10^5 30 0.10	1.6×10^5 26 0.21	2.0×10^5 31 0.08	0.02	Yes
7	0 21 0.61	3.0×10^4 25 0.25	6.0×10^4 31 0.07	1.0×10^5 31 0.07	0.00	Yes

*Where a significant difference exists, estimates of the treatment ratings are displayed below the total scores.

bitterness is detectable. The bitter factor is the lactone form of limonin. The method used for extracting limonin has not been very reliable.

As the original tests appeared promising, some additional tests should be made using oranges known to be of root stock susceptible to bitterness development and storing the juice for sufficient periods for bitterness to develop.

b. Tests with Meats

(1) Canned Beef: Ranking by Means of Paired Comparisons. A good grade of Sirloin roast beef was obtained from the Food Service Department of the University of Michigan. This beef was canned using the raw-pack canning procedure described in Home and Garden Bulletin No. 6, Revision of AWI-110, USDA, for beef packed in No. 2 cans. The canned beef was given the following dosages of irradiation: 1×10^5, 3×10^5, 5×10^5, 6×10^5, 7×10^5, 8×10^5, 1.0×10^6, 1.2×10^6, 1.4×10^6, 1.6×10^6, 1.8×10^6, and 2.0×10^6 rep. Several cans of beef were also set aside for controls.

The statistical treatment of the taste-panel results may be found in Table 19. Canned beef could be given between 1.0×10^6 and 1.2×10^6 rep without affecting texture or flavor; but at dosages higher than 1.2×10^6 the lean meat became softer and more stringy, whereas the fat, although still white, was almost completely liquefied. The fat in the cans of beef given high dosages of irradiation, 1.6×10^6 to 2.0×10^6 rep, rose to the top of the cans; whereas the fat in the cans of control beef was distributed throughout the can. This phenomenon has not been explained.

(2) Canned Pork: Ranking by Means of Paired Comparisons. Stew-size pieces of lean loin of roast pork were obtained from Food Service. The pork was canned using the canning procedure for raw pork in No. 2 cans described in Home and Garden Bulletin No. 6, Revision of AWI-110, USDA. The canned pork was given the following dosages of irradiation: 2×10^5, 4×10^5, 6×10^5, 8×10^5, 1.0×10^6, 1.2×10^6, 1.4×10^6, 1.6×10^6, and 1.8×10^6 rep. Several cans of pork were set aside for controls. The taste-panel results for canned pork were similar to those for canned beef. At radiation dosages between 1.2×10^6 and 1.4×10^6 rep, definite changes in texture and flavor start to take place. Only a few taste-panel sittings were held on irradiated canned pork; thus the data are of limited value statistically.

(3) Ground Raw Pork: Ranking by Means of Paired Comparisons. As reported by H. J. Gomberg and S. E. Gould in Progress Report No. 4, a dose of 20,000 rep is adequate to prevent the maturation and reproduction of encysted trichinae. However, only limited data on the flavor of irradiated raw pork were available.

TABLE 19

RANKING OF CANNED BEEF BY
MEANS OF PAIRED COMPARISONS

Sitting No.	*Dose and Total Score for Each Dose				P	Significant Difference?
1	0 22	$1.0x10^5$ 20	$3.0x10^5$ 26	$5.0x10^5$ 22	-0.31	No
2	0 19	$6.0x10^5$ 21	$7.0x10^5$ 25	$8.0x10^5$ 25	0.17	No
3	0 25	$1.0x10^6$ 26	$1.2x10^6$ 31	$1.4x10^6$ 26	0.33	No
4	0 22	$1.6x10^6$ 25	$1.8x10^6$ 22	$2.0x10^6$ 21	0.69	No.
5	0 15 0.46	$5.0x10^5$ 16 0.33	$8.0x10^5$ 18 0.18	$1.0x10^6$ 23 0.03	0.02	Yes
6	0 22 0.21	$5.0x10^5$ 23 0.17	$8.0x10^5$ 18 0.56	$1.0x10^6$ 27 0.06	0.05	Yes
7	0 23	$1.6x10^6$ 26	$1.8x10^6$ 31	$2.0x10^6$ 28	0.15	No
8	0 21 0.62	1.6x10 27 0.17	1.8x10 32 0.06	$2.0x10^6$ 28 0.14	0.01	Yes

*Where a significant difference exists, estimates of the treatment ratings are displayed below the total scores.

To determine whether or not the flavor of pork is affected by the radiation dosages necessary to break the trichinosis cycle, ground lean loin pork of uniform quality was obtained from Food Service. Ground meat has a shorter refrigerator shelf-life than standard meat cuts and generally spoils within a few days. Therefore, ground meat was considered to be the most severe test for pasteurization.

(a) First Series of Tests on Pork: In the first series of tests with ground pork, relatively lean loin of pork was obtained from the butcher shop in the Food Service Building of the University of Michigan and was put through a conventional meat grinder twice. The ground pork was divided into four portions. One portion was stored in closed glass containers in a refrigerator at 40°F. The other three portions were placed in closed glass containers and irradiated in the radiation cave at approximately 45°F with dosages of 30,000, 60,000 and 100,000 rep respectively, after which they were placed in the refrigerator with the controls.

The meat was prepared for tasting by the director of the taste-panel tests, who is trained in the art of cooking and experienced in the evaluation of foods for the food industry. The ground pork was made into patties and fried slowly in a neutral hydrogenated vegetable shortening until well done. Each sample was fried separately and care was taken to use the same degree of cooking in each case. The observation was made that the irradiated samples had a slightly different odor, most noticeable when the glass containers were first opened. However, with the low radiation dosages used, this odor was not objectionable and disappeared on cooking. The raw irradiated meat had a slight color change, appearing to lose some of its redness immediately after irradiation and the outer surfaces of the raw ground pork in particular developed a grayish tinge. The cooked meat had no off color or off flavor.

Table 20 shows the statistical analysis of the first pork-storage experiment; no significant difference in flavor preference was detected by the panel until the fifth day of storage, at which point the control was definitely inferior to the irradiated food, especially to that given the higher doses. Fresh control samples were substituted for the remaining sittings. After eight days of storage there appeared to be no significant difference in preference between the fresh control and the pork given the two highest doses, but the 30,000-rep sample appeared to have developed an undesirable flavor. Therefore, based on these data it is concluded that ground pork given a dose of irradiation between 60,000 and 100,000 rep will keep for eight days at 40°F and perhaps longer.

(b) Second Series of Tests on Pork: A second series of taste-panel tests was made with ground pork as before except that a fifth portion of the ground pork was frozen and set aside for use as the control after spoilage of

TABLE 20

RANKING OF PORK BY MEANS OF PAIRED COMPARISONS

(FIRST SERIES OF TESTS)

Sitting No.	Storage Time (Days)	*Dose and Total Scores for Each Dose				P	Significant Difference?
		0	3.0×10^4	6.0×10^4	1.0×10^5		
1	1	26	30	24	28	0.37	No
2	1	26	26	29	27	0.87	No
3	3	22	23	20	25	0.49	No
4	3	25	18	23	24	0.12	No
5	5	36 0.00	29 0.04	21 0.55	22 0.41	Less Than 0.01	Yes
6	5**	30	30	25	23	0.11	No
		23 0.04		17 0.27	14 0.69	0.01	Yes
7	8	21 0.28	28 0.05	19 0.45	22 0.22	0.02	Yes
			20 0.00	12 0.60	13 0.40	0.01	Yes
		14		14	17	0.61	No
8	8	20	19	15	18	0.40	No

*Where significant differences exist estimates of the preference ratings are displayed below the total scores.

**At this point the control food was definitely spoiled; hence frozen control samples were used for the remaining sittings.

the raw control. Irradiation dosages of 30,000, 60,000, and 100,000 rep were also used in this experiment. The data treated statistically are shown in Table 20. Some variations in the keeping quality of ground meat were expected because of differences in the age of meat obtained from the butcher shop and differences in initial contamination with microorganisms. The control spoiled on the third day; subsequently the three treatment levels did not develop significant differences in preferences as compared to fresh control through the tenth day of storage (at which time the supply of experimental material was exhausted).

Additional tests with pork should be made, including studies on irradiated pork fat and on various fat-meat mixtures. Some tests should also be made with standard cuts of pork such as chops and roasts. The tests to date indicate that packaged and irradiated fresh pork ground at the packing house might be expected to have a refrigerator storage life of around two weeks using a radiation dose between 60,000 and 80,000 rep. Although no tests have been made with standard cuts of pork, it is expected that the refrigerator shelf-life would be as long or longer than that of ground meat because of freedom from contamination by microorganisms below the surface.

(4) Raw Ground Beef: Ranking by Means of Paired Comparisons. Ground beef was used for the next series of tests. An attempt was made to prepare a typical hamburger mixture. Ground round of beef with about 25 percent fat was obtained from Food Service and passed through the grinder twice. The ground meat was divided into five portions and the same procedure was used as in the second series of experiments with pork. The irradiation dosages used were 50,000, 80,000, and 110,000 rep.

Unfortunately, the first panel tests indicated that this entire batch of ground meat did not possess the typical flavor; but the reason was not known. Therefore, there may be some question as to the significance of these tests, but they were continued with the idea of repeating the tests with another batch of ground beef. The results of this storage experiment appear in Table 21. The panel showed no decided preference between the raw control and the irradiated sample stored at refrigerator temperature for one through four days. The supply of raw control was exhausted after four days and the frozen control was used thereafter. Spoilage apparently was not detected by the panel in the raw control after four days storage, which was contrary to expectation. Food Service uses ultraviolet lamps in the cold-storage rooms for beef, which may explain the long refrigerator life of the raw ground beef and possibly the flavor of this batch of ground beef.

After eleven days of storage the panel detected a significant difference between the fresh control and the treated samples, as shown in Table 21. Also, it appeared to most panel members that the raw ground beef given 5×10^4 and 8×10^4 rep had developed decided off flavors. However, these off flavors were not

TABLE 21

RANKING OF PORK BY MEANS OF PAIRED COMPARISONS

(SECOND SERIES OF TESTS)

Sitting No.	Storage Time (Days)	*Dose and Total Scores for Each Dose				P	Significant Difference?
		0	3.0×10^4	6.0×10^4	1.5×10^5		
1	1	29	24	28	27	0.58	No
2	1	24	30	27	27	0.39	No
3	3*	23	22	26	19	0.20	No
4	3	20	27	19	24	0.05	?
5	5	30	23	27	28	0.24	No
6	5	30	24	26	28	0.37	No
7	6	28	18	16	20	0.61	No
8	6	21	26	22	21	0.36	No
9	7	29	24	29	26	0.44	No
10	7	26	27	32	23	0.07	No
11	10	22	27	21	20	0.12	No
12	10	21	26	21	22	0.36	No

*At this point the control food was definitely spoiled; hence frozen control samples were used for the remaining sittings.

so evident in the beef given 5 x 10^4 and 8 x 10^4 rep and stored for fourteen days, as shown in Table 22. Again there was a decided preference for the non-irradiated frozen ground beef, but the irradiated beef given 5 x 10^4 rep had a good flavor and was definitely better liked than the beef given 8 x 10^4 and 1.1 x 10^5 rep. The beef given the highest dosage of irradiation was rancid to most of the panel members after fourteen days of storage. Thus the fourteen-day storage results do not agree with the eleven-day storage data.

One day after irradiation the color of the irradiated ground beef samples was still a bright red, whereas the color of the nonirradiated samples had changed to a medium reddish brown. After the second day color changes were noticeable in the irradiated samples. The outer surface of the ground beef given 5 x 10^4 and 8 x 10^4 rep became progressively browner throughout the 14 day storage period; the inner portions remained a medium reddish brown. This was not true of the sample given 1.1 x 10^5 rep which retained its bright red color through five days of storage. After the fifth day the outer surfaces of the sample started to turn brown, but even after eleven days this sample had more red color than the samples given 5 x 10^4 and 8 x 10^4 rep. The color changes observed in irradiated beef do not conform to what had happened to irradiated fruits and vegetables. For example, the outer and inner portions of apples and peaches became progressively browner with increasing doses of irradiation.

(5) Raw Chicken Legs and Thighs: Ranking by Means of Paired Comparisons. Cut-up whole frying chicken and chicken breasts and legs have become a popular item in meat markets, but the refrigerated storage life of cut-up chicken is appreciably shorter than that of undrawn fowl. Legs and thighs of frying chicken were obtained from Food Service for the first series of tests with chicken. The legs were divided into five portions as in the previous tests; one portion was used as a raw control, a second portion was used as a frozen control, and the other portions were given radiation dosages of 80,000, 150,000 and 200,000 rep.

In cooking the chicken, the legs were separated from the thighs and then simmered in slightly salty water until tender. No preference was shown by the panel through eight days of storage. After the eighth day of storage, the control chicken had developed a very obnoxious odor and flavor even though this chicken was stored in the frozen state. Without good control chicken for a standard of comparison with the irradiated chicken, it was difficult for the judges to come to any conclusion about the flavor of the irradiated chicken. The statistical treatment of the taste-panel results is presented in Table 23. There was no significant difference between the nonirradiated and irradiated chicken.

The chicken legs and thighs used for this experiment were received by Food Service in the frozen condition and it is considered that this treatment

TABLE 22

RANKING OF BEEF BY MEANS OF PAIRED COMPARISONS

Sitting No.	Storage Time (Days)	*Dose and Total Scores for Each Dose				P	Significant Difference?
		0	5.0×10^4	8.0×10^4	1.1×10^5		
1	1	22	20	25	23	0.49	No
2	1	26	29	28	25	0.70	No
3	4	22	22	22	24	0.94	No
4	4	23	20	25	22	0.49	No
5**	7	29	24	28	27	0.58	No
6	7	27	25	25	31	0.29	No
7	11	18 0.47	28 0.03	26 0.02	18 0.47	0.0004	Yes
8	11	20 0.71	34 0.02	29 0.08	25 0.19	0.0002	Yes
9	14	19 0.83	23 0.17	33 -	33 -	Less Than 0.0001	Yes
10	14	22 0.49	23 0.39	31 0.07	32 0.05	0.002	Yes

*Where significant differences exist estimates of the preference ratings are displayed below the total scores.

**Frozen control was substituted at this point.

may have had some effect on our experimental results, even though care was taken to see that the chicken used for frozen controls did not thaw before it was used. In future experiments fresh chicken of the same breed, age, and weights should be used. The keeping quality and flavor of refrigerated irradiated legs, which have a tight covering of skin, should be compared with those of the breasts, which only have an outside layer of skin.

TABLE 23

RANKING OF CHICKEN BY MEANS OF PAIRED COMPARISONS

Sitting No.	Storage Time (Days)	Total Scores for Each Dose				P	Significant Difference?
		0	8.0×10^4	1.5×10^5	2.0×10^5		
1	1	29	23	27	29	0.29	No
2	1	27	26	27	28	0.99	No
3	4	26	24	30	28	0.37	No
4	8	23	30	26	29	0.18	No

5. DISCUSSION

Taste panels using the triangle test with fresh fruit exposed to gamma radiation were difficult to conduct, as the judges were influenced by changes in appearance and texture. The dark red color of cherries was somewhat bleached by higher doses of irradiation, and the cherries were softer and juicier; the flavor of cherries was not affected by irradiation, however. With peaches, changes in texture were not so noticeable, but the flavor, although not objectionable, resembled peaches flavored with almond extract. Peaches were the only fruit tested where a significant difference in flavor existed between the nonirradiated and irradiated samples, as shown in Table 16.

Plums and cantaloupe exhibit texture and flavor changes after irradiation; but the flavor of both was not too well liked by the taste-panel judges. Color and texture changes of irradiated apples, and applesauce made from irradiated apples, were visibly apparent to the judges; therefore, it was found necessary to irradiate cooked applesauce. The activity of enzymes responsible for color changes was prevented by cooking. Although the triangle

tests revealed that irradiation produced a change in flavor at a level detectable by the panel members, the results of the preference tests showed that there was no significant difference between the flavor of irradiated and nonirradiated applesauce To some of the judges the irradiated applesauce tasted sweeter and more full-bodied. It was though that the results obtained with taste panels conducted using the preference test were more reliable, since the judges were not as concerned about being correct in their selection of the irradiated or odd sample as they were when using the triangle test.

The results obtained with Navel oranges were inconclusive due to the fact that it was not recognized that bitterness would develop on standing to such an extent that it could be tasted Low doses of irradiation may produce desirable flavor changes in the Navel orange juice that has been extracted from fruit of root stock susceptible to bitterness development. Storage of the extracted juice for varying lengths of time before and after irradiation should indicate whether gamma radiation has any effect on the bitterness factor, the lactone form of limonin.

Results of the refrigerator storage experiment with whole irradiated peaches packaged in polyethylene and saran were not successful. Control peaches in saran looked like fresh peaches after two months of storage, and none of the irradiated peaches had as good an appearance as the controls. Peaches given 5×10^5 and 1×10^6 rep still had an almond flavor after storage for two months, but this is not true of peaches given 1.5×10^6 and 2.0×10^6 rep, which were flat and flavorless. Peaches packaged in polyethylene, both irradiated and non-irradiated, were very unpalatable due to fermentation and overripening.

More successful results were obtained with sliced peaches stored in syrup Without evacuation the control peaches and peaches given 5×10^5 and 1×10^6 rep fermented in a short period of time; this, however, was not true of peaches given 1.5×10^6 and 2.0×10^6 rep. The latter peaches retained a good color and flavor after four months of storage at room temperature.

Some preliminary work was done on the irradiation of canned roast sirloin beef and canned loin pork. It was thought that a combination of heat and irradiation might not have as drastic an effect on the texture of meat as the use of either heat or radiation alone and that lower doses of irradiation might produce less change in flavor of the meat. It was found that canned beef could be given about 1×10^6 rep without affecting texture or flavor. Similar results were found for canned irradiated pork.

Interest in the pasteurization of raw ground pork was prompted by the finding of H. J Gomberg and S. E. Gould that 20,000 rep of gamma radiation could break the trichinosis cycle. Taste-panel tests were conducted using irradiated pork because little previous work had been done on the flavor of pork. Along

with the tests on flavor, irradiated and nonirradiated pork was stored at refrigerator temperature, 40°F, since it was hoped that low doses of irradiation might prolong the storage life of refrigerated meat without undesirable flavor changes. Results obtained show that the storage life of gamma ray pasteurized pork given a dose of 60,000 to 80,000 rep can be lengthened to ten days at refrigerator temperature and possibly longer. Also it was found that no noticeable flavor change occurred in this irradiated pork within that period.

The method for the pasteurization of raw ground beef with low doses of irradiation was similar to the pork experiment. However, beef was given slightly higher doses of irradiation. Ground round of beef with 25 percent fat was obtained from Food Service, where ultraviolet lamps are used in their cold-storage rooms. The use of ultraviolet lamps may have had an undesirable effect on the flavor of beef which might not have been present if freshly killed beef had been used. Further, there was no control on the age and quality of the beef. In future experiments beef should be obtained as fresh as possible in order to obtain more consistent results. In the beef experiment reported, taste-panel results for beef eleven days old did not confirm the results for beef fourteen days old; but at both times the flavor of the fresh frozen control was better than that of the irradiated beef. Further tests will be required to determine whether or not it is possible to store irradiated beef at refrigerator temperature any longer than eight days. However, nonirradiated beef cannot be kept in a refrigerator longer than about four days; hence it appears evident that the refrigerator shelf-life of beef has been at least doubled by pasteurization.

The work reported on chicken is not conclusive because the chicken legs and thighs received from Food Service were in the frozen condition and of undetermined age. No significant differences in flavor were observed in a comparison of irradiated and nonirradiated chicken stored up to four days at refrigerator temperature. Fresh chicken of known age, weight, and breed subjected to pasteurizing dosages of gamma irradiation may possibly have a longer storage life.

6. SUGGESTIONS FOR FUTURE WORK

It is planned to undertake more research on the pasteurization of beef, pork, and chicken, including a study of ground pork and beef and pasteurization of different cuts of pork and beef such as chops, steaks, and roasts. Organoleptic tests have shown that pasteurized ground pork may be kept for ten days or longer in the refrigerator; it is planned therefore, to supplement and substantiate this finding by bacterial counts at definite intervals. Studies on canned pork and beef will also be extended. Different cooking times will be combined with various doses of irradiation.

The effects of gamma irradiation on different varieties of wheats used for making bread flour will be studied by incorporating the flour milled from bread wheat into recipes specifying cake or pastry flour.

7. CONCLUSIONS

The taste-panel studies have been shown to be a sensitive and statistically reliable method of determining flavor changes and flavor preferences in food subjected to gamma irradiation. Ranking by means of paired comparisons was found to give more reliable results than the triangle test.

In the taste-panel experiments on fresh fruits the most promising results were obtained with fresh peaches and dark sweet cherries, for their flavors were the least affected by gamma irradiation. The flavor of cooked applesauce was not changed by irradiation. Studies with Navel oranges indicated that low irradiation doses may improve the flavor of orange juice which has been extracted from fruit of undesirable root stock.

Saran was found to be a very good material for maintaining nonirradiated peaches fresh. Nonirradiated peaches packaged individually in saran kept for 2 months at refrigerator temperature, 40°F. Peaches sliced in a 40° Brix syrup and 0.1% ascorbic acid and irradiated with 1.5×10^6 and 2.0×10^6 rep kept for four months at room temperature without changes in texture and flavor and since no evacuation equipment was necessary, this may be a new method of preserving fruit.

Experiments on irradiated canned beef and pork showed that radiation dosages of about 1×10^6 rep or less do not significantly alter the flavor and texture of canned beef and canned pork. Experiments with irradiated ground raw pork showed that ground lean pork given a radiation dose of 60,000 rep will keep ten days, or possibly longer whereas nonirradiated pork will keep only about four days when stored at approximately 40°F. Also, nonirradiated ground beef will keep three or four days at 40°F, but irradiated ground beef given a dose between 70,000 and 90,000 rep will keep for eight days and possibly longer. This indicates a promising possible future for pasteurization of prepackaged meat by gamma irradiation at the packing plants, a new method which should appreciably increase the shelf-life of refrigerated prepackaged fresh meat.

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B. RADIATION PASTEURIZATION OF FRESH FRUIT

Personnel:

L. E. Brownell, Associate Professor of Chemical Engineering and Director of Fission Products Laboratory, J. V. Nehemias, Assistant Director of Fission Products Laboratory, Health Physicist and Research Associate.

Miss H. A. Harlin, Research Assistant and Food Chemist.

1. INTRODUCTION

If the radiation dosage is sufficient, it is possible to achieve complete sterilization of foods. (See Kempe, L. L., et al., page 222 of this report.) Certain very real problems remain to be solved however before the process can be applied to commercial production.

First, it must be clearly demonstrated that no toxins, i.e., induced radioactivity, toxic radiation degradation products, or carcinogens are produced by the required dose of radiation, and that no dietary deficiencies are caused by component degradation. Long-term animal-feeding experiments are now in progress in this laboratory. In the pilot studies of the program there were no indications of any deficiencies or toxic products of radiation in the completely irradiated diet (2,000,000 rep) fed exclusively to successive generations of rats. Long term feeding and breeding experiments are being continued using 124 parent albino rats.

Second, general palatability must be maintained; that is, the product must be acceptable to the public. Presumably a certain amount of flavor change would be permitted. Canned produce definitely tastes different from fresh produce and yet has achieved wide public acceptance. Taste-panel analyses are now underway in this laboratory to evaluate the degree of flavor change and also to study preference ranking as a function of dose. The results to date vary sharply from product to product. With cherries, for example, even the lowest doses produced detectable differences, mostly in texture; however, the changes were not always considered undesirable.

Third, the process must be economically feasible; that is, radiation-pasteurized produce, although possibly a premium product, must compete generally with other produce on the market. No detailed cost analyses are yet available, because the cost and availability of the large amounts of radioactive materials required have yet to be determined. An analogous study, however, has been undertaken at this laboratory to evaluate the economic feasibility of irradiating 2000 hogs per day with 30,000 rep to control trichinosis. Based on the best cost data available, such a plant could be operated for substantially less than one-half cent per pound of marketable pork. Sterilization doses, however, are 100 times as high as those required for trichinosis control, and the economic feasibility of radiation sterilization of low-unit-cost produce is still an open question.

If, on the other hand, it is possible to "pasteurize" at lower doses, that is, to inhibit mold formation, fermentation, etc., so that a substantial increase in shelf-life results, all three of these problems become proportionately less difficult to solve. Experiments have, therefore, been undertaken to determine the degree of pasteurization as a function of dose and its dependence on container material and storage temperature.

2. PROCEDURE

a. Selection

Peaches and cherries for the experiment were purchased during the produce season on the open market through the facilities of the University Food Service. Samples selected for each experiment were made as representative as possible of the lot from which they were selected with reference to size, color, and degree of ripening. All bruised or visibly damaged fruit was rejected. Stems were left intact whenever possible. Thus, any differences in the rate of mold formation which appear as a function of dose should be attributable to differences in treatment.

b. Packaging

Each cherry and peach used in the course of this work was individually packaged for the dual purposes of yielding meaningful statistics on the molding characteristics of individual fruit and of preventing cross contamination. Three different packaging materials were used to determine the effect of container properties on molding characteristics. Polyethylene bags,* heat-sealed at this laboratory, provide a water-tight gas-permeable container. Saran bags,**

*Purchased from the Visking Corporation.
**Courtesy of Dow Chemical Company, Plastics Technical Service.

electronically sealed at the Dow Chemical Company plant, provide a water-tight relatively gas-tight container. Lusteriod tubes* stoppered and wax-sealed at this laboratory, also provide a water-tight gas-tight container, but with a somewhat different geometry. Lusteroid contains a chlorine-bearing component.

It was desired to compare these three types of barriers with reference to their possible effect on rate of mold formation subsequent to radiation treatment. No attempt was made to evaluate the packaging material on any other basis. A spot-check sample of the finished packages was subjected to a water leak test. As no faulty packages were discovered, it is assumed that the proportion of leaky bags used in the experiment was small enough to be considered negligible.

c. Treatment

As soon as possible after packaging, the samples were irradiated, dosage varying in steps of 200,000 from 0 to 2,000,000 rep. Irradiation at all levels was conducted concurrently as far as possible. This can be done in the 10,000-curie gamma cave by irradiating simultaneously at different dosage rates. From fifteen to thirty samples were irradiated for each experiment at each dose to reduce the effect of random variations in fruit and mold on the total data. The actual number used in a particular experiment was determined by the packaging material, fruit, manpower for sealing, irradiation volume, and storage space available.

d. Storage

Following irradiation the fruit was immediately stored, either at room temperature (70°F) or at refrigerator temperature (40°F), in such a way as to facilitate visual inspection. The stored fruit was then inspected daily for the appearance of mold. Whenever mold was first detected on a sample of fruit, that sample was removed and destroyed. No other criterion was used in this series of experiments. The effects of radiation on texture, color, odor, and taste are being studied in separate experiments.

3. RESULTS

Figure 31 is a plot of the percent of cherry samples remaining without visible mold formation, after 5, 10 and 20 days of storage at room temperature as a function of the radiation dose received. Approximately 50 percent of the

*Purchased from International Equipment Company.

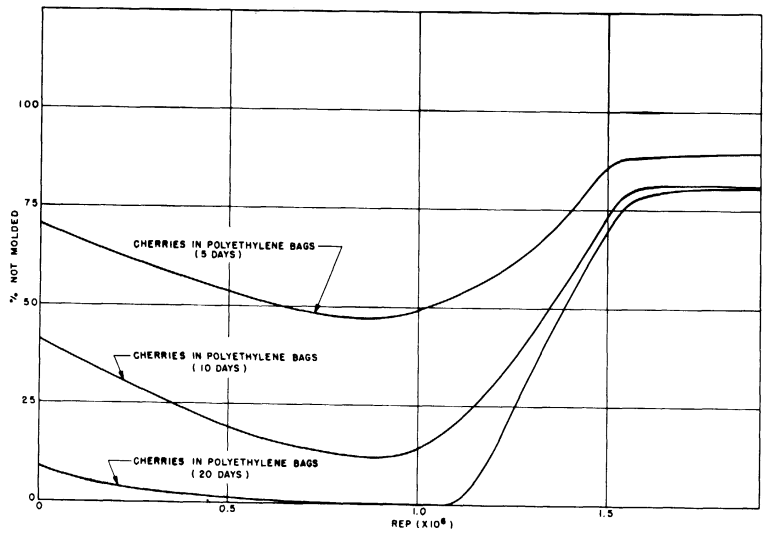

Fig. 31. Percent of Cherries Stored at Room Temperature Which Did Not Show Mold Formation, as a Function of Dose Received after Various Storage Periods.

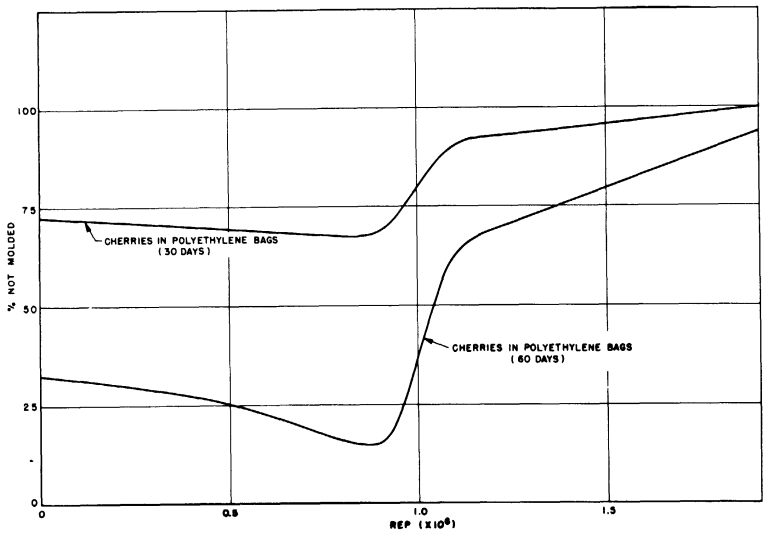

Fig. 32. Percent of Cherries Stored at Refrigerator Temperature Which Did Not Show Mold Formation as a Function of Dose Received after Various Storage Periods.

unirradiated (control, 0-rep) samples in this experiment had molded after 8 days. At doses above 1.5×10^6 rep a substantial majority of the cherries were still free of mold even after 20 days at room temperature. At doses below 1×10^6 rep, however, the irradiated fruit actually molded more rapidly than the unirradiated fruit.

Figure 32 is a similar plot for cherries stored at refrigerator temperature. These curves are similar in form to those for storage at room temperature, but of course molding occurred much more slowly at the lower temperature. Again it is observed that for doses less than 1×10^6 rep the rate of mold formation is greater than for untreated fruit. Figure 33 is a photograph of these cherries after 90 days of refrigeration. The column of cherries on the extreme right has received 2×10^6 rep, the second column from the right 1.8×10^6 rep, and so on across the board to the extreme left column, which was untreated. Each column was initially of equal length and cherries were removed as soon as visible mold formation occurred. Thus the cherries shown in the photograph had not developed mold after 90 days. A line through the tops of these columns can be compared with the curves in Fig. 32 for an indication of how much molding took place between 60 and 90 days.

Figure 34 illustrates the comparison between polyethylene bags and lusteriod tubes for storage at room temperature. Much less mold occurred on the samples in lusteriod tubes than in polyethylene bags at all dosages. In fact, the rate of mold formation on cherries in lusteriod tubes was, over much of the range studied, approximately independent of dose. It is further seen by comparing the bottom two curves of Fig. 34 that the observed molding phenomena for cherries are approximately duplicated by peaches.

Figure 35 clearly demonstrates the marked difference in molding characteristics displayed by fruit in polyethylene bags and fruit in saran bags, both at refrigerator temperature and at room temperature. It is interesting to note that the unirradiated samples in saran bags stored at 40°F actually molded more slowly than the treated samples, even up to 2×10^6 rep.

4. CONCLUSIONS

These studies indicate that at least 1×10^6 rep are required to effect any marked decrease in the rate of mold formation on fresh fruit. Lower doses, in fact, have the effect of increasing the rate of mold formation.

If, increasing the radiation dosage decreases the number of organisms present, an apparent anomaly exists. One explanation might be that radiation causes a change in the fruit, either physical or chemical, which enables the mold to grow more readily. This effect at lower doses apparently stimulates

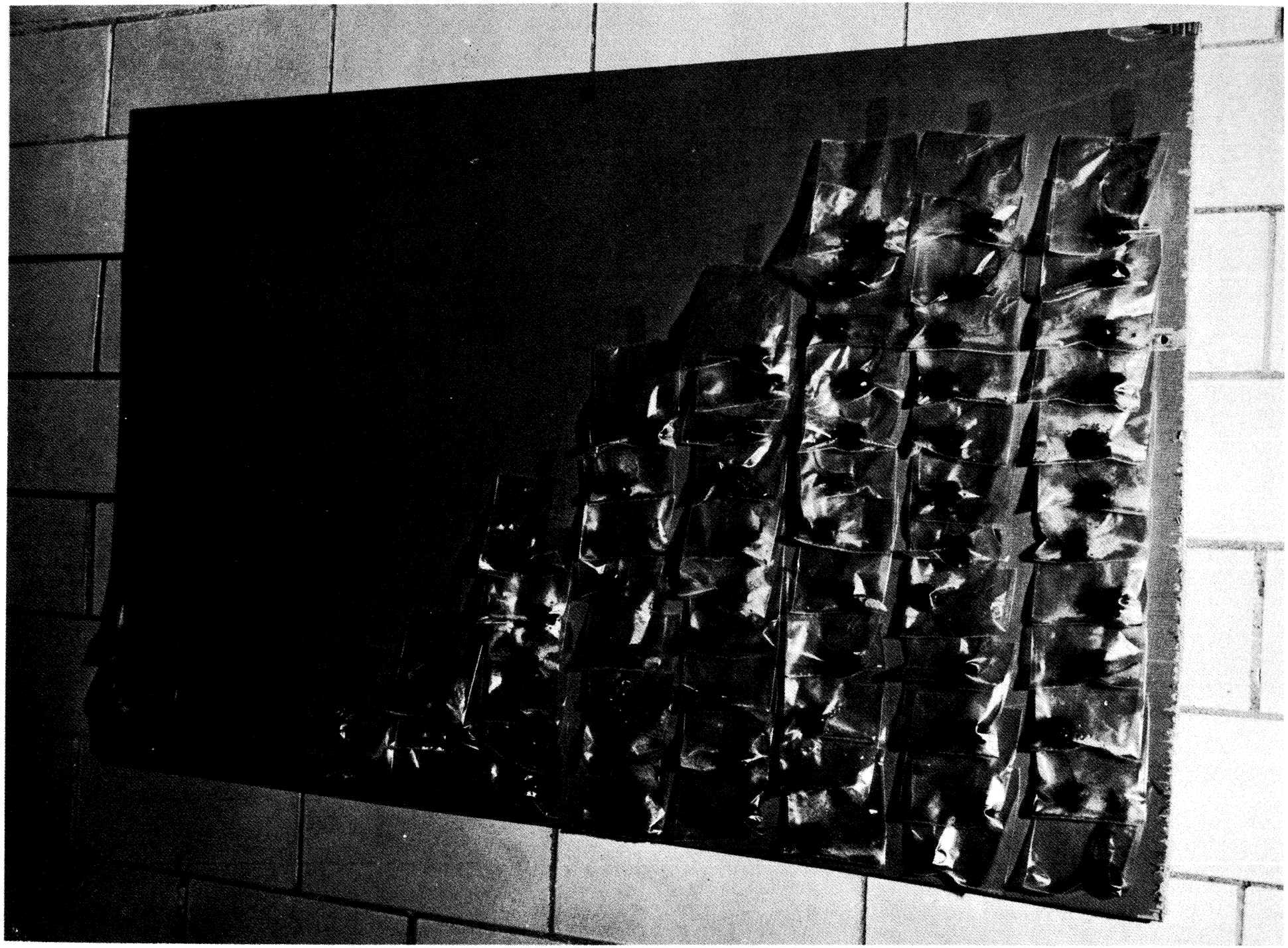

Fig. 33. Number of Cherries Left Unmolded after 90 Days Cold Storage. Dosage Varies in Steps

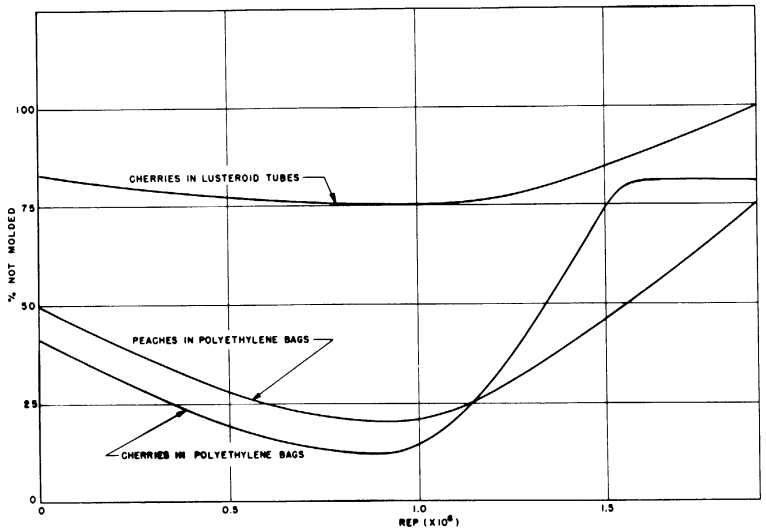

Fig. 34. Percent of Peaches and Cherries at Room Temperature Which Did Not Show Mold Formation in Polyethylene Bags and Lusteroid Tubes as a Function of Dose Received after Various Storage Periods.

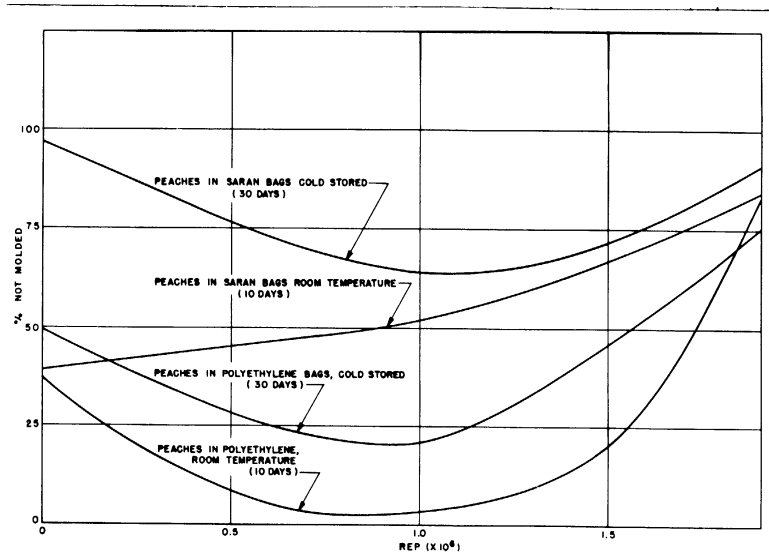

Fig. 35. A Comparison of Storage Properties of Peaches in Saran Bags and Polyethylene Bags at Room Temperature and Refrigerator Temperature.

growth rates enough to more than compensate for the fact that fewer organisms are present initially. Finally, of course, at higher radiation doses the probability of killing completely all mold organisms on a particular sample of fruit increases, and more and more samples will be free of mold formation regardless of growth-promoting factors. At doses above 1×10^6 rep under most of the experimental conditions studied, radiation definitely retarded mold formation, as compared to untreated samples stored at both 40°F and 70°F.

At doses above 1.5×10^6 rep undesirable changes have been observed in most fruit, independent of the rate of mold formation. Peaches turn brown; cherries become "mushy"; undesirable "off" flavors develop. Other experiments are in progress to evaluate these effects and their relation to the dose received, and to seek means of preventing their occurrence.

Differences in the rate of mold formation in various container materials seem to depend primarily on the relative efficiency of the materials as gas barriers, the better gas barrier apparently inhibiting mold formation more efficiently.

5. SUMMARY

In summary, "radiation pasteurization" of fresh fruit, i.e., an effective increase in the satisfactory storage life of the fruit with a radiation treatment substantially less than that required for sterilization, is possible but only at doses greater than 1×10^6 rep. At these doses certain undesirable changes occur in the fruit which must be studied further. Those packaging materials which present the best gas barrier appear best for inhibition of mold growth.

C. PROPOSED NEW METHOD OF WHOLESALING FRESH MEAT

Personnel:

L. E. Brownell, Associate Professor of Chemical Engineering and Director of Fission Products Laboratory; J. V. Nehemias, Assistant Director of Fission Products Laboratory, Health Physicist and Research Associate; and R. C. Dennis, Mathematician and Resarch Assistant.

1. INTRODUCTION

A new method of wholesaling fresh meat is proposed for consideration by some of the larger packing houses and some of the larger retailers of fresh meat. This proposed new method consists of preparing packaged standard cuts of fresh meat, packaged fresh ground meat, packaged cut-up chicken, etc. in retail-size portions at the packing house rather than at the retail meat market and of pasteurizing the packaged meat at the packing house by means of a relatively small dose of gamma radiation prior to shipping to the retailer. Radiation pasteurization extends the refrigerator shelf-life of fresh meat, which should make this new method feasible. Some of the reasons for considering this method of handling meats are the recent trends in retailing meats and the economics involved.

a. Recent Trends in Retailing Meat

The meat departments of many supermarkets have found a consumer preference for purchasing weighed and packaged cuts of meat, cut-up chicken, ground meat, etc. One manager of a local supermarket who was consulted on the subject stated the store under his management had doubled its meat sales in about one year after installing prepackaging of meat. This method of merchandizing is popular with the majority of the customers probably because it avoids the necessity of the customer waiting to be served by a butcher and permits the customer to inspect the various cuts and to select a purchase at leisure without being hurried by a busy butcher. This practice also permits the cutting and prepackaging of meat in advance of the busiest market hours and results in more efficient

use of the meat cutter's time. This method of retailing meat is popular with both the retailer and the consumer and is becoming more or less standard practice in the larger meat markets. A photograph of a typical meat counter in a modern supermarket using prepackaging is shown in Fig. 36. The prepackaged meat is shown in the foreground. In the background of the photograph, behind the glass panels, is a large room devoted to the cutting, weighing and packaging of the meat. Much of this area could be saved if the meat were prepackaged at the packing house.

One disadvantage of prepackaging fresh meat is the necessity of selling the cut-up meat rapidly so as to prevent loss by spoilage, which has limited the practice to the larger meat markets that have a rapid turnover. Cut-up chicken and ground fresh meat can be kept only for a few days even under refrigeration, whereas the uncut carcasses can be kept under refrigeration much longer. The uncut flesh of an animal that was in good health at the time of slaughter is relatively sterile, but when the flesh is cut the surface becomes contaminated with a variety of microorganisms from the air and from the hands and implements of the butcher. This contamination can become quite high in the case of ground meat since meat trimmings that often have a high surface contamination of microorganisms are frequently used for ground meat and quite often the meat grinder itself is not free of contamination. This results in the incorporation of a large number of microorganisms into an excellent growth medium and accounts for the short shelf-life of refrigerated ground fresh meats. Rapid spoilage practically prohibits the preparation of ground meat at the packing house except in the frozen state.

b. Advantages of Cutting and Prepackaging Fresh Meat at the Packing House

(1) Advantages to the Retailer. If cut and prepackaged retail-size portions of fresh meats having sufficient refrigerator shelf-life were available to the retailer, a number of economies would be possible.

(a) Fewer butchers would be required at retail stores and more would be required at packing houses where they could be used more efficiently.

(b) Some retailers might dispense entirely with meat cutting.

(c) Handling of bones and meat scraps would be eliminated.

(d) Shipping costs would be reduced.

(e) Packaging of meat would be avoided.

Fig. 36. Photograph Showing a Typical Meat Counter Display in a Modern Supermarket (By Courtesy Kroger Supermarket, Packard Street, Ann Arbor, Michigan).

(f) Less floor space would be required to handle meats.

(g) Less refrigerator space would be required per pound of meat sold.

(h) Total cost of packaged meat could be reduced by mass-handling methods at packing houses, which could increase the national consumption of meat.

(i) Fresh pork would be free of danger of contamination by trichinae,[2] which could increase the national consumption of pork.

(j) Loss of meat by spoilage could be reduced.

(k) Satisfying consumer preference could increase trade.

(2) Advantages to the Packing House. The packing house would benefit in a number of ways.

(a) Mass-production methods of cutting could be used at packing houses and a profit realized thereby.

(b) Mass-production methods of prepackaging could be used at packing houses and a profit realized thereby.

(c) Packing houses could handle bones and meat scraps at a profit more easily than the retailer.

(d) Demand for specialty items such as radiation-pasteurized prepackaged meat could increase trade of packing houses using this method.

(e) Total trade also could be increased by reduction of handling costs through mass-handling methods at packing houses.

(f) Sale of pork could be increased by eliminating danger of trichinae contamination.

(g) Great savings could be realized in tonnage of meats shipped and in shipping space in public and private carriers.

(h) Labor of handling would be reduced (70 lbs of cut meat is equivalent to 100 lbs of carcass meat).

(3) Advantages to the Consumer. The consumer would also benefit from this method.

(a) Pasteurized pork free from the causative agent of trichinosis, would be of even greater importance to the consumer than to the retailer and packing houses because it would remove the threat of trichinosis to his health.

(b) Pasteurized meat has a longer refrigerator shelf-life, which would be of advantage to housewives who shop at a supermarket only once or twice a week.

(c) Cost of meat may be reduced by savings realized in mass handling techniques.

(d) Other less common parasites such as tapeworm and certain pathogenic microorganisms which are sometimes found in meat could be rendered harmless (this idea should be investigated).

2. EXPERIMENTAL RESULTS ON PASTEURIZATION OF RAW MEAT

a. Microbiological

The personnel of Michigan Memorial-Phoenix project 41 have made a limited microbiological study of gamma-ray-pasteurized raw meat. The results of this study are reported in Part IV of this report, page 222.

In these experiments raw ground lean beef was obtained from the University Food Service and was innoculated with a psychrophilic organism isolated from raw meat. After innoculation the meat had an initial count of about 5×10^5 microorganisms per gram. The meat was divided into six portions. Two portions were used as controls and the other four were given irradiation dosages of 20,000, 40,000, 80,000, and 160,000 rep. After irradiation the meat was stored in a refrigerator at a temperature of 4°C. The growth of microorganisms was measured by making four separate counts on each sample at regular intervals up to a total of 13 days of storage.

Growth of the organism in the irradiated samples showed a lag, whereas there was no lag in the growth observed with the controls. This lag increased with dosage. The count of the microorganisms in the controls increased as a logarithmic function of time from the start of the test. After the initial lag the count in the irradiated samples increased in a similar manner. The lag was 0

for the controls, approximately 1/2 day for the sample receiving 20,000 rep, 1-1/2 days for the sample receiving 40,000 rep, 3-1/2 days for the sample receiving 80,000 rep, and 5 days for the sample receiving 160,000 rep.

Spoilage as evidenced by off odor after 13 days of storage was detected only in the controls and in the 20,000-rep sample. No off odor was detected after 13 days of storage in the samples receiving 40,000, 80,000, and 160,000 rep. The samples receiving 80,000 and 160,000 rep showed a brownish color after two days of storage.

There is no exact relationship between the count of microorganisms and "spoilage"; however, a large population of certain microorganisms results in certain types of spoilage. Taste-panel tests are required to evaluate the quality of the food properly.

At the American Meat Institute[1] a study of the microbiology of fresh beef stored at 2°C under aerobic conditions and high humidity was conducted. The following summary of a portion of this study is taken from AEC Bulletin: TID-3046, "After 3 to 7 days large numbers of microorganisms develop and cause considerable slime as well as a characteristic musty odor. A detailed physiological study of 20 bacterial cultures isolated from the slimy meat identified virtually all as belonging to the genus Pseudomonas. The most common species seems to be P. geniculata. In broth culture these bacteria grow moderately well at 0°C. Their maximum temperature limit for growth is approximately 37°C NaCl. In broth culture they are able to tolerate 4 but not 6% NaCl, therefore would be of no importance in most cured meats, but would definitely influence the storage life of any unfrozen fresh meat if kept under conditions that would maintain a moist surface (e.g., prepackaged fresh meats). The lethal dose of γ rays for two representative cultures, P. geniculata and an unidentified species, in suspensions of 10^5 to 10^7 cells/ml in nutrient broth, was found to be approximately 30,000 rep. The addition of 0.1% Na nitrite to the broth at pH values of 5.0, 6.0, 7.0 and 8.0 had no significant effect on the sensitivity of the bacteria to irradiation. The survivor curve was exponential. These organisms are considerably more resistant to radiation when incorporated into ground fresh beef, and the protective agent in the ground beef seems to reside in the meat juices rather than in the particulate matter. In one experiment in which only 13,000 rep were required to destory 99% of the cells in nutrient broth, 40,500 rep were required when the cells were in raw meat, 28,500 rep when in washed cooked meat, and 55,000 rep when in the juice from cooked meat."

b. Taste-Panel Studies

Taste-panel studies on gamma-ray-pasteurized fresh meat are reported in a previous section of this report, pages 96-106. In these studies two series of tests were made on irradiated raw ground lean pork, one series on raw

raw ground beef (with 25% fat), and one series on chicken legs. The controls for the ground pork and the ground beef, stored at refrigerator temperature of 40°F, spoiled after approximately 4 days of storage. For taste-panel tests after 4 days of storage, controls which had been stored in the frozen state were used for all three meats.

Taste-panel tests on ground pork and ground beef definitely showed an increase in refrigerator shelf-life as a result of radiation pasteurization. Doses of the order of 60,000 to 100,000 rep prevented spoilage of ground pork through 10 days of storage. (The supply of samples was exhausted at 10 days and the experiment therefore ceased.) Taste-panel studies on ground beef stored at 40°F for 7 days indicate no preference between frozen control and the irradiated samples receiving radiation dosages from 50,000 to 110,000 rep. When the storage at 40°F was extended to 14 days, the frozen control was preferred to the irradiated samples; however, there was little preference between the samples receiving 50,000 rep and the frozen control.

These results are interesting in view of the fact that the unfrozen control of all ground meats tested spoiled after about 4 days of storage at 40°F, but the results are much too meager. Additional data on the meats tested and on other types of fresh meat, fish, etc. are necessary to establish the advantages and limitations of a process for gamma-ray pasteurization. Such a study is being made by the American Meat Institute. The limited data available to this laboratory indicate that such a process may be quite promising. On the other hand, some recent articles in the literature[3,4] might be interpreted as discouraging the use of gamma radiation to process foods from the standpoints of uniformity of dose, efficiency of absorption, cost, etc. Since these opinions are not shared by this laboratory, it seems advisable to consider a design of a radiation facility for gamma-ray pasteurization of meat to demonstrate the feasibility of gamma-ray processing.

3. PROPOSED METHOD OF PREPARING CUT-UP PREPACKAGED MEATS FOR IRRADIATION AT THE PACKING HOUSE

a. General Plan

The proposed method of handling cut-up, prepackaged and pasteurized meat consists of the usual slaughtering procedures through chilling. Chilling prior to cutting is desirable so as to firm the meat for easier cutting and also because there is less growth of microorganisms if the meat is cut while chilled and kept at refrigerator temperature until used by the consumer. Instead of refrigerated quarter and half carcasses of beef, pork, and lamb being shipped from the packing house to the retailer, it is proposed that the chilled carcasses be cut, prepackaged, weighed, packed in cartons, and pasteurized by gamma

radiation at the packing house and then shipped under refrigeration to the retailer. The pasteurization treatment with gamma radiation might be used to destroy about 95 percent of the microorganisms without developing off flavors in the packaged meat and thereby increasing the refrigerator shelf-life of the meat.

b. Cutting, Prepackaging, and Weighing

In the method proposed, the meat cutting would be performed as a mass-handling operation with each cutter performing a special function such as the cutting of steaks, chops, roasts, trimming, etc. All cuts of meat on such a cutting floor would be of retail size and would be conveyed from the cutting tables to packaging machines. Here the meat would be packaged using plastic films such as polyethylene, saran, and plio film; cellophane; paper; perhaps aluminum foil or combinations of foil, paper, and plastic; etc.

Films of polyethylene have very good properties as water-vapor barriers, are odorless and strong, and can be heat-sealed. One of the disadvantages of polyethylene film is that it is not a very satisfactory oxygen barrier. Also, many of the aromatics, flavor substances such as the esters, and volatile oils pass through polyethylene film. Saran film is more satisfactory as a barrier to oxygen and flavor substances but cannot be heat-sealed easily. Cellophane is inexpensive and has good properties as a barrier to oxygen, but is a poor barrier to water vapor and loses its strength when wet. Aluminum foil has excellent properties as a barrier to all substances, providing that it is free from tiny holes, but has poor strength when used alone. Laminated packaging material can be used so as to take advantage of the properties of each of the components. Thus, a laminated material with an outer layer of heavy Kraft paper, a center layer of thin aluminum foil, and an inner thin layer of polyethylene has the strength and economy of the paper, the barrier effectivensss of aluminum foil, and the heat-sealing properties of polyethylene. A single transparent film may be preferred, however, to permit visual inspection of the contents. As a compromise a transparent plastic window might be placed in each cut of meat packaged with a laminated material.

Immediately following the packaging by machine, it is proposed that the individual packages be weighed and the weight stamped on the package by machine. This would avoid the tiresome and expensive operation of weighing and recording the weight manually, which is usually necessary when the packaging is performed by the retailer. As retail prices vary, the final pricing would be done by the retailer. However, this operation is rather simple for weighed and packaged products and is necessary for nearly all grocery items. If desirable, the packaged and weighed cuts could be selectively sorted as to weight by machine in such a mass-handling operation. Thus, all roasts of a certain cut weighing 3 lbs, 0 oz could be separated by machine from those of

all other weights and shipped to retailers asking for cuts of exact weights. Such a degree of specialization probably would not be desirable for most retailers, but might be of advantage in the largest supermarkets.

c. Cartons for Irradiation and Shipping

The packaged and weighed cuts of meat must be packed into cartons of suitable size for shipping. Such cartons probably would be made of Kraft paperboard, which is more or less standard material for cartons used to ship other packaged food products. The cartons should have sufficient thickness to handle the larger cuts of meat and yet be of economical and convenient size.

The maximum-size cut of fresh meat normally sold is a fresh ham of pork or a rib roast of beef; such cuts usually do not exceed 8 inches in thickness. A carton 8 inches in thickness therefore should be of sufficient thickness to hold the larger cuts and would not be so thick as to give difficulty in providing a uniform irradiation dose. A carton weighing about 100 to 150 lbs is the maximum weight that any one man can lift safely (portland cement and chemicals are usually packaged in 100-lb bags, partly for this reason). For easy stacking in refrigerated carriers and cold rooms, a carton 2 feet by 1-1/2 feet by 8 inches thick will be considered and a 20-percent loss in effective thickness as a result of voids caused by irregularities in the shape of the cuts will be allowed. Using a density of 70 lbs/ft for the packaged cuts of meat, the cartons are estimated to have an approximate weight of $2 \cdot 1\text{-}1/2 \cdot 8/12 \cdot 0.8 \cdot 70 = 112$ lbs/carton.

In buying from a packing house a retailer might order by the carton in anticipation of market sales. For example, the retailer might place an order for 6 cartons of steaks, 10 cartons of beef roasts, 8 cartons of ground beef, etc. This would have a definite advantage in that some markets have a large demand for some cuts such as steaks, whereas other markets find a greater demand for roasts, ground beef, or some other cut. With present practice the retailer is supplied meat by the half or quarter and must sell a rather fixed percentage of all cuts. If local demand is not in this exact proportion the retailer is forced to dispose of surplus cuts by grinding or by lowering the price on those cuts. With the new method proposed for wholesaling meat, the retailer could simply order fewer cartons of those cuts for which the demand happened to be low in his area. As the wholesaler would serve many retail markets, local differences would tend to be averaged out and the relative demand for the different cuts would fix the relative prices as it does today, but on an overall basis.

4. SELECTION OF A GAMMA SOURCE

A design of a facility for irradiating whole hogs for the purpose of breaking the cycle of trichinosis was presented in Progress Report 5 of this project. In that design cesium-137 was used as the source of radiation, partly because there were some meager cost data available on cesium-137 and partly because no satisfactory study had been made of the advantages and disadvantages of using mixed fission products. The "mixed" fission products are defined as the mixture of radioactive elements resulting from the fission of uranium after separation from process wastes while the "gross" fission products are considered to be the "mixed" fission products before separation from process wastes. The analysis and activity of the gross fission products vary with each process used and therefore a more general analysis of the problem can be made on the basis of "mixed" fission products than on "gross" fission products.

In selecting a source of gamma radiation for this design, mixed fission products of various ages are therefore considered and compared with cesium-137. In general, the mixed fission products have the advantage of being available in much larger quantities than cesium-137, which constitutes only about 6.2 percent by weight of the mixed fission products at the time of removal from the reactor. The other important gamma emitters,[2] zirconium-95, its daughter product columbium-95, and cerium-144, have much greater gamma activity than cesium-137. However, using cesium-137 instead of mixed fission products has numerous advantages which will be pointed out by comparing (a) the activity of mixed fission products and cesium-137, (b) the absorption characteristics of gamma radiation from mixed fission products, and cesium-137, and (c) the economics of using mixed fission products and cesium-137.

a. The Activity of Mixed-Fission-Product and Cesium-137 Sources

The specific radiation flux R_o in r/hr at a distance of 1 cm from a 1.0-mc point source was determined as a function of the gamma energy in mev by Marinelli[5] as shown in Fig. 37.

The radiation coefficient α, for a source of infinitesimal area expressed in r/hr per unit area of emitting surface at unit distance from the emitter, can be calculated from R_o using the following relation:

$$\alpha = R_o \rho S t = \frac{r}{\text{hr-curie}} \frac{\text{gm}}{\text{cc}} \frac{\text{curie}}{\text{gm}} \text{cm at 1 cm}$$

$$= \frac{r}{\text{hr-cm}^2} \text{ at 1 cm}$$

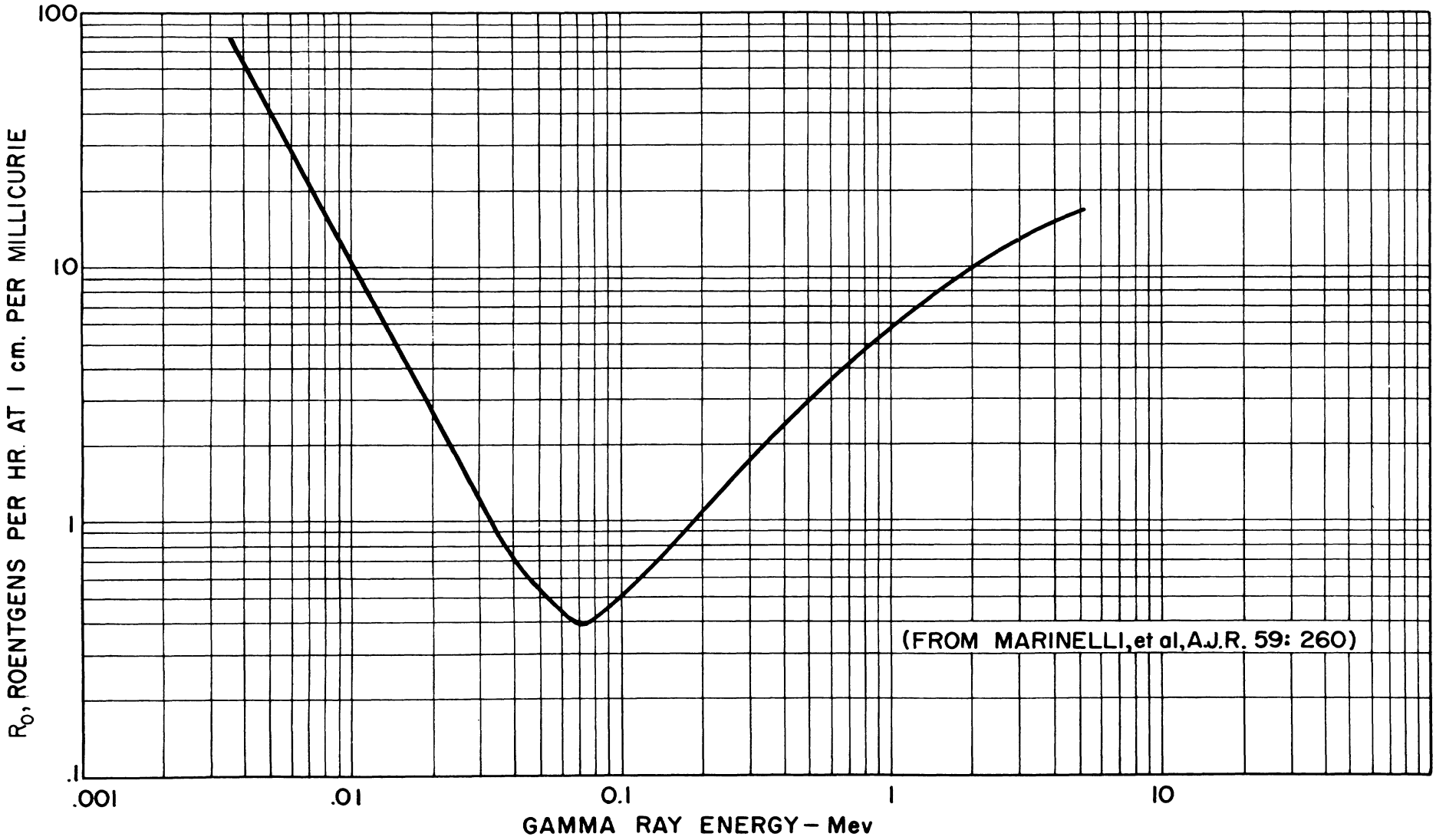

Fig. 37. Specific Radiation Flux R_o as a Function of Energy in mev of Gamma Radiation.

$$= \frac{r}{\text{hr-in.}^2} \text{ at 1 in.}$$

$$= \frac{r}{\text{hr-ft}^2} \text{ at 1 ft ,}$$

or

$$= \frac{r}{\text{hr}}$$

where

R_o = specific radiation flux, r/hr-curie at 1 cm,

ρ = density of emitter, gm/cc,

S = specific activity of emitter, curies/gm, and

t = thickness of emitter, cm.

As the radiation flux at a given distance from the source of infinitesimal area is inversely proportional to the square of the distance from the source and directly proportional to the area of the source, the dimensions of distance squared and of area cancel each other. Thus, the radiation coefficient, α, is independent of the units used for distance and area providing that consistent units are used. To calculate the flux for a source of finite area the following integration must be performed over the area of the source:

$$I = \alpha \int\int \frac{da}{r^2} .$$

The constituents of mixed fission products at three months, six months, one year, and two years after removal from a reactor, including all isotopes present in excess of 1 percent are listed in Tables 24-27. The second column of these tables lists the percent of the total activity contributed by each radioisotope. The contribution of cesium-137 is less than 1 percent of the total activity until the fission products are almost one year old and therefore does not appear in the second columns of Tables 24 and 25. Cesium-137 contributes 1.5 percent of the activity of one-year-old fission products and 4.0 percent after two years as shown in Tables 26 and 27 respectively. The radiation coefficients, α, are listed in the last columns of Tables 24-27.

The factor limiting specific activity is the rate of heat absorption within the source material. This heat absorption is chiefly the result of beta-particle activity. An upper limit of 10,000 total curies per pound has been suggested from these thermal considerations.

TABLE 24

COMPOSITION OF MIXED FISSION PRODUCTS
THREE MONTHS AFTER REMOVAL FROM REACTOR

Isotope	Percent[6] Contribution	Gamma[6,7] Energy	Gamma Specific Activity, curies/pound	R_o[5]	α
Cb^{95}	18	0.758	1800	4.4	70,000
		0.216	1800	1.2	19,000
Zr^{95}	14	98% 0.708	1372	4.2	51,000
		2% 0.216	28	1.2	300
Sr^{89}	13	none	---	---	---
Y^{91}	11	none	---	---	---
Ce^{141}	8.6	0.14	860	0.75	5700
Ru^{103}	7	0.3, 0.5	700	2.3	14,000
Rh^{103}	7	none	---	---	---
Ce^{144}	6	none	---	---	---
Pr^{144}	6	0.7	600	4	21,000
La^{140}	2	10% 1.6	20	8	1,400
		20%, 0.5, .1, 1.6	40	11.5	4,000
		70%, .8, .1, 1.6	140	15.5	19,000
Ba^{140}	2	40%, .3, .2	80	3	2,100
Pr^{143}	2	0.7	200	4	7,000
		Total	7,640		214,500

TABLE 25

COMPOSITION OF MIXED FISSION PRODUCTS
SIX MONTHS AFTER REMOVAL FROM REACTOR

Isotope	Percent[6] Contribution	Gamma[6,7] Energy	Gamma Specific Activity, curies/pound	R_o[5]	α
Cb^{95}	25	0.758	2500	4.4	97,000
		0.216	2500	1.2	26,400
Ce^{144}	12	none	---	---	---
Pr^{144}	12	0.7	1200	4	42,000
Zr^{95}	15	98% 0.708	1470	4.2	54,000
		2% 0.216	30	1.2	320
Y^{91}	11	none	---	---	---
Sr^{89}	8.5	none	---	---	---
Ru^{103}	4.2	0.3, 0.5	420	2.3	8,600
Rh^{103}	4.2	none	---	---	---
Ce^{141}	2.5	0.14	250	0.75	1,650
Pm^{147}	2.0	none	---	---	---
Ru^{106}	1.0	none	---	---	---
Rh^{106}	1.0	0.5, 0.7	17	3.5	530
		Total	8387		230,000

TABLE 26

COMPOSITION OF MIXED FISSION PRODUCTS
ONE YEAR AFTER REMOVAL FROM REACTOR

Isotope	Percent[6] Contribution	Gamma[6,7] Energy	Gamma Specific Activity, curies/pound	R_o[5]	α
Ce^{144}	27	none	---	---	---
Pr^{144}	27	0.7	2700	4	95,000
Cb^{95}	15	0.758	1500	4.4	58,000
		0.216	1500	1.2	16,000
Zr^{95}	7	98% 0.708	686	4.2	25,000
		2% 0.216	14	1.2	150
Pm^{147}	6	none	---	---	---
Y^{91}	4	none	---	---	---
Sr^{89}	3	none	---	---	---
Ru^{106}	2.5	none	---	---	---
Rh^{106}	2.5	0.5, 0.7	42	3.5	1300
Sr^{90}	2	none	---	---	---
Y^{90}	2	none	---	---	---
Cs^{137}	1.5	none	---	---	---
Ba^{137}	1.5	0.7	300	4	10,000
		Total	6742		205,500

TABLE 27

COMPOSITION OF MIXED FISSION PRODUCTS
TWO YEARS AFTER REMOVAL FROM REACTOR

Isotope	Percent[6] Contribution	Gamma[6,7] Energy	Gamma Specific Activity, curies/pound	R_o[5]	α
Ce^{144}	30	none	---	---	---
Pr^{144}	30	0.7	3000	4	105,000
Pm^{147}	14	none	---	---	---
Sr^{90}	5.2	none	---	---	---
Y^{90}	5.2	none	---	---	---
Cs^{137}	4	none	---	---	---
Ba^{137}	4	0.7	400	4	14,000
Ru^{106}	3.5	none	---	---	---
Rh^{106}	3.5	0.5, 0.7	60	3.5	1850
			3460		120,850

If mixed fission products are concentrated to 10,000 curies per pound, the useful gamma-emitting isotopes will be present to the extent determined by the percent composition as listed in the second columns of Tables 24-27. The specific activity in curies per pound of each gamma-emitting isotope is listed in the fourth columns. The total gamma specific activity is plotted in Fig. 38 as a function of time after removal from the reactor. This graph shows that the optimum storage time, from the point of view of useful gamma activity per 10,000 curies of total activity, is approximately six months.

It may be seen from Tables 24-27 that even for the optimum case (six months), the coefficient α is appreciably lower than that previously computed for cesium-137 (340,000). Using identical geometrical configurations, a gamma source containing six-month-old fission products would have to be 1-1/2 times as active (including both beta and gamma activity) as a gamma source containing cesium-137 to produce the same levels of gamma radiation in air.

b. The Absorption Characteristics of Radiation from Mixed Fission Products and Cesium-137

The absorption characteristics in meat of the complex of gamma energies from mixed fission products must be considered. Figure 39 illustrates the absorption characteristics of each important component as well as the total absorption effects, using broad-beam absorption coefficients.[8] The resultant total absorption does not differ appreciably from that found for cesium-137. A gamma source containing six-month-old mixed fission products would approximate the absorption efficiency of a source containing pure cesium-137.

c. The Economics of Using Mixed Fission Products and Cesium-137

Perhaps the most important consideration is the rate at which the radiation flux from such fission-product radiation sources decreases with time. Figures 40, 41, and 42 illustrate respectively the component and total time decay to be expected from the compositions calculated in Tables 25-27.

As a practical consideration in the operation of a radiation facility, the radiation flux must be kept from dropping excessively in order to prevent a decrease in capacity. Assuming that a 10 percent drop in flux is permissible, then 1/6 of a source containing six-month-old mixed fission products must be replaced every week. For year-old fission products this minimum replacement period is lengthened to 2 weeks and for 2 year-old material to 5 weeks. Replacement of high-level gamma sources is expensive, time-consuming, and dangerous and therefore must be minimized. Prolonged storage of the gross fission products, however, tends to increase the cost per curie for two reasons: first, expensive storage facilities must be constructed and maintained; and second, the "natural"

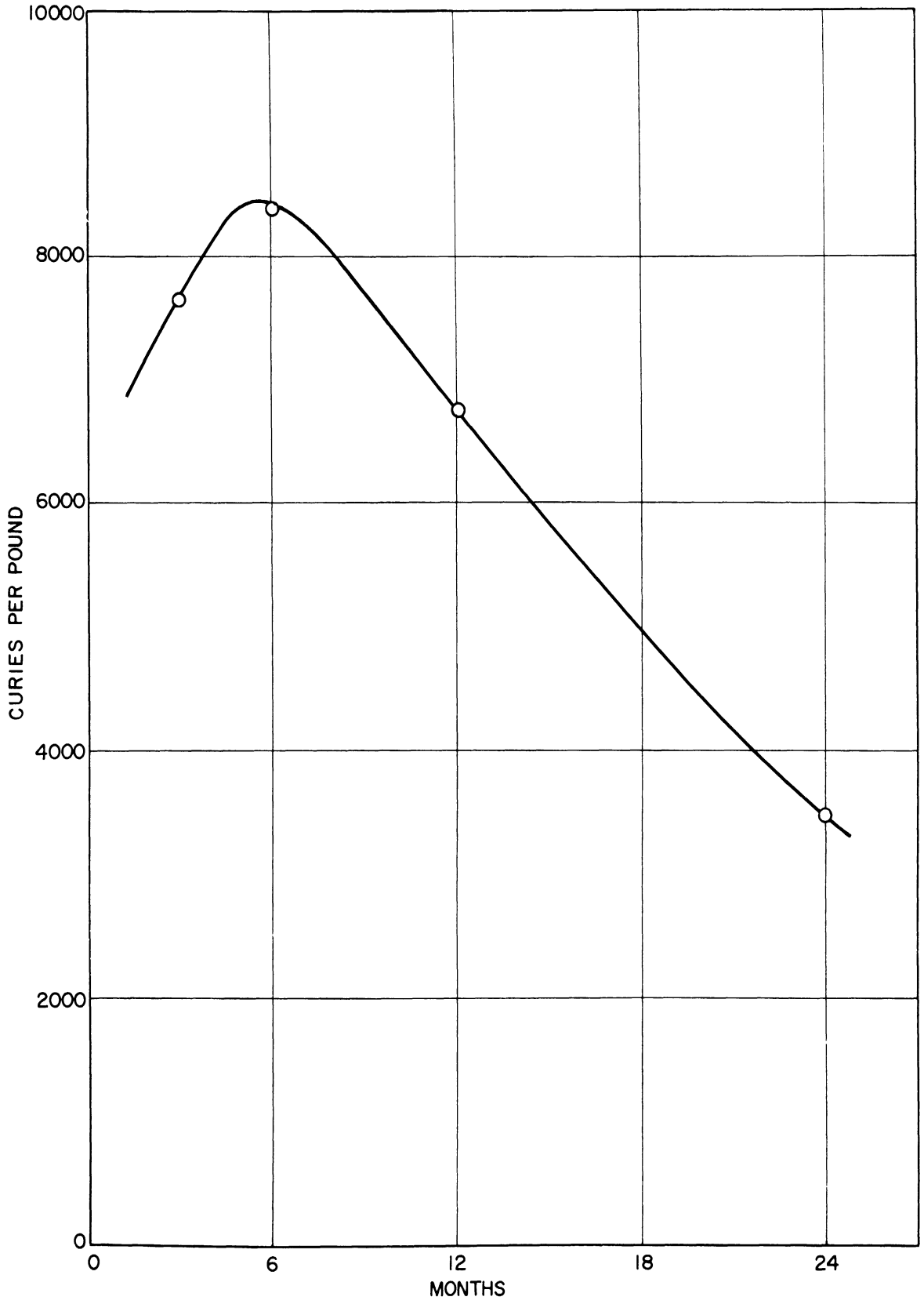

Fig. 38. Gamma Specific Activity in Curies per Pound as a Function of Age for Mixed Fission Products.

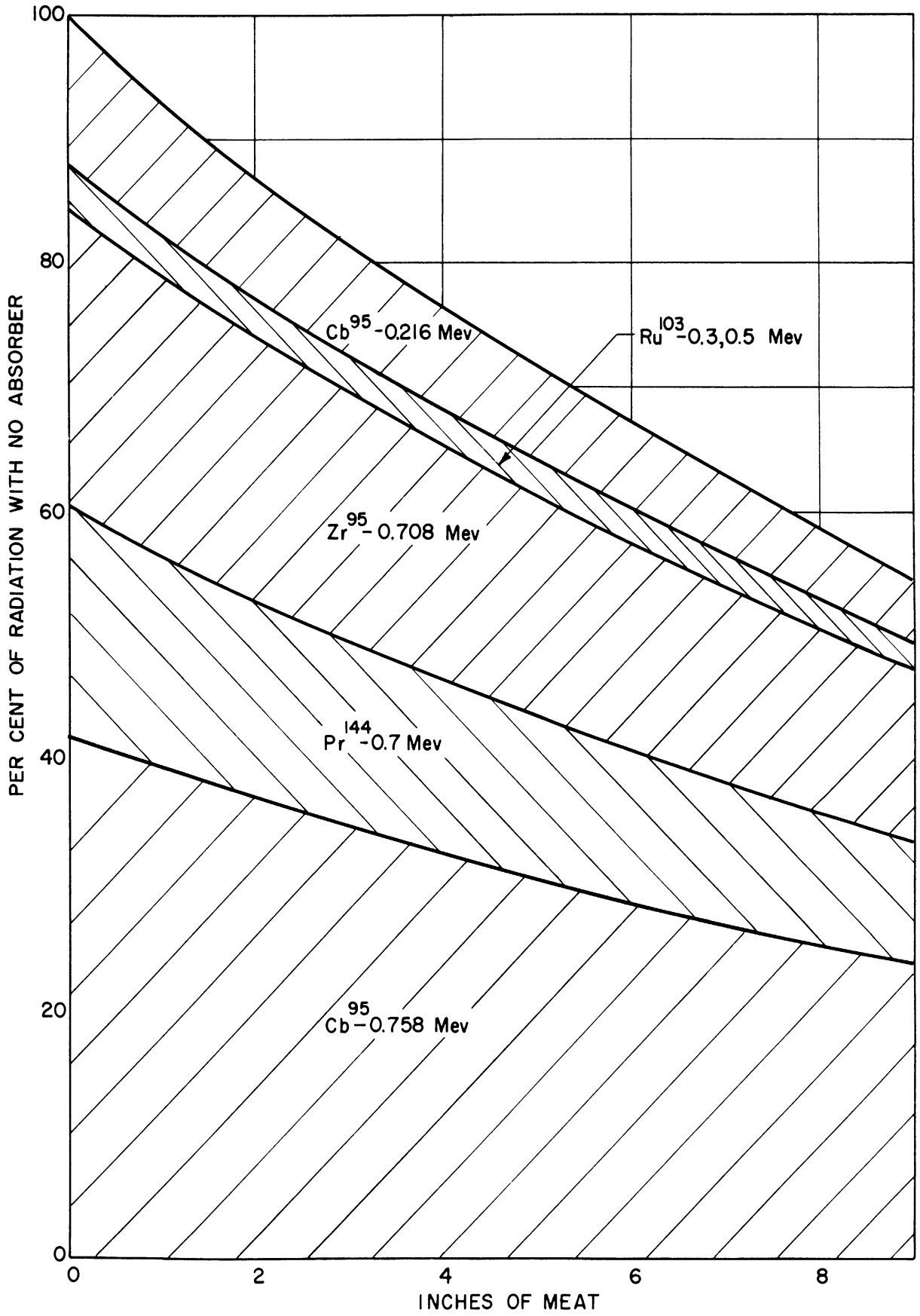

Fig. 39. Absorption in Meat of Gamma Radiation from Mixed Fission Products Six Months Old.

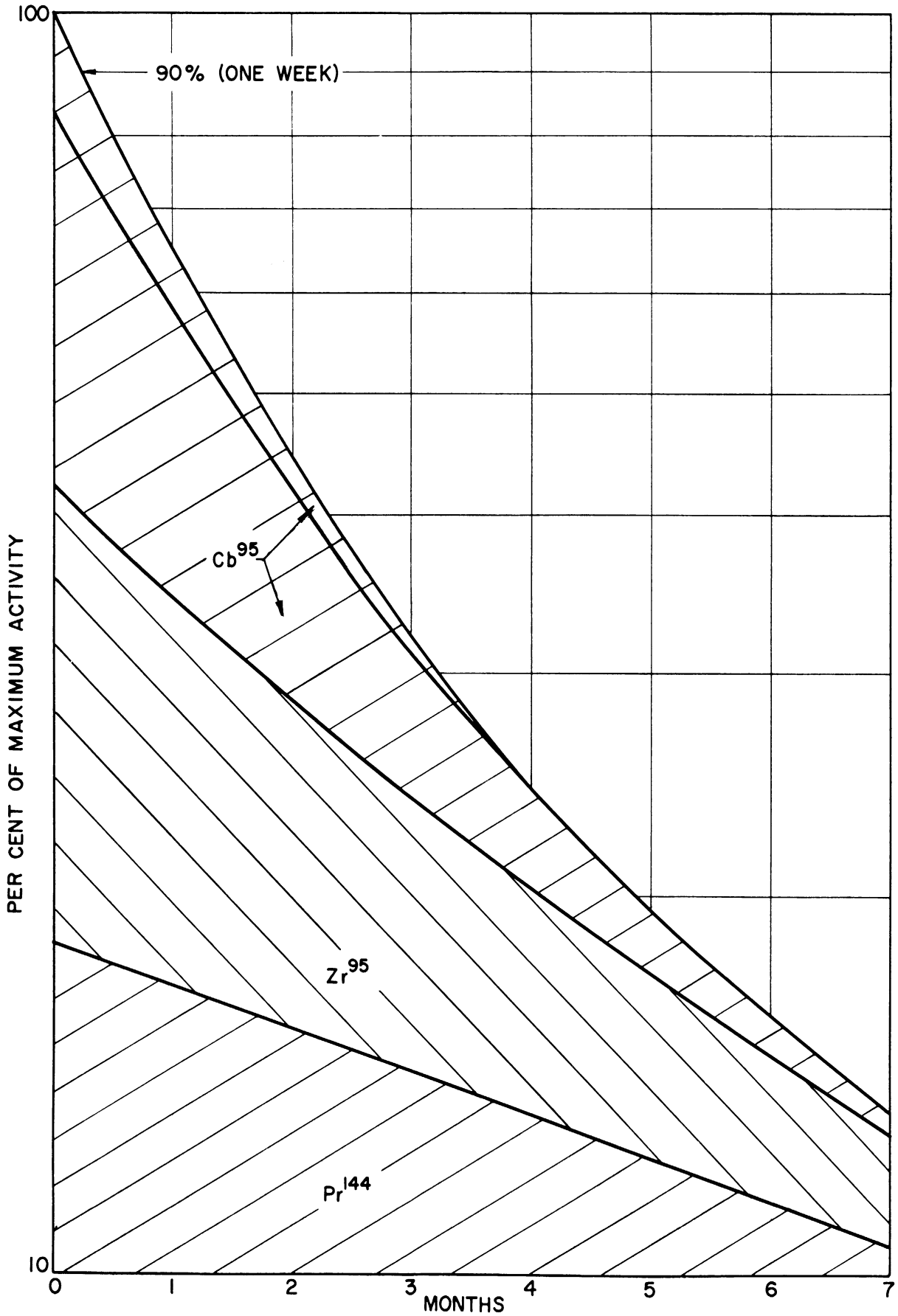

Fig. 40. Time Decay Characteristics of Six-Month-Old Mixed Fission Products.

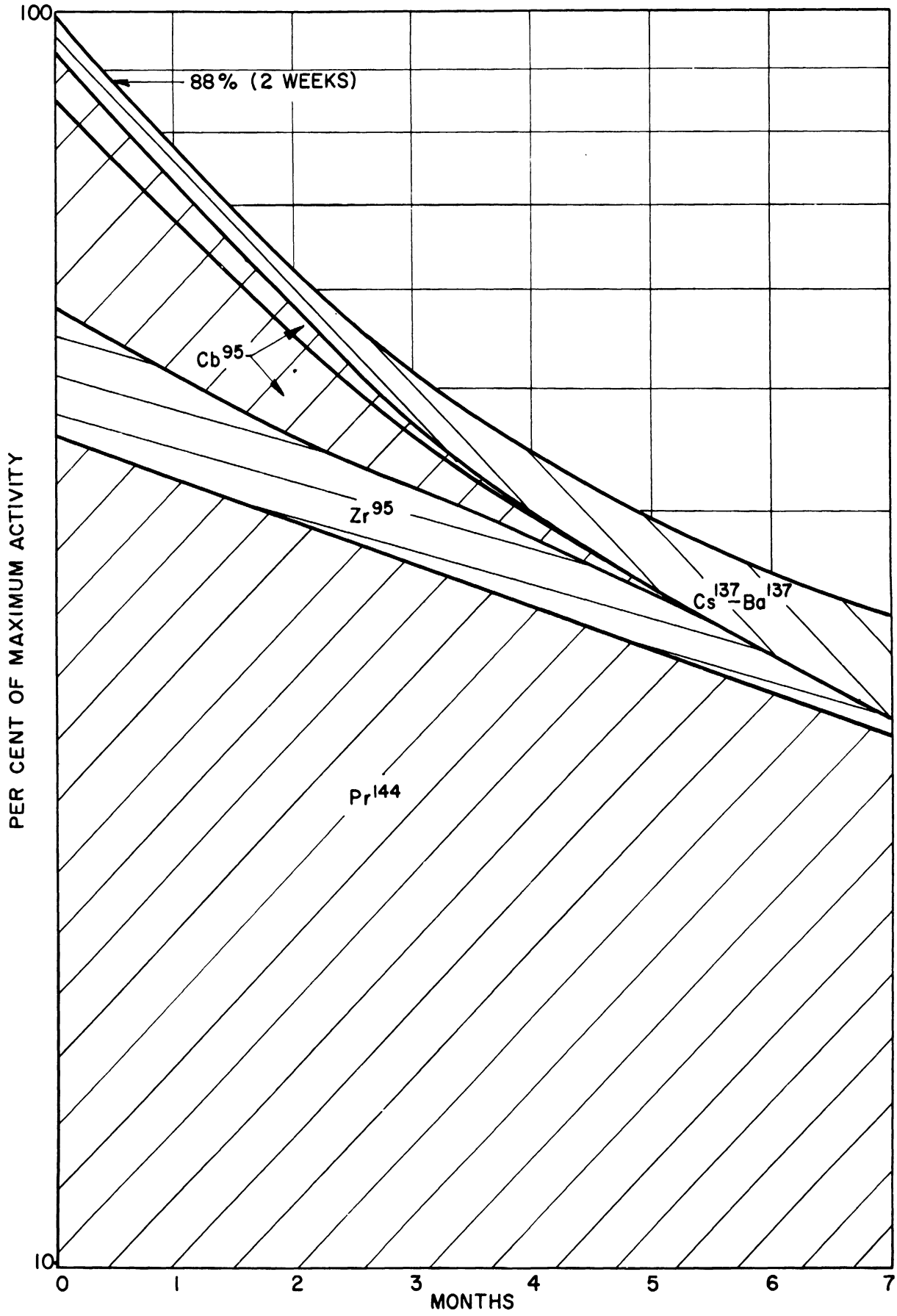

Fig. 41. Time Decay Characteristics of One-Year-Old Mixed Fission Products.

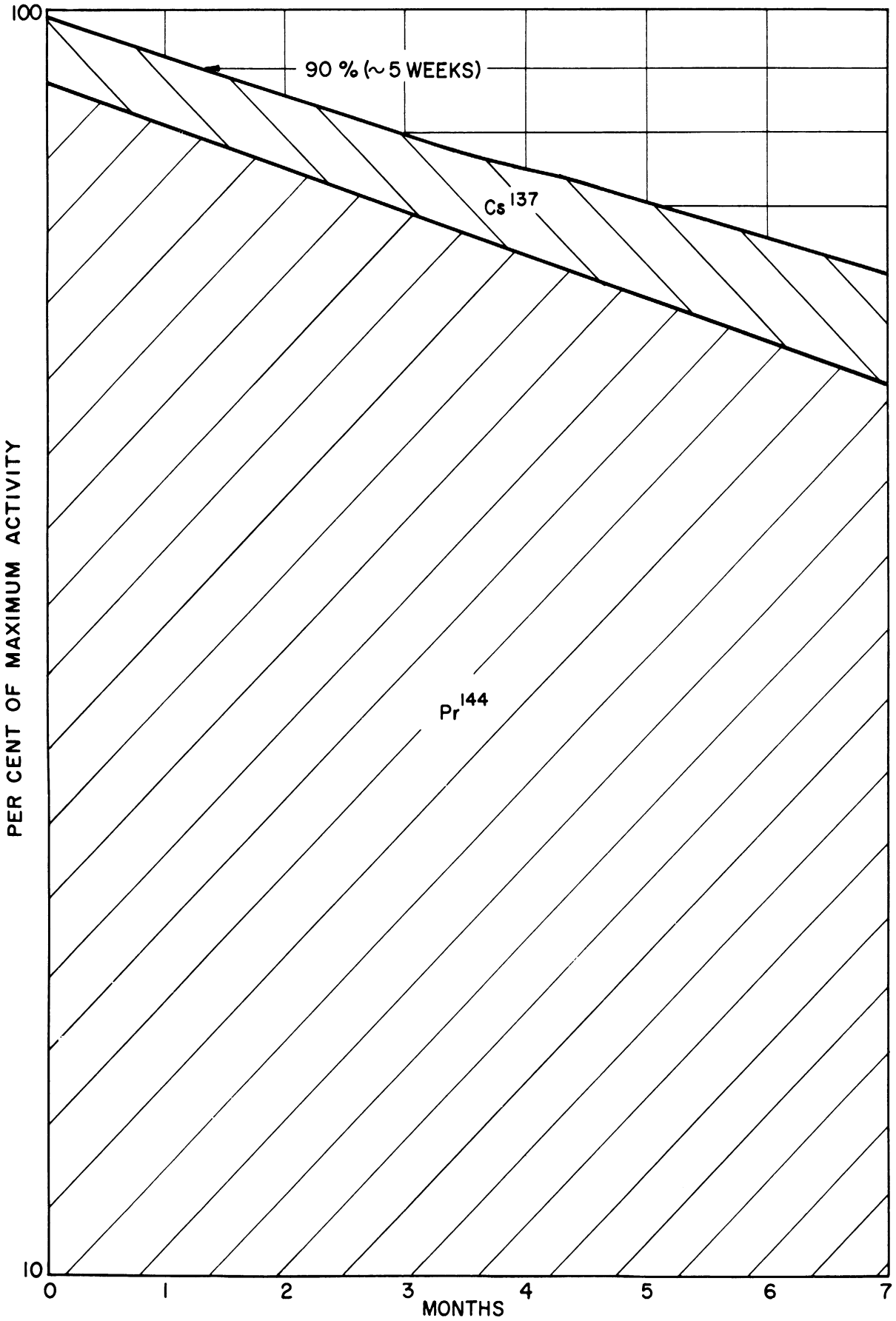

Fig. 42. Time Decay Characteristics of Two-Year-Old Mixed Fission Products.

specific activity decreases tremendously and the degree of processing required to achieve 10,000 curies per pound is increased proportionately.

However, if cesium-137 were used rather than the mixed fission products the problem of what to do with the remaining fission products, particularly the beta emitters with long half-lives such as strontium-90 would not be solved. One of the main objectives of investigating uses of radiation from fission products is to utilize either the mixed or the gross fission products and thereby eliminate the expensive storage of these materials.

The mixed fission products have a short effective half-life during the first two years of decay, when they have high gamma activity. If the mixed fission products are allowed to decay for four years, about half the gamma radiation will be from cesium-137; however, such a source of four-year-old mixed fission products would have only about 10 percent of the gamma activity of separated cesium-137 as a result of dilution by the other fission products and their decay products.

As a means of analyzing costs, the value of 1-1/2 megacuries of mixed fission products can be compared to the 1-1/2 megacuries of cesium-137 described in the irradiation facility design of Progress Report 5. As six-month-old fission products have the optimum radiation coefficient, α, they will be considered in this comparison.

A weekly replacement of 1/6 of a six-month-old fission-product source amounts to 8-2/3 total replacement each year. Cesium-137 would require replacement of 1/6 of the source every 5 years to prevent the activity from dropping more than 10 percent. Thus for a 1.5-megacurie source the total activity of six-month-old mixed fission products required in a 5-year period as compared to cesium-137 would be:

For Mixed Fission Products:

(1.5 megacuries)(8-2/3 replacements/1 yr)(5 yr) = 65 megacuries of six-month-old fission products required

For Cesium-137:

(1.5 megacuries)(1/6 replacements/5 yr)(5 yr) = 0.25 megacurie of cesium-137 required

In other words, in a five-year period 65/0.25 or 260 times as many curies of six-month-old mixed fission products would be required as of cesium-137.

If the values of the two sources are compared on the basis of activity replacement without regard to shipping and installation costs or to the differences in interest on the investment, the cesium-137 will have a value as a source

of radiation 260 times as great as the six-month-old mixed fission products. Thus, if the 1.5 megacuries of cesium-137 used in the pork irradiation facility described in Progress Report 5 has an initial value of $483,000 as estimated, the corresponding value for the six-month-old mixed fission products would be $483,000/260 or $1,860 for 1.5 megacuries of six-month-old mixed fission products.

It appears very unlikely that 1.5 α megacuries of mixed fission products could be concentrated and prepared into a suitable source for $1,860. Furthermore, the shipping and installation cost associated with replacing 0.25 megacurie every week would in itself exceed $1,860 appreciably.

Another approach to estimation of cost may be made on the basis of prices for cesium-137 thereapy sources, which are now $100 for the first curie and $25 per curie for additional activity. Using the same scaling-factor approach for transition from small- to large-scale operations[9] and using a base price of $100 per curie, the cost of 1.5 megacuries would be $507,600. On the same basis, the total cost would be $253,800 at $50 per curie and only $126,900 at $25 per curies.

A third approach to price was made with the aid of experts* in the field of uranium processing. It is estimated that through the use of modern large-scale techniques cesium-137 can be separated in megacurie amounts for about $0.30 per curie. The cost of a 1.5 megacurie source would then be $450,000.

Considering the range of values obtained from these varied approaches, it is felt that the value chosen, $483,000, represents a reasonable estimate.

d. Conclusions Regarding the Selection of a Gamma Source

The previous discussion indicates that from the standpoint of the potential user of high-level gamma sources, cesium-137 would be much more satisfactory than six-month-old mixed fission products. The older mixed fission products have some advantages over the six-month-old material. For instance, one-year-old mixed fission products could be kept 2 weeks, and two-year-old material could be kept 5 weeks without exceeding the stipulated 10-percent drop in activity. This rather small increase in replacement time would tend to increase the relative value of the older mixed fission products, but this advantage would be offset in part by decrease in activity.

The limited consideration given to these problems suggests that the most satisfactory method of disposing of the gross fission products might be to separate the radioisotopes with a long half-life, namely, cesium-137, cerium-144, and strontium-90. The cesium-137 then could be used as a very satisfactory long-

*Private communication from Dr. Harold Ohlgren, formerly chief engineer of Chemical Processing Plant, American Cyanamid Company, Idaho Falls, Idaho.

half-life gamma source. Cerium-144 might be used where a shorter-half-life radioisotope would be satisfactory. In some applications these two gamma emitters might be used together. Because of the long half-life of strontium-90 and because of its biological hazard, this beta emitter would have to be stored until some satisfactory use is found for it. Use as a power source for "permanent" electric batteries is a possible use which might require large amounts of strontium-90. However, strontium-90 is present in the mixed fission products to only 5.3 percent but weight[10] is not a gamma emitter, and can readily form insoluble salts, so that the safe storage of large quantities of this radioisotope should be much easier and cheaper than storing the gross fission products. Such a separation would leave only the radioisotopes with a short half-life and the process wastes in the gross fission products; this remaining mixture, which constitutes the major bulk of the gross fission products, could safely be discarded after storage for a relatively few years.

This suggestion is the result of a very limited analysis. Additional and more extensive studies of this complex problem are needed to provide a basis for determining the most satisfactory disposition of the gross fission products and selecting suitable gamma sources for industrial use. For this design, however, it is considered best to select cesium-137 as the gamma source.

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5. DESIGN OF RADIATION CHAMBER

a. Selection of Number of Passes

The radiation chamber should be designed to irradiate packaged meat in cartons on a mass scale. This will involve the use of a conveyor to pass the cartons of meat into the radiation chamber, through the field of radiation, and out of the radiation chamber. For high efficiency in utilizing the radiation a sufficient total thickness of meat should be used to absorb most of the radiation.

The thickness of pork necessary to absorb half the gamma radiation (half-value thickness) has been measured for gamma radiation from cobalt-60 and from spent reactor fuel rods (essentially gamma radiation from cerium-144) and found to be about 10 inches (8). Cobalt-60 emits gamma rays of 1.33 and 1.17 mev, while cesium-137 emits gamma rays of 0.67 mev. On the basis of the experimental measurements and the difference in energy the half-value thickness for cesium-137 radiation in meat is estimated to be 8 inches. For design purposes the prepackaged meat will be considered to be packed to a thickness of 8 inches in cartons conveyed so as to present an 8-inch-thick absorber over 80 percent of the area through which the conveyor passes. The cartons would have some voids because of the irregular shapes of meat cuts. However, for a given conveyor speed the minimum total dose per pound of meat will result when the cartons are packed to a full 8 inches with meat, which would be possible with ground meat.

One pass of the meat conveyor would theoretically result in the absorption of 50 percent of the incident radiation normal to the carton surface. The distribution of the meat within the cartons and the free space between cartons reduces this value. However, this is somewhat compensated for by the greater total thickness the meat offers to all the radiation not normal to its surface, such as that from the extremities of the source. With these considerations the meat conveyed as shown in the following design, was considered to be 85 percent efficient as an absorber. Therefore, each path of travel would absorb 50 percent (half-value thickness) times 85 percent (efficiency) equals 42.5 percent of the radiation in the area through which the conveyor passes.

The first pass should be at least 1-1/2 to 2 feet from the source to provide a relatively uniform radiation dosage. Subsequent passes must be further away from the source; but the distances should be kept to a minimum to decrease the radiation loss as a result of space attenuation. If the first conveyor pass is located 21 inches from the source as in the design used in Progress Report No. 5, the radiation absorbed by the meat at this position may be calculated. The radiation flux at the second pass will be decreased by absorption in the meat of the first pass and by distance from the source. As successively less radiation is available to each additional pass a practical limit to the number of passes will be reached. Figures 43a, 43b, 43c, 44 and 45 show a design with four passes on either side of the source.

b. Description of Design

Figure 43a shows an elevation view of the radiation chamber and conveyor system and Figs. 43b and 43c show some of the details. Prepackaged meat cuts packed into cartons 8 inches by 24 inches by 18 inches are brought by belt conveyor A from the meat cutting and packaging areas. The cartons move down slide B into tray 1 of the irradiation conveyor while this conveyor is in the stationary phase of its intermittent operation. As the irradiation conveyor moves, the cartons are carried down into the radiation chamber through opening C and past concrete shield D. Four vertical passes, E, F, G, and H are made on the right side of the source, and four passes J, K, L, and M are made on the left side of the source. This arrangement permits irradiation of the cartons from both sides so as to produce a more uniform radiation dose.

Well N is filled with water and is used to hold the source when the radiation must be shut off to permit entry to the radiation chamber for maintenance, routine inspection, or replacement of a portion of the source. If the radiation chamber is located above grade as shown in Fig. 43a, a concrete wall, P, which is 3 feet by 4 inches thick, would be used for shielding. If the radiation chamber is placed below grade, the wall thickness may be reduced to that required for structural strength alone, as the earth will act as a radiation shield. A labyrinthine entrance to the radiation chamber is provided at the lower left as shown in Fig. 43a.

The radiation chamber is maintained at refrigerator temperature to reduce the rate of reproduction of the microorganisms during irradiation. After irradiation the cartons of "pasteurized" meat pass concrete shield D, travel through opening C out of the radiation chamber, and are dumped into slide Q. Conveyor R carries the cartons of pasteurized meat to refrigerator storage for subsequent shipping.

A plan view of the radiation chamber is presented in Fig. 44. This view shows the simple labyrinth used as an access passage for routine inspection and maintenance. The conveyor in the radiation chamber may be driven by

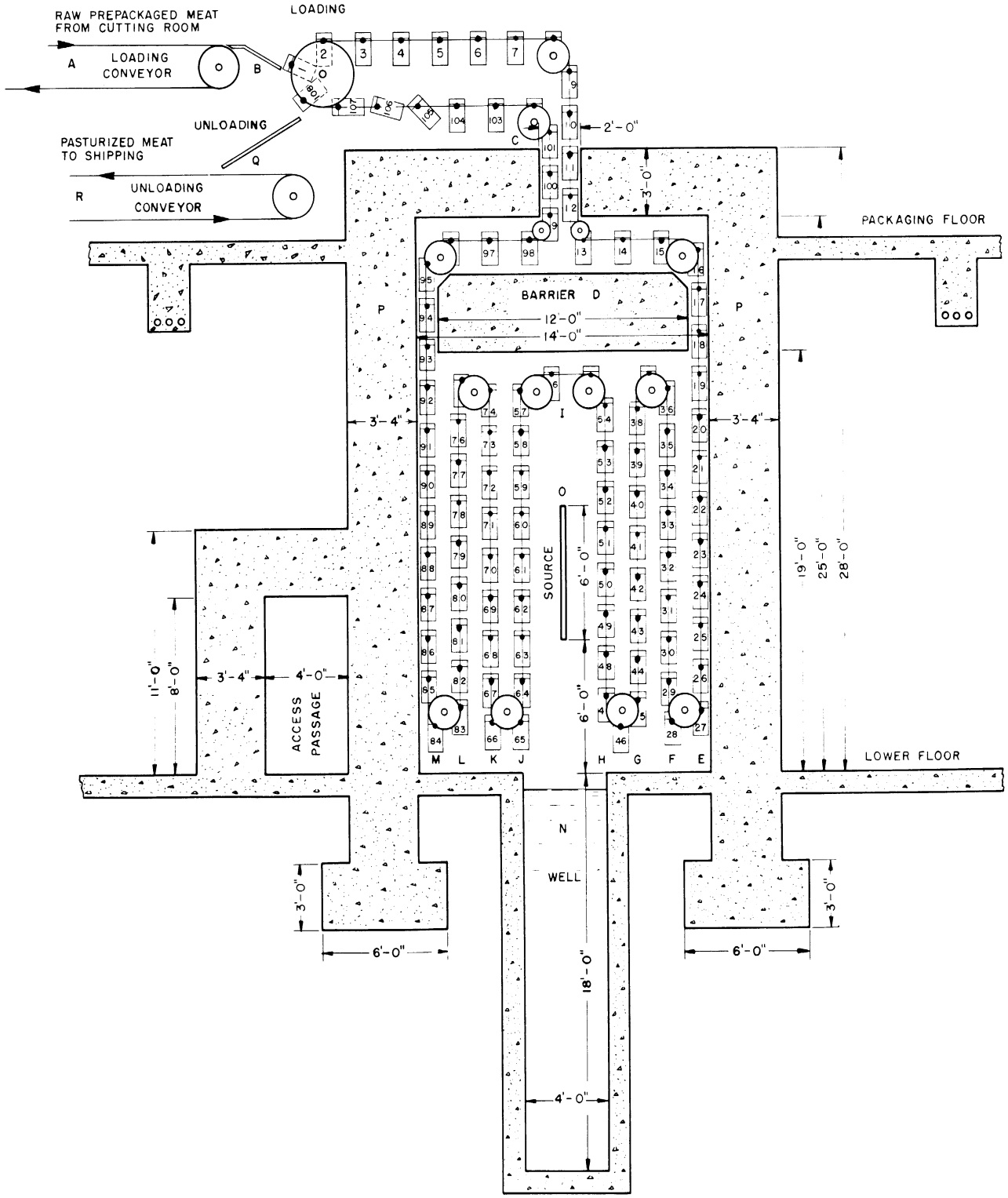

Fig. 43a. Elevation View of Radiation Chamber for Prepackaged Meat.

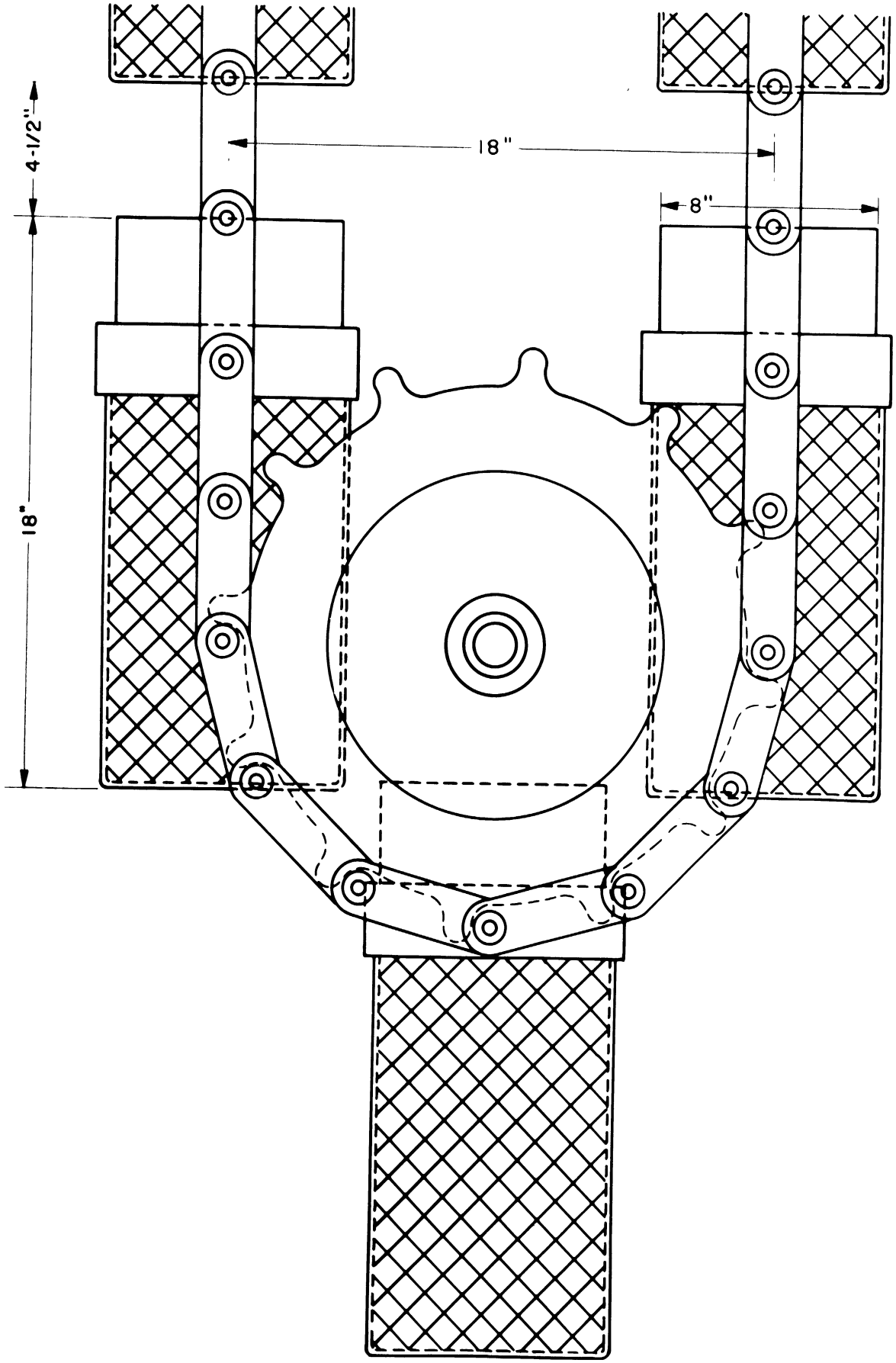

Fig. 43b. Sprocket and Tray Detail for Radiation Chamber for Prepackaged Meat.

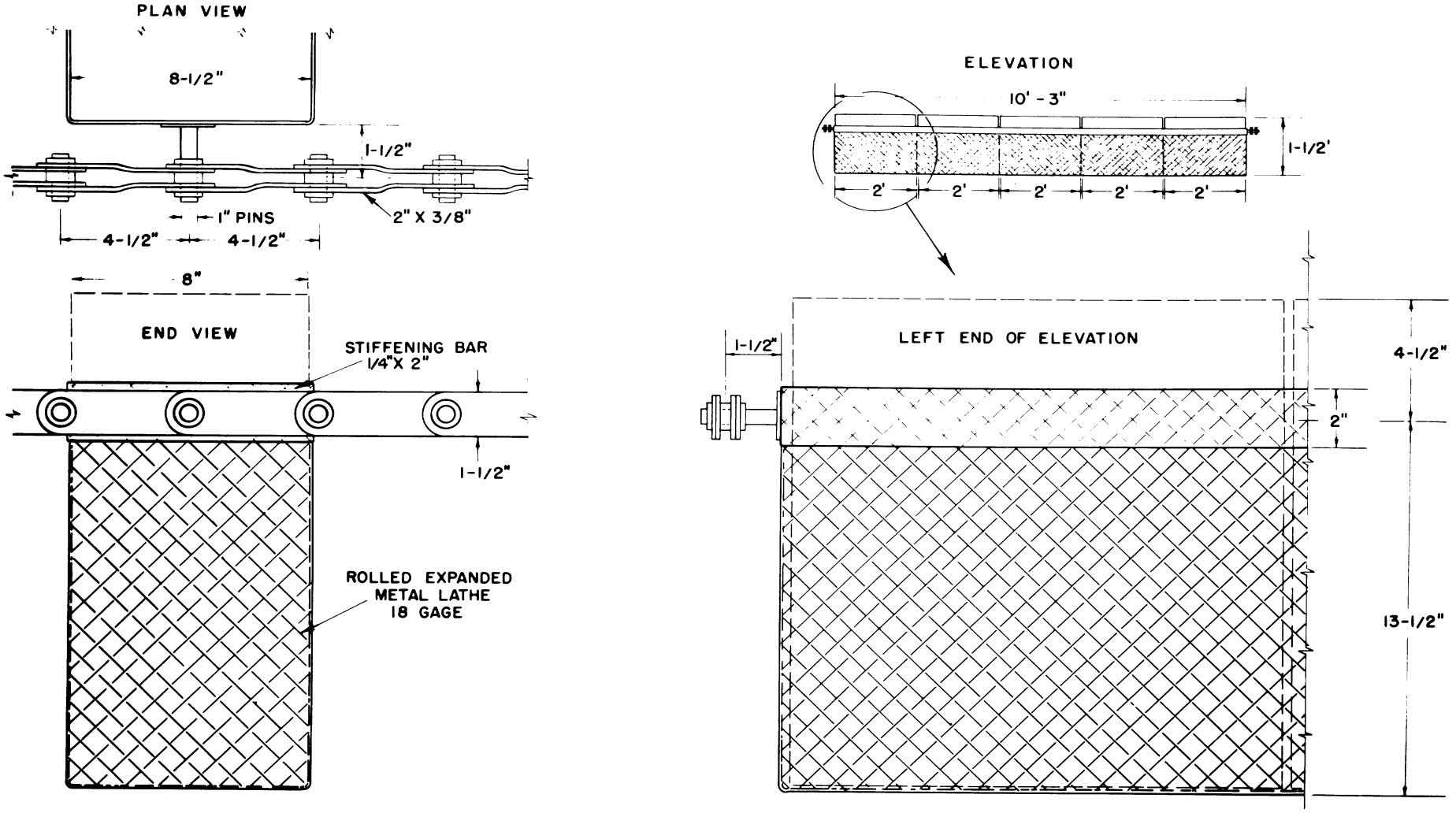

Fig. 43c. Tray and Chain Detail for Radiation Chamber for Prepackaged Meat.

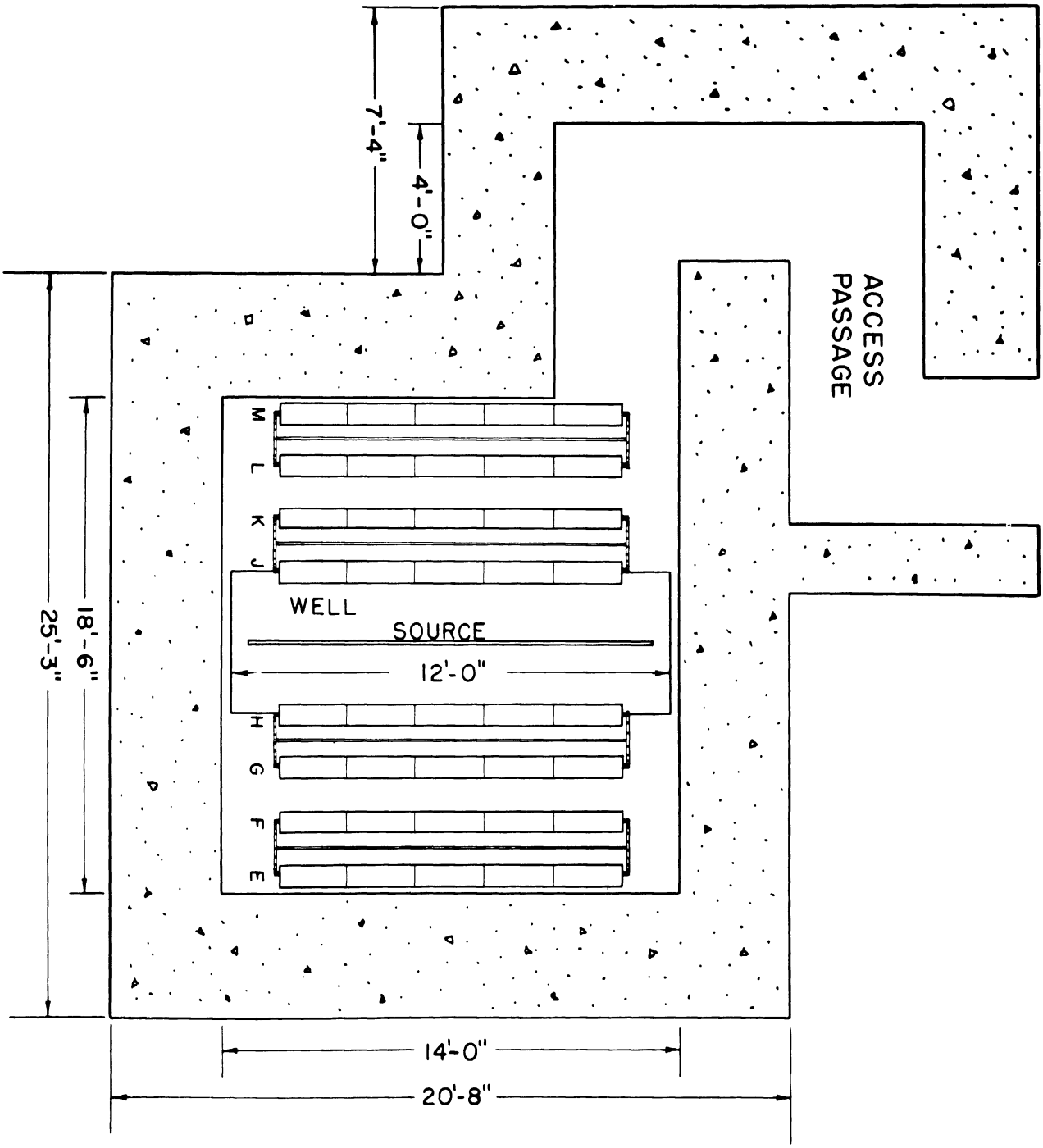

Fig. 44. Plan View of Radiation Chamber for Prepackaged Meat.

sprockets on stub shafts. Some of the sprockets may be "idlers", but more than one sprocket should be used as a "driver" so as to reduce the total tension in the conveyor. All drivers could be connected by either a common shaft or a chain drive to keep them synchronized.

As the trays of cartons pass around the arc at the end of each vertical pass, a sufficient arc must be maintained in order to prevent interference between the top of one tray of cartons and the bottom of the following tray of cartons. When the spacing between trays is decreased, a larger radius must be used on the sprockets to prevent interference. The optimum design for continuous absorption, however, requires minimum spacing between trays and, to minimize attenuation with distance, minimum spacing between passes. Thus some compromise must be made between minimum spacing of the trays and minimum spacing of the passes. The detail of the trays passing around a sprocket is shown in Fig. 43b. Using the 4-1/2 inch free space between the trays shown in Fig. 43b will require a sprocket having a minimum diameter of about 18 inches to prevent interference between successive trays. The spacing between passes would be 18 inches at the lower limit between passes E and F, and G and H. As the trays would not interfere between passes F and G, the spacing between these passes at the lower limit may be less than 18 inches; a distance of 12 inches was selected. Similarly, the spacing at the upper limit can be 12 inches between passes E and F, and G and H, whereas the spacing between F and G must be 15 inches to avoid interference.

The details of the proposed tray and conveyor chain are shown in Fig. 43c. The tray shown is fabricated of 16-gauge rolled expanded steel lathe with a reinforcing steel strip 3 inches by 1/4 inch around the top. The trays are pivoted 13-1/2 inches from the bottom of the trays and 4-1/2 inches from the top of the cartons. The center of gravity is placed well below the pivot point, which aids in maintaining a vertical position. As an additional measure, guides may be used to keep the trays vertical while in the radiation chamber and while passing around the sprockets. Guides will be used outside the radiation chamber to unload the trays at position Q and to hold the trays at an angle while being loaded at position B. Figure 45 shows a cut-away view of the radiation chamber in perspective.

c. Optimum Activity Distribution in a Plaque Source

Consider the radiation field associated with an infinite strip of uniformly distributed radioactive material, such as represented in Fig. 46. Along any line parallel to the long axis (x) of the source, the dosage rate would remain constant. Such a plaque would provide a radiation field admirably suited for the irradiation of packaged meat traveling on a conveyor system in a path perpendicular to the long axis of the source. While the meat would pass through a continuously varying field of radiation, each carton of meat would have accumulated the same total dose after passing through the radiation chamber since

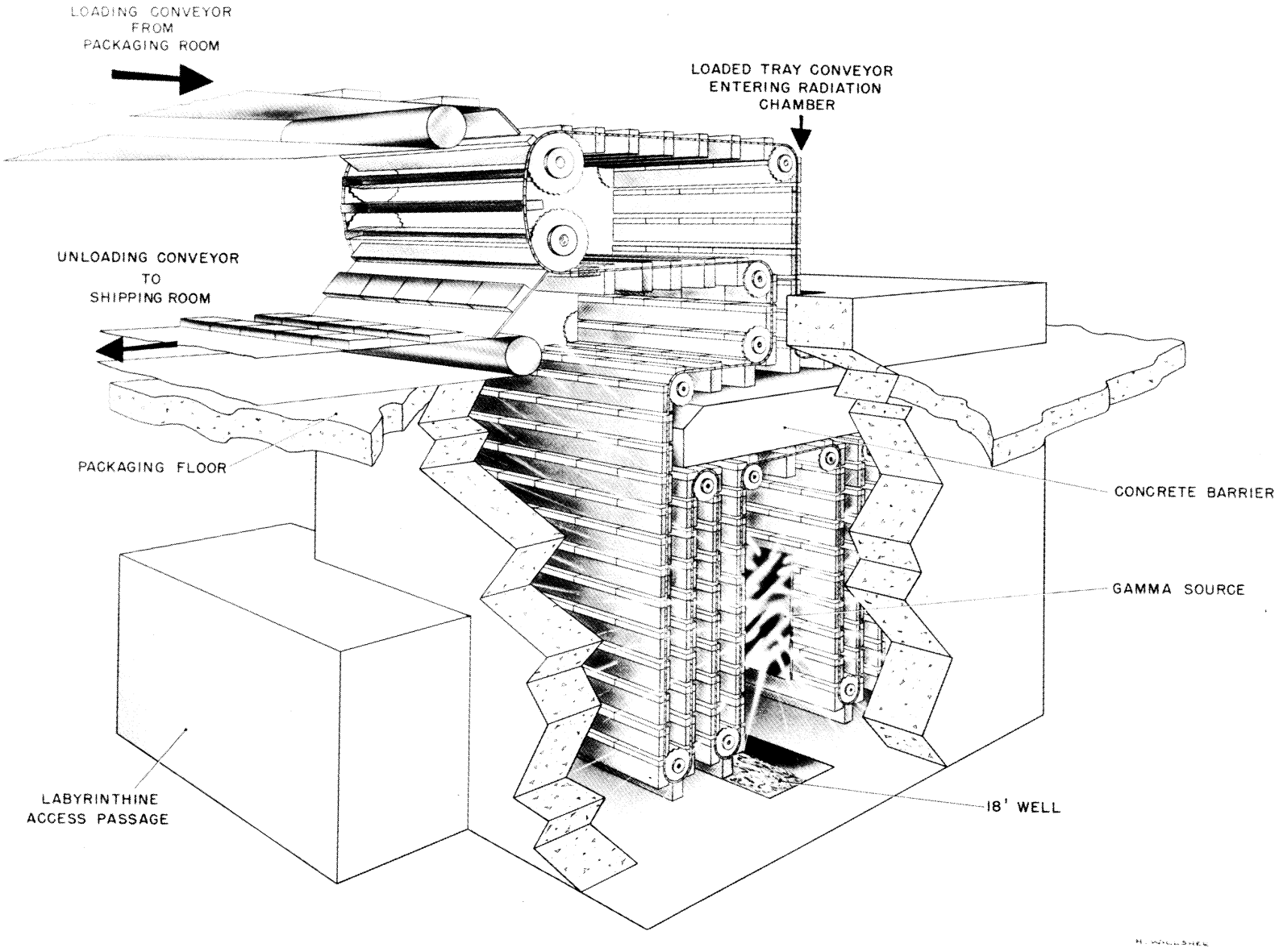

Fig. 45. Cut-away View of Radiation Chamber for Prepackaged Meat.

each row of meat would be oriented with its long axis parallel to the long axis of the source.

Infinite plaques, of course, cannot be realized. Figure 47 shows the radiation field associated with a plaque of finite length and uniform concentration. The design of such a plaque was presented in Progress Report 5, pp. 91-100. However, if a uniform radiation dose is to be given to each carton of prepackaged meat, only the field directly opposite the middle section (about one-third of the total width) should be used; even this portion of the field varies approximately 10 percent. Thus, in order to develop a uniform field 10 feet wide, a plaque at least 30 feet wide would be necessary. Such an installation would be quite inefficient since well over two-thirds of the radiation from the plaque would be wasted.

By varying the specific activity over the length of the plaque, the radiation field can be made more uniform and the length of the plaque can be reduced with a resultant gain in efficiency. This procedure was employed in the following design. The plaque is represented in Fig. 48. It is composed of nine sections, of different uniform concentrations as shown in the figure. The lowest concentration is approximately two-thirds that of the highest.

The procedure for calculation of the dose rate at an arbitrary position about the source is similar to the procedure described in Progress Report 5; however, in this case a further complication is introduced by the nonuniform concentration of radioactive material. The procedure employed is best illustrated by means of an example.

Consider the dose rate existing at the point p = (40, 24, 21) shown in Fig. 49. The flux contributed by the shaded portions of the plaque is given by

$$I_1 = \alpha_1 (H_1 + H_2) ,$$

where α_1 = the concentration coefficient for this portion of the source (see previous section for discusstion of α)

$$H_1 = \int_8^{32}\int_0^{12} \frac{dx\,dy}{x^2 + y^2 + 21^2} = \int_8^{32} \frac{1}{\sqrt{x^2 + 441}} \tan^{-1} \frac{12}{\sqrt{x^2 + 441}}\,dx ,$$

and

$$H_2 = \int_8^{32}\int_0^{60} \frac{dx\,dy}{x^2 + y^2 + 21^2} = \int_8^{32} \frac{1}{\sqrt{x^2 + 441}} \tan^{-1} \frac{60}{\sqrt{x^2 + 441}}\,dx .$$

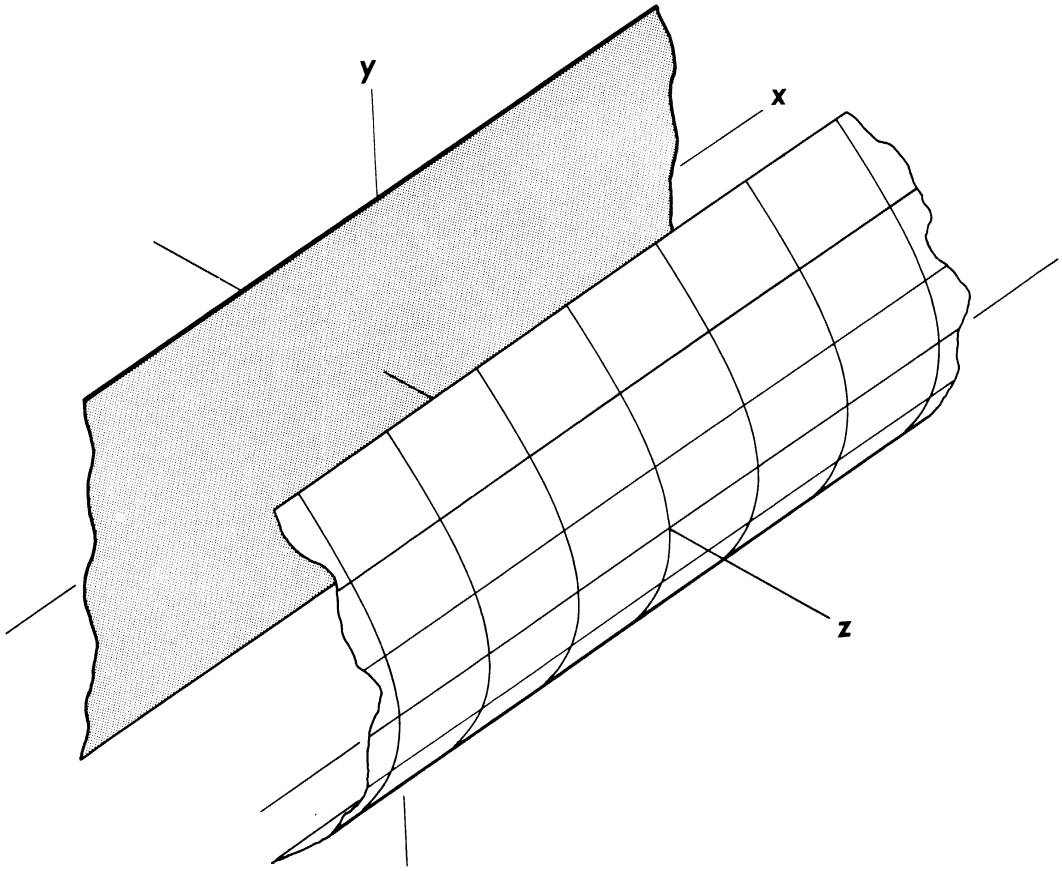

Fig. 46. A Section of a Typical Isodose Surface Generated by a Semi-infinite Strip Plaque of Uniformly Concentrated Activity.

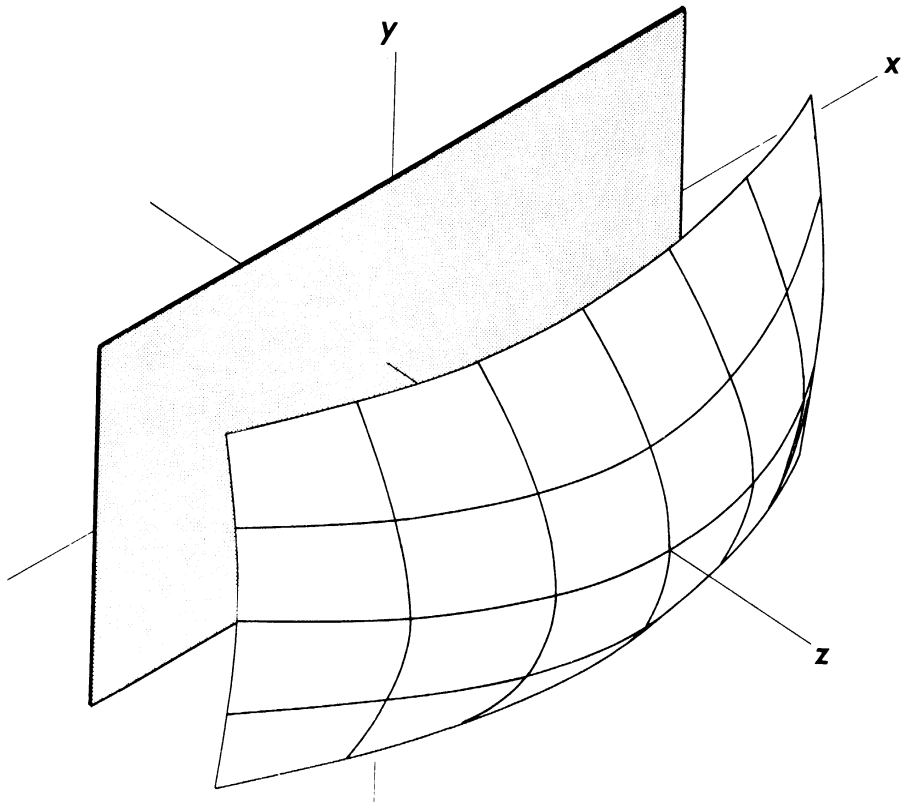

Fig. 47. A Section of a Typical Isodose Surface Generated by a Finite Plaque Source of Uniformly Concentrated Activity.

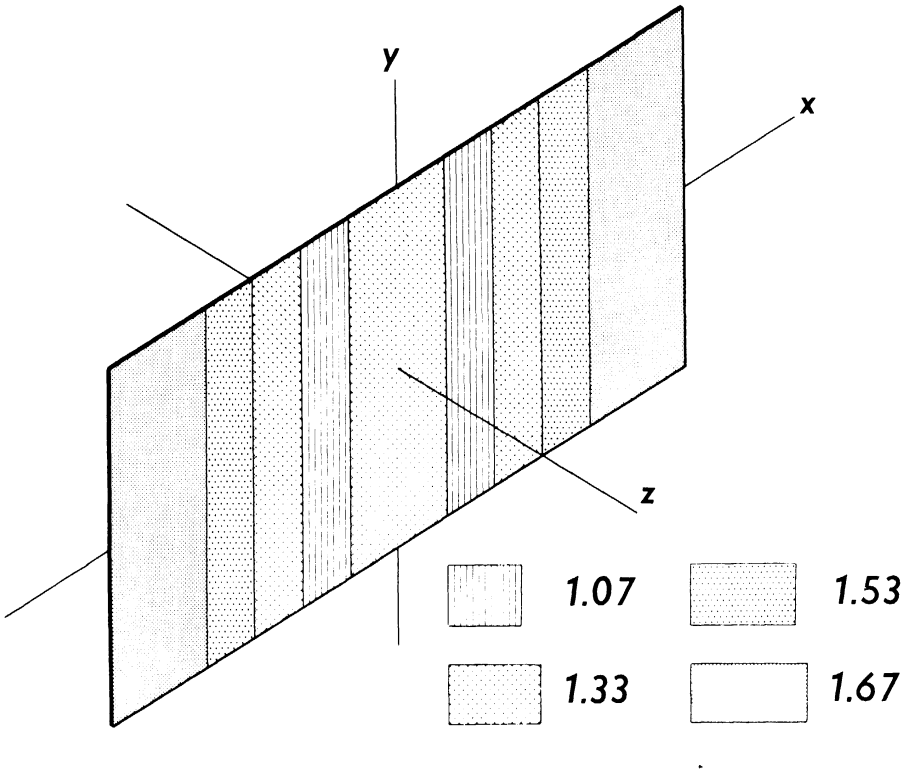

Fig. 48. A View of the Plaque Source Consisting of Several Strips, Each of Uniformly Concentrated Activity. (Relative Concentrations are Shown in Lower Right.)

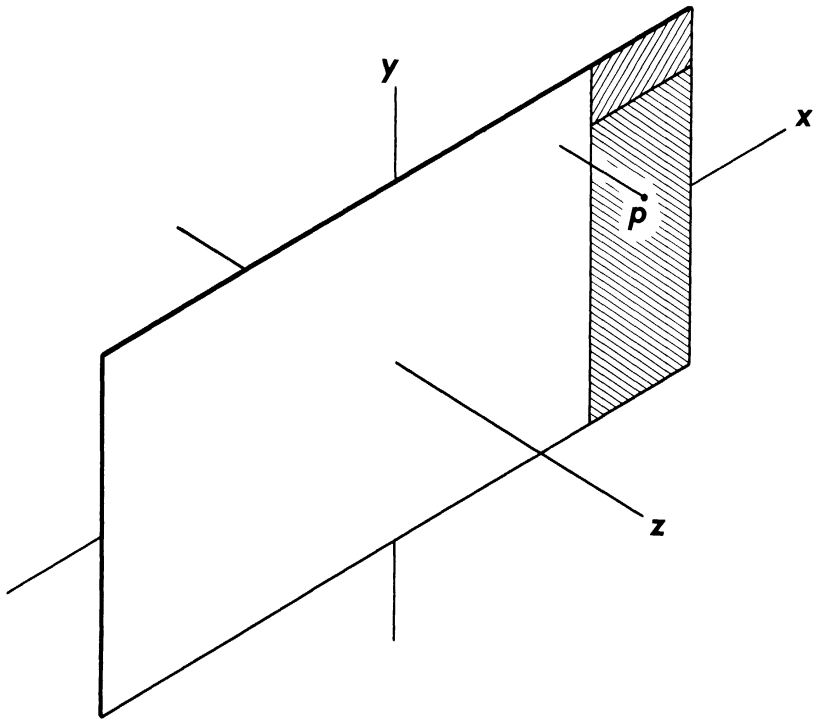

Fig. 49. The Portion of the Source under Consideration in the Sample Calculation of the Radiation Flux at a Typical Position, p.

These integrals cannot be formally integrated; hence Euler's quadrature formula was used to numerically approximate the integrals.

This procedure is followed for each of the nine portions of the plaque to obtain the total dose rate at the point (40, 24, 21) as

$$I = I_1 + I_2 + \ldots + I_9 .$$

d. Capacity Calculations

The capacity of the radiation chamber shown in Figs. 43a-45 will depend on the activity of the source and the dose required. The total activity of the source has been set at 1.5 megacuries so as to compare this design with that presented in Progress Report 5. Fixing this activity fixes the magnitude of the radiation field in a plane perpendicular to the face of the source.

The radiation flux in a plane perpendicular to the face of the source at its center line was calculated for the lines 21 inches from the source and 83 inches from the source and for the points at the center line 36, 51, and 66 inches from the source. The values determined are shown in Fig. 50. This figure shows that the radiation flux along the horizontal axis parallel to the source can be made approximately uniform by varying the activity of the source along the horizontal axis. The flux may be considered constant along the horizontal axis and this dimension may be eliminated from the calculations of radiation field, permitting the plotting of the radiation field in two dimensions.

As the conveyor passes are spaced at 21, 36, 51, and 66 inches from the source, the dose rates (in air) were calculated at these distances as a function of the vertical distance above the center line of the source. The dose rate curves at 21, 36, 51, and 66 inches as calculated are shown in Fig. 51. These data were cross plotted to give the isodose curves shown in Fig. 52. The dose rates shown in Fig. 51 are for radiation in air and must be corrected for absorption in the meat. This correction was made using 8 inches as the half-value thickness for absorption in meat for gamma radiation from cesium-137 with an absorber efficiency of 85 percent. The isodose curves corrected for absorption are shown in Fig. 53.

For purposes of comparison, a dose of 30,000 rep, the dose selected for the pork irradiation facility of Progress Report 5 will be used in capacity calculations, also, capacities will be compared for a dose of 80,000 rep, which is considered a more suitable dose for pasteurization of prepackaged fresh meat.

Figure 54 shows a plot of the radiation flux in rep/hour at the center of meat after corrections are made for absorption plotted as a function of the length of path traveled through the first half of the radiation chamber. This figure was obtained by use of Fig. 41 and Fig. 53. The plot of radiation flux

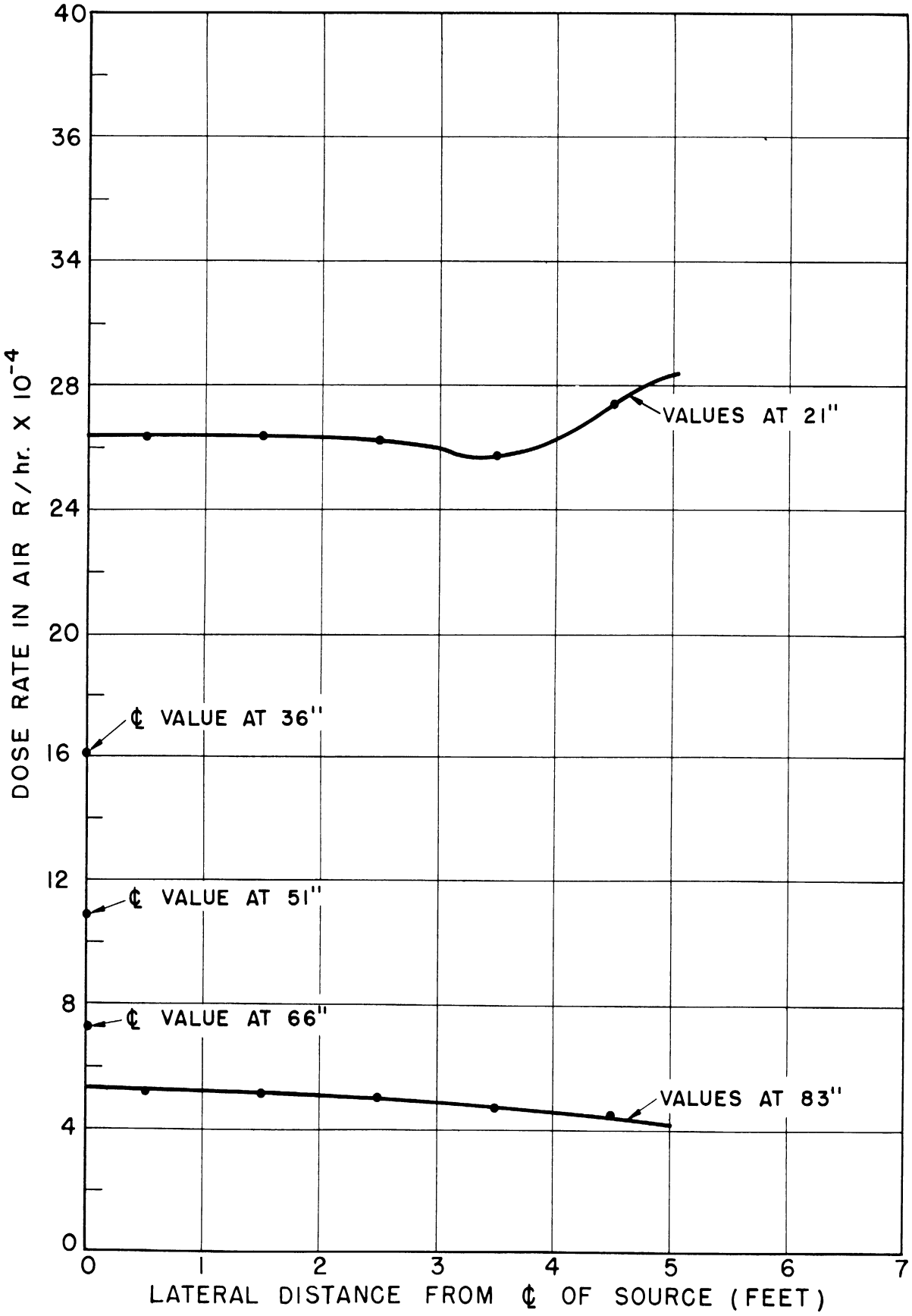

Fig. 50. Radiation Flux (r/hr in Air) in a Horizontal Plane at Center Line of Source for One Quadrant of Radiation Chamber.

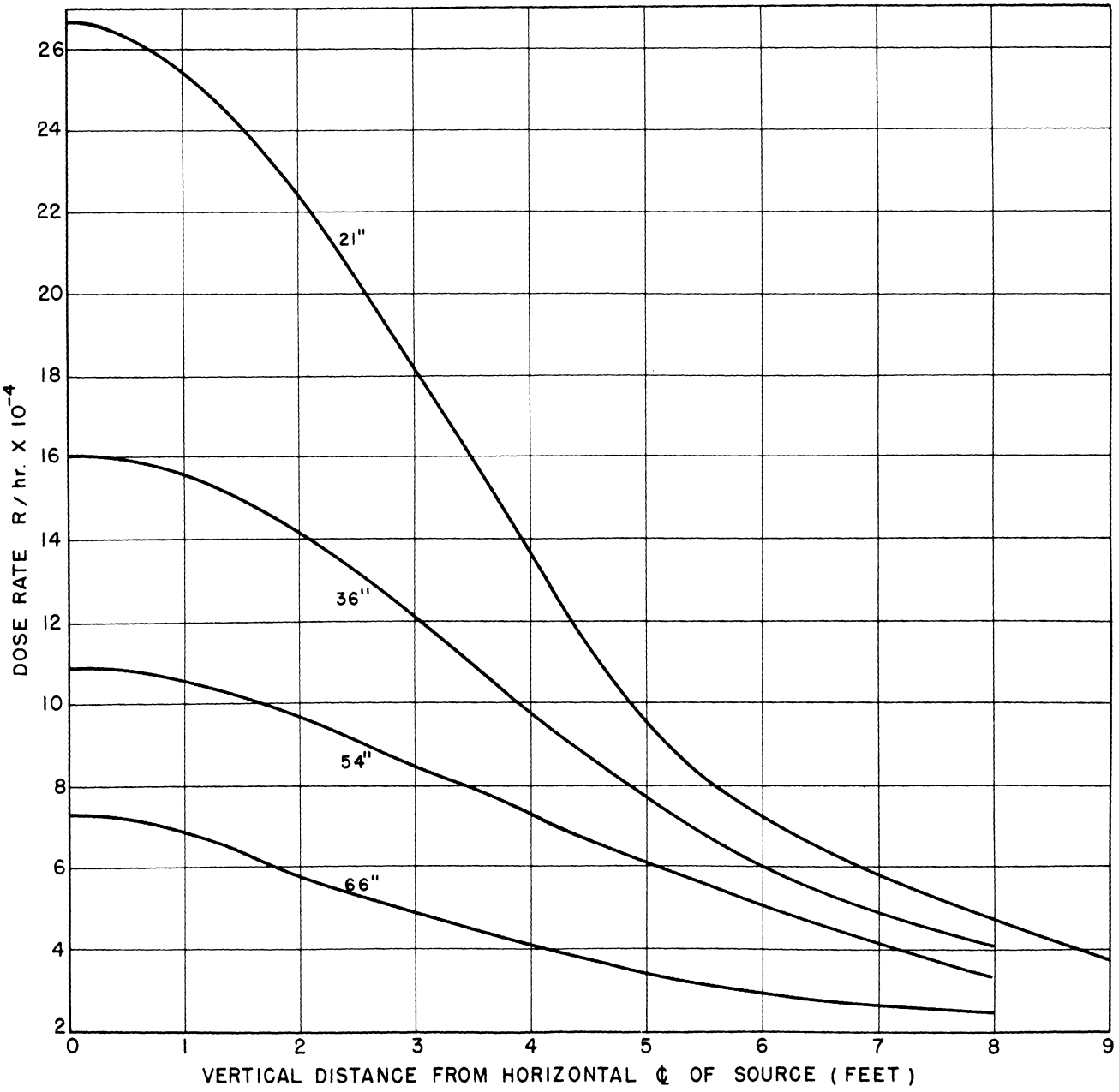

Fig. 51. Radiation Flux (r/hr in Air) in Vertical Plane (Perpendicular to Source at Center Line) for One Quadrant of Radiation Chamber.

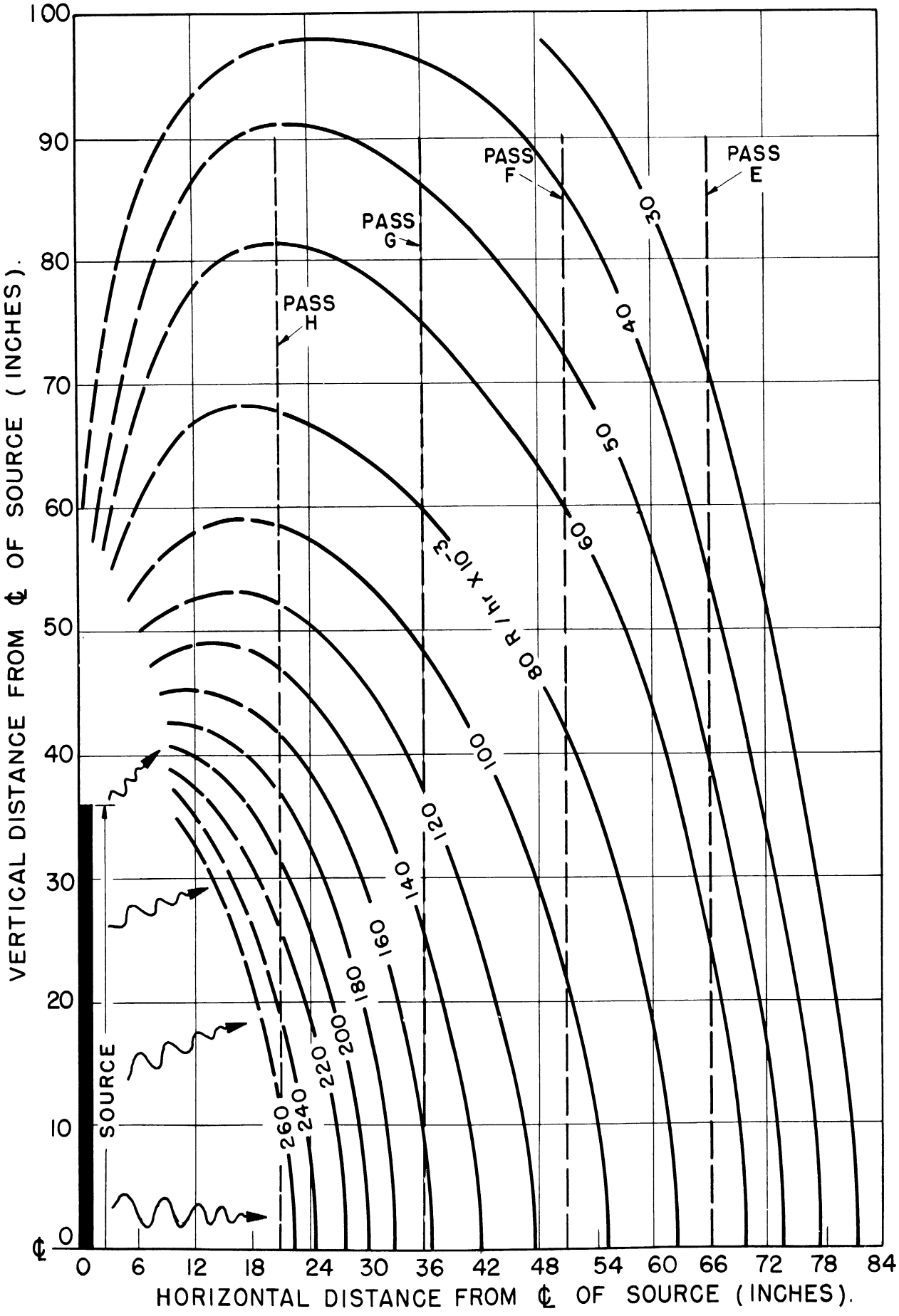

Fig. 52. Isodose Curves (r/hr in Air) in Vertical Plane Perpencidular to Source at Center Line for One Quadrant of Radiation Chamber.

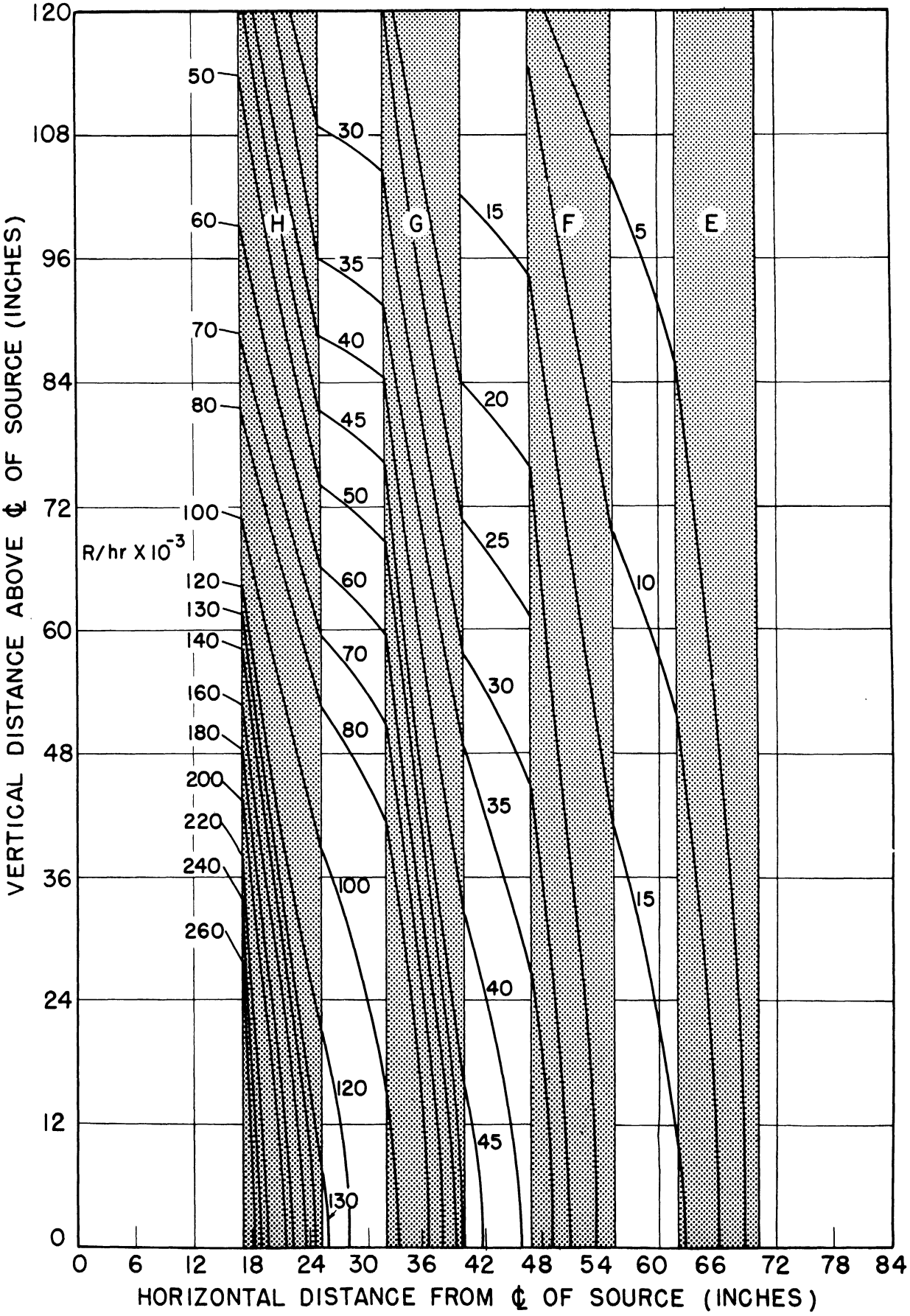

Fig. 53. Isodose Curves (rep/hr) from Fig. 52 Corrected for Absorption.

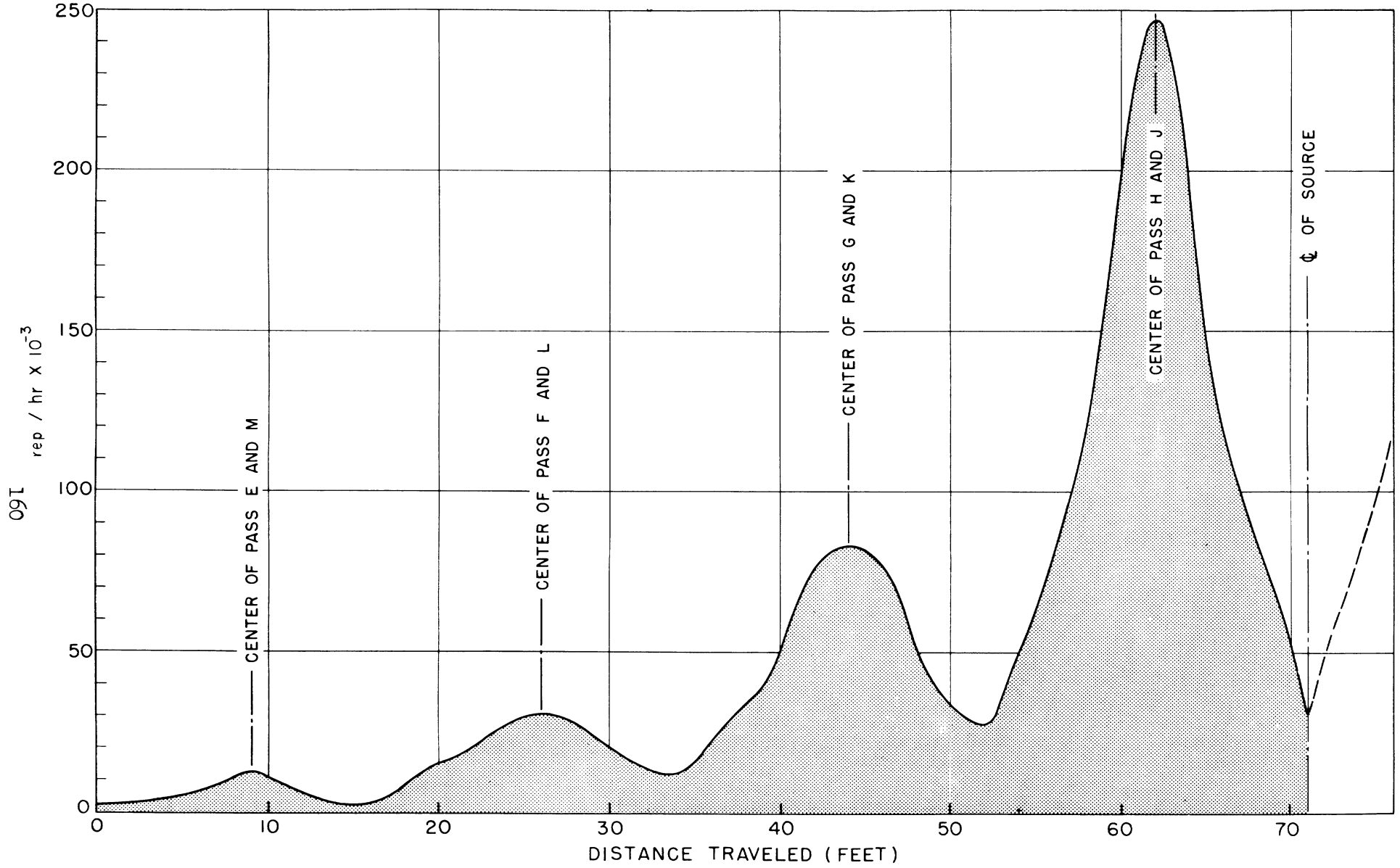

Fig. 54. Dosage Rate Received at Center of 8-Inch Carton of Meat as a Function of Distance along Path in Radiation Chamber.

for the second half of travel through the radiation chamber would be a mirror image of this curve and therefore is not shown. To determine the time required to absorb a given dose, the curve must be integrated graphically. This was accomplished by employing the Trapezoidal rule. Twice this integral gives the accumulated dose for the meat in the center of the carton as a function of the tray speed through the chamber. The radiation dose received by the meat near the surface of the carton is calculated to be approximately 10 percent higher than the dose received by the meat at the center of the carton as a result of less absorption. The accumulated dose may be expressed as:

$$\text{Accumulated Dose in rep} = 2\,\frac{\text{rep ft/hr}}{\text{x ft/hr}}$$

where: x = tray speed in feet per hour.

From the plot of dose rate versus length of path traveled, Fig. 54, the accumulated dose at a tray speed of 1 foot per hour was determined to be $7(10)^6$ rep.

Dividing the specified dose by the accumulated dose as a function of tray speed yields a value which has the reciprocal units of tray speed. Multiplying this value times the length of path traveled in feet gives the time required per cycle for the meat to obtain a specified dose.

$$\text{Radiation Time per Cycle (for } 3 \times 10^4 \text{ rep Dose)} = \frac{3\,(10)^4 \text{ rep}}{7\,(10)^6 \text{ rep ft/hr}}\ 140 \text{ ft} = 0.60 \text{ hrs}$$

$$= 36 \text{ minutes}$$

There are 74 trays within the chamber with 5 cartons of meat per tray, each weighing approximately 116 pounds. The capacity in pounds of meat per cycle would then be:

$$\text{Capacity/Cycle} = \frac{74 \text{ trays in chamber}}{\text{cycle}} \times \frac{5 \text{ cartons}}{\text{tray}} \times \frac{116 \text{ lbs}}{\text{carton}}$$

$$= 4.3\,(10)^4 \text{ pounds/cycle}$$

The capacity in pounds per hour may be obtained by dividing by the exposure time required per cycle.

$$\text{Capacity/Hour} = 4.3\,(10)^4 \frac{\text{pounds}}{\text{cycle}} \cdot \frac{1}{0.6 \text{ hrs/cycle}}$$

$$= 7.16\,(10)^4 \frac{\text{pounds}}{\text{hour}}\ \frac{1}{2000 \text{ pounds/ton}}$$

$$= 35.8 \frac{\text{tons}}{\text{hour}} \text{ (for } 3 \times 10^4 \text{ rep dose)}$$

Similar calculations may be performed to determine the radiation time required to obtain 80,000 rep. A new computation of the capacity of the radiation facility may then be made utilizing the different exposure time.

With the same source design and source strength any increase in dose would be a function of the exposure time for the same path of travel through the chamber. The time required to obtain 80,000 rep would then be equal to:

$$\text{Radiation Time per Cycle (for } 8 \times 10^4 \text{ rep Dose)} = \frac{80{,}000 \text{ rep}}{30{,}000 \text{ rep}} \times 0.6 \text{ hours}$$

$$= 1.6 \text{ hours}$$

The capacity of the plant will be reduced due to the longer radiation time necessitated by the larger dose.

$$\frac{\text{Capacity}}{\text{Hour}} = \frac{30{,}000 \text{ rep}}{80{,}000 \text{ rep}} \quad 35.8 \frac{\text{tons}}{\text{hour}} = 13.4 \frac{\text{tons}}{\text{hour}} \text{ (for } 8 \times 10^4 \text{ rep dose)}$$

The design of the new radiation facility for the pasteurization of meat is obviously greater in efficiency as evidenced by the increased capacities. For a similar dose of 30,000 rep an increase of over 5 times the production is realized with the new chamber design as compared to the design described in Progress Report 5. For a dose of 80,000 rep the new facility has double the capacity of the previous pork irradiation facility designed to give a radiation dose of 30,000 rep.

e. Cost Estimates

(1) Estimated Cost of Radiation Facility. To compare this design with the design for the pork irradiation facility reported in Progress Report 5, a source of 1.5 megacuries of cesium-137 will be used, as in that design. Although this design is more elaborate than that prepared for the irradiation of hog carcasses, the greater efficiency in absorbing radiation results in a more economical design. Using similar cost figures, the cost of the radiation facility was estimated to be $82,500.00 as shown in Table 28.

(2) Estimated Cost of Irradiation of Meat Based on 5-Year Amortization. Comparison with pork irradiation facility: For purposes of comparison the costs will be taken as $483,000 for 1.5 megacuries of cesium-137, $25,000 for a shipping container, and $30,000 per year for operation and maintenance including two health physicists. These costs are the same as those estimated for the pork irradiation facility described in Progress Report 5. The cost of the radiation chamber was estimated to be $82,500 as shown in Table 28.

TABLE 28

ESTIMATED COST OF RADIATION FACILITY

Excavation and shoring for footings and well	$ 800	
Concrete for 12-1/2 x 18 x 3-ft well (60 yds at $20/yd)	1200	
Reinforcing for well (4000 lb at $0.10/lb)	400	
Asphalt lining for well	200	
Forms for well (1300 bd ft at $100/M)	130	
Labor for forming and pouring well	600	
Concrete for walls and footings (300 yds at $20/yd)	6000	
Forms for wall (6000 bd ft at $100/M)	600	
Labor for forming and pouring wall	2200	
Concrete for access passage (50 yds at $20/yd)	1000	
Forms for access passage (1000 bd ft at $100/M)	100	
Labor for forming and pouring access way	800	
Concrete for floor (6 yds at $20/yd)	120	
Reinforcing for floor (500 lb at $0.10/lb)	50	
Labor for pouring floor	100	
Concrete for barrier wall (20 yds at $20/yd)	400	
Forms for barrier wall (1000 bd ft at $100/M)	100	
Reinforcing for barrier wall (2000 lbs at $0.10/lb)	200	
Labor for forming and pouring barrier wall	400	
Concrete for roof (70 yds at $20/yd)	1400	
Forms for roof (1000 bd ft at $100/M)	100	
Reinforcing for roof (3000 lb at $0.10/lb.)	300	
Labor for forming and pouring roof	600	
Elevator mechanism	5600	
Ion-exchange system for well water	3000	
Monitoring equipment	4000	
Chain conveyor (244 ft x 2 at $5.00/ft)	2440	
Conveyor sprockets and stud shafts	3000	
Conveyor drive	6200	
Conveyor trays (110 trays) $20/tray	2200	
Dump mechanism	3000	
Loading mechanisms	5600	
Conveyor control mechanism	4200	
Access doors (with safety interlock)	1400	
Ventilating and refrigeration of air	6000	
Wiring	600	
Water lines and labor for pipe fitting	800	
Backgrading	200	
Painting	600	
Subtotal for labor and materials		63,640
Miscellaneous contingencies (10% of subtotal)		6,360
Engineering costs (7% labor and materials)		5,000
Contractors fee (10% of costs)		7,500
Total		82,500

Cost of radiation chamber	$ 82,500
Shipping container	25,000
1.5-megacurie source installed	483,000
Total investment	$590,500

Amortizing the investment on a 5-year basis and including 6 percent interest on the investment, the annual cost of such a plant would be

$$\frac{1.18}{5}\ \$590,500) = (\$139,000) \ .$$

Annual costs including operation:

$$(\$139,000) + \$30,000 = (\$169,000) \ .$$

Annual capacity for a dose of 30,000 rep:

$$(7.16(10))^4 \text{ lbs/hr (20 hrs operation/day)(260 days/year)} = 3.72(10)^8 \text{ lbs/yr.}$$

Cost per lb to be added to meat for a radiation dose of 30,000 rep:

$$\frac{(\$169,000)/\text{yr}}{(3.72(10)^8)\text{lbs/yr}} = \$.000458/\text{lb} = .458 \text{ mills/lb} \ .$$

Comparing this estimated cost with the figure of 2.3 mills per lb estimated for the pork irradiation facility, the present design may be considered 2.3/(.46) or 5 times as efficient in utilizing radiation.

Using a pasteurization dose of 80,000 rep: On the same basis but using a dose of 80,000 rep the annual capacity becomes:

$$(3.72(10)^8)3/8 = 1.4(10)^8 \text{ lbs/yr} \ .$$

Cost per lb to be added to meat for a radiation dose of 80,000 rep would be

$$.46 \text{ mills/lb } (8/3) = 1.23 \text{ mills/lb} \ .$$

(3) Estimated Cost of Irradiation of Meat Based on Rental of Source and Shipping Container. The rapid strides made by industry in developing new techniques necessitates amortization of new porcesses over a short period, usually 5 years. This is true because a process which is successful today may be obsolete and have to be abandoned in less than 5 years in the interests of new developments. However, it is not realistic to amortize a cesium-137 source in 5 years, as this radioisotope has a half-life of 33 years. Thus, it is believed that the fission product sources must be rented either by the AEC or by a contractor rather than sold if an amortization realistic with the actual useful life of the source is to be realized.

After two half-lives or a total of 66 years, 75 percent of the cesium-137 will have decayed. Therefore, it would be more realistic to amortize the source over say a 33-year or a 50-year period or a rate of 3 or 2 percent, respectively, rather than 20 percent per year. Also, interest on a long-term investment might be 4 percent rather than 6 percent making the annual cost of the source only 6 or 7 percent of the initial cost.

In Progress Report 5, it was estimated that the shipping and installation costs would be approximately 1/6* of the installed costs, or in the case of the 1.5 megacurie cesium source the cost of installation would be approximately $80,500 and the cost of the source itself would be $402,500. If the source were rented, the annual rental should include the use of the shipping container. Therefore, the annual cost for rental would be 0.06 x (402,500 + 25,000) = $25,650/yr. The installation cost would then be included in the cost of the chamber and this total would be amortized in a 5-year period the annual amortization would thus be:

$$(82,500 + 80,500)\ \frac{1.18}{5} = \$38,450/\text{yr}$$

On this basis the total annual cost for rental of source, including operating costs and 5-year amortization would be

$$\$25,650 + 30,000 + 38,450 = \$94,100/\text{yr}$$

and the cost per lb to be added for an irradiation dose of 80,000 rep would be:

$$\frac{\$94,100/\text{yr}}{(1.4 \times 10^8)\ \text{lb/yr}} = \$.000672/\text{lb} \quad \text{or} \quad 0.67\ \text{mills/lb}$$

f. Discussion

There are many problems that must yet be solved before it will be possible to pasteurize prepackaged meat on a commercial basis with gamma radiation. In the opinion of the personnel of this laboratory the chief problem to be solved before gamma radiation may be used by the food industry is the establishment of the wholesomeness of irradiated food. There are sufficient applications of gamma radiation to the food industry in which flavor is not a major problem to support this opinion; i.e., pasteurization of prepackaged meats, irradiation of grains to prevent insect damage, etc.

*This ratio is undoubtedly high for a 1.5 megacurie source

After conducting pilot animal feeding experiments for about one year the personnel of the Phoenix Project No. 41 (see Part IV of this report) have yet to find any reason why irradiated food should not be considered wholesome. However, long term feeding studies with significant numbers of animals must be completed before the wholesomeness (or toxicity) of irradiated food can be reliably established. It is gratifying to learn that the Surgeon General's Office is considering the investigation of this problem.

Many other problems exist such as color changes which are particularly noticeable on the surface of irradiated raw beef. More taste panel studies are required on different types and cuts of meat including fish and other seafoods. More data are needed on the increase in shelf life that can be expected using different radiation dosages and different storage conditions.

In addition to the need for more information on irradiated products there also should be more information on the sources of radiation, the design of radiation facilities, and the cost of irradiation. This design was made with the intention of exploring some of these questions. It is obvious to those who have worked with this design that it could be improved considerably. More attention should be given to using separated cesium-137 and possibly cerium-144 as this appears more feasible than attempting to use the gross fission products. If the radioisotopes with long half life such as these two and strontium-90 could be put to industrial use the remainder of the gross fission products could be discarded after storage for a limited time. Different designs of sources should be made so as to determine the optimum conditions for preparing and handling the sources.

Although the design shown was intended for the pasteurization of meat, it might be used for the irradiation of bags of potatoes and onions to prevent sprouting or for the sterilization of canned food. The cartons could be filled with No. 10 cans of food rather than prepackaged meat. If a radiation dose of 3.2×10^6 rep were used for sterilization rather than the 8×10^4 rep used for pasteurization, the capacity would be reduced by a factor of 40 or to about 1/3 tons/hr or about 8 tons per 24 hour day. The cost per pound for irradiation would be increased to about $0.03 per pound if a rented source were used. If the capacity were tripled by using 4.5 megacuries the cost per pound for irradiation would be approximately halved. These figures show that radiation sterlization at least is economically feasible.

PART IV. COOPERATIVE RESEARCH WITH MICHIGAN MEMORIAL-PHOENIX PROJECTS

A. INTRODUCTION

As explained in the preface, this report includes a description of various research projects supported by the Michigan Memorial-Phoenix Project in which the irradiation facilities in the Fission Products Laboratory have been used. This subject material has been included because it is believed to have considerable bearing on the possible utilization of the waste fission products. Most of the material presented in this manner in this report will appear at some later date in the technical literature. No portion of this report dealing with work supported by the Michigan Memorial-Phoenix Project is to be reproduced without permission of the authors.

B. ANIMAL-FEEDING EXPERIMENTS

Personnel:

Supervisors and Consultants: H. C. Eckstein, Professor of Biological Chemistry; L. E. Brownell, Director of Fission Products Laboratory and Associate Professor of Chemical and Metallurgical Engineering; L. L. Kempe, Assistant Professor of Bacteriology and Assistant Professor of Chemical and Metallurgical Engineering.

Laboratory Personnel: H. O. France, Research Associate; B. W. Uhlendorf, Research Assistant; R. Dennis, Statistican; E. Ambo, Laboratory Assistant; R. Rose, Laboratory Assistant.

1. Introduction

An experiment is now underway on the growth, reproduction, and general health of rats fed a diet in which the protein and carbohydrate constituents have been exposed to approximately 4 million rep of cobalt-60 gamma irradiation, a dose 10 to 100 times above the pasteurization level and somewhat above the sterilization level. Results of some preliminary experiments on the effects of irradiation of all or part of the diet with 2- and with 20-megarep doses are presented.

Indications to date are that as compared with changes in palatability and vitamin content, toxicity appears to be an unimportant aspect of food irradiation even at much higher levels than would be encountered in future commercial pasteurization or sterilization with gamma radiation.

2. Experimental Procedures, Methods, and Results

a. Experimental Studies on Long-Term Feeding and Reproduction of Albino Rats. The general purpose and procedures of these studies have been described in Progress Reports 4 and 5. The principal changes in procedure result from attempts to simplify the design in order to avoid some of the many difficulties encountered in work of this nature. The following factors have been taken into account in designing the experiment: First, the number of animals will steadily increase during the first year to almost twice the initial number. Second, the necessity for the daily preparation and irradiation at a high level of a diet made up of 22 ingredients limits the number of variables which can be studied. Third, loss of animals can be expected to result from the high incidence of respiratory infection in animals supplied during the winter months. Fourth, frequent personnel changes can be expected in an experiment of this nature. Fifth, short-term experiments can supply answers to questions not answered by this experiment.

(1) Animals: Fourteen litters of Holtzman rats were obtained as weanlings on January 13, 1954. The 124 animals were divided to include 31 males and 31 females in each of two groups. One group receives a nonirradiated diet, and the other group receives the partially irradiated diet in which essentially all of the proteins and the carbohydrates, and a part of the fat have been irradiated.

All variables not studied in the experiment should be both minimized and randomized, i.e., any local environmental differences in temperature, illumination, ventilation and exposure to infection or to distracting influences should be kept to a minimum. For this reason the control and experimental cages are placed in an alternate horizontal arrangement and males and females in an alternate vertical arrangement. Two nonlittermate animals are housed in each cage except for the odd animal in each group.[55]

The animal room, which was described in Progress Report 5 and is shown in Fig. 55 was cleaned and fumigated during the week preceding the arrival of the animals, and is reserved exclusively for this experiment. Only individuals and materials essential to the experiment are allowed entry. Possibly deleterious environmental conditions such as insufficiently shielded ultraviolet lights and overly rapid air movement have been corrected. A permanent record of temperature and humidity, which are maintained at constant levels by an air-conditioning unit is provided by means of a hyrothermograph.

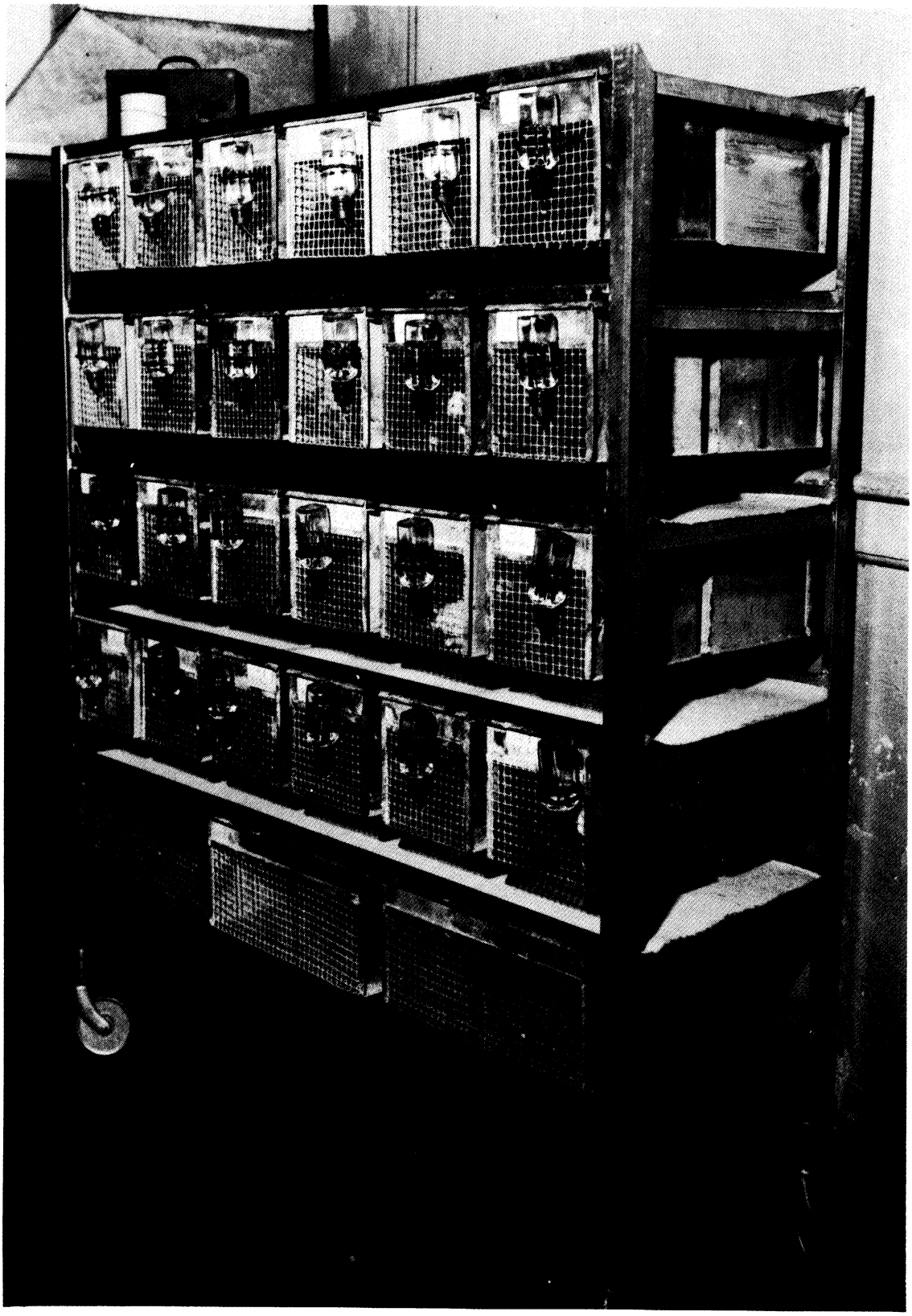

Fig. 55. New Rack and Cages for Experimental Rats.

As was foreseen, some of the animals developed symptoms of respiratory infection shortly after arrival. Inasmuch as rats of the same age which were raised in the colony and which have been continuously exposed to the disease have not shown similar symptoms, and in view of the fact that animals in the previous conducted experiments in this laboratory were remarkably disease-free even at 10 months of age, the infection must have been present in the animals on their arrival and may have been incurred or enhanced during shipping. The only measure of disease control planned is the removal from the experiment of the most heavily infected animals and attempts to avoid the introduction of new infection. The present number of animals is considerably in excess of that required for the successful completion of the experiment.

The effort to eliminate vermin by extermination before commencing the experiment followed by preventing their ingress has thus far been successful. No insecticides are used in the animal room and racks are sterilized by exposure to gamma radiation.

The routine care of the animals is described in a detailed handbook of procedure. The carefully trained personnel are closely supervised by investigators with long experience in animal nutrition studies. Animals are regularly fed once daily in the late afternon, shortly before an automatic time switch turns out the lights for 12 hours. Inspection at 8 to 12 hour intervals to ensure an adequate supply of food and fresh water at all times is part of a continuing effort to maintain optimal nutritional status prior to and during the reproduction studies.

(2) Diet: Table 29 lists the quantities of the dietary constituents per kilogram of diet, as well as the stimated quarterly requirements for the first year. Sufficient quantities of the ingredients will be kept on hand to fulfill requirements for one to three months. A perpetual inventory type of bookkeeping system provides complete readily accessible data on the source, rate of consumption, supply on hand, and other pertinent information on the 22 ingredients of the diet and the various premixes.

A vitamin premix consisting of all the water soluble vitamins except choline, an oil premix consisting of alpha tocopherol, corn oil and cod liver oil, and a radiation premix composed of the canned meat, casein, starch and cellulose are combined with the other ingredients and fed within two days of preparation. The premixes are stored at 4°C for not longer than 7 days. The oils are also refrigerated.

The irradiated portion of the diet includes all of the protein except any present in the liver and yeast preparations, all of the starch except a negligible amount added with the vitamin premix, about 12 percent of the fat, about 20 percent of the niacin, and small amounts of the various other vitamins and the salts which are added with the canned meat preparation.

TABLE 29

REQUIREMENTS FOR DIET INGREDIENTS

Constituent	Amount per Kgm of Diet	Amt. per Quarter from March, 1954 - March, 1955			
		Apr.-June	July-Sept.	Oct.-Dec.	Jan.-March*
1. Alpha cellulose	30 gm	45 lb	60 lb	70 lb	70 lb
2. Ascorbic acid	16 mgm	45 gm	15 gm	20 gm	20 gm'
3. Biotin	0.125 mgm	0.1 gm	0.1 gm	0.1 gm	0.1 gm
4. Calcium pantothenate	0.25 mgm	20 gm	25 gm	25 gm	25 gm
5. Casein	75 gm	110 lb	150 lb	185 lb	185 lb
6. Choline chloride	1.8 gm	1.5 kgm	2.0 kgm	2.2 kgm	2.2 kgm
7. Cod liver oil	30 gm	5 gal	8 gal	10 gal	10 gal
8. Folic acid	.44 mgm	0.30 gm	0.40 gm	0.5 gm	0.5 gm
9. Inositol	0.62 gm	400 gm	500 gm	700 gm	700 gm
10. Liver-concentrate	9.4 gm	12 lb	15 lb	20 lb	20 lb
11. Menadione	1.6 mgm	1 gm	1.5 gm	2 gm	2 gm
12. Niacin	31 mgm	20 gm	25 gm	35 gm	35 gm
13. P. Amino-benzoic acid	.31 gm	200 gm	250 gm	350 gm	350 gm
14. Pyridoxine HCl	6.2 mgm	4 gm	5 gm	7 gm	7 gm
15. Riboflavin	6.2 mgm	4 gm	5 gm	7 gm	7 gm
16. Thiamin HCl	3.1 mgm	2 gm	2.5 gm	4 gm	4 gm
17. α-Tocopherol	0.3 gm	200 gm	300 gm	400 gm	400 gm
18. Yeast, brewers	9.4 gm	12 lb	15 lb	20 lb	20 lb
19. HMW salt mixture	24 gm	35 lb	50 lb	60 lb	60 lb
20. Corn oil	30 gm	5 gal	8 gal	10 gal	10 gal
21. Corn starch	290 gm	420 lb	575 lb	715 lb	715 lb
22. Swift's beef for babies	500 gm	145 ctns	200 ctns	250 ctns	250 ctns
Totals		650 kg	850 kg	1100 kg	1100 kg

*During the 12 months to March 1956, requirements will decrease to about one-half to two-thirds of amount required in this quarter.

The vitamin content of the diet, in particular the content of vitamins A and E, is considerably in excess of requirements for the rat. The principal reason for exceeding minimum requirements, as well as for adding ascorbic acid and menadione, which are not exogenous nutritive essentials in this species, is to detect possible toxicity from these sources in completely irradiated diets.

Slight adjustments have been made in the diet described in Progress Report 5. Roughage content was increased to 5 percent on a dry basis in order to eliminate occasional episodes of diarrhea which occurred in some of the animals in the exploratory studies. A decrease in fat content from the 17 percent level used in the preliminary studies to 12 percent on a dry basis was made when the project's consultant, Professor H. C. Eckstein, found that even in the presence of large amounts of lipotropic factors a fat accumulation occurred in the livers of rats on a high fat diet. The Hubbel-Mendel-Wakeman salt mixture is now added at a 4 percent level on a dry basis, and the supplementary salts are omitted.

The paired-feeding technique has been abandoned as being impractical, inasmuch as variable degrees of evaporation and loss of diet from the dishes through the wire screen floors of the cages prevent accurate determination of food intake. The water which the diet contains as a result of the incorporation of the canned meat products amounts to 38.5 percent. A small additional amount of water is added to improve the consistency of the diet. A record is kept of the amount of food put in each cage and of the total which remains the following day in order to ensure adequate allowances. Additional food is supplied during the night to cages to which the food has been consumed or spilled.

(3) Irradiation: Bacteriological studies now in progress indicate that the radiation dose necessary to destory high concentrations of resistant spore-forming organisms may be in the neighborhood of 3×10^6 rep. In order to allow a reasonable margin of safety, the food is irradiated with approximately 4×10^6 rep (cobalt-60 gamma radiation). The "radiation flavor" induced by this dose can be expected to make the diet somewhat less acceptable to rats, a fact which must be taken into account in interpreting the data on the weight of the rats.

Calculations based on dietary requirements when the number of animals in the experiment is at a maximum indicate that 6 kgm of diet per day must be irradiated. This will be accomplished by placing one row of 3 by 3 by 10-inch packages on end and tangent to the cylindrical source, with a second row behind the first. Paraffin wedges between the boxes in the first row will ensure uniform flux in the second row. Approximately 2 days will be required for the material in the first row to accumulate 4×10^6 rep and 5 days for that in the second row. The packages will be rotated 180° halfway through the exposure. A 4-7-day supply of irradiated diet is maintained. The high-flux position in the center well of the source is available for emergency use. Dosimetry measurements are made

periodically by incorporating chemical or glass dosimeters calibrated against a ferrous sulfate standard in the packaged diet during irradiation. Corrections for self-absorption, for decay, and for the effect of the presence of other materials in the radiation field are made in calculating exposure time.

(4) Reproduction and Longevity Studies: From each of the two groups of 62 animals, 16 female and 8 male animals will be selected for breeding. Freedom from respiratory infection and approximation of body weight to the mean and range for the group will be the criteria for the selection of the breeders.

The second litters of the first and second filial generations will provide additional data on reproductive capacity and maintenance of lactation. The design of the experiments on reproduction is described in detail in Progress Report 5.[1] Longevity data will be provided over a period of two years by the 124 animals in the parent generation.

(5) Pathological and Hematological Studies: Experience gained from the preliminary studies has shown that the range of variation in red- and white-cell counts is so great that only marked differences between the groups would be apparent in these studies. Hematocrit and hemoglobin determinations will be relied on for routine determinations of hematological status, with cell counts being made only at 6-month intervals.

Animals that die or are removed from the experiment because of conditions such as severe chronic respiratory infection will be autopsied. The lung, heart, liver, spleen, kidney, and a section of small intestine will be preserved for histopathological examination. The animals that survive to the end of the experiment will undergo similar examination.

(6) Biochemical Studies: If marked differences develop between the control and experimental groups, appropriate biochemical studies to determine the cause and nature of the changes can be undertaken in conjunction with concurrent investigations on the nature of radiation-induced changes in foodstuffs and in animals which consome food irradiated at high levels. Such studies would be in line with the recommendation of Lehman et al.[2] that organ function and enzyme activity determinations be made in experiments on appraisal of foods for toxicity.

(7) Growth Data: Inasmuch as body weight is a sensitive measure of the nutritional adequacy and toxicity of a diet, as well as of its acceptability, growth curves are expected to afford valuable information. Consequently, emphasis is placed on the acquisition of reliable data. Weekly weighings at the same hour of the same day on an accurate scale will continue for the duration of the experiment.

Weight data obtained for the first 6 weeks of the experiment are shown in Fig. 56. The slightly lower weight gain of the experimental males, if significant, is believed to be due to a difference in palatability rather than to changes in nutritional value or the occurrence of toxicity in the irradiated diet, if the conclusions drawn from the results of the high-level exploratory studies described below are correct.

A qualified statistician is available for consultation on the analysis of the data, and for recommendations on questions of animal selection as well as on overall experimental design.

b. Pilot Studies with Albino Rats and Mice. (1) Introduction: Work has been continued on preliminary small-scale experiments which, as stated in Progress Report 5, have the functions of (1) determining the presence or absence of acutely toxic effects of irradiated diets on rats, (2) training of personnel, and (3) providing preliminary data to be used as a guide in setting up the long-term experiment. Results are reported in this section of growth and reproduction studies on small groups of rats fed diets partly and completely irradiated with cobalt-60 gamma radiation at 2- and 20-megarep levels. In addition, short-term high-level experiments involving tube feeding of rats, and growth and reproductive studies on mice, are being undertaken. No evidence was found of a deleterious action of irradiation other than changes in palatability except that at the 20-megarep level, vitamin destruction also occurred.

(2) Experimental: The initial results of an experiment involving a total of 27 Holtzman rats fed diets partly and completely irradiated with 2 megarep were described in Progress Report 5. These animals had at 3 months of age been divided into three groups which received the following diets prepared according to the formula of Progress Reports 4 and 5: nonirradiated control diet A; diet B, in which protein and carbohydrate ingredients alone were irradiated; and completely irradiated diet C. The experiment was continued until the females had weaned their second litters. Figures 57 and 58 show the growth curves during the breeding period, and Table 30 presents the reproduction data for the second litters. Weights of only the 24 young that were assigned to the second high-level experiment were recorded. Figures 59 and 60 depict the parents and young of a representative litter fed the completely irradiated diet. No differences were observed between the control group and those fed the partly and completely irradiated (2 megarep) diets.

In a study designed to test the wholesomeness of food irradiated at higher levels, 24 six-week-old members of the first litters of the animals in the low-level study were placed on diets in which part or all of which had been irradiated with 20 megarep. Three groups of 8 animals each, divided equally as to sex and littermates, received diets ad libitum prepared as previously described, XA being the control diet, XB the partly irradiated diet, and XC the completely

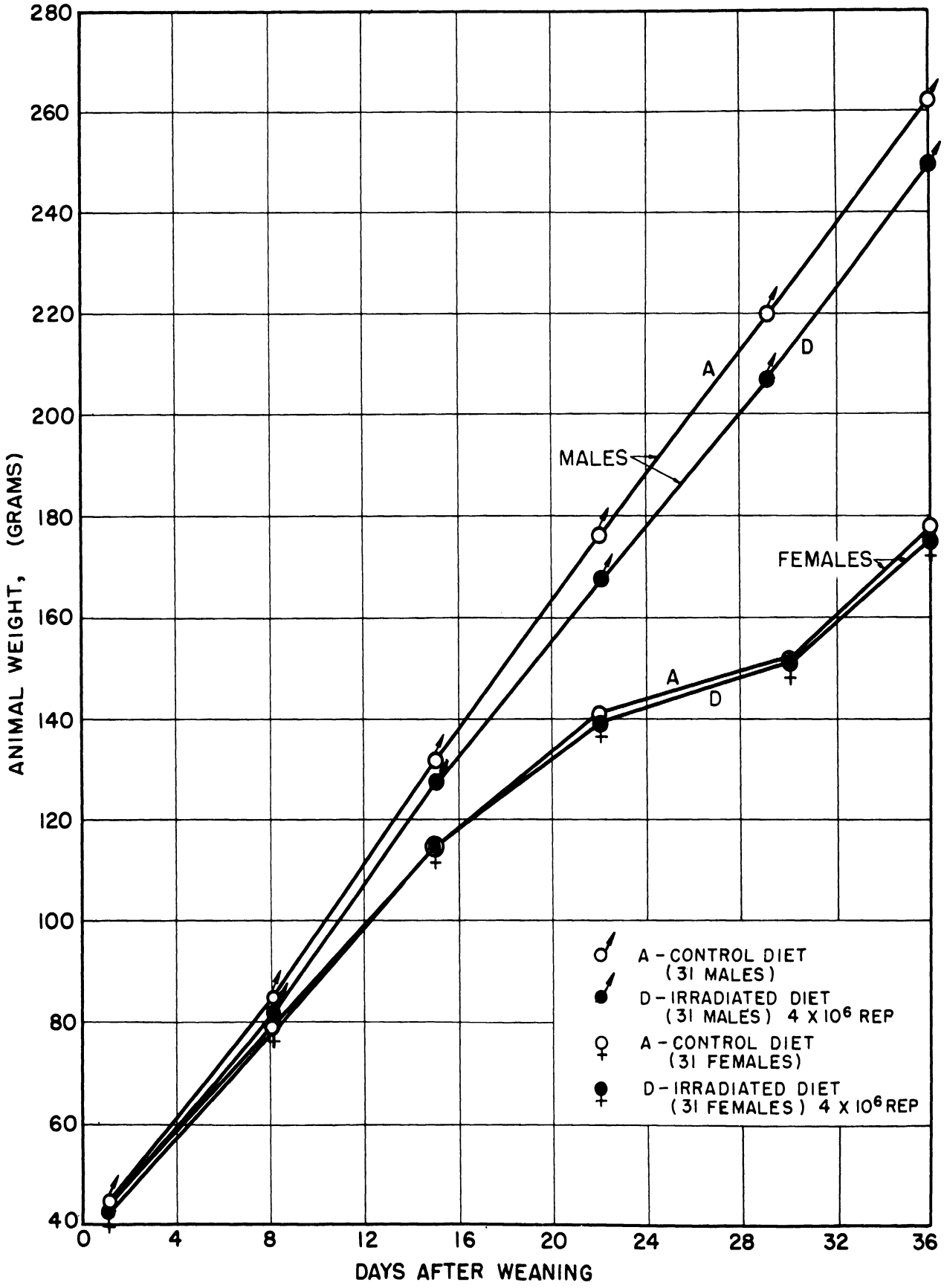

Fig. 56. Growth curves of male and female rats in the long term experiment. The experimental diet was irradiated at the 4 megarep level.

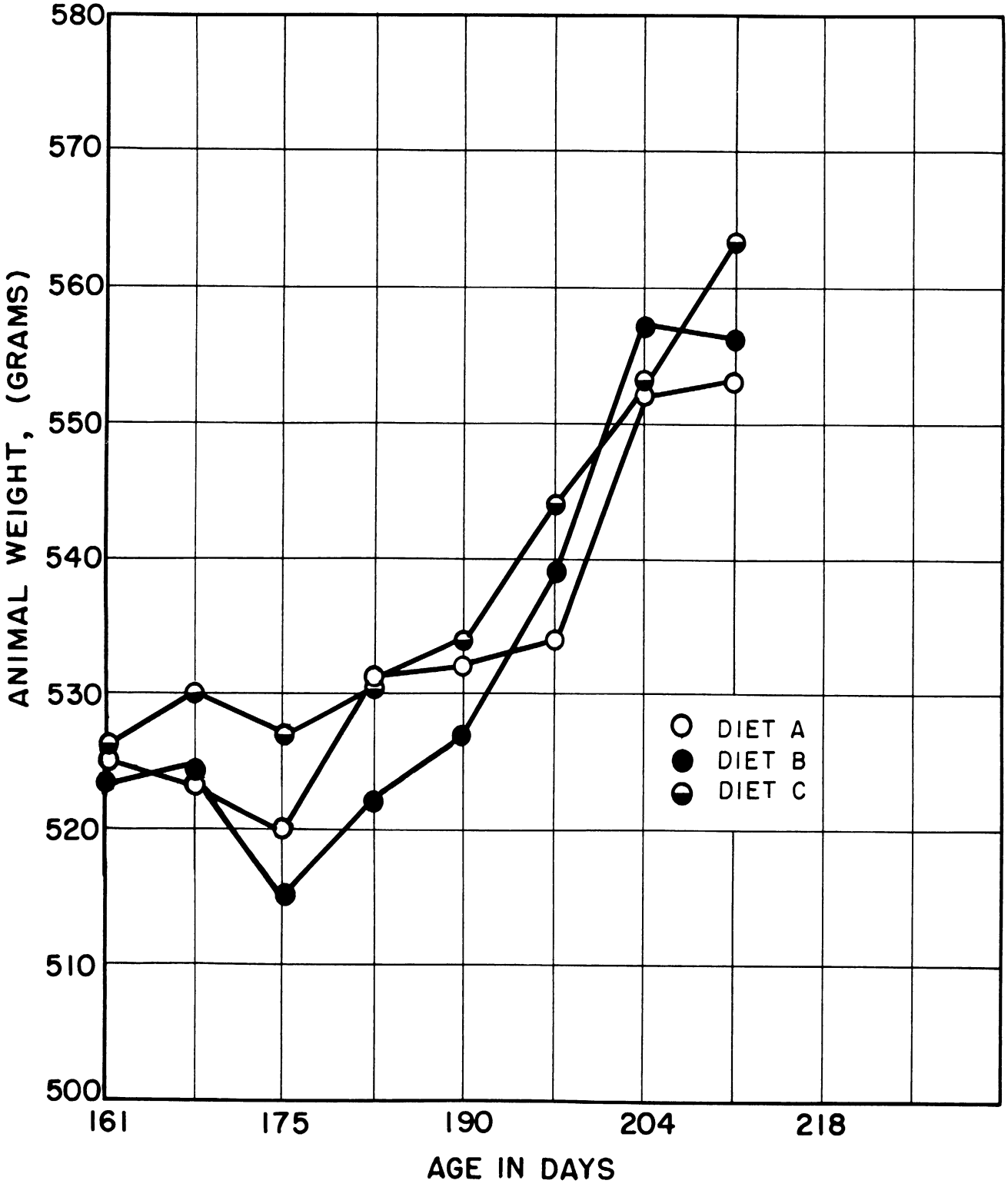

Fig. 57. Growth curves of male rats during second breeding period, after having been fed diets partly and completely irradiated with 2 megarep. The animals received these diets for approximately 3 months.

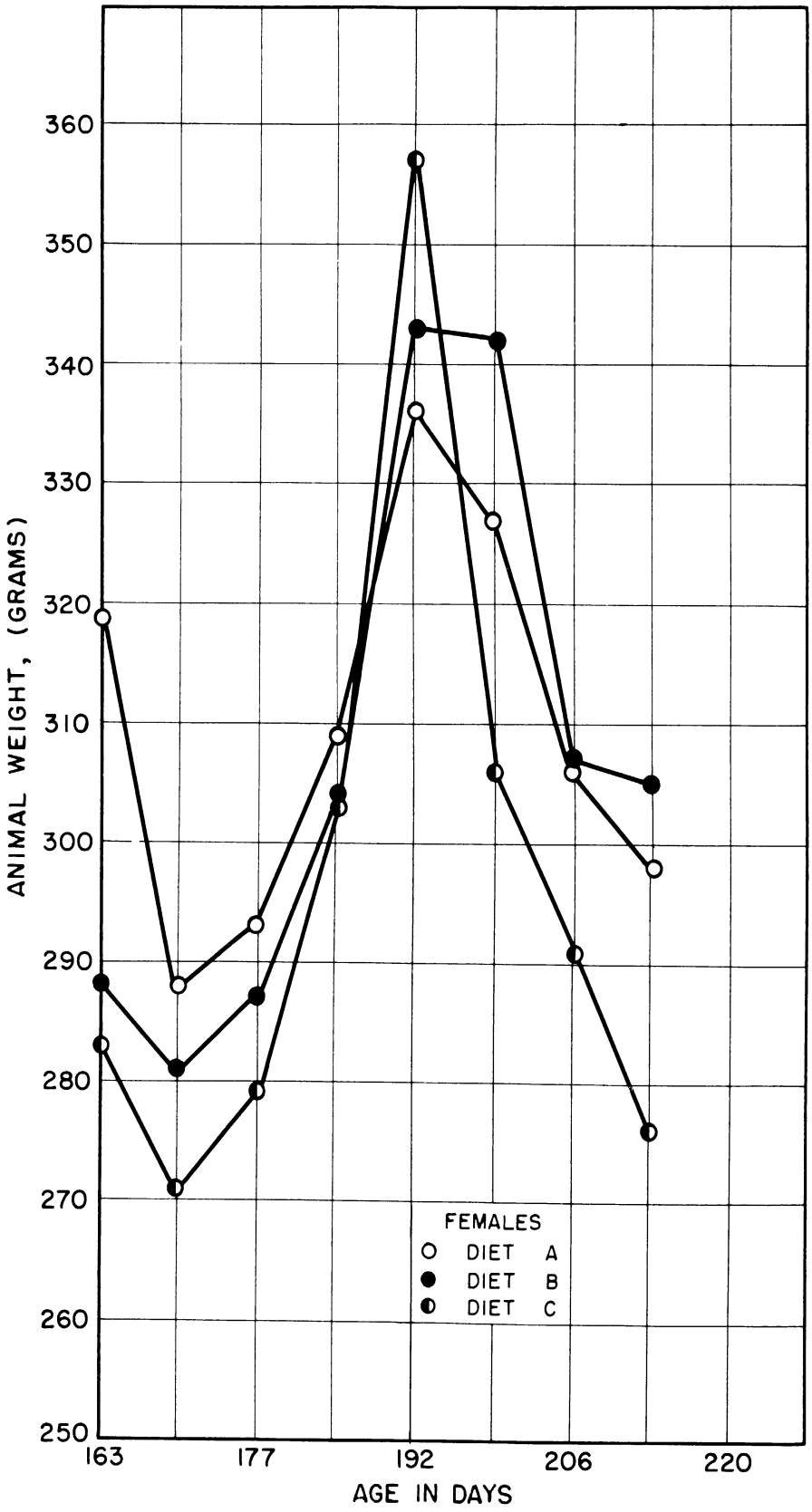

Fig. 58. Growth curves of female rats during second breeding period, after having been fed diets partly and completely irradiated with 2 megarep. The animals received these diets for approximately 3 months.

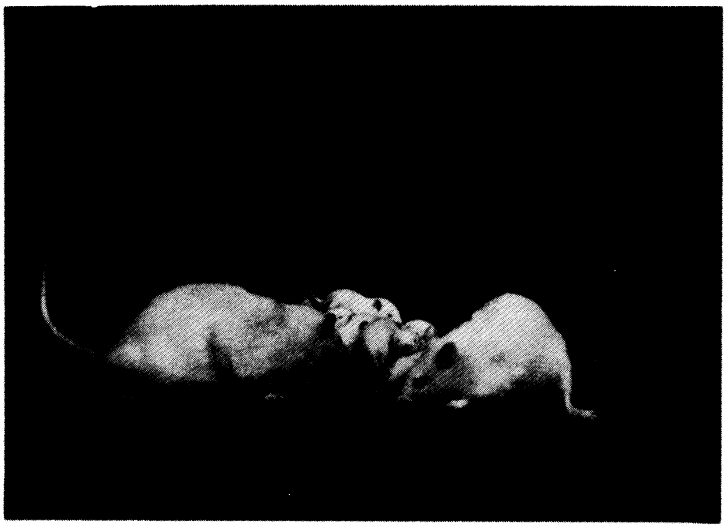

Fig. 59. Parents and Young from Group Fed a Diet Completely Irradiated with 2 Megarep.

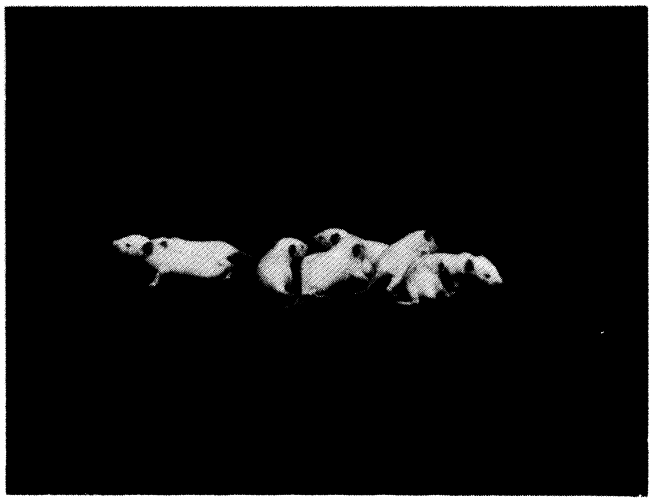

Fig. 60. Young Rats from Group Fed a Diet Completely Irradiated with 2 Megarep.

irradiated diet. The growth curves for the males, Fig. 61, indicate a marked reduction in rate of weight gain for the animals on the partly and completely irradiated diets.

In an attempt to determine whether the decreased growth was caused by a change in the nutritional adequacy or in the palatibility of the irradiated diets, a second high-level experiment was initiated with 24 young rats of second litters of the animals used in the 2 megarep experiment. The rancidity of the diet completely irradiated with 20 megarep (XC diet, containing 17 percent fat) and the strong radiation odor and soupiness of the separately irradiated canned-meat constituent of the XB diet were accompanied by a lowered food intake in the rats fed these diets. In order to control the factor of palatability in the new experiment all the animals were restricted in food intake to the amount consumed by the group with the lowest intake. An additional experimental group of animals were fed a diet in which all the protein and carbohydrate constituents were irradiated after mixing (the XB diet), for comparison with the group fed XB diet, in which the canned Swift's Beef for Babies and the dry protein and carbohydrate ingredients were irradiated separately.

The weight changes of the four groups (each containing 3 males and 3 females except the XC group, which had 2 males and 4 females, with littermates distributed equally among the groups) on restricted dietary intake were followed for the first 3 weeks after weaning. The four groups gained weight at an approximately equal rate during this period (Figs. 62 and 63 a finding which suggests that the retardation in weight gain observed in the group fed 20 megarep irradiated diet ad libitum was due to lack of acceptability rather than to impaired nutritional value of the 20-megarep diets.

An interruption of the exploratory experiments at this point, which resulted from diversion of radiation facilities to preparation of diet for the proposed long-term study as well as personnel changes, delayed further growth and reproduction studies temporarily. When, after four weeks, the regular weekly weight determinations were renewed as a result of a decision to postpone the long-term experiment, marked differences between the groups were found, and reproduction among the control animals of the first high-level (20 megarep) study was below normal. Records kept during this period as well as subsequent observation indicated that several variables other than the experimental ones were operating during the interval. The problem of irradiating sufficient food for the two high-level groups was solved by discontinuing the ad libitum experiment. Reproduction failure in the animals on the nonirradiated as well as the irradiated diets was probably due in part to vitamin E insufficiency. An examination of the diet mixing procedures revealed that the α-tocopherol, essential to reproduction in rats, had been added, in anticipation of initiation of the long-term experiment, to a stock mixture of the cod liver oil and corn oil which had been stored at elevated room temperatures for a prolonged period. The water-soluble vitamins

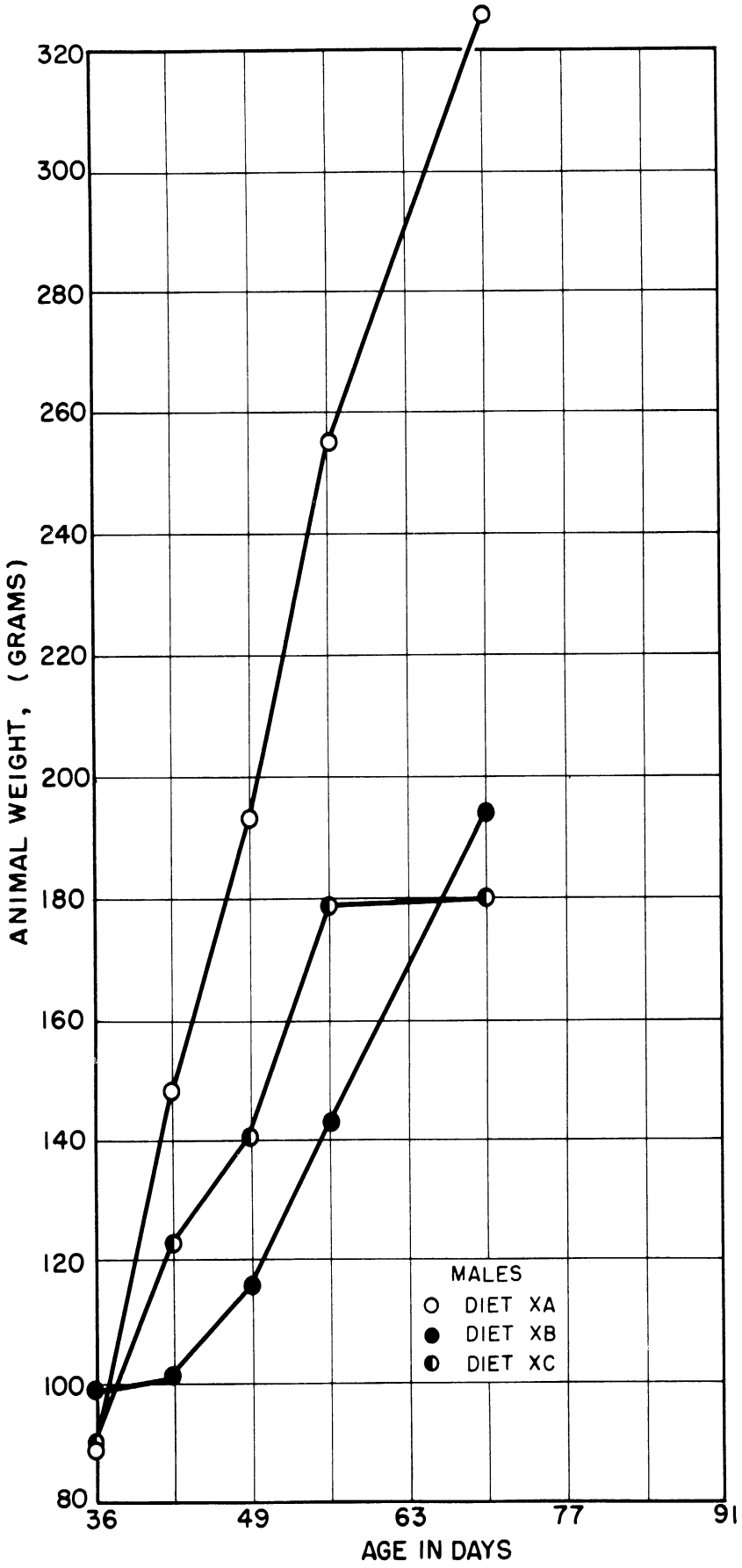

Fig. 61. Growth curves of male rats which had been fed diets partly or completely irradiated with 20 megarep. Diets fed ad libitum.

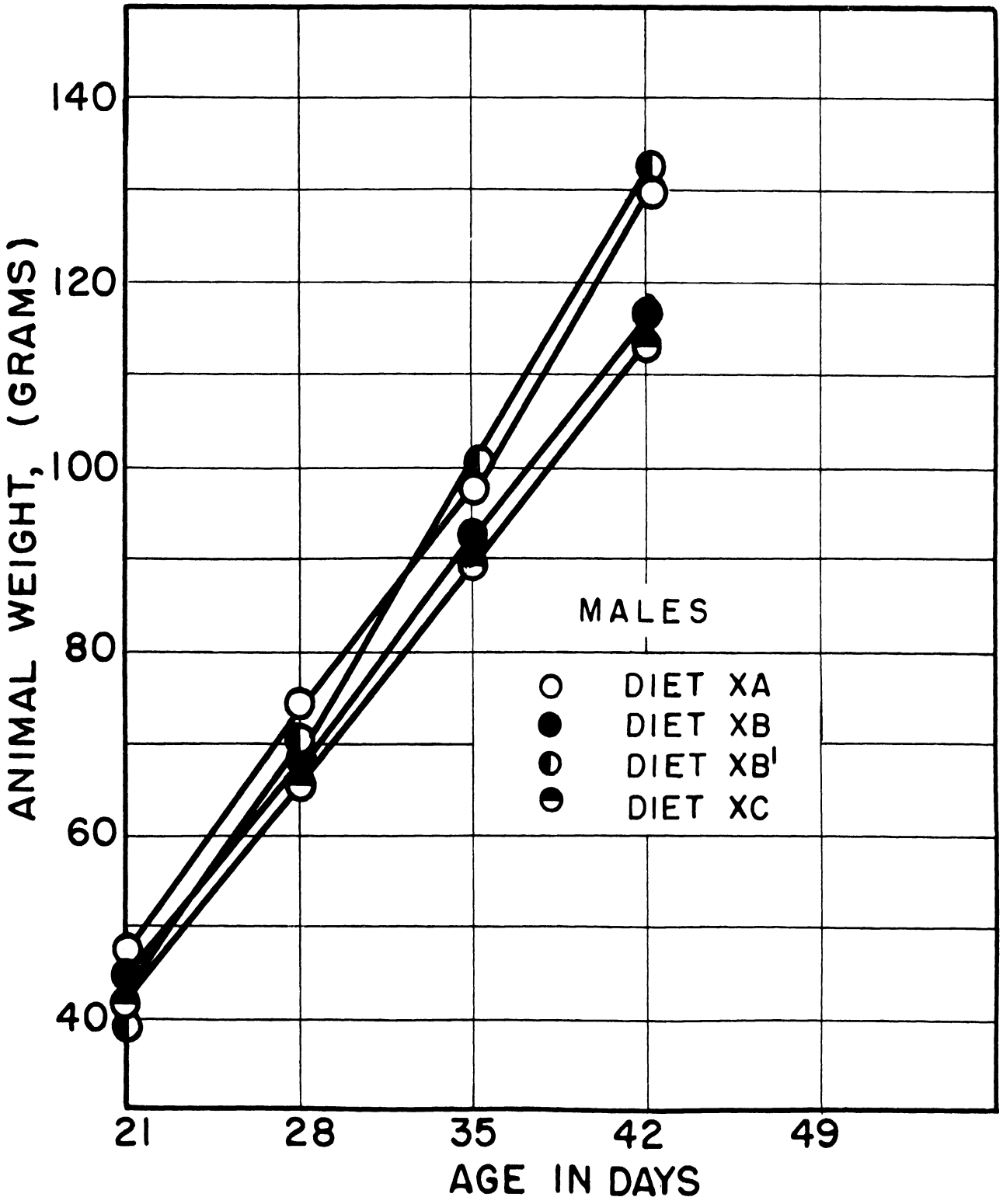

Fig. 62. Growth curves for the first 3 weeks after weaning of male rats fed diets partly and completely irradiated with 20 megarep dose.

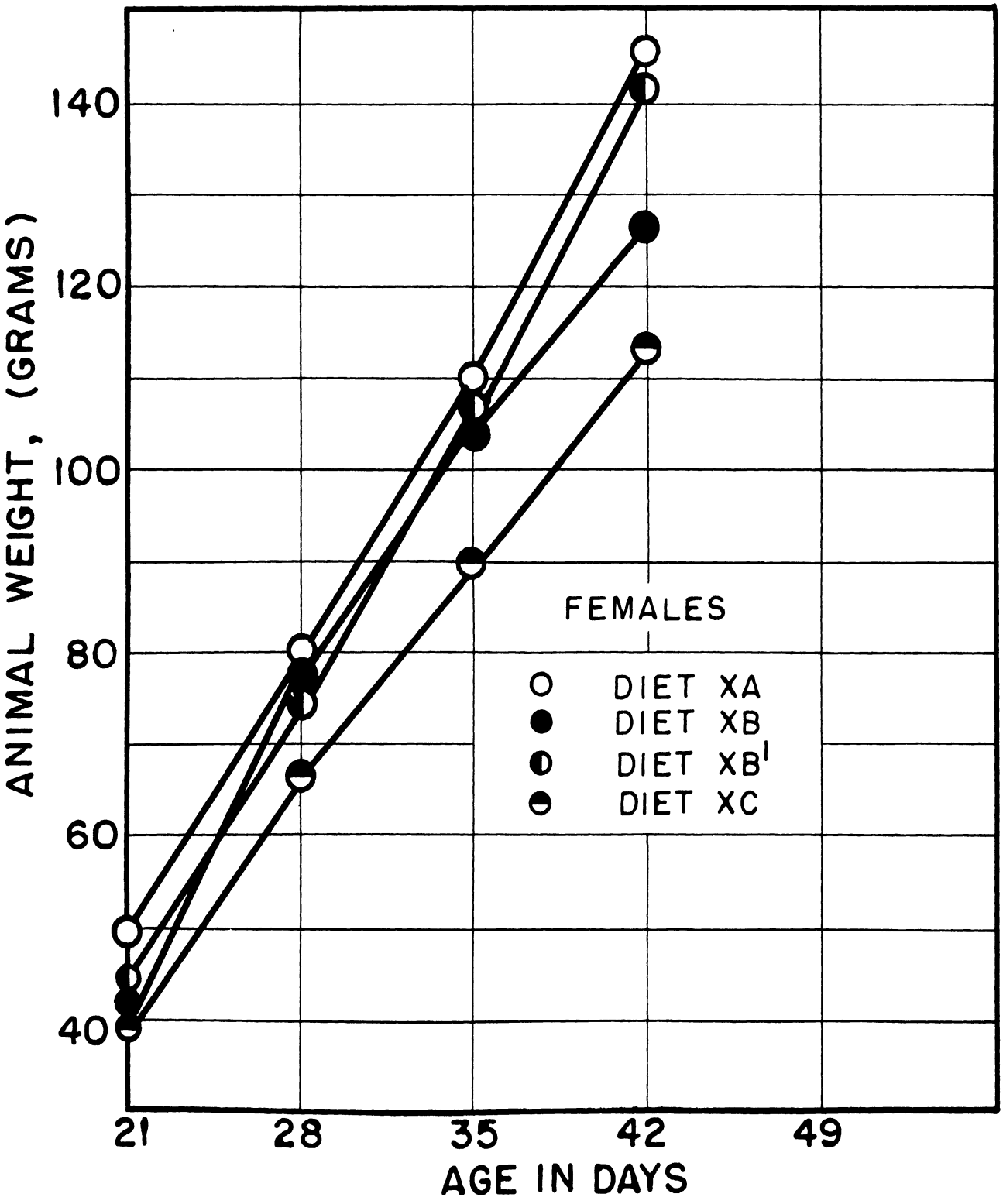

Fig. 63. Growth curves for the first 3 weeks after weaning of female rats fed diets partly and completely irradiated with 20 megarep dose.

were also being added from a stored stock mixture. These mixtures were discarded. The results of the breeding studies are shown in Table 31. Despite the many deleterious influences to which they were subjected during a critical period of their development, the XB animals show reproductive capacities that compare favorably with the control animals.

Figures 64-69 show representative male and female animals and their litters from the groups receiving the nonirradiated and the two partially irradiated 20 megarep diets. The young were all born within 2 days of each other. Figures 70 and 71 which were made at the same time as Figs. 64-69, depict the effects of exclusive feeding for 10 weeks of diet completely irradiated at 20 megarep (animals from the same litters as the other adult rats).

In the case of the XB[1] animals, limited radiation facilities made necessary the feeding of diet which had been stored after irradiation in sealed cans in the cold for periods up to 6 to 8 weeks. As noted in Progress Report 5, peroxides and other compounds are formed as a result of irradiation of fats and their effects may not appear until after an appreciable storage period. Since peroxides from fats destory α-tocopherol, necessary for reproduction in the rat, and since the canned meat contains 3 percent fat, the lowered reproductive efficiency in these animals (only 2 of the 3 females had young, although all became pregnant) may be attributable to storage. The stored diet supported normal growth, as seen from the growth curves and photographs of the adult rats and as confirmed by a short-term experiment in which mice were fed the same diet.

The vaginas had not opened in the XC females at 110 days of age, and no pregnancies occurred. The downhill course of these animals indicated by the weight curve was accompanied by the development of typical symptoms of thiamine deficiency. Figure 72 shows a male in this condition. This male and one female were at this point placed on a completely irradiated diet to which nonirradiated water-soluble vitamin mixture was added in the original quantities. The animals immediately started eating the diet despite its extreme rancidity (the fat content was at the original level of 17 percent). In less than 8 weeks the 4 month old male increased in weight from 116 to 316 gram, and the female from 139 to 232 gram; 5 weeks after vitamin supplementation began the female gave birth to 7 young, of which 2 survived to weaning age.

The necessity for completing the pilot studies and starting the long-term experiment prevented the maintenance of a freshly irradiated supply of diet in sufficient quantity to feed more than this one pair of XC animals. The striking recovery of these two rats when the water-soluble vitamin premix was added to the completely irradiated 20 megarep diet (see Figs. 70 and 71) suggests that vitamin destruction rather than the formation of toxic substances in this high-fat ration is responsible for its adverse effect on growth and reproduction.

TABLE 30

REPRODUCTION DATA ON SECOND LITTERS
OF FEMALE RATS IN (2 x 10^6 rep) PILOT EXPERIMENT

Group	No. Females	No. Litters	Average No. per Litter at Birth	Average of Recorded Weights at 21 Days
A	5	Not recorded		
B	4	4	10.	44.5 gm
C	4	4 4	9.5	42.4 gm

TABLE 31

REPRODUCTION OF RATS FED NONIRRADIATED DIET AND DIETS PARTIALLY AND COMPLETELY IRRADIATED WITH 20 x 10^6 REP SINCE WEANING

Group	Diet	No. of Females	No. of Successful Pregnancies	No. of Offspring at Birth	Total No. of Offspring at 21 Days	Mean Weight of Offspring at 21 Days
XA	Nonirradiated	3	3	26	24	43.0 gm
XB	Partially Irradiated*	3	3	16	16	31.8 gm
XB'	Partially Irradiated**	3	2	20	20	26.9 gm
XC	Completely Irradiated	2	0	0	0	------
XC'	Completely Irradiated; Nonirradiated Water-Soluble Vitamin, Premix added.	1	1	7	2	42.0 gm

*Canned meat and mixed dry protein and carbohydrate constituents irradiated separately.

**All protein and carbohydrate constituents mixed before irradiation. Irradiated mix stored up to two months before feeding.

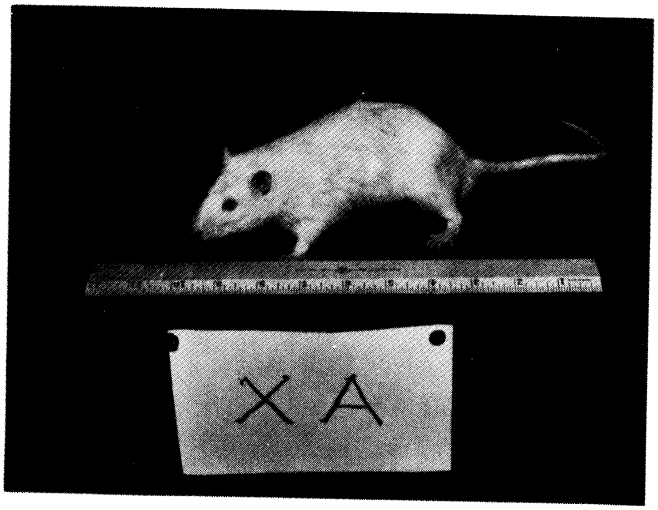

Fig. 64. Male Fed Non-irradiated Diet.

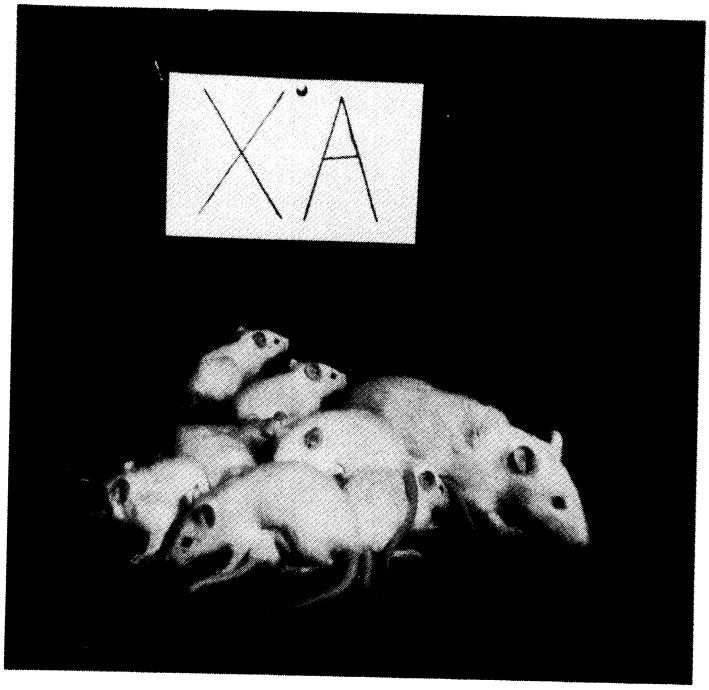

Fig. 65. Female Fed Non-Irradiated Diet with Young at 21 Days after Birth.

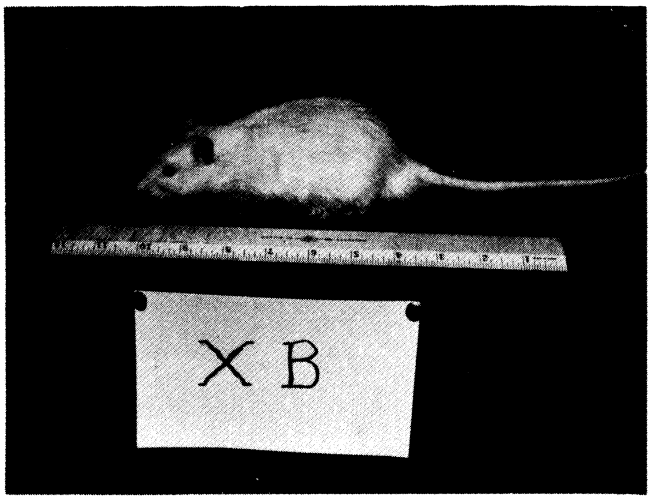

Fig. 66. Male Fed Diet Partly Irradiated at 20 Megarep Dose.

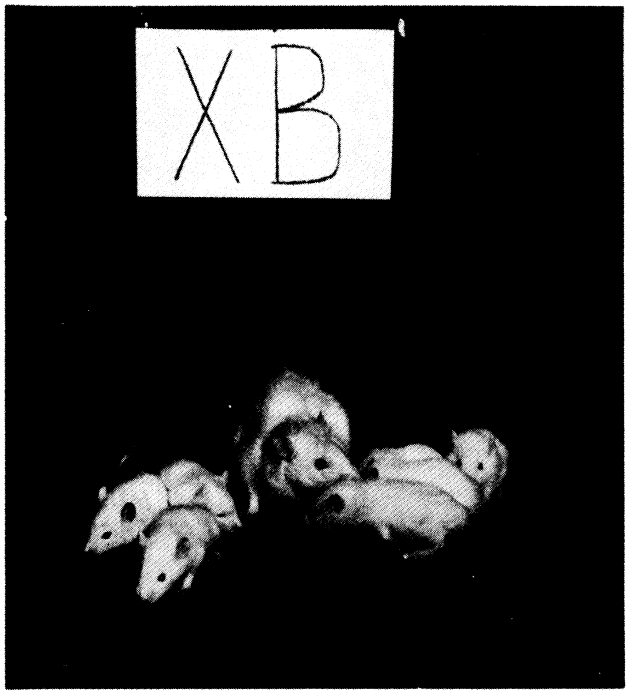

Fig. 67. Female Fed Diet Partly Irradiated at 20 Megarep Dose with Young at 20 Days after Birth.

Fig. 68. Male Fed Diet Partly Irradiated at 20 Megarep Dose and Subsequently Stored Up to 2 Months.

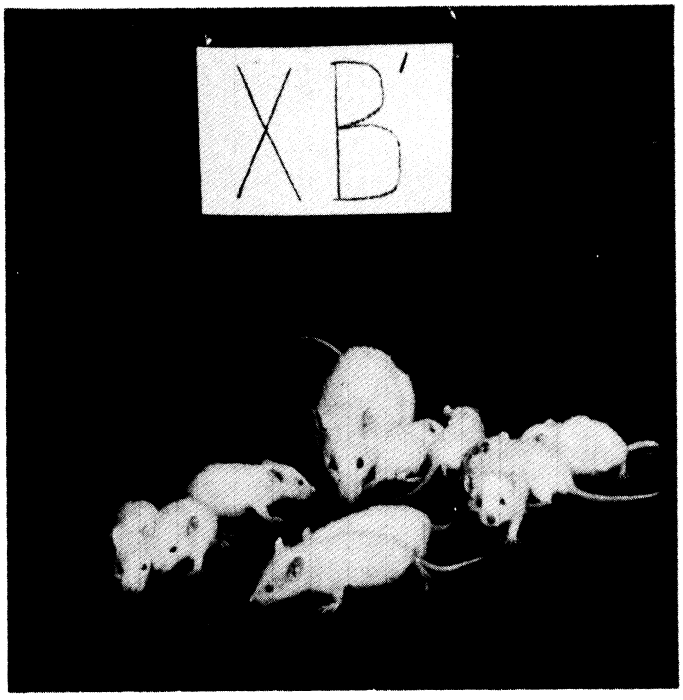

Fig. 69. Female Fed Diet Partly Irradiated at 20 Megarep Dose and Subsequently Stored Up to 2 Months (with Young at 21 Days after Birth).

Fig. 70. Two Males on Left, Female on Right, Showing Effects of Feeding Diet Completely Irradiated at 20 Megarep Dose (Note Characteristics of Thiamin Deficiency).

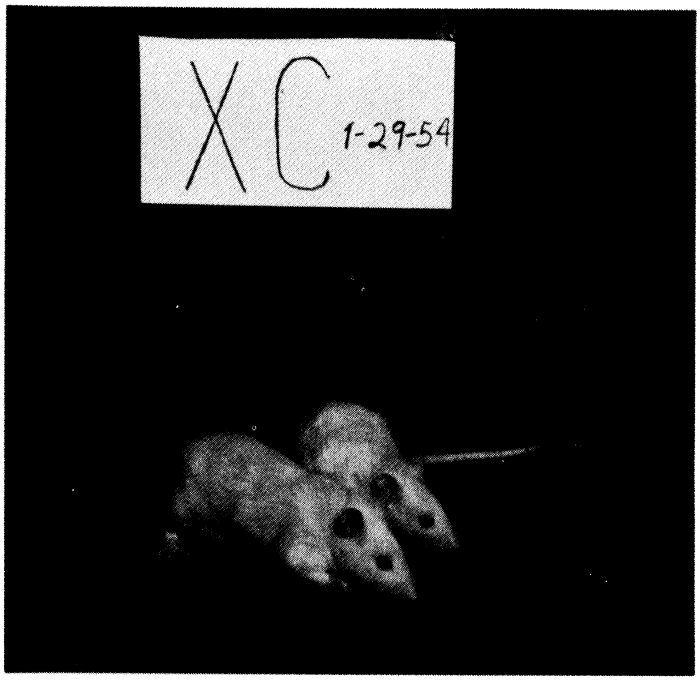

Fig. 71. Male (Left) and Female Fed Exclusively Completely Irradiated Diet at 20 Megarep for 10 Weeks, Then Fed the Same Diet Supplemented with the Water Soluble Vitamin Premix for 3 Weeks (Note: These Animals are the Same as First Two in Fig. 70 Above and Show Recovery from Vitamin Deficiency).

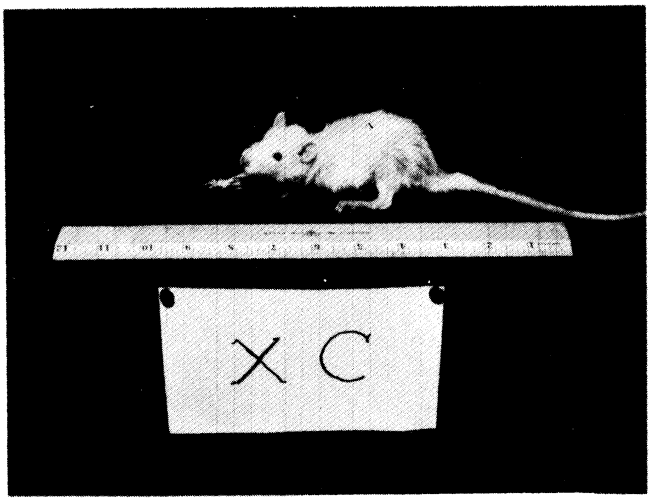

Fig. 72. Two Views of Male Fed Diet Completely Irradiated at 20 Megarep Dose before Vitamin Supplementation of Diet, Showing Characteristic Symptoms of Thiamin Deficiency.

Hematological studies were performed on most of the parent rats in the high-level (20 megarep) experiment. The mean values of the red and white cell count in the blood are shown in Table 32. The sample size, however, was too small for any definite conclusions to be drawn.

After the young were weaned, the parent animals were sacrificed and the organs preserved for histopathological studies.

In contrast to the observations on the blood of rats on standard commercial diets an apparent lipemia occurred in a number of the animals on experimental diets which contained 17 percent of fat on a dry basis.

A number of other corrective steps were found to be necessary, among which were (1) an increase in the number of feedings and a change to an ad libitum schedule to counteract loss of diet by spilling; (2) addition of more roughage to the diet to bring it from 4.5 percent to 7.5 percent, a level at which the diarrhea initially observed has not recurred; (3) removal of insufficiently shielded ultraviolet lights; and (4) elimination of vermin.

The growth curves (Figs. 73 and 74) show that the XB animals, whose diet contained canned meat irradiated before it was mixed with the other ingredients, underwent the most decided change. Their increase in growth rate took place at the same time as marked improvement in odor and consistency of the irradiated meat occurred. The practice had been to remove the meat from the small cans in which it was supplied before irradiation and to transfer it to a larger can to make the irradiation more convenient. In view of the high temperatures which prevailed in the diet preparation room, this practice was discontinued. A possibility that the temperatures of the cave containing the cobalt-60 source, which ranges from a low of 38°F in the winter to perhaps 80°F or more in the summer, might have influenced the effects of the radiation on the food is under investigation.

The animals on the completely irradiated XC diet showed a cessation of growth after the study was renewed. Inspection of the diet records indicated that substitutions with control rations had been made on several occasions. The necessity for this arose in part from the fact that insufficient radiation time was available for satisfying the requirements of both this experiment and other demands.

With due allowances for any irregularities which may have occurred, the weight curves show that normal or near-normal growth is possible on diets containing protein and carbohydrate constituents irradiated at 20 megarep dose, but that growth is not maintained on a diet that is completely irradiated at this level. At 110 days of age the animals in the second high-level experiment were mated, each mate being rotated among the females with transfers at weekly intervals.

TABLE 32

RED AND WHITE BLOOD CELL COUNTS OF RATS FED NONIRRADIATED DIET AND DIET PARTIALLY AND COMPLETELY IRRADIATED WITH 20 x 10^6 REP DOSE

Group	Diet	Sex	Red Cells per cu mm	White Cells per cu mm
XA	Nonirradiated	M	9.2×10^6 (3)*	13.3×10^3 (3)
		F	9.1×10^6 (3)	14.1×10^3 (3)
XB	Partially irradiated canned meat, and mixed dry protein and carbohydrate constituents irradiated separately	M	8.2×10^6 (3)	17.5×10^3 (3)
		F	9.4×10^6 (3)	13.1×10^3 (3)
XB'	Partially irradiated protein and carbodydrate mixed before irradiation, stored up to two months	M	9.4×10^6 (1)	18.5×10^3 (1)
		F	8.7×10^6 (3)	12.4×10^3 (3)
XC	Completely irradiated	M	9.6×10^6 (3)	13.1×10^3 (3)
		F	7.9×10^6 (2)	9.2×10^3 (3)

*Number of Animals

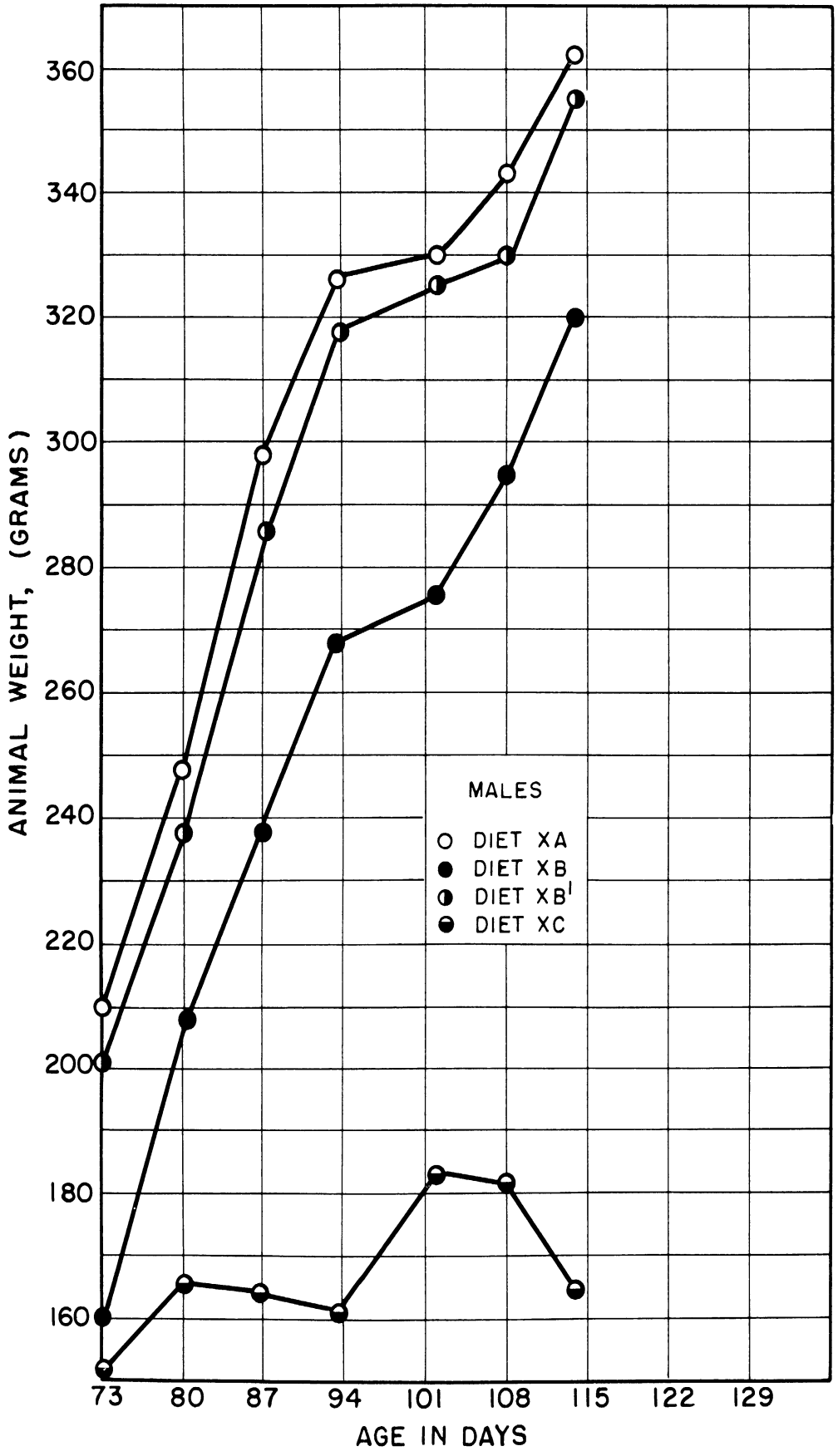

Fig. 73. Growth curves of male rats fed diets partly and completely irradiated with 20 megarep dose. Food intake was restricted during first 7 weeks after weaning, then allowed ad libitum.

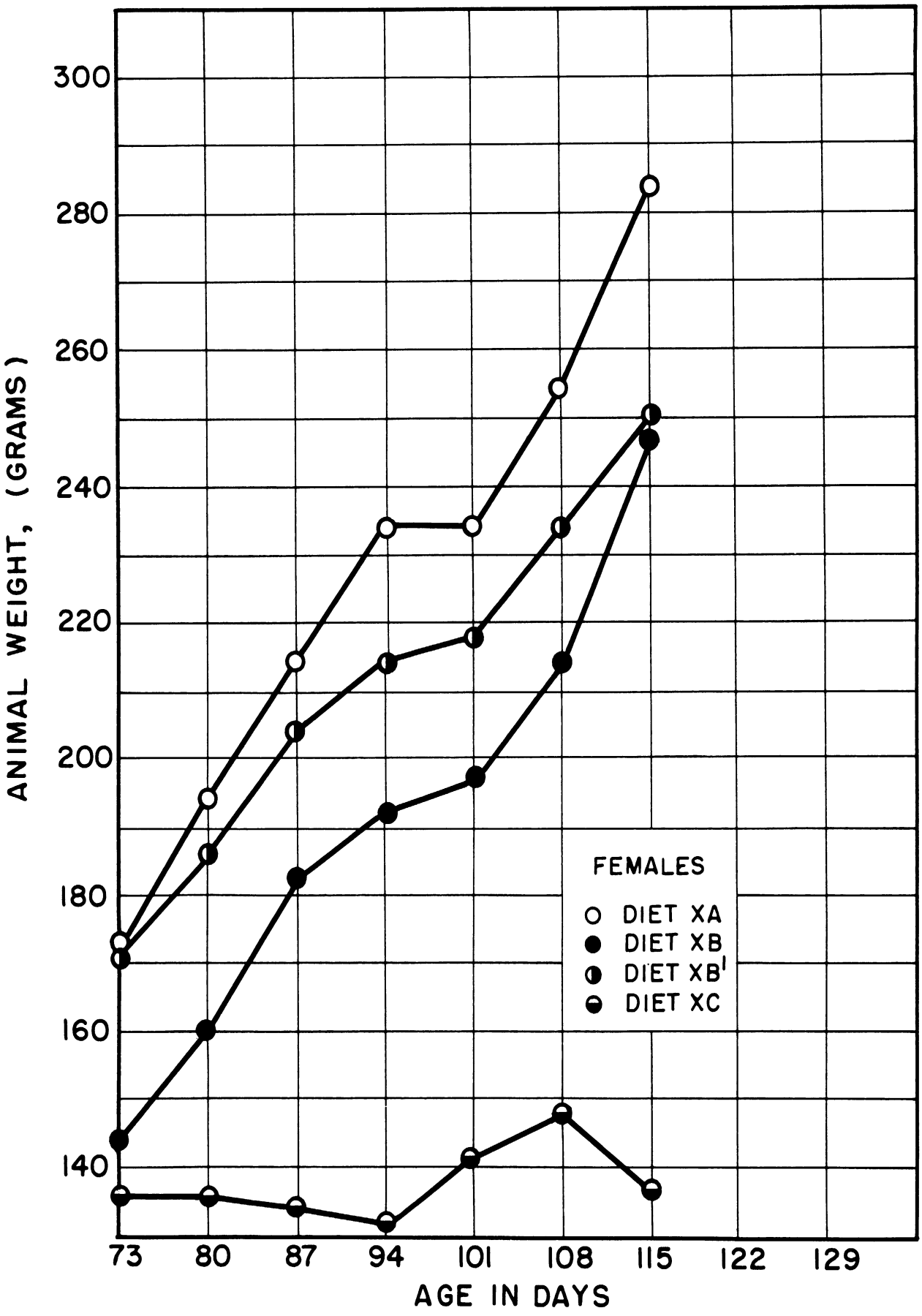

Fig. 74. Growth curves of female rats fed diets partly and completely irradiated with 20 megarep dose. Food intake was restricted during first 7 weeks after weaning, then allowed ad libitum.

3. Discussion

The factors which were considered in setting up these studies on the wholesomeness of irradiated food have been discussed in previous reports. A recent paper of Lehman and Laug[4] contains several suggestions which apply to an investigation of the sort now underway. The recommendations that there be a "deliberate exaggeration of dosage," that the animal tests be applied chiefly to the protein ingredients of the diet, and that a relative excess of protein be incorporated in the diet for toxicity studies are met in the long-term experiment. The suggestion of Lehman and Laug that investigations of toxicity and of nutritional adequacy require mutually exclusive tests brings into focus the difficulty of attempting to answer too many questions with one experiment. The diet being used is rich in protein (28 percent), so that conceivably any borderline change in availability of essential amino acids could be masked by the presence of an excess of protein. This possibility suggests the desirability of testing in another experiment the effects of irradiation on diets containing minimal amounts of essential ingredients.

That the present long-term experiment will leave many questions unanswered is fully realized, as is indicated in the previous progress reports. A principal goal in setting up the experiment in its present form has been to keep it within the limits of practicality. The present approach of setting up short-term studies to investigate such problems as the radiation threshold, if any, for the development of acutely toxic substances in various dietary constituents appears at present to involve less interference with the long-term studies than the inclusion therein of additional groups.

Concerning the results obtained thus far in the exploratory high-level studies, vitamin destruction at a 20 megarep dose appears to be the principal deleterious effect insofar as growth and reproduction of rats is concerned. Elsewhere in this report experiments with Tetrahymena are described in which destruction of a few essential nutrilites at high levels of irradiation rather than formation of any toxic substances appears to be the principal consequence of irradiation.[3]

Current studies in which rats receive their fully dietary intake quantitatively by stomach-tube administration indicate that exposure of a canned-beef preparation to 45 million rep does not produce poisons which have an immediately harmful effect on rats even when the irradiated meat is the chief dietary constituent. Feeding this material over a longer period, or, as Lehman and Laug[4] suggest, impairing the capacity of the animals for detoxification by concurrent treatment with liver poisons, may of course indicate that changes of a toxic nature can occur at very high levels of irradiation.

4. Summary

An experiment is described in which the growth and reproductive capacities of rats fed a diet containing irradiated proteins, carbohydrates, and a small amount of fat and vitamins contained in the canned meat over a period of two years and for three generations are being determined.

Results are presented which indicate that the feeding of the irradiated diet (4 megarep dose from cobalt-60 gamma rays) for the first 6 weeks after weaning has little effect on growth when compared with the growth of a control group on a similar but nonirradiated diet.

The final data from an exploratory study in which small groups of rats fed partly and completely irradiated diets given 2-megarep exposures are presented. Animals fed these diets for 4 months (ultil they were 7 months old) showed normal growth and reproduction.

The effects of feeding a diet irradiated at 20 megarep in part or in its entirety on the growth of young rats allowed to eat ad libitum were compared with effects of limiting the food intake to the extent that control and experimental animals received approximately equal amounts. The animals fed the irradiated diet ad libitum starting at the age of 7 weeks showed a marked reduction of weight gain when compared with animals fed the control diet. Groups of animals fed a partially irradiated diet on a restricted basis for 3 weeks after weaning and then allowed food ad libitum from the seventh week on showed no significant differences either in growth or reproduction from littermate controls.

Animals which were fed a freshly prepared diet completely irradiated at a 20-megarep level from 7 weeks to 17 weeks after weaning, weighed only one-third to one-half as much as littermate controls at the end of this period. Addition of the water-soluble nonirradiated vitamin premix to the completely irradiated diet restored, in part at least, the capacity for growth and reproduction.

The evidence from these experiments and others still in progress appears to indicate that no acutely toxic substances are produced in diets exposed to 20 megarep and more of gamma irradiation. Vitamin destruction reduces the nutritional value of the diets completely irradiated at high levels. Otherwise, the principal effects of high levels of gamma-ray irradiation, which are apparent during the first few months of feeding can be ascribed to changes in palatability of the irradiated foods and the resulting lower food intake.

5. References

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C. THE USE OF TETRAHYMENA FOR THE EVALUATION OF THE EFFECTS OF GAMMA RADIATION ON ESSENTIAL NUTRILITES

Personnel:

A. M. Elliott, Associate Professor of Zoology; L. E. Brownell, Associate Professor of Chemical Engineering; J. A. Gross, Research Assistant, Department of Zoology.*

1. Introduction

The research on the use of Tetrahymena for the evaluation of the effects of gamma radiation on essential nutrilites has been supported by Michigan Memorial-Phoenix project Number 73. This report is taken from a manuscript prepared by A. M. Elliott, L. E. Brownell, and J. A. Gross to be submitted for publication in an appropriate scientific journal. No parts of the following report are to be reproduced without permission of the authors.

Proposed studies on the effect of gamma-ray irradiation of nutrilites in conjunction with growth requirements for Tetrahymena pyriformis, a ciliate protozoan whose nutritional requirements closely simulate those of mammals, have been described in Progress Report 5.[15]

*Present Address: Zoology Department, University of California at Los Angeles.

The effects of x-rays and of alpha and beta rays on naturally occurring foods, individual amino acids, and vitamins have been recorded in the literature; but the effects of gamma rays on nutrilites, in general, have not as yet been adequately observed. The purpose of the experiments discussed in this section is to add to present knowledge in this field through studies on the effect of gamma rays on indiviaual amino acids, nucleic acid derivatives, and vitamins required for the growth of Tetrahymena. In addition, the possibility of the formation of toxic or decomposition products during the radiation process was investigated by means of growth curves of Tetrahymena, and, finally, organoleptic (odor and color) observations before and after radiation were recorded as evidence for or against changes in the chemical structure of the individual nutrilites.

2. Materials and Methods

Stock cultures of the ciliated protozoan, Tetrahymena pyriformis E, were grown in a 1 percent tryptone-proteose-peptone broth. The inoculum for experimental studies was obtained by harvesting cells from 48-hour stock cultures by centrifugation and aseptically washing the cells three or four times in redistilled water. Washed cells were resuspended in distilled water and diluted to give an optical density of 0.032 in the Lumetron colorimeter, model 401, with the blue (420 mμ) filter. Such a suspension contains approximately 25,000 cells/ml. Subsequently, 0.2 ml of this suspension was pipetted into 5-ml amounts of sterile, chemically defined medium[9,10,11] in 15- by 125-mm pyrex culture tubes. Following inoculation the cultures were incubated vertically at 25°C in the dark. Daily growth measurements were made turbidimetrically with the Lumetron, by adapting the method described by Elliott.[8] The pH of the medium was routinely set at 7.6 before autoclaving, after which the readings were 7.2 to 7.4, except in irradiated complete liquid medium where the pH dropped to as low as 6.7 at the highest radiation level. The significance of this pH decline is considered in the discussion.

All media were prepared with glass-redistilled water, and the glassware was routinely given a final rinse in glass-redistilled water before drying for use in an experiment.

Results reported are the averaged data from at least two experiments, each run in duplicate or triplicate. Any deviation from this procedure is discussed with the indiviual experiments in question.

3. Results

a. Irradiation of Complete Defined Medium. A preliminary irradiation of the medium was performed to determine the range of radiation effects and the comparative radiation effects on dry and liquid media. The defined medium used

to culture Tetrahymena pyriformis E was irradiated in solution and in the dry state (except for thioctic acid, which was used as a concentrated solution of sodium-Dx, L-6-thioctate*). Dry, crystalline amino acids, nucleic acid components, glucose, sodium acetate, and inorganic salts were mixed and irradiated in aluminum-foil-capped 50-ml Erlenmeyer flasks. Vitamins were not included in the dry mix because of the mechanical difficulties of weighing the small amounts which are required. Therefore, except for thioctate they were separately irradiated in 5-ml glass screw-cap vials, made up in concentrated solution, and dispensed into the media. The liquid medium was prepared, sterilized, and irradiated in 500- or 1000-ml pyrex reagent bottles. At selected increments of irradiation, aliquots were aseptically removed and transferred to culture tubes for inoculation.

A range of irradiation from 1×10^5 to 4×10^6 rep was employed in the initial screening experiment for both the dry and liquid medium and for the medium prepared from irradiated dry constituents. The highest radiation dose had no apparent effect on the dry components since the protozoa grew equally well in a medium prepared from the irradiated mix and in the nonirradiated medium. On the other hand, the irradiated liquid medium failed to support growth of the ciliates when the irradiation dose was 5×10^5 rep or over. When nonirradiated medium was added to these inhibited cultures, growth recovery was achieved at a rate which was inversely related to the radiation level; i.e., recovery was greater when fresh medium was added to that which received only 5×10^5 rep than when added to medium irradiated by 4×10^6 rep. The smaller growth recovery at the higher irradiation level may be due to: (a) increased toxicity with increased irradiation, or (b) destruction of more nutrilites at higher doses of radiation. A dose-response curve (Fig. 75a) based on experimental evidence, shows that the critical range of irradiation for the complete liquid medium is from 1.5×10^5 to 3×10^5 rep. The complexity of events which may occur in complete medium makes it difficult to identify the components being affected. Therefore, the direct approach of irradiating indiviual medium components was undertaken.

b. Irradiation of Individual Components of the Medium. In the first experimental series in which separate components were to be tested for the effects of irradiation, each was irradiated in the concentration which usually is employed in the preparation of the medium (Table 33). Media were prepared by omitting one or several of the components in groups. The five groupings used were: (1) amino acids, (2) vitamins, (3) nucleic acid components, (4) glucose, and (5) sodium acetate. Each component was individually irradiated for 1×10^6 rep. The irradiated compounds were then pooled in the described groups and added to that

*Na-D, L-6-thioctate was generously supplied by the Lederle Laboratories, Pearl River, New York.

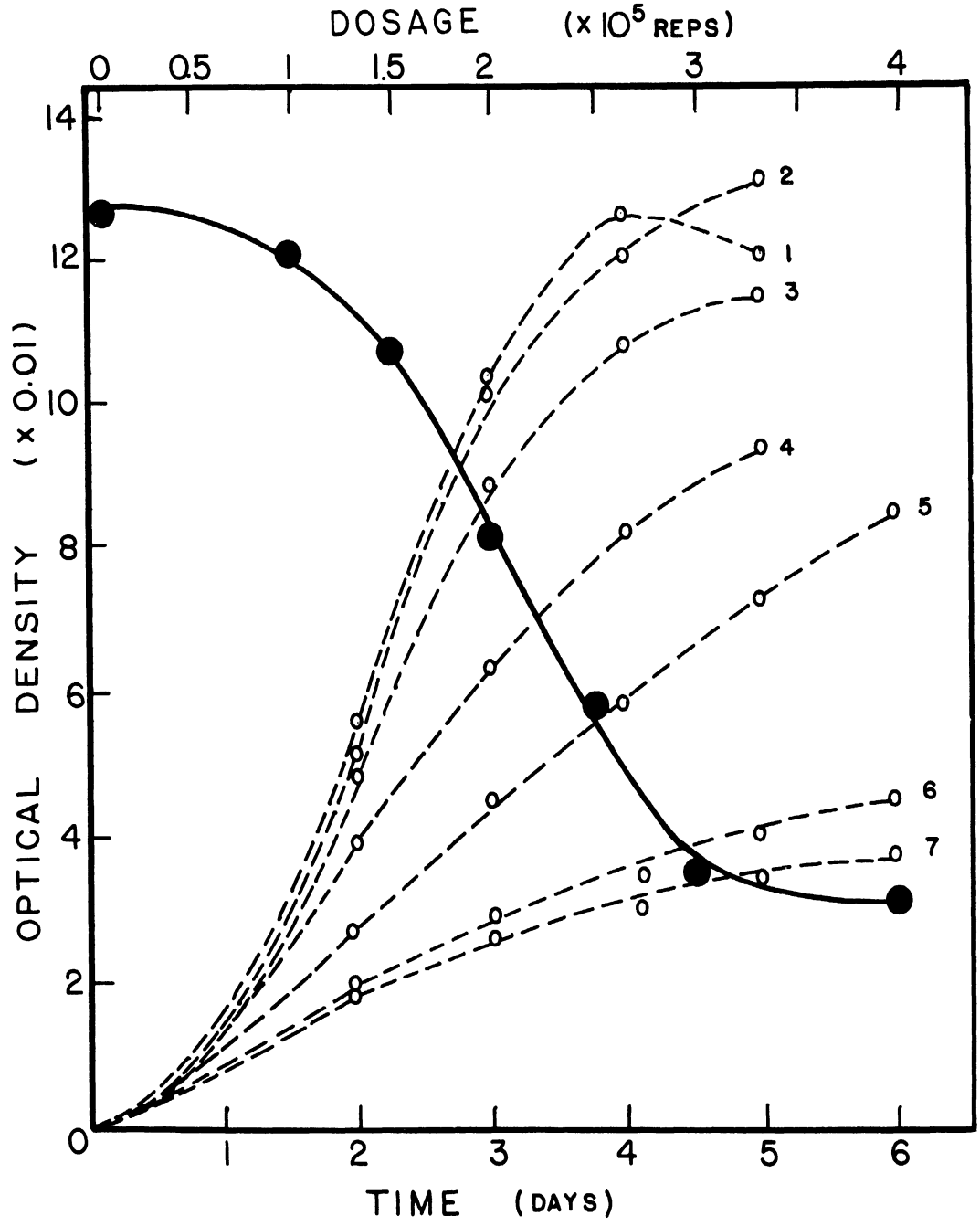

Fig. 75a. Growth inhibitions caused by irradiation of the chemically defined medium used for culture of T. pyriformis E. Broken lines indicate growth in optical density plotted against time in days. 1, control; 2, 1 x 10^5 rep; 3, 1.5 x 10^5 rep; 4, 2 x 10^5 rep; 5, 2.5 x 10^5 rep; 6, 3 x 10^5 rep, 7, 4 x 10^5 rep. ●-●, dose-response in optical density at peak growth of control plotted against radiation dose in rep.

TABLE 33

COMPOSITION OF THE BASAL CHEMICALLY DEFINED MEDIUM

Compounds	Molarity	Final Concn. in Medium, mg/l
Amino Acids		
L-Arginine · HCl	0.172	150
L-Histidine · HCl	0.142	110
D_xL-Isoleucine	0.152	100
L-Leucine	0.106	70
L-Lysine	0.048	35
D_xL-Methionine	0.046	35
D_xL-Phenylalanine	0.061	100
D_xL-Serine	0.316	180
D_xL-Threonine	0.343	180
L-Tryptophan	0.019	20
D_xL-Valine	0.102	60
Carbon Sources		
Glucose	1.4	1000
Na acetate	3.0	1000
Nucleic Acid Components		
Adenylic acid	0.0088	25
Cytidylic acid	0.0096	25
Guanylic acid	0.0087	25
Uracil	0.0223	25
Vitamins		
Thiamine-HCl	8.25×10^{-4}	1
Riboflavin	7.5×10^{-5}	0.1
Ca pantothenate	0.96xx 10^{-4}	0.1
Niacin	2.03×10^{-4}	0.1
Pyridoxine · HCl	2.33×10^{-3}	2
Folic acid (PGA)	5.75×10^{-6}	0.01
Thioctic acid	4.3×10^{-8}	0.001
Inorganic Salts		
K_2HPO_4	0.144	100
$MgSO_4 \cdot 7H_2O$	8.1×10^{-3}	10
$Zn(NO_3)_2 \cdot 6H_2O$	3.9×10^{-3}	5
$FeSO_4 \cdot 7H_2O$	4.2×10^{-4}	0.5
$CuCl_2 \cdot 2H_2O$	7.3×10^{-4}	0.5

experimental medium which was deficient in the test group. On testing the growth of Tetrahymena in such media it was found that only the medium containing irradiated vitamins failed to support growth. All other media yielded normal growth except a control medium prepared entirely from irradiated components, in which growth also was negative.

In order to determine toxicity effects in the irradiated vitamins, another experiment was devised using complete nonirradiated synthetic medium to which the irradiated vitamin group was added. Growth of the protozoa in this medium was equal to the control growth, demonstrating that the irradiated vitamins were not toxic, although they might have been destroyed by the gamma rays.

At this point in the investigation it became clear that certain organoleptic changes occurred in the irradiated compounds (Table 34) although some of the compounds retained normal biological activity; hence it was found necessary to run dose-response curves on all the components of the medium to determine at which level of irradiation each nutrilite was rendered incapable of serving as a nutrient for Tetrahymena. It was decided to restrict irradiation dosage to the range where a linear relationship occurs. As indicated by Allsopp,[2] this effect takes place in dilute aqueous solutions of not less than 10^{-4}M. Therefore, the medium components were prepared and irradiated in the concentrations listed in Table 35. The following irradiation levels were used: 1×10^5, 2.5×10^5, 5×10^5, 7.5×10^5, 1×10^6, 1.5×10^6, 2×10^6, 3×10^6, and 4×10^6 rep.

c. Irradiation of the Vitamins. Preliminary experiments had shown that media containing irradiated vitamins fialed to support growth of the ciliate. In order to determine which of the vitamins were destroyed at this level of irradiation (1×10^6 rep), an omission experiment was performed in which single vitamins prepared as shown in Table 33 were left out of the medium. The omitted vitamin was then replaced by the irradiated vitamin. Growth experiments in this series indicated that pyridoxine and thiamine did not decrease in efficiency as a result of irradiation; whereas thioctate, folic acid, riboflavin, niacin, and pantothenate were all partially destoryed. A lack of toxicity in all the irradiated vitamins was demonstrated by supplementing the nonirradiated control medium with single irradiated vitamins and obtaining growth which was equivalent to that in the control medium.

The determination of the dose levels at which each of the vitamins was rendered useless to Tetrahymena as a growth factor was considered important. Employing the concentrations described in Table 35 media were again prepared with single-vitamin omissions. After the omitted vitamin had received the series of irradiation doses prescribed, it was added to the medium. Tubes of media were then sterilized, inoculated, and checked for growth of the organism. Positive damage to all vitamins except thiamine and thioctate was observed.

TABLE 34

ORGANOLEPTIC CHANGES IN AQUEOUS SOLUTIONS OF VITAMINS, AMINO ACIDS, AND NUCLEIC ACID COMPONENTS SUBJECTED TO GAMMA RADIATION FROM COBALT-60

Component	Irradiation, rep	Color	Odor
L-Arginine · HCl	1 x 10^6	none	none
	1 x 10^7	none	none
	2.3 x 10^7	none	faintly sweet
L-Histidine · HCl	1 x 10^6	none	none
	1 x 10^7	light amber	none
	2.3 x 10^7	light amber	dilute ethyl alcohol
D_XL-Isoleucine	1 x 10^6	none	faintly sweet
	1 x 10^7	none	musty fruit
	2.3 x 10^7	none	musty fruit
L-Leucine	1 x 10^6	none	faintly sweet
	1 x 10^7	none	musty fruit
	2.3 x 10^7	none	musty fruit
L-Lysine · HCl	1 x 10^6	none	none
	1 x 10^7	none	none
	2.3 x 10^7	yellow	faintly sweet
D_XL-Methionine	1 x 10^6	none	boiled cabbage
	1 x 10^7	turbid	boiled cabbage
	2.3 x 10^7	turbid-light straw	boiled cabbage
D_XL-Phenylalanine	1 x 10^6	none	none
	1 x 10^7	yellow	mild geranium
	2.3 x 10^7	dark amber	geranium
D_XL-Serine	1 x 10^6	none	none
	1 x 10^7	straw	faintly sweet
	2.3 x 10^7	none	none
D_XL-Threonine	1 x 10^6	none	none
	1 x 10^7	light straw	faintly sweet
	2.3 x 10^7	light straw	dilute ethyl alcohol
L-Tryptophan	1 x 10^6	light brown	none
	1 x 10^7	dark brown	mild indole
	2.3 x 10^7	dark brown	indole
D_XL-Valine	1 x 10^6	none	faintly sweet
	1 x 10^7	none	sweet
	2.3 x 10^7	none	musty fruit

TABLE 34 (cont.)

Component	Irradiation, rep	Color	Odor
Guanylic acid	1 x 10^6	none	none
	1 x 10^7	none	none
	2.3 x 10^7	light straw	none
Adenylic acid	1 x 10^6	none	none
	1 x 10^7	light straw	none
	2.3 x 10^7	light straw	none
Cytidylic acid	1 x 10^6	none	none
	1 x 10^7	light straw	none
	2.3 x 10^7	light straw	none
Uracil	1 x 10^6	none	none
	1 x 10^7	none	none
	2.3 x 10^7	none	none
Thiamine · HCl	1 x 10^6	turbid	H_2S
	2 x 10^6	turbid	H_2S
Riboflavin	1 x 10^6	faded yellow	faintly sweet
	2 x 10^6	none	faintly sweet
Ca pantothenate	1 x 10^6	none	none
	2 x 10^6	none	none
Niacin	1 x 10^6	none	none
	2 x 10^6	none	none
Pyridoxine · HCl	1 x 10^6	none	none
	2 x 10^6	none	none
Folic acid	1 x 10^6	none	none
	2 x 10^6	none	none
Na-D_xL-5-Thioctic	1 x 10^6	none	none
	2 x 10^6	none	none

TABLE 35

MOLAR EQUIVALENT CONCENTRATIONS OF MEDIUM COMPONENTS

Component	Molarity	Amount of Concentrate to be used, ml/l
Amino Acids		
L-Arginine · HCl	0.05	14.2
L-Histidine · HCl	0.05	10.6
D_xL-Isoleucine	0.05	15.2
L-Leucine	0.05	10.7
L-Lysine	0.048	5.0
D_xL-Methionine	0.046	5.0
D_xL-Phenylalanine	0.05	12.1
D_xL-Serine	0.05	34.4
D_xL-Threonine	0.05	30.3
L-Tryptophan	0.05	1.96
D_xL-Valine	0.05	10.3
Vitamins		
Thiamine · HCl	1.98×10^{-4}	15.00
Riboflavin	2.0×10^{-4}	1.37
Ca Pantothenate	2.0×10^{-4}	1.95
Niacin	2.03×10^{-4}	4.00
Pyridoxine	2.0×10^{-4}	50.00
*Folic acid (PGA)	2.0×10^{-5}	0.21
Thioctic acid	2.0×10^{-4}	0.045
Nucleic Acids		
Adenylic acid	8.8×10^{-3}	10
Cytidylic acid	9.6×10^{-3}	10
Guanylic acid	8.7×10^{-3}	10
Uracil	9.1×10^{-3}	25

*Folic acid could not be prepared at the concentration of the other vitamins, since such a concentration is above its limit of solubility.

The inconsistencies noted for pyridoxine and thioctate between the preliminary study and these results can be explained on the basis of the different molar concentrations at which the vitamins were irradiated. Since subsequent studies were performed at the concentrations listed in Table 35, the latter results were of prime interest. It was reasoned, therefore, that a lack of effect on thiamine and thioctate was due to an insufficient irradiation dose or to a retention of these vitamins in the cells to such an extent that enough was present to produce normal growth despite damage to the extracellular vitamin. Therefore, cells which were subsequently used for determining thioctate and thiamine damage were passed through two serial transfers in thioctate- and thiamine-deficient media respectively before inoculation into the experimental media containing the irradiated vitamins. When this method was used, damage to these two vitamins was indicated. All results are recorded in Figs. 75b, 75c, 76, and 77.

Figure 75b shows the curves obtained by culturing Tetrahymena in a medium containing niacin which has received progressively increasing levels of irradiation. The plotted dose-response curve was obtained by comparing growth of the cultrues at the different levels of irradiation at the time when control growth reached its peak. In Figs. 75c, 76, and 77 the dose-response curves for the other vitamins are shown. These curves were obtained from data similar to those shown for niacin. In all cases the critical radiation range was found to be between 2.5×10^5 and 1×10^6 rep, except for niacin which is affected between 1×10^6 and 2×10^6 rep. This indicates that of the vitamins tested niacin is the most stable. The plots shown are not calculated curves; but they represent the investigators' interpretation of the experimental data.

When single vitamins irradiated at 1×10^6 rep supplemented the complete nonirradiated medium, normal growth occurred. This indicates that radiation damage to the vitamins does not result in the formation of substances toxic to Tetrahymena.

d. Irradiation of the Amino Acids and Nucleic Acid Components. The usual method of preparation was employed and found suitable for these experiments since media deficient in any amino acid of the medium would not support growth of Tetrahymena. However, the only nucleic acid component that could be shown to be required for the growth of this organism was guanylic acid which has been shown by Kidder et al.[12] to be essential for strain W. Five serial transfers of the ciliate through media deficient in any one of the other three nucleic acid components still yielded normal growth. The conclusion was that irradiation effects on these compounds could not be determined with Tetrahymena in the basal synthetic medium utilized in this research. Nevertheless, adenylic and cytidylic acids as well as uracil were irradiated to determine color and odor changes (Table 34) and to test for toxicity.

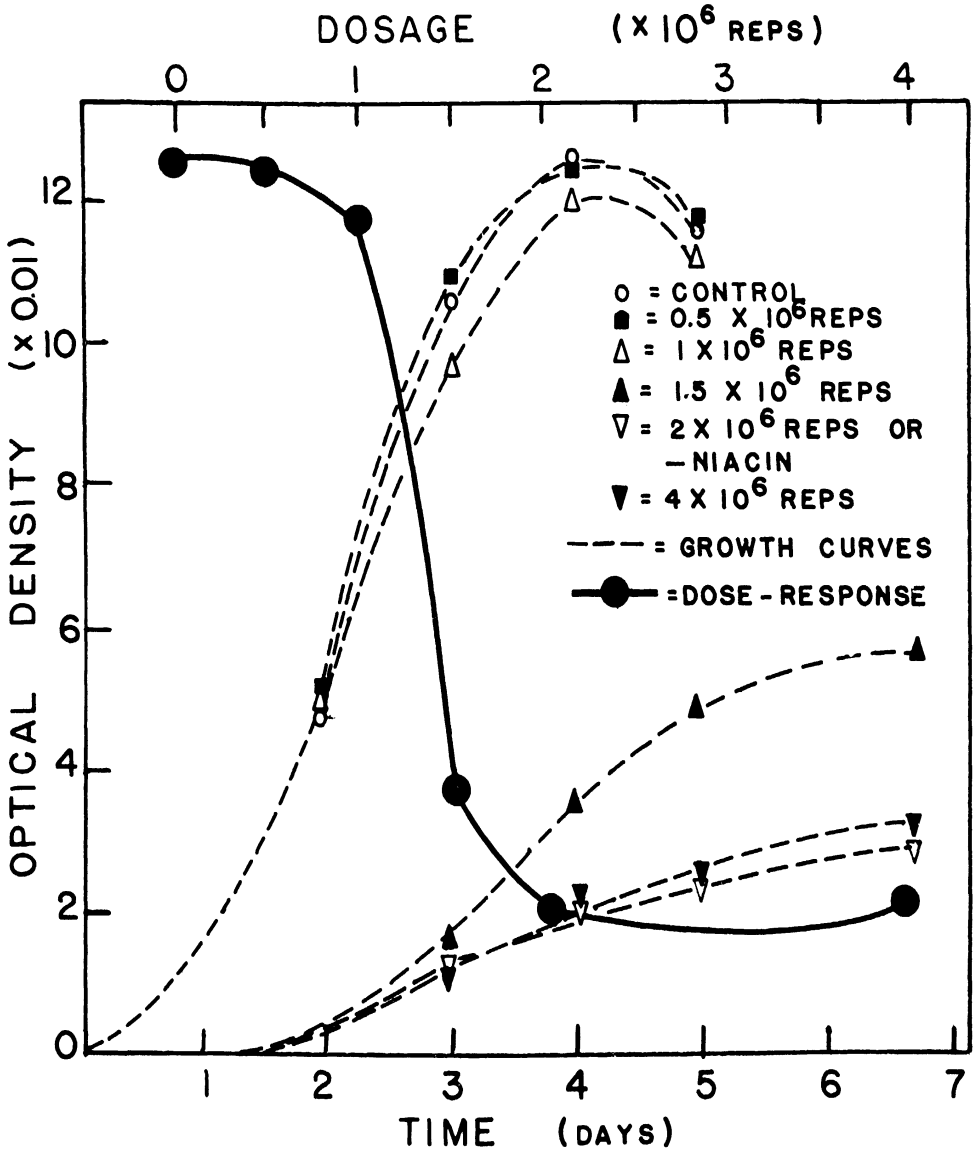

Fig. 75b. Dose-response and growth curves in chemically defined medium prepared with irradiated niacin.

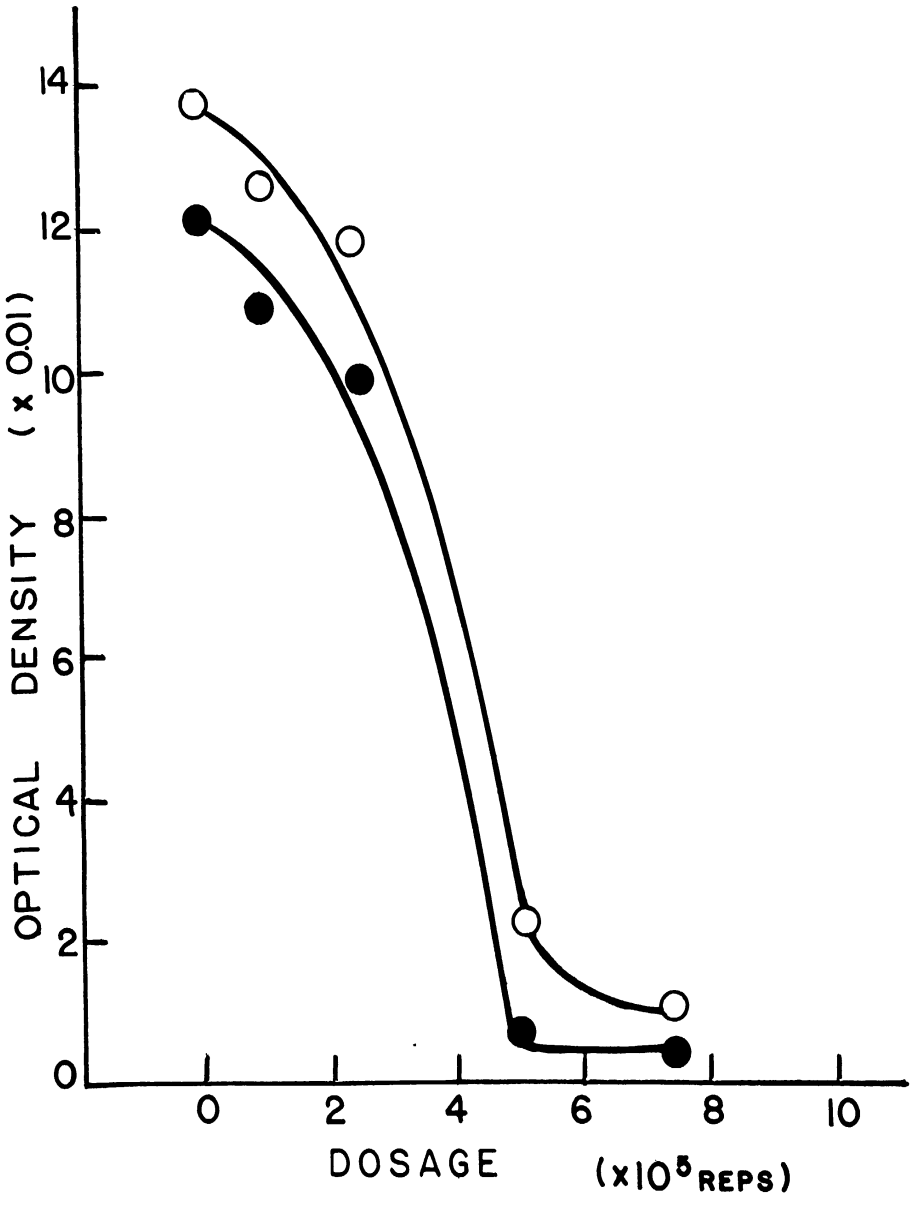

Fig. 75c. Dose-response in Chemically Defined Medium Prepared with Irradiated Thiamine and Pyridoxine, Respectively. O-O, Thiamine; ●-●, Pyridoxine.

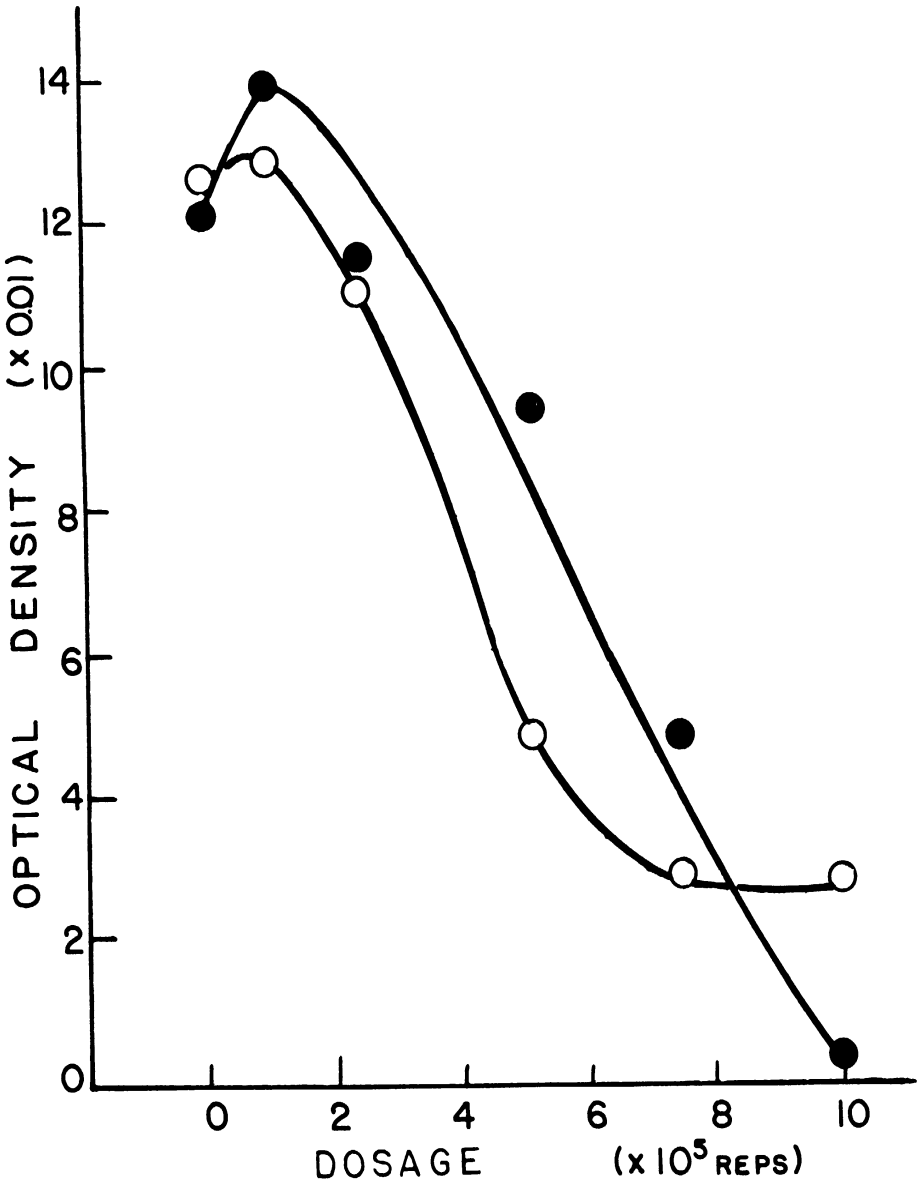

Fig. 76. Dose-response in Chemically Defined Medium Prepared with Irradiated Riboflavin and Thioctic Acid, Respectively. O-O, Riboflavin; ●-●, Thioctic Acid.

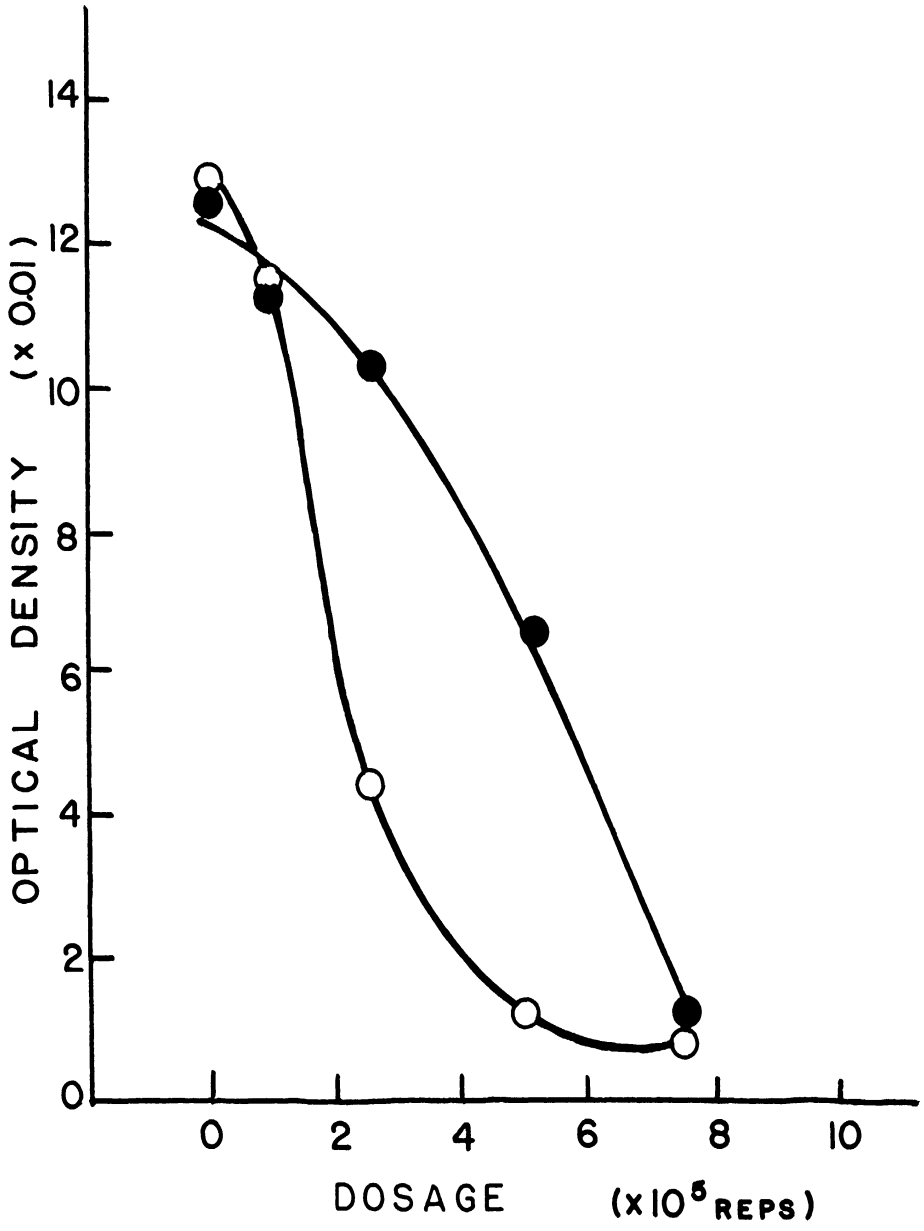

Fig. 77. Dose-response in Chemically Defined Medium Prepared with Irradiated Pantothenate and Folic Acid. O-O Pantothenate; ●-●, Folic Acid.

The amino acids and guanylic acid were tested by the method of single omissions described previously for the vitamins. All irradiations were made on components at the concentrations given in Table 35. Neither the amino acids nor guanylic acid was damaged at any irradiation level up to 4×10^6 rep. However, at 1×10^7 rep serine activity was completely destroyed (Fig. 78). Growth recovery was effected by adding nonirradiated serine to the cultures inhibited by irradiated serine. The other amino acids and guanylic acid were not damaged at this level of irradiation.

When the amino acids were irradiated at 2.3×10^7 rep, methionine as well as serine was damaged (Fig. 79). Guanylic acid was also almost completely destroyed at this irradiation dose (Fig. 80). There were indications that some of the other amino acids were slightly damaged at this high irradiation level; but the extent of damage was not conclusive. At the highest irradiation dose only one single experiment was performed at the highest irradiation dose; the results should, therefore, be confirmed in future tests.

Lack of toxicity of the irradiated components was demonstrated in the usual manner by supplementing a complete nonirradiated medium with irradiation-damaged component. In all cases growth was normal.

e. Organoleptic Observations on Irradiated Materials. Early in this study it became obvious that color and odor changes occurred in irradiated materials. Progressively higher irradiation doses caused increasingly greater changes. Although organoleptic tests revealed radical changes in some of the irradiated compounds, the biological activity of most of the substances as determined by growth of Tetrahymena, remained unimpaired. On the other hand, biological damage was incurred by some of the vitamins; yet their color and odor remained unaltered.

The organoleptic observations listed in Table 34 were recorded by several persons. All the nonirradiated solutions were essentially odorless and colorless, except for riboflavin which is yellow and folic acid which is a light greenish-yellow. Therefore, all the recorded changes are the results of irradiation. These observations may afford some insight into the type of radiation damage which has been shown to occur in growth experiments.

4. Discussion

Earlier in this paper it was reported that irradiation of the complete liquid defined medium resulted in a drop in pH corresponding to the radiation dose. At 4×10^6 rep the pH was lowered from 7.6 to 6.7. This variation is well within the range of optimal growth of Tetrahymena as demonstrated by Elliott[17] and Slater.[18] Therefore, the observed pH decline in irradiated medium may be considered to have had little effect. The acid production was possibly caused

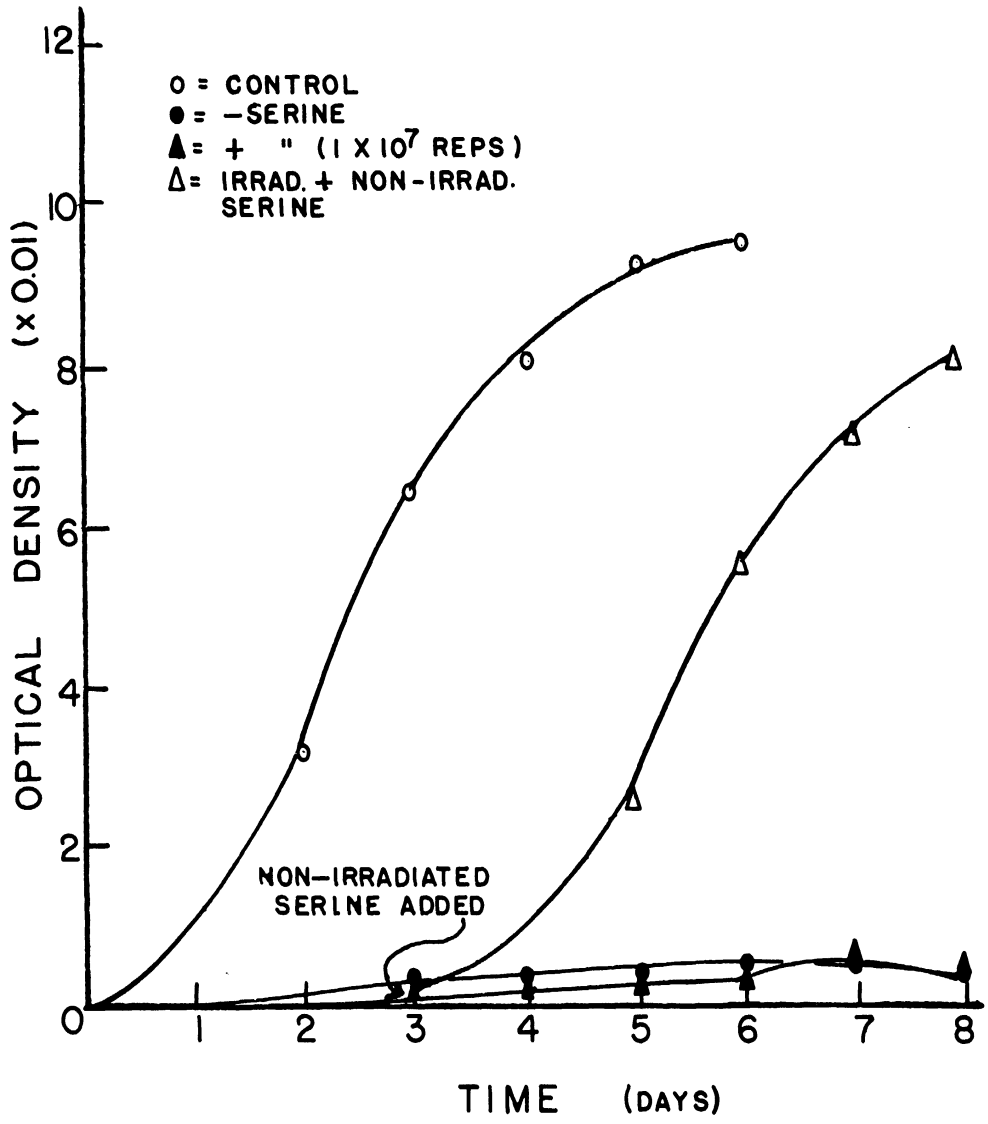

Fig. 78. Growth Inhibition in Chemically Defined Medium Prepared with Irradiated Serine, and Growth Recovery upon Addition of Non-irradiated Serine at 3 Days.

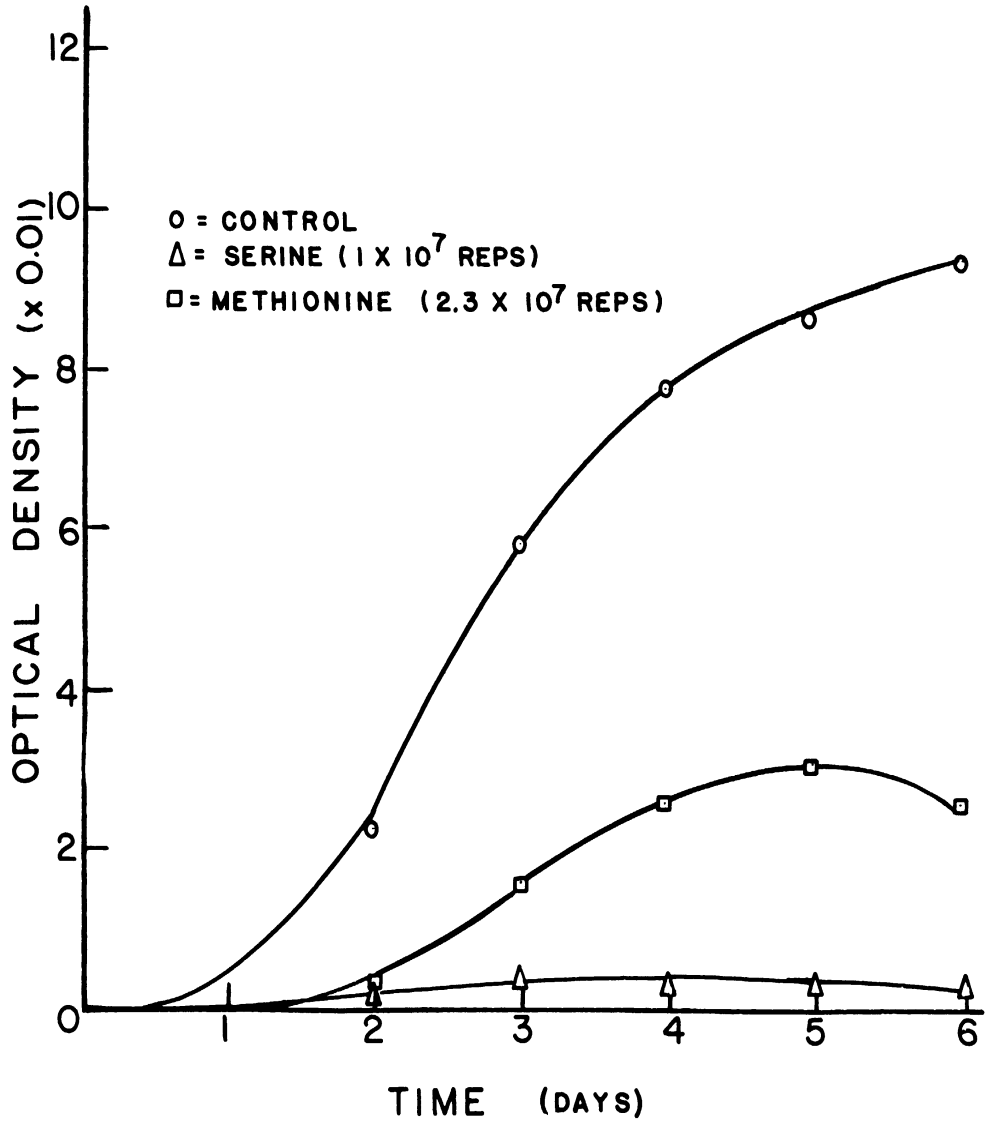

Fig. 79. Growth Inhibition in Chemically Defined Media Prepared with Irradiated Methionine and Serine, Respectively.

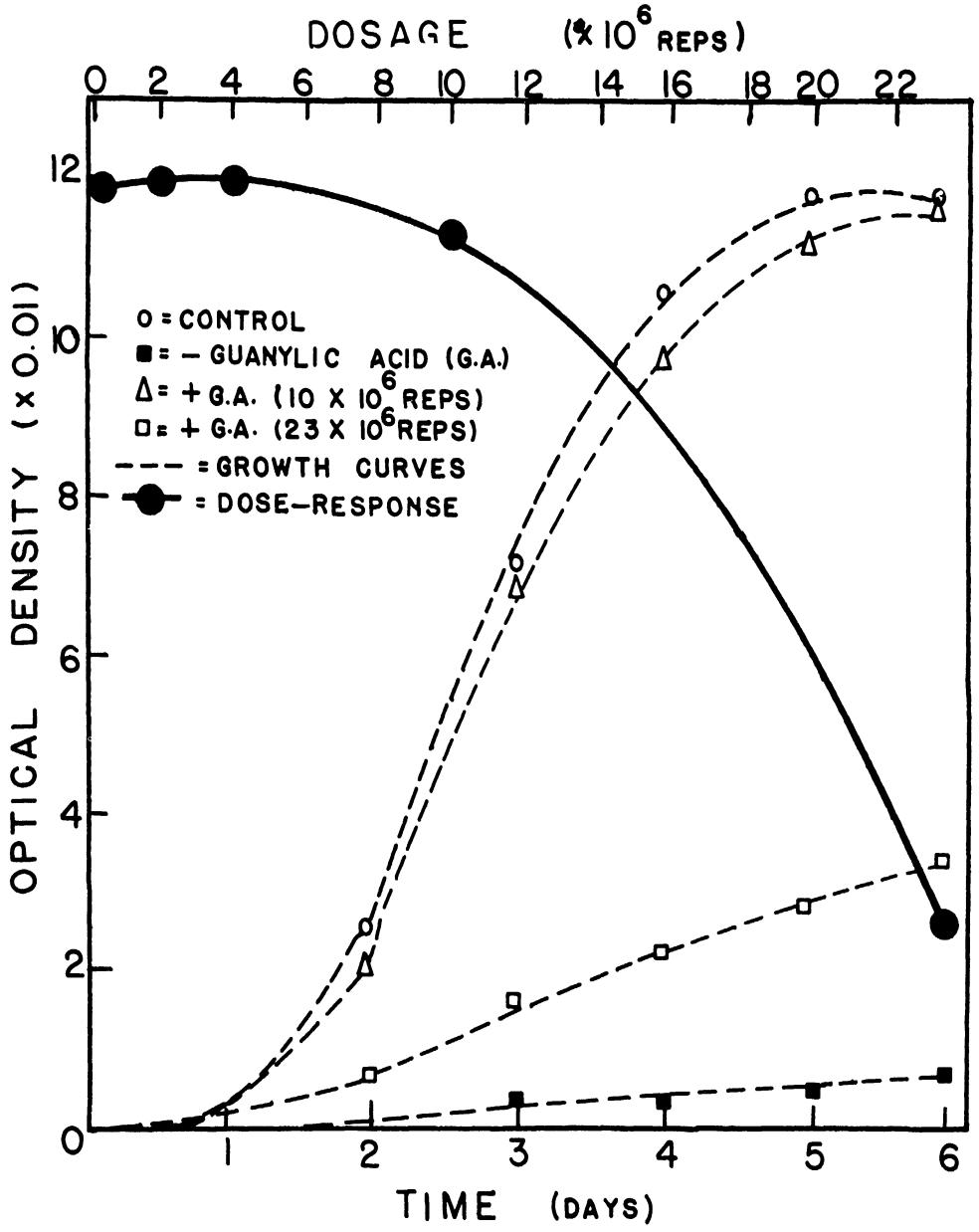

Fig. 80. Dose-response and Growth-Inhibition Curves in Chemically Defined Media Prepared with Irradiated Guanylic Acid.

by the breakdown of glucose. Baumgartner[4] showed that bacteria failed to grow on irradiated carbohydrate media due to the production of acid by irradiation. Addition of base to the medium readjusted the pH and normal bacterial growth was resumed.

The lack of effect of irradiation on dry materials was to be expected, since in general, compounds in the dry state are more stable than the same compounds are in solution. Lea[13] states that the enzyme ribonuclease in the dry state was inactivated by x-rays only between the levels of 1.5×10^7 and 8×10^7 r. The highest radiation dose received by the dry defined medium used in this investigation was about one-tenth of the dose required to damage dry ribonuclease.

Taylor et al.[20] demonstrated that x-ray radiation of a yeast medium resulted in a change from adequacy for growth of the ciliate Colpidium. Data from this report also indicate that an irradiated aqueous medium given 5×10^5 rep is unable to support growth of another ciliate, Tetrahymena.

Since the total concentration of the complete liquid medium is of the order of 10^{-2} M, each of the constituent compounds is present in far greater dilution. The low radiation dose necessary to inactivate the medium in this form can be attributed to the instability of dilute solutions and the radiation sensitivity of the vitamins. Lea[13] states that dilute solutions are readily inactivated by radiation doses which are so small that they may have little or no effect at all on dry preparations or more concentrated solutions. Furthermore, in considering the radiation effects on the complete medium, a complex situation arises which involves a protective interaction among the various compounds of the medium. Such protection has been reported in solutions of two compounds. For example, in pure solution ascorbic acid is known to be highly radiosensitive; but Proctor and Goldblith[14] have reported that niacin at 10 μg/ml can protect as much as 500 μg/ml of ascorbic acid against the effects of ionizing radiations. Obviously such factors complicate the situation in a complex medium. For these reasons it was though advisable to discontinue studies with the complete medium and instead investigate radiation effects on pure solutions of the medium components.

It is well known that vitamins are affected by various types of radiations.[1,16,17] It has been demonstrated, for example, that ultraviolet radiation changes riboflavin to hemiflavin, which separates out from the original aqueous solution because of its extremely low solubility.[16] On the other hand, cobalt-60 radiation caused riboflavin to become colorless; but no precipitate was formed. Robinson[16] describes the reduction of riboflavin to a colorless compound when treated with sodium dithionite. It seems likely, therefore, that gamma radiation from cobalt-60 reduces riboflavin rather than decomposes it to hemiflavin. The one difference between the actions of cobalt radiations and sodium dithionite is that color is not restored by aeration of the irradiation solution.

Niacin was shown to be the most radiation-resistant of the vitamins. This observation supports the contention of Proctor and Goldblith[14] that niacin is a relatively radiostable substance.

The dose-response data for thioctate seems to indicate activation of growth at low radiation levels. This stimulation may be real and, if so, may be strengthened by the report that photoradiation of enzymes causes an initial increase in enzyme activity, followed by a decrease.[17]

The effects of radiations on proteins and amino acids have been studied for many years. The work done up to 1936 has been excellently reviewed by Arnow[3]. A more recent review with a comprehensive bibliography is that by Sparrow and Rubin.[19] Other reports also emphasize the radiation sensitivity of amino acids.[6,22]

In the light of previous studies it was considered likely that cobalt-60 radiations would decompose amino acids. It was shown in the present research that serine is inactivated and apparently is the most radiosensitive of all the amino acids that were irradiated in this investigation. The destruction is probably similar to that found by Dale and Davies.[6] These studies also showed that methionine appeared to be the next most radiosensitive amino acid. No information was found in the literature regarding radiation studies on methionine solutions.

Several reports emphasize that nucleic acids are degraded by various types of radiations both in irradiated living cells[13] and in vitro.[19,21] The effects of irradiation of nucleotides are not known; however, it was shown in this investigation that guanine nucleotide loses its biological value for Tetrahymena at high radiation doses.

5. Conclusions

A complete synthetic medium for the growth of Tetrahymena pyriformis E was subjected to gamma radiation from cobalt-60. A dose-response curve indicated the range of radiation which damaged the medium so that growth of Tetrahymena was inhibited.

The essential vitamins and amino acids which comprise the medium were individually irradiated in solution. Media were prepared with single irradiated components and tested for their ability to support growth of the protozoa.

Thiamine, riboflavin, pantothenate, pyridoxine, folic acid, and thioctic acid were shown to be altered structurally by less than 1×10^6 rep, while 2×10^6 rep were required to produce changes in niacin. Most amino acids proved to be relatively radiation-resistant. At the high radiation levels of 1×10^7 rep

and 2.3 x 10^7 rep respectively, only serine and methionine were damaged. All other amino acids remained biologically active even after receiving 2.3 x 10^7 rep, the highest level of radiation employed. However, organoleptic observations showed radiation caused color and odor changes in many of those amino acids whose biological activity for Tetrahymena remained unaffected. The nucleotide guanylic acid was also inactivated at 2.3 x 10^7 rep.

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D. MICROBIOLOGICAL STUDIES FOR PHOENIX PROJECT 41

Personnel:

L. L. Kempe, Assistant Professor of Bacteriology, Medical School, and Assistant Professor of Chemical and Metallurgical Engineering, Engineering School; J. T. Graikoski, Research Assistant; and R. A. Gillies, Research Assistant, Phoenix project 41.

Note:

The results reported here were presented at the local section meeting of the Society of American Bacteriologists in Detroit, Michigan, on December 5, 1953.

1. Gamma-Ray Sterilization of Canned Meat Inoculated with Spores

a. Introduction. The interest in the killing of spores with gamma radiation is occasioned by the possibility of using fission products for sterilizing foods. This study was inaugurated to investigate the effectiveness of gamma radiation for sterilizing foods packed in tin cans of commercial size. Previous work has shown that gamma radiation will kill spores suspended in various substrates contained in nonmetallic vessels.[1,2]

However, there is reason to believe that metallic containers may alter the rate at which spores are killed by reducing the quantity of radiation delivered and by inducing secondary radiations. It is also reasonable to expect that the nature of the suspending medium, as well as other factors such as oxygen tension, temperature, etc., will affect the lethality of gamma radiation on spores. Since meat is one important class of foods that has been considered for sterilization with gamma radiation, tests are presently being made on the rate at which these radiations will kill spores of anaerobic bacteria present in meat packed in No. 2 tin cans.

b. Equipment and Methods. The organisms presently being used in this study are Putrefactive anaerobe NCA 3679 and Clostridium botulinum 62A. The first organism was obtained from the National Canner's Association, while the latter came from the Hooper Foundation. Spore suspensions are prepared from cultures grown according to National Canners Association techniques.[3] The Putrefactive anaerobe is cultured in pork infusion broth and Clostridium botulinum in casitone broth.

Inoculated meat packs are prepared in the following manner: Ground beef, purchased on the open market, is first precooked at 15 psig steam pressure for 15 minutes. After the resulting broth is drained off, No. 2 tin cans are packed to within 1/2 inch of the top with hot meat, covered loosely with their lids, and steam-sterilized at 15 psig for 1 hour. The cans of meat are then removed from the autoclave, one at a time, and inoculated with a titrated spore suspension into the approximate geometrical center of the meat. Following this, the cans are immediately sealed in a Western-type closing machine and then cooled in running tap water at about 14°C. This treatment develops a vacuum of about 27 inches of mercury in the cans.

For irradiation, the cans are placed in the radiation cave on turn tables that rotate at one revolution per minute. The turntables are set up in the radiation chamber, which is maintained at a temperature of about 15°C. Normally, four cans are irradiated at each dosage level. Also, two sets of controls are used: one set tests the heat-sterilization process and the other establishes the viability of the spores in meat. The first set is neither inoculated nor irradiated; the second set is inoculated but not irradiated.

Subsequent to irradiation, the cans are marked and placed in an incubator at 37°C. Those cans that swell are removed from the incubator and recorded as positive for gas production. The odor of the gas from all cans is observed in order to compare it with the characteristic odor usually produced by anaerobic bacteria growing in meat. In addition, representative cans from the group that develop gas are aseptically opened and approximately 20 grams of meat is removed for subculturing in veal infusion broth at 37°C. Stained slides are made from the subcultures to verify the presence of anaerobic spore-forming bacteria. Representative cans that do not swell are likewise checked for sterility by subculturing after suitable periods of incubation have elapsed.

c. Results. Tables 36 and 37 show the effect of increasing dosages of gamma radiation on canned meat inoculated with spores of Putrefactive anaerobe NCA 3679 and Clostridium botulinum 62A respectively.

Figure 81 is a graph showing the combined effects of increasing spore concentration and increasing gamma irradiation dosages on the killing of Clostridium botulinum spores.

Samples of meat taken from cans are being examined microbiologically for the pressure of the organism inoculated. To date, such organisms have not been recovered from the unswollen cans. This is at least reasonable evidence that the cans which show no gas production after 30 days incubation are sterile.

d. Discussion and Conclusions. The sterility dosage for canned meat increases from 2.5 to 4 million rep as the concentration of Clostridium botulinum spores is increased from 4 to 40,000 per gram.

There appears to be a difference in radiation sensitivity between the spores of Putrefactive anaerobe NCA 3679 and Clostridium botulinum 62A when irradiated in canned meat.

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TABLE 36

RADIATION STERILIZATION OF CANNED MEAT INOCULATED WITH SPORES OF PUTREFACTIVE ANEROBE NCA 3679-CONC. 40,000/GM

Sample	Dose (Rep) x 10^6	% Swells
Control not inoculated not irradiated	0	0
Control inoculated not irradiated	0	100
1	2.0	100
2	2.3	0
3	3.0	0
4	3.5	0
5	4.0	0
6	4.5	0
7	5	0

TABLE 37

RADIATION STERILIZATION OF CANNED MEAT INOCULATED WITH SPORES OF CL. BOTULINUM 62A CONCENTRATION ≠ 40,000/GM MEAT

Sample	Dose (Rep) x 10^6	% Swells
Control not inoculated not irradiated	0	0
Control inoculated not irradiated	0	100
1	2.0	100
2	2.6	100
3	3.0	50
4	3.5	50
5	3.9	0
6	4.5	0
7	5.4	0

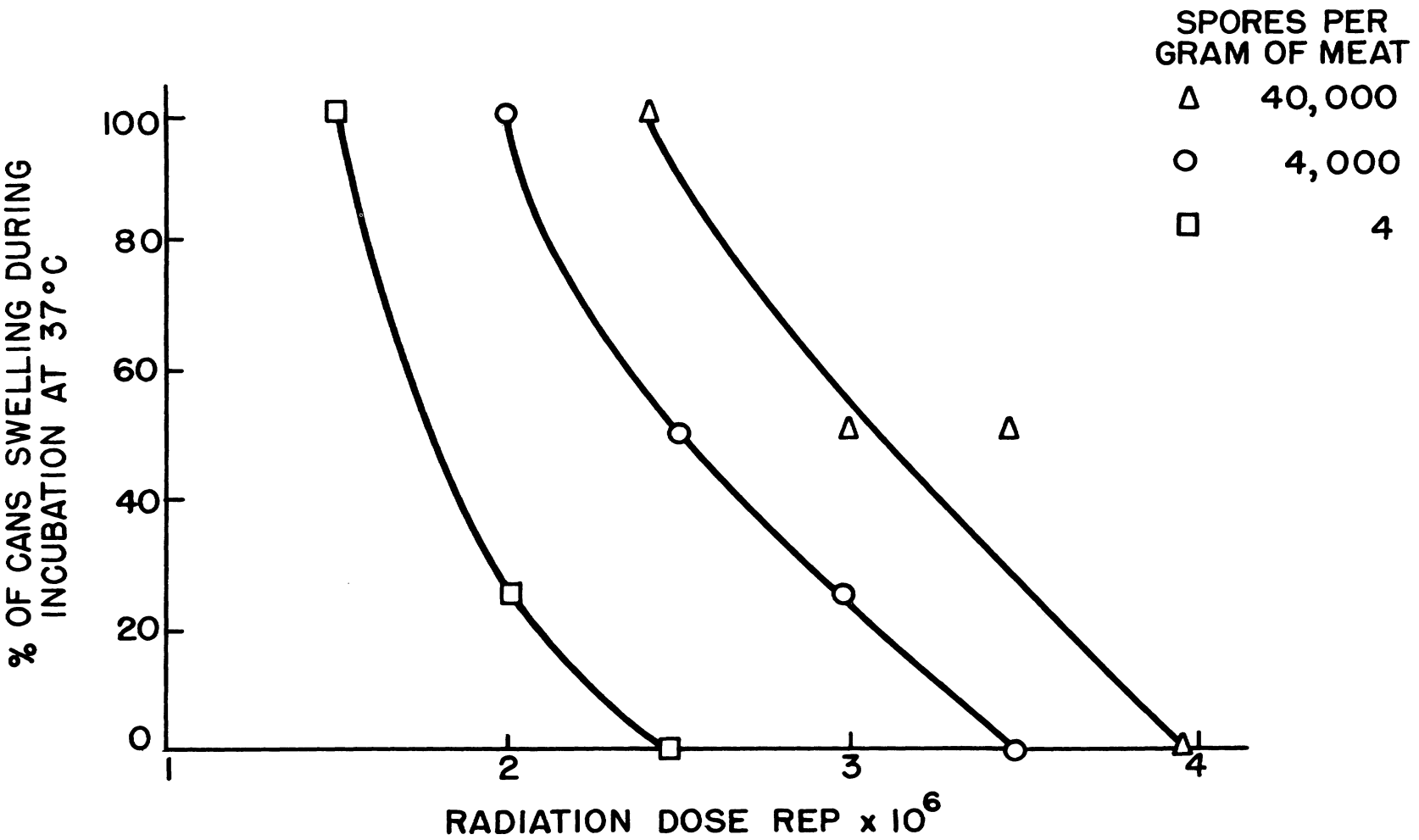

Fig. 81. Effect of Spore Concentration on Radiation Dose Required to Prevent Can Swelling during Incubation at 37°C.

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2. Aerobic Growth of Microorganisms in Ground Beef at 4°C after Exposure to Sublethal Dosages of Gamma Radiation

a. Introduction. Since microorganisms play a major role in the spoilage of meat and meat products, the storage life at refrigerator temperatures should be increased by keeping the microbial flora of the meat at a minimum. Rigid sanitation practices by meat packers and meat processing plants have done much to increase the storage life of meats; but the subsequent exposure of meat to air and contaminated surfaces makes the control of the microbial population a difficult problem.

Radiation dosages needed to inactivate vegetative forms of microorganisms are much lower than those required for bacterial spores. Decreasing the vegetative microbial flora sufficiently to prolong the storage life of meat at refrigerator temperatures should require a much smaller dose of gamma radiation than is required for sterilization and destruction of spores.

The following study was initiated to determine what effect low dosages of gamma radiation would have on the aerobic microbial population of ground beef and what relation this effect would have to the storage life of the meat at 4°C.

b. Experimental Studies. For this experiment 25 grams of fresh lean ground round steak were transferred to 250-ml Erlenmeyer flasks containing sterile glass beads and sand. Meat was handled aseptically in all operations. Prior to irradiation the meat was inoculated with 1 ml of a suspension of a psychrophilic gram-positive bacillus originally isolated from spoiled ground beef. This organism grew readily at a temperature of 4°C. The concentration of organisms in the inoculation was 8×10^5 per ml. An uninoculated control sample was kept to establish the normal population of the meat.

The inoculated samples were placed at various distances from the source in the radiation cave and irradiated simultaneously. Five samples each were irradiated with a total dosage of 20,000, 40,000, 80,000, and 160,000 rep. Samples were rotated 180° after receiving half the total dosage in order to insure uniform dosage. The total time for irradiation was 2 hours and the temperature of the cave was 7°C. Controls, both inoculated and uninoculated, were held at 4°C during irradiation. Immediately after irradiation counts were made on one sample each of the untreated control and the inoculated control and on one sample from each level of radiation dosage.

For purposes of counting 100 ml of sterile cold physiological saline containing 0.1% gelatin and soluble starch was added aseptically to each flask. The flasks were then shaken rapidly on a New Brunswich shaker (190 1-inch-diameter oscillations/minute) for 30 minutes. By this method samples were homogenized quite rapidly except for the fat present. Decimal dilutions were then made from this sample in physiological saline containing 0.1% gelatin. Beef extract peptone agar was used for plating. The plates were counted by means of a Quebec counter after incubation at room temperature (22-25°C) for 3 days.

All other samples were stored at 4°C. On the days indicated in the tables, one flask of each sample was removed and plated as outlined above.

c. Results. Table 38 shows the aerobic microbial counts on the meat immediately after irradiation. No differences in counts are apparent up to a dosage of 40,000 rep. Table 39 shows counts per gram of meat after subsequent storage at 4°C. The data are plotted on semilogarithmic paper in Fig. 82 and on plain coordinate paper in Fig. 83 as total counts per gram.

TABLE 38

EFFECT OF GAMMA RADIATION
ON AEROBIC MICROBIAL FLORA OF GROUND BEEF
INOCULATED WITH 3.2×10^4 PSYCHROPHILIC BACTERIA PER GRAM

Sample	Microorganisms Per Gram	% Survivors
Control	8.0×10^4	100.0
20,000 rep	1.4×10^5	100.0
40,000 rep	6.8×10^4	85.0
80,000 rep	7.5×10^3	9.0
160,000 rep	9.5×10^3	12.0

No detectable differences in either color or odor were noted in the samples immediately after irradiation. After five days of storage, both the inoculated and uninoculated controls had a pronounced putrid odor. Initiation of spoilage in the sample receiving a radiation dose of 20,000 rep was indicated

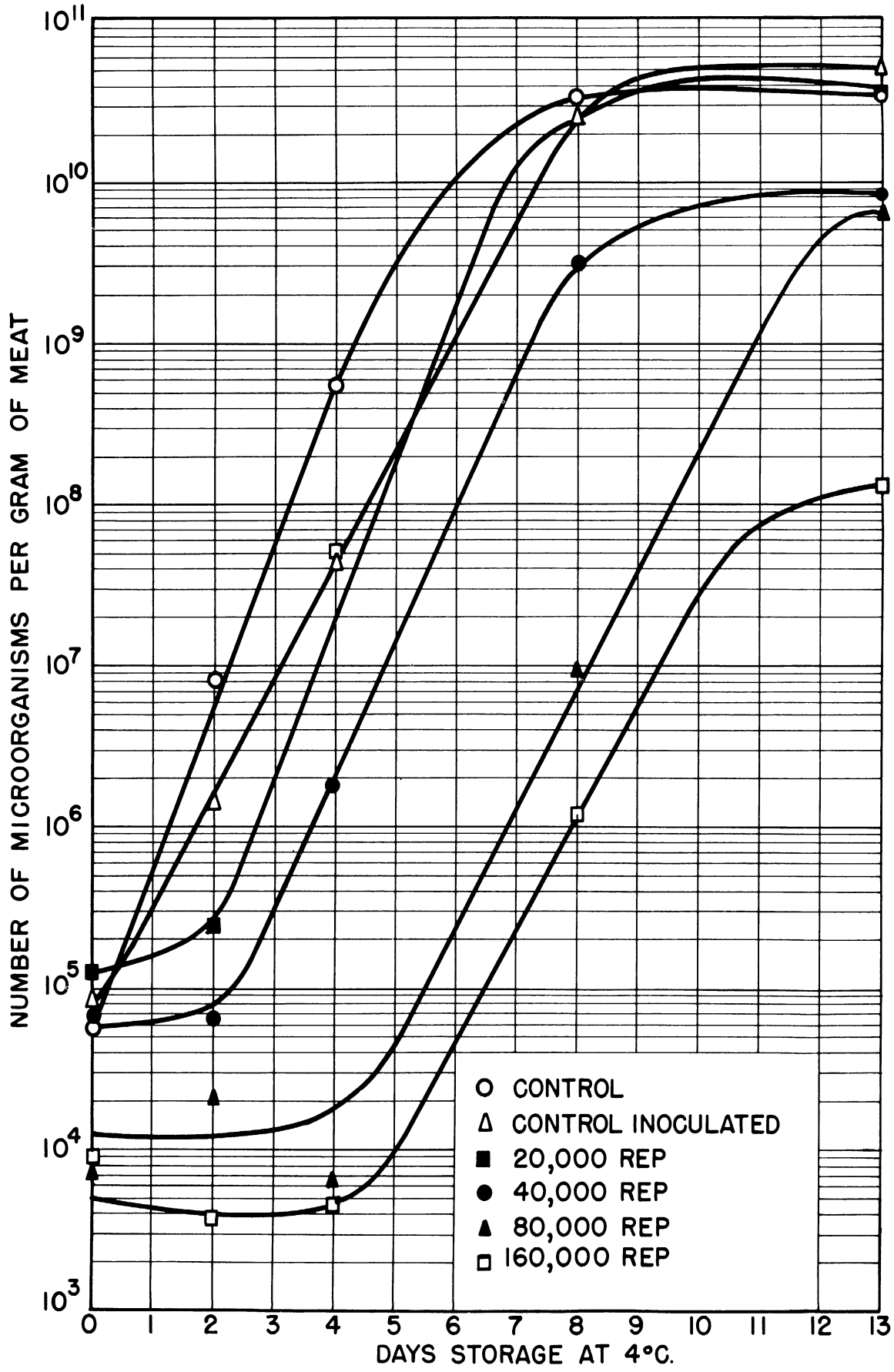

Fig. 82. Aerobic Count of Microorganisms in Ground Beef Stored at 4°C. After Receiving Low Dosage Gamma-Radiation.

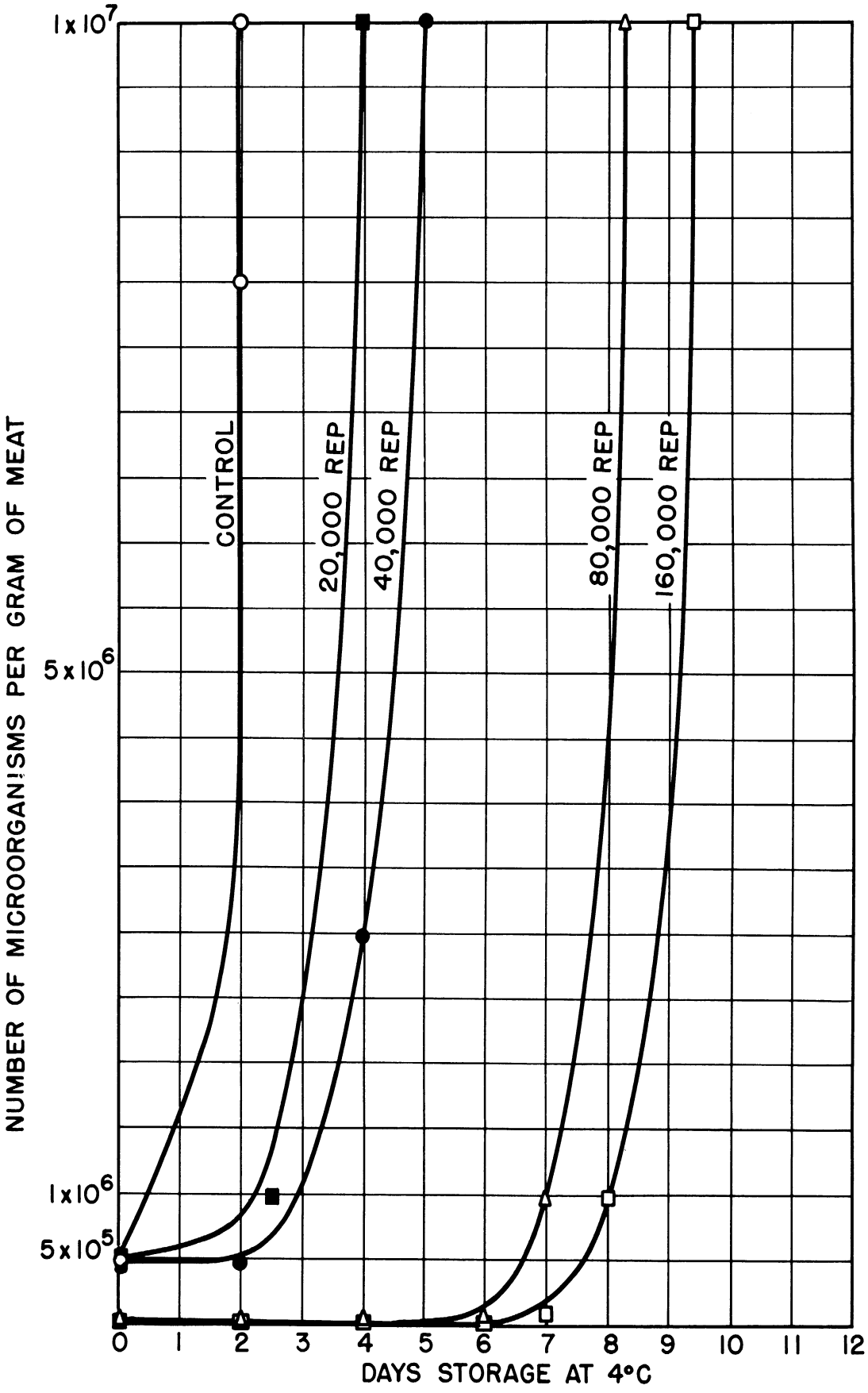

Fig. 83. Aerobic Count of Microorganisms in Ground Beef Stored at 4°C. After Receiving Low Dosage Gamma-Radiation.

by a slight off odor, whereas none of the other irradiated samples had an off odor. After 13 days of storage all the irradiated samples except that which received a dose of only 20,000 rep were still free of off odors and there was no indication of spoilage (Table 40).

The irradiated samples developed a brown color during storage. This color change was apparent after 4 days and was more pronounced in the samples receiving 80,000 and 160,000 rep; the samples receiving 40,000 rep were discolored but to a lesser extent. A color change similar to that occurring between 40,000 and 80,000 rep was also observed in the samples stored less than 4 days after the flasks were left at room temperature subsequent to plating of samples. After 13 days of storage the controls were deep red and slimy in appearance. The 20,000 rep samples were also red, the 40,000 rep samples were reddish brown, and the 80,000- and 160,000-rep samples were brown.

TABLE 39

EFFECT OF SUBLETHAL DOSES OF GAMMA RADIATION ON THE AEROBIC MICROFLORA OF GROUND BEEF AFTER STORAGE AT 4°C

Sample	Number of Microorganisms per Gram of Meat after Indicated Number of Days of Storage at 4°C				
	0	2	4	8	13
Control	5.5×10^{4}	8.0×10^{6}	5.5×10^{8}	3.3×10^{10}	2.8×10^{10}
Control Inoculated	8.0×10^{4}	1.5×10^{6}	4.3×10^{7}	2.5×10^{10}	4.5×10^{10}
20,000 rep	1.3×10^{5}	2.2×10^{5}	5.0×10^{7}	----	2.8×10^{10}
40,000 rep	6.8×10^{4}	6.5×10^{4}	1.8×10^{6}	3.0×10^{9}	8.3×10^{9}
80,000 rep	7.5×10^{3}	2.0×10^{4}	6.3×10^{3}	9.3×10^{6}	6.5×10^{9}
160,000 rep	9.5×10^{3}	2.8×10^{3}	4.3×10^{3}	1.2×10^{6}	1.3×10^{8}

d. Discussion. A time lag occurs in the growth of microorganisms with increasing dosages of irradiation, suggesting that an initial decrease in population occurred. The action of the medium (meat) on microorganisms should also be considered since radiation may alter its ability to support microbiological growth. A complete correlation between number of organisms and degree of

TABLE 40

CHARACTERISTICS OF GROUND BEEF STORED AT 4°C FOR 13 DAYS AFTER IRRADIATION

Sample	Color	Odor
Untreated Control	Red	Putrid
Inoculated Control	Red	Putrid
20,000 rep	Red	Putrid
40,000 rep	Red-brown	Good
80,000 rep	Brown	Good
160,000 rep	Brown	Good

spoilage cannot be made since, with the probable exception of the 160,000 rep sample, the population density in all the samples reached that of the controls when the controls had developed a putrid smell.

e. Conclusions. On the basis of the experiment reported here, it may tentatively be concluded that irradiation dosages of about 40,000 rep prevent the development of an off odor of putrid meat in ground beef stored for 13 days at 4°C, but an off odor of putrid meat and a slimy growth develop in samples receiving a radiation dosage of approximately 20,000 rep or less under similar storage conditions.

E. STUDIES ON THE USE OF TISSUE CULTURE MEDIA IRRADIATED BY GAMMA RAYS FROM COBALT-60*

Personnel:

D. J. Merchant, Assistant Professor of Bacteriology; L. L. Kempe, Assistant Professor of Bacteriology, Medical School, and Assistant Professor of Chemical

*This study was initiated under Phoenix project 41 and is being continued at the present time as a new Phoenix project 74.

and Metallurgical Engineering, Engineering School; R. D., Stewart, Research Assistant; J. T., Graikoski, Research Assistant, Phoenix project 41.

1. Introduction

Tissue cultivation is proving to be an increasingly valuable tool for the investigation of a variety of problems in the biological and medical sciences. This is borne out by the publication entitled, A Bibliography of the Research in Tissue Culture, which presents some 15,000 references on studies employing culture techniques. These papers report work done in such varied fields as virology, cancer research, embryology, histology, and pathology.

Numerous attempts have been made to produce a synthetic medium for tissue-cell cultivation so that the complex biological substances such as blood serum, blood plasma, and embryo extracts need not be used. While progress has been made, no synthetic medium has yet been devised which can sustain a culture indefinitely. Most tissue-culture workers feel that ultimately a suitable synthetic medium will be produced, but until that time, tissue culture must depend on naturally produced biological constituents for media.

The use of these natural constituents in media is generally satisfactory, but their preparation for use is far from ideal. There is no satisfactory method at present for the sterilization of blood plasma, blood serum, or embryo extract; hence tissue-culture media must still be prepared by tedius, time-consuming aseptic methods. Furthermore, even occasional contamination may represent a considerable loss of time and money.

2. Feasibility of Gamma Ray Sterilization of Tissue-Culture Media

Preliminary studies were designed to test the feasibility of using tissue-culture media sterilized by gamma radiation. Several factors required particular attention: first, chain reactions are known to be induced by radiation; second, toxic products such as organic peroxides may be formed; third, new substances capable of stimulating abnormal growth may be formed; and fourth, growth essentials in the irradiated material could be destroyed.

The use of gamma radiation for sterilization of a variety of materials has been the subject of intensive investigation. The data from the work of Lawrence, Brownell, and Graikoski of the University of Michigan* indicate that the dosage required to destory the sporeformer Bacillus subtilis is nearly ten times as great as that which will destroy the nonsporeformers. Since spore-forming bacteria are seldom encountered in tissue-culture media, the common

*Nucleonics, 11 No. 1, 9-11 (1953).

contaminant Staphylococcus aureus was selected for the preliminary study. When this organism was suspended in saline solution and exposed to gamma radiation, the effect was found to be almost identical to that previously shown for Escherichia coli.

3. Experimental Studies

The problem consists of determining the radiation dosage necessary to effect destruction of Staphylococcus aureus various medium components, and evaluating alterations of these materials which might result from such treatment.

Plasma is primarily used in tissue-culture work to provide mechanical support or anchorage for tissue explants for which its usefulness depends on its clotting or coagulation time. Secondarily, plasma serves as a source of nutriment. To determine whether alterations in these properties would occur as a result of irradiation, 5-ml samples both in the liquid and dehydrated states were subjected to gamma radiation.

The dehydrated plasma samples irradiated at dosages ranging from 250,000 to 1,000,000 rep showed far less alteration in clotting time than those samples irradiated in the aqueous state. One month later, clotting times were rechecked, since a chain reaction set up at the time of irradiation could conceivably have altered the plasma clotting properties. No such change was noted.

One series of plasma samples was contaminated with 7.5×10^8 Staphylococcus aureus per ml and subjected to gamma radiation. The Staphylococci were destroyed between 100,000 and 150,000 rep in both the dehydrated and aqueous plasma samples.

To determine whether the irradiated plasma might be either toxic or stimulatory to tissue growth, explants from various chick embryonic tissues were embedded in plasma clots and incubated at 37°C for 48 hours. These cultures were nourished by a fluid medium consisting of 20% embryo extract, 40% horse serum, and 40% Hank's balanced salt solution. The percentage increase in area of the outgrowth in 48 hours was determined by measurements with an ocular micrometer, a procedure which fails to take into consideration height increase of the explant. The mitotic and amitotic figures are another indication of growth characteristics. The data obtained indicate that no toxic substances were present in high enough concentration to inhibit growth for the time interval studied. The data available at present are too limited to determine if growth was stimulated by the irradiated plasma.

Embryo extract is the major source of growth-promoting factors for cell cultivation. In addition it is used to induce plasma clot formation. To ascertain the effect of gamma rays on these properties, 5-ml samples of embryo

extract wer subjected to dosages of gamma radiation ranging from 250,000 to 1,000,000 rep. The results indicate that the incorporation of irradiated embryo extract in tissue-culture medium does not appear to alter the growth-promoting properties of the medium. No difference could be detected between the irradiated extract and its control in the induction of clot formation. Staphylococci of a concentration of 7.5×10^8 per ml were destroyed by 150,000 rep.

The next experiment undertaken was the cultivation of chick embryonic tissue in a medium composed of chicken plasma, chick embryo extract, and horse serum, each of which had been exposed to 1,000,000 rep of gamma radiation. The embryonic heart explants cultured in the irradiated medium showed outgrowth equal to that of the controls. Moreover, no significant change in the mitotic and amitotic figures was observed.

4. Conclusions

The observations presented here suggest that it may be possible to use tissue-culture media exposed to gamma radiation for cell cultivation. It has been established that nonspore-forming organisms present in tissue-culture medium are destroyed by relatively low dosages of gamma radiation and that 1,000,000 rep is not detrimental to the growth-promoting and tissue-sustaining properties of tissue-culture media as measured over relatively short time intervals.

PART V. COOPERATIVE RESEARCH WITH INDUSTRY

A. EFFECT OF GAMMA RADIATION ON GLASS FIBERS

In accordance with arrangements made by Mr. Burton M. Palmer of the Owens-Corning Fiberglas Corporation, glass fibers were irradiated in the Fission Products Laboratory; two tests were made by J. V. Michener of the Physics Research Laboratory of Owens-Corning Fiberglas Corporation to determine the effect of gamma radiation on (1) the tensile strength and (2) the modulus of elasticity of glass fibers. No part of this report is to be reproduced without the permission of J. W. Michener, the author.

1. Tensile Strength of Gamma-Irradiated Glass Fibers

a. Purpose. It was desired to determine the effect of high-intensity gamma-ray irradiation on the strength of two commercial textile glasses, A and B.

b. Method. Four tubes of fiber glass were prepared. One tube of each glass was irradiated for approximately 20 hours by the 10-kilocurie cobalt-60 gamma-ray source. The samples received a total radiation dose of 5.9×10^5 rep and were returned to Newark, where tensile strength measurements were made.

c. Results. The results are given below:

	Nonirradiated	Irradiated
Sample A	$478 \pm 42.1 \times 10^3$ psi	$421 \pm 48.6 \times 10^3$ psi
Sample B	$388 \pm 41.7 \times 10^3$ psi	$357 \pm 44.6 \times 10^3$ psi

These results indicate a statistically significant change on the 1-percent level for A and on the 12-percent level for B.

d. Interpretation. Theory, together with earlier experiments on alkali halides and metals, indicates that gamma radiation changes only the electronic energy levels and does not cause any atomic rearrangement. This is borne out by the effect on glass in that Young's modulus is unchanged by an irradiation 10 times greater than that received by these samples; hence the difference in

strength is probably due to surface changes, possibly caused by the higher humidity in the chamber where the irradiation took place. It is also possible that the surface reaction with moisture was accelerated by the gamma irradiation.

e. Conclusions. Under the conditions of this experiment the irradiated fibers were significantly weaker. It is noted that for 20 hours the irradiated fibers were in a more humid atmosphere; but the possibility of increased surface damage to the fiber due to the irradiation cannot be ruled out. If an application for Fiberglas should be found where the fibers will be subjected to high-intensity gamma irradiation, a further test should be conducted before a recommendation is made. Note that these fibers were not subjected to neutron irradiation; therefore, these data are not valid for application to fiber in a pile where high-intensity gamma and neutron irradiation are present. It is expected that neutron irradiation will definitely weaken fibers.

2. Young's Modulus of Gamma-Irradiated Glass Fibers

a. Purpose. It was desired to determine the effect of high-intensity gamma radiation on the Young's modulus of glass.

b. Method. Eight samples each of two commercial glasses, B and C, were used. These samples were cylindrical rods about 1/4 inch in diameter by 3 inches in length. The resonant frequency of each rod was measured using a matched quartz resonator in a series circuit. After the initial measurement the rods received the following gamma irradiation from the cobalt-60 source:

2 rods of each glass received a dosage of 5.9×10^6 rep.

2 rods of each glass received a dosage of 5.9×10^5 rep.

2 rods of each glass received a dosage of 5.9×10^4 rep.

2 rods received no irradiation, and were used as controls.

After the irradiation the resonant frequency of each rod was measured again.

c. Results. None of the samples showed any significant change in Young's modulus. The highest dosage corresponds to a very high intensity of x-ray irradiation for 5000 hours and was sufficient to darken the glass considerably.

d. Interpretation. As is to be expected, the gamma irradiation does not cause any measurable structural change in glass. However, it does change the electronic energy-level system, causing a noticeable coloration of the glass.

e. Conclusions. The Young's modulus of glass is not appreciably affected by large amounts of gamma irradiation. The glass does undergo a coloration, however.

It should be noted that these data are not sufficient to indicate that glass may be used in a pile, since it would also receive high-intensity neutron irradiation. Neutron irradiation would be expected to cause serious structural changes in glass.

B. PRELIMINARY STUDIES ON THE USE OF GAMMA RADIATION TO PASTEURIZE BEER

Messrs. N. F. Richey and K. B. Zint of the Reynolds Metals Company arranged for the gamma irradiation of bottled, canned, and keg beer to investigate the use of gamma radiation for the pasteurization of beer. The Fission Products Laboratory provided the gamma irradiation through the use of the 10-kilocurie source. All the beer was analyzed at the Goebel Brewery by Messrs. H. H. Noffze and E. P. Pomaville of the Goebel Brewing Company, and no part of this report is to be reproduced without their permission.

In the first irradiation tests a spectrum of radiation doses was used to survey the effects of radiation on a bottled draft (unpasteurized) beer. The doses used are listed in column 2 of Table 41 and the observations made on the irradiated samples are given in the subsequent columns. Considerable bleaching occurred, as shown by the results listed in the third column. Samples receiving doses of 10,000 rep or more had off odors and off tastes.

In the next series of tests the radiation doses were limited to 10,000 rep in view of the off flavors developed at the higher doses in the first series of tests. The radiation doses used are listed in the first columns of Tables 42 and 43, and the observations and results are given in the subsequent columns of these tables.

A final series of tests was made using cans and an aluminum half-barrel as well as the bottles. Figure 84 is a photograph showing the cans, bottles, and half-barrel used in these tests. The samples were located about 4 feet from the source so as to reduce the radiation field sufficiently for the low doses used. The results of these tests are given in Table 44.

The conclusion made by the Goebel personnel is "It appears, after this preliminary investigation of the use of cobalt-60 irradiation as a substitute for pasteurization, that in dosages which will not harm the beer in odor, clarity, color, and taste, brewery organisms are not inactivated."

TABLE 41

THE EFFECT OF GAMMA RADIATION ON pH, COLOR, ODOR, TASTE, AND BACTERIAL COUNT IN DRAFT BEER

Code	Radiation Dose, rep	pH	Color	Bacterial Count	Immediate Comment	After One Week
L	0 (control)	4.33	2.6° Lov.	3 yeasts	normal	normal
	0 (pasteurized control)	4.33	2.6	0	normal	normal
	5,000	4.3	2.4	1 yeast	taste, odor normal	clear, odor and taste normal
	10,000	4.37	2.3	1 rod	taste, odor off a little	clear, odor and taste off
A	20,000	4.36	2.3	0	off taste	off taste
B	40,000	4.33	2.0	2 yeasts	odor, taste off	slightly hazy, odor off
C	80,000	4.33	1.4	0	bad odor, taste off	odor, taste off
D	160,000	4.35	1.0	0	bad odor	odor, taste off
E	200,000	4.33	1.0	0	odor faint	odor off
F	300,000	4.33	0.9	0	skunky	skunky
G	400,000	4.32	0.6	0	skunky	skunky
H	500,000	4.32	0.6	0	skunky	skunky
I	600,000	4.32	0.6	0	skunky	skunky
J	1,000,000	4.32	0.8	0	skunky	skunky
K	2,000,000	4.28	hazy	0	skunky	skunky

TABLE 42

BACTERIAL COUNT OF IRRADIATED BEER IN BOTTLES FROM SECOND TESTS

Radiation Dose, rep	Within 24 Hrs	After One Week	After Two Weeks	After Three Weeks
0 (control)	0	0	138 yeasts	4000 plus yeasts
0 (pasteurized beer)	0	0	0	0
1000	0	0	160 yeasts	3 rods 4000 plus yeasts
2000	1 yeast	0	88 yeasts	4000 plus yeasts
3000	1 rod 3 yeasts	0	42 yeasts	4000 plus yeasts
4000	0	0	36 yeasts	4000 plus yeasts
5000	0	0	64 yeasts	4000 plus yeasts
6000	0	0	44 yeasts	4000 plus yeasts
7000	3 yeasts	0	30 yeasts	4000 plus yeasts
8000	2 yeasts	0	42 yeasts	4000 plus yeasts
9000	0	0	120 yeasts	4000 plus yeasts
10,000	0	0	110 yeasts	4000 plus yeasts

Beer was innoculated into Bacto W. L. Nutrient Agar and incubated at 25°C for 3 days; these tests were conducted between January 27, 1954, and February 22, 1954. Beer stored at 22°C 1 ml samples plated.

TABLE 43

GOEBEL BREWERY COMMENTS ON IRRADIATED BEER IN BOTTLES IN SECOND TESTS

Radiation Dose, rep	pH	Color	Within 24 Hours			After One Week			After Two Weeks			After Three Weeks		
			Clarity	Odor	Taste	Clarity	Odor	Taste	Clarity	Odor	Taste	Clarity	Odor	Taste
0 (control)	4.37	2.7	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
0 (pasteurized beer)	4.31	2.7	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1000	4.37	2.6	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2000	4.37	2.6	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Off
3000	4.37	2.6	Normal	Normal	Off	Normal	Normal	Off	Normal	Normal	Off	Normal	Normal	Off
4000	4.37	2.6	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Off	Off
5000	4.37	2.8	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Off	Off
6000	4.37	2.5	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Off	Off
7000	4.37	2.5	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Off	Off
8000	4.37	2.5	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Off	Normal	Off
9000	4.37	2.5	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Normal	Off
10,000	4.37	2.5	Normal	Normal	Off	Normal	Normal	Off	Normal	Off	Off	Normal	Normal	Off

TABLE 44

RESULTS OF TESTS ON BEER IRRADIATED IN CANS, BOTTLES, AND ALUMINUM HALF-BARRELS
BEER IRRADIATED (WITH 5000 REP) ON FEBRUARY 10, 1954

Brown Bottle -	Plated: 1 rod and 7 yeasts per ml of beer
	Taste: Normal Odor: Off
White Bottle -	(Was Pasteurized Beer): Beer was skunky
	1 Bottle - 300,000 reps: bottle turned to smoky color
	1 Bottle - 150,000 reps: bottle turned to smoky color
Canned Beer -	Plated: 1 rod and 4 yeasts per ml of beer
	Taste: Very definitely off
	Odor: Very definitely off
	Clarity: Clear
Aluminum Half-Barrel -	Control Plated: 14 yeasts per ml of beer
	Irradiated: (stored at 35°F and plated 5 days after·1120 yeasts per ml of beer)
	Odor and taste of irradiated beer was definitely off.

Fig. 84. Photograph Showing Arrangement of Beer in Cans, Bottles, and Half-Barrel with Relation to Gamma Source.

C. PRELIMINARY STUDIES ON THE USE OF GAMMA RADIATION TO PRESERVE CUT FLOWERS

Mr. Richard M. Baltic of the Shaw-Baltic Florists in Cleveland, Ohio, arranged for the irradiation of cut roses and gardenias at the Fission Products Laboratory. The first treatment yielded the following results:

Dose	Room Temperature	Refrigerated
1×10^3	package broken - no test	molded after 30 days
	package broken - no test	perfect after 30 days
1×10^4	molded after 8 days	perfect after 30 days
1×10^5	package broken - no test	perfect after 30 days

1×10^6	flowers turned brown	brown
2×10^6	flowers turned brown	brown

The failure of the packaging materials supplied by the florist limited the value of the first experiment.

In subsequent experiments, the flowers were heat-sealed in polyethylene bags. The second treatment yielded the following results:

Dose	Room Temperature	Refrigerated
0	molded after 7 days	perfect after 30 days
1×10^3	molded after 12 days	perfect after 30 days
1×10^4	molded after 12 days	perfect after 30 days
1×10^5	molded after 14 days	perfect after 30 days

The results of the second experiment indicate beneficial effects of radiation and of polyethylene packaging on the storage qualities of cut roses.

Further experiments are proposed and the above-mentioned refrigerated samples are being kept under observation. Packaging material, dosage received, age of flowers at irradiation, temperature of storage, and type of flower are parameters to be studied in future experiments.

PART VI. SUBPROJECT M943-7, OPERATION OF THE FISSION PRODUCTS LABORATORY

Personnel:

L. E. Brownell, Director, and Associate Professor of Chemical and Metallurgical Engineering; J. V. Nehemias, Assistant Director and Health Physicist; S. Pedersen, Research Associate; D. E. Harmer, Chemist; and E. Ambo, Laboratory Assistant.

Advisors:

H. J. Gomberg, Associate Professor of Electrical Engineering; W. W. Meinke, Assistant Professor of Chemistry, L. Thomassen, Professor of Chemical and Metallurgical Engineering.

A. Routine Use of the High Level Gamma Source

The high level gamma (10-kilocurie) source has been in routine operation for one year. During this period, large-scale irradiations for the animal-feeding experiments and for studies on the sterilization and pasteurization of food and on the promotion of chemical reactions have been performed almost continuously. In addition, exploratory and service irradiations have been performed for a variety of Phoenix research groups and for some studies of commercial interest. These experiments are described in detail in the appropriate section of this report.

B. Safety

The proposed safe operating procedures described in previous reports have been maintained. The only case of significant radiation exposure recorded in connection with routine use of the source was the result of hanging a lab coat, complete with film badge, near the entrance to the cave. Even this one reading was substantially below the tolerance value.

Periodic resurveys of the radiation level in areas surrounding the laboratory verify that the radiation facility presents no hazard in these areas and that the radiation levels are decreasing with the half-life of cobalt-60 (5.3 years). A communication has been received from the Division of Biology and Medicine, AEC, regarding the problem of permissible levels in these areas.

"There appears to be little doubt that permissible exposure to general populations (that is, non-occupational exposure) will be limited to one-tenth of the permissible level for occupational exposure. The permissible level for occupational exposure is set at 300 milli-rem per week, so that the recommendation of the Radiation Policy Committee of the University of Michigan - 30 milli-rem per week to persons in adjoining areas - is valid.

A certain amount of "peaking" is permissible. That is, exposures might amount to as much as 90 mr in any one week provided the total averages out to 30 mr/week over a period of 3 months. Note also that these are actual exposures to persons in the area - not simply area exposures - and that absence of the inhabitants for appreciable periods during the day may be taken into account in performing the experiments."

C. Radiation Effect

Ordinary plate-glass mirrors used for remote observation of the source room have proven to be quite unsatisfactory. The mirror which was directly exposed to radiation darkened noticeably within a few days and after 10 days of normal operation became too dark to permit a clear view of the source. The mirror in this position received approximately 1000 rep/hr.

A mirror of nonbrowning glass was purchased from the Pittsburgh Plate Glass Company. It has been in routine service for several months with no significant darkening of the glass. For reasons of economy, ordinary plate glass is still used in the outer mirror, which is not in direct line of radiation from the source; this outer mirror has not darkened appreciably in over one year of operation.

A wooden table, on which samples were placed to be irradiated, was installed around the source and was in routine use for approximately one year. It was constructed of 5/8-inch plywood, and was exposed almost continuously to a radiation flux varying up to 150,000 rep/hr at the highest level. After one year of operation without incident, the table collapsed under a normal load. On inspection, the portions of the wood which had been exposed to the highest radiation flux were found to be dry and rather crumbly, as in "dry rot." The wood fibers had lost much of their tensile strength and the portion exposed to the highest flux (see left side of Fig. 85) failed with very little splintering. A new table of more rigorous construction was designed and is now in use. (Fig. 86)

D. Control of Water in Well for the High Level Gamma Source

Successful control of the pH, visibility, and inhibitor content of the water of the shielding well for the high level gamma source has been continued

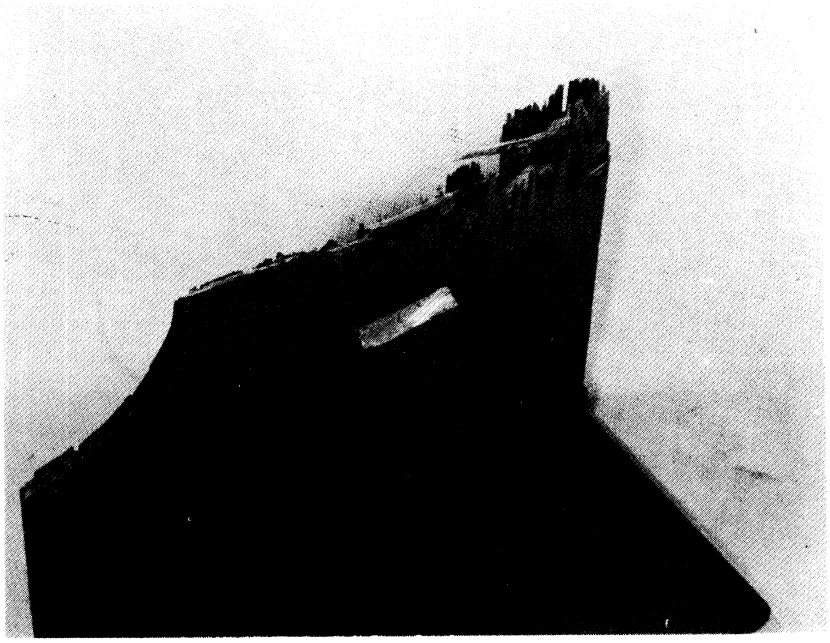

Fig. 85. The Effect of Gamma Radiation on Wooden Table Support Showing Definite Signs of Brittleness and Crumbling (Highest Radiation Exposure Occurred at Left of Section Shown).

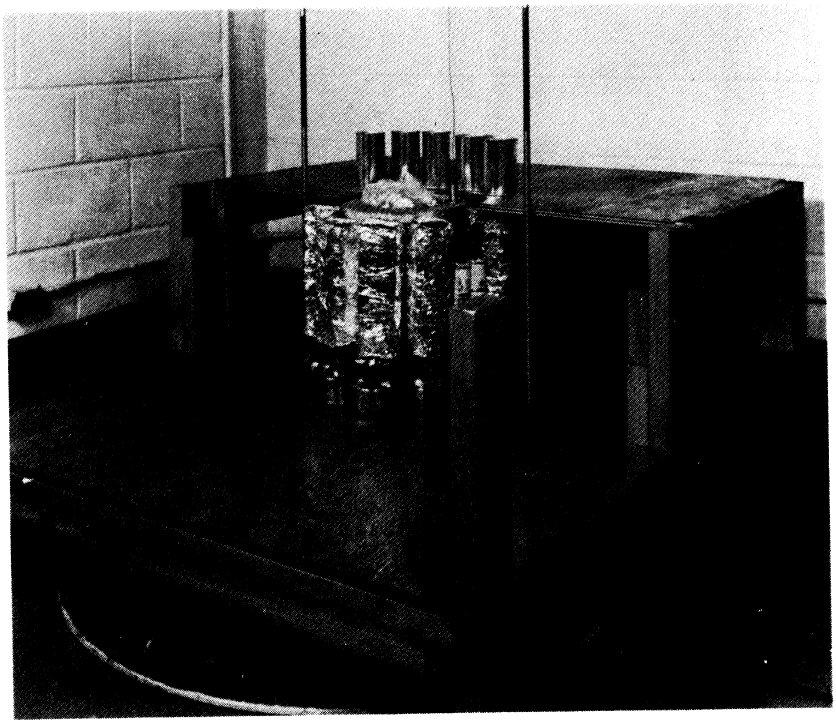

Fig. 86. New Wooden Support Table Shown with Packages of Rat Diet Wrapped in Aluminum Foil in Position around the High Level Gamma Source.

using the methods described in the Progress Report 5.[1] The small ion-exchange system shown in this previous report has been replaced by a larger, more convenient system. Figure 87 shows this system in its present form, while Fig. 88 is a diagram showing the functions of individual parts of the system. With this arrangement all control and treatment of the well water is carried out from the laboratory outside the radiation cave, thus minimizing interruptions of irradiation experiments in progress and eliminating the task of removing the steel well cover for frequent water sampling and treatments.

The water, which comes through copper tubing from the bottom of the well, is drawn into and pumped through the system by a centrifugal pump driven by a 1/4 hp motor. The water then passes through one of two filters. The smaller one of these is very fine and has a relatively small throughput, while the larger contains a coarser filter element and has a greater throughput. Ordinarily the two filters are used in parallel; but when it is especially desired to remove a very fine precipitate rapidly, all the water flow is put through the finer filter. From the filters the water flows to a series of valves which permit the stream to be run through the ion-exchange columns or directly back to the well.

An outlet is provided near the entrance to the filters, from which samples of untreated well water may be drawn for pH measurement, and another outlet is located at the exit of the ion exchange section to sample the treated water. A funnel into the discharge line allows buffers or inhibitors to be added as necessary. Storage bottles on the top of the rack contain solutions for regeneration of the resin beds. City water is available for backwashing of the resin beds and for replacement of well water lost by evaporation.

In its completed form, the water treatment system will include three columns of ion-exchange resin, although only one has been assembled at the present time (see Fig. 87). These three columns will consist of two cationic exchange beds ("Nalcite HCR") and one anionic exchange bed ("Nalcite SAR"). In ordinary usage, a portion of the well water will flow through a cationic exchange column continually. The alternate cationic column can then be regenerated at the operator's convenience. The anionic column will be used in conjunction with one of the cationic columns for complete removal of all ions from the well water, which may be necessary when extremely good visibility is required or when the total ionic concentration of the water becomes too high. Salts are continuously leached from the well walls, and high concentrations of these would encourage electrolytic corrosion even though pH is properly controlled. When completely installed,

[1] Brownell, L. E., et al. "Utilization of the Gross Fission Products, Progress Report 5 (COO-196), Univ. of Mich., Eng. Res. Inst., Proj. M943, September, 1953, pp. 170-172.

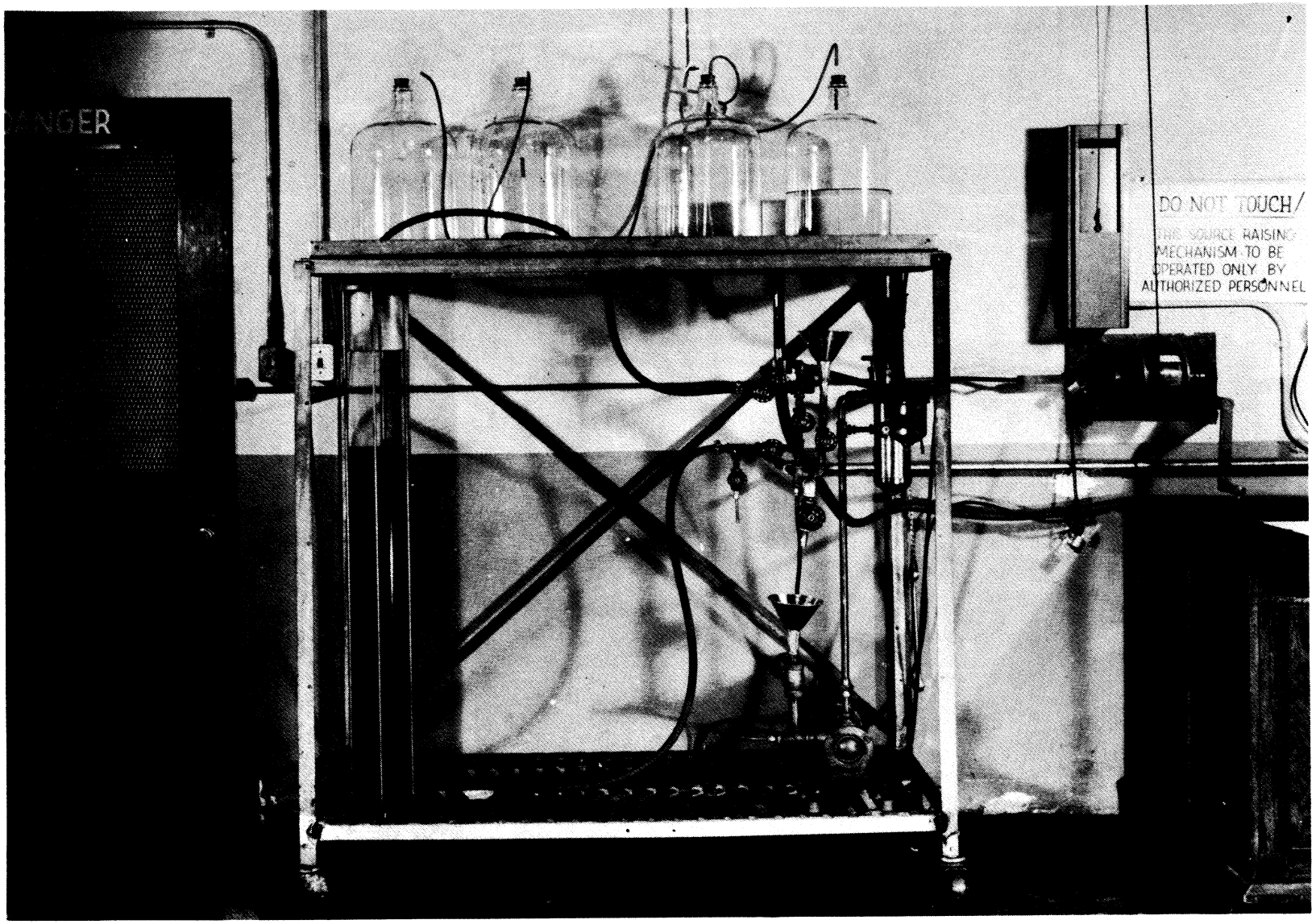

Fig. 87. Photograph of the Water Control System for the Well of the High Level Gamma Source.

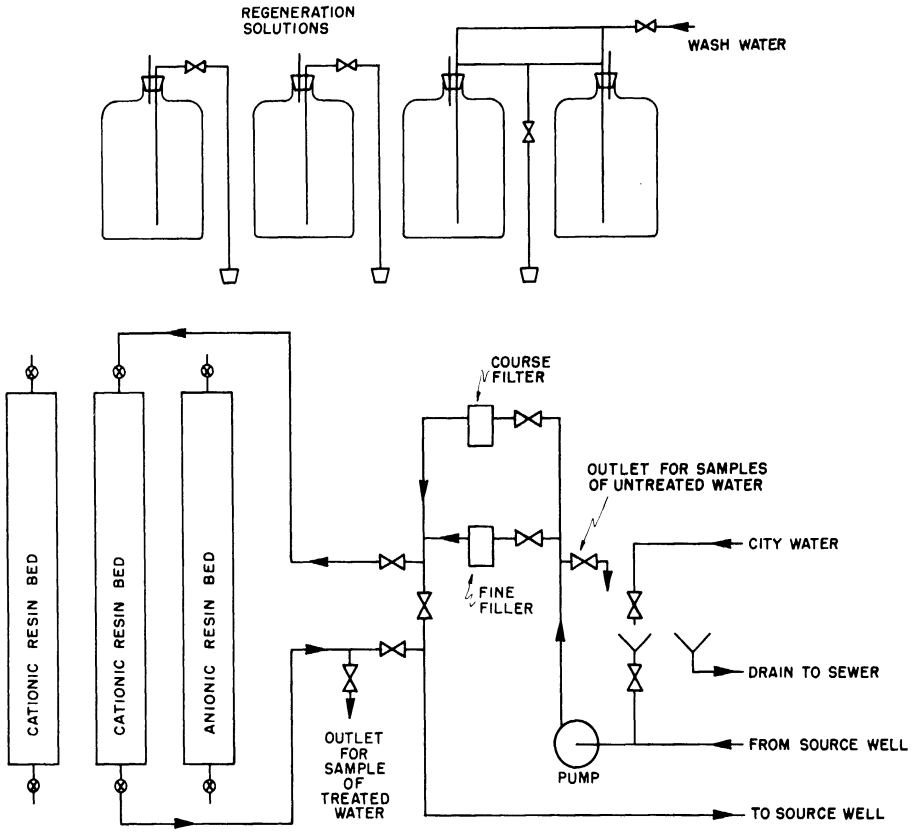

Fig. 88. Schematic Diagram of the Components of the Well Water Control System.

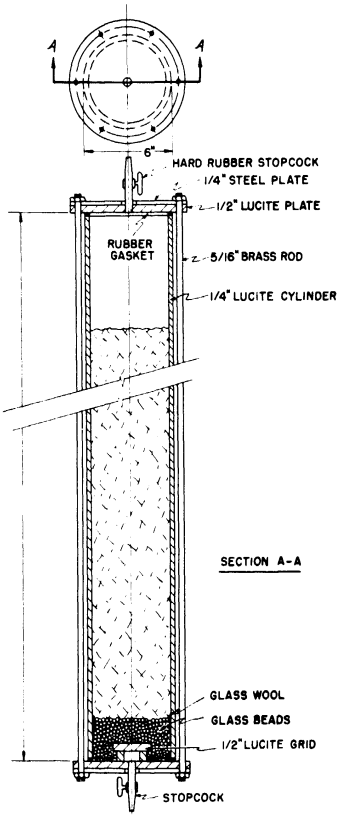

Fig. 89. Sectional View of Ion Exchange Column Used for Water Treatment of the Source Well.

this water control system should provide an efficient and effective means for maintaining well-water conditions which will minimize possible corrosion of the source rods.

The ion-exchange columns are constructed in the following manner: Cylindrical Lucite tubes 6 inch O.D. by 52 inches with 1/4-inch walls are fitted with flat 3/8-inch Lucite plates drilled and threaded in the center for 3/8-inch hard-rubber stopcocks. A 1/4-inch steel backing plate is used over the Lucite plates to provide the necessary mechanical strength. Rubber gaskets between the Lucite and caps and the column assure a water-tight seal. The whole assembly is held together by means of six brass rods which extend through the Lucite and steel plates. The ion-exchange resin is supported on glass beads which in turn are kept from entering the exit hole in the bottom plate by a small Lucite plate cut in the form of a grill. Figure 89 is a drawing of these columns.

E. Redisposition of Laboratory Space

Since the laboratory programs involving the use of radioactive isotopes have been discontinued, that portion of the laboratory has not been in use. The exigencies of the animal-feeding program, however, demand that maximum use be made of all available laboratory space, therefore, this portion of the laboratory is now in use as an auxiliary animal room.

APPENDIX

RANK ANALYSIS OF INCOMPLETE BLOCK DESIGNS BY A METHOD OF PAIRED COMPARISONS

The following is an outline of a method of analysis developed by Bradley and Terry.[31] When only two items are to be compared in a ranking experiment, a test of the null hypothesis of "no difference" between these items with respect to some characteristic may be based on the binomial distribution. An example is afforded by the modified triangle test employed in this laboratory (see Section 3b of this report). The method proposed by Bradley and Terry is a generalization of the binomial model and distribution.

Consider t treatments in an experiment involving paired comparisons, each with true ratings (or preferences), $\pi_1, \ldots, \pi_t$, on a particular subjective continuum throughout an experiment. For convenience, the further conditions that every $\pi_i = 0$ and

$$\sum \pi_i = 1$$

are imposed. Further, the probability that treatment i will be judged preferable to treatment j is

$$\frac{\pi_i}{\pi_i + \pi_j}$$

Let r_{ijk} = the rank of the i^{th} treatment in the k^{th} repetition of the block in which treatment i appears with treatment j. Note that $r_{ijk} = 3 - r_{jik}$. Estimates of $\pi_1, \ldots, \pi_t$ will be denoted by $p_1, \ldots, p_t$ respectively, and n will indicate the number of repetitions of the design; a repetition is defined to be a set of all pairs of treatments.

The probability of the observed rankings in the k^{th} repetition for the block in which treatments i and j are compared is given by

$$\left(\frac{\pi_i}{\pi_i + \pi_j}\right)^{2-r_{ijk}} \left(\frac{\pi_j}{\pi_i + \pi_j}\right)^{2-r_{jik}} = \frac{\pi_i^{2-r_{ijk}} \pi_j^{2-r_{jik}}}{(\pi_i + \pi_j)} .$$

For if the i^{th} treatment obtains top ranking, $r_{ijk} = 1$ and $r_{jik} = 2$, and the expression above becomes $\pi_i/(\pi_i + \pi_j)$; alternatively, $r_{ijk} = 2$, $r_{jik} = 1$, and the probability is $\pi_j/(\pi_i + \pi_j)$. Multiplying the appropriate expressions for

all comparisons within a repetition and for all n repetitions, we reach the likelihood function in the general form

$$L = \prod_i \pi_i^{2n(t-1)-\sum_{j\neq i}\sum_k r_{ijk}} \prod_{i<j} (\pi_i + \pi_j)^{-n}$$

To test the hypothesis "no π_i is assumed equal to any π_j $(i \neq j)$" against the hypothesis "$\pi_i = \pi_j$ for all $i, j \leq t$" the test statistic becomes

$$B_1 = n \sum_{i<j} \log (p_i + p_j) - \sum_i \left[2n\,(t-1) - \sum_{j\neq i} \sum_{k=1}^{n} r_{ijk} \right] \log p_i \ .$$

The estimates, p_i, may be obtained from the following equations

$$\frac{a_i}{p_i} - n \sum_{j\neq i} (p_i + p_j)^{-1} = 0 \quad (i, j = 1, \ldots, t) \ ,$$

$$\sum_i p_i = 1 \ ,$$

$$a_i = 2n\,(t-1) - \sum_{j\neq i} \sum_{k=1}^{n} r_{ijk} \ .$$

It is possible to generate all combinations of treatment sums of ranks for any given number of treatments and repetitions of the paired comparison design. The probability of each such combination may be obtained under the null hypothesis of equality of true treatment ratings. If three items are compared in a single repetition, the possible sets of rank sums are 2, 3, 4 and 3, 3, 3. Each of the six permutations of the elements of the first set has a probability 1/8, while the probability of the second set is 2/8. The treatment sums of ranks for two repetitions and three treatments are obtained by adding 2, 3, 4 and 3, 3, 3 component-wise to each of the sets of sums of ranks consisting of all permutations of 2, 3, 4 and to 3, 3, 3. The probability of a given new permutation is obtained by multiplying the basic probabilities of the combination and the permutation used to produce the given permutation. The probability of a given new combination of rank sums is obtained by adding the probabilities obtained for each permutation of the elements of the combination. The procedure is presented in Table 45. The generation of treatment sums of ranks and probabilities for three treatments and two repetitions. For example, adding the probabilities corresponding to the various permutations of 5, 6, 7 the probability, 36/64, of the combination is obtained.

TABLE 45
THE PROBABILITIES OF RANK SUMS

Probabilities		1/8	1/8	1/8	1/8	1/8	1/8	2/8
	Rank Sums	2,3,4	2,4,3	3,2,4	3,4,2	4,2,3	4,3,2	3,3,3
6/8	2,3,4	4,6,8	4,7,7	5,5,8	5,7,6	6,5,7	6,6,6	5,6,7
2/8	3,3,3	5,6,7	5,7,6	6,5,7	6,7,5	7,5,6	7,6,5	6,6,6

Bradley and Terry have included tables which contain for each possible combination of rank sums the value of the likelihood ratio statistic, B_1, and the likelihood estimates of the true treatment ratings $p_1, \ldots, p_t$, together with probabilities, P, that B_1 will not be exceeded if the null hypothesis is true. P is obtained by accumulating the individual probabilities of the individual sets, beginning with small values of B_1 which are most discordant under the null hypothesis.

For a more detailed discussion of this test as well as application of this method to the testing of other more general hypotheses see (31).

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Kaydon Engineering Corporation	J. D. Fitzpatrick	1
Walter Kidde Nuclear Laboratory, Inc.	J. Silverman	1
Knolls Atomic Power Laboratory	L. Dorfman	1
John Labatt, Ltd.	W. F. Read	1
Lawler-Wilson		1
Libby, McNeill and Libby	G. W. Beach	1
Little, George A.		1
Low Temperature Research Station	R. S. Hannan	1
Manufacturing Chemists' Association, Inc.	D. W. Dresden	1
Massachusetts Institute of Technology	B. E. Proctor; S. A. Goldblith	2
Mathieson Chemical Corporation	C. G. Spatorico; J. B. O'Hara	2
Medical Nutrition Laboratory (U.S. Army)	H. F. Kraybill; T. E. Friedemann	2
Memorial Center for Cancer and Allied Diseases	J. S. Laughlin	1
Merck and Company, Inc.	H. B. Matthews	1
Michigan Department of Health	G. D. Cummings; H. D. Anderson	2
Michigan State College	K. Wilson	1
Minneapolis-Honeywell Regulator Company	W. E. Belcher, Jr.	1
Minute Maid Corporation	J. E. Melvin	1
Monsanto Chemical Company	L. Widdoes	1
Nash-Kelvinator Corporation	T. J. Ammel	1
National Cancer Institute	A. W. Pratt	1
National Dairy Research Laboratory, Inc.	F. W. Barber; F. J. Carleton	2
National Research Corporation	F. Maslan; J. Duffy	2
City of New York, Department of Health	G. M. Lacerre	1
New York University	S. Z. Lewin	1
North American Aviation, Inc.		1

Nuclear Development Association		1
Nucleonics	C. J. Mosbacher, Jr.	1
Oak Ridge National Laboratory	C. Hochanadel; A. F. Rupp; N. T. Bray	3
Office of Ordnance Research, U.S. Army	W. E. Wilson	1
Owens-Corning Fiberglass Corporation	B. M. Palmer, J. V. Michener	2
Pasteuray Corporation	H. W. Abshire	1
Pennsylvania Salt Manufacturing Company	Librarian	1
Philco Corporation	L. A. Staebler; H. W. Schaefer	2
Princeton Radiation Chemical Laboratory, Inc.	G. C. Akerlof	1
Purdue University	J. E. Christian	1
Office of the Quartermaster General		1
Quartermaster Food and Container Institute, U.S. Army	B. H. Morgan, R. Pomerantz K. T. Swartz; D. K. Tressler	4
Radiation Instrument Development Laboratory		1
Radio Corporation of America	R. G. Picard	1
The Rath Packing Company	G. Christianson	1
Reynolds Metal Company	N. F. Richey, K. B. Zint	2
Reaction Motors Inc.	R. L. Wehrli	1
Refrigerator Research Foundation	W. J. Hoover	1
Rensselaer Polytechnic Institute	L. G. Bassett	1
Rohm and Hass Company	L. C. Eagleton	1
Seeger Refrigerator Company	R. M. Henrickson	1
Shaw-Baltic Florists	R. M. Baltic	1
Shell Development Company	Chief Librarian	1
Southwest Research Institute	J. P. O'Meara	1
Stanford University, Microwave Laboratory	C. Susskind	1
Wm. J. Stange Company	R. E. Morse	1
Stirling and O'Brien, Inc.	D. B. Stirling	1

Surgeon General's Office, U.S. Army	T. Huber	1
Swedish Institute for Food Preservation Research	G. Borgstrom	1
Swift and Company	W. M. Urbain	1
Syracuse University	B. P. Burtt	1
Technical Enterprises, Inc.	A. Redniss	1
Tracerlab, Inc.	R. D. Zentner	1
The University (Leeds, England)	F. S. Dainton; H. P. Hood	2
Union Oil Company Research Center	J. E. Sherborne	1
United Shoe Machinery Corporation		1
U.S. Department of Health, Education and Welfare	J. D. Faulkner	1
U.S. Naval Supply Depot	J. A. Corrick	1
University of Chicago	L. S. Skaggs	1
University of Chicago, American Meat Institute Foundation	H. R. Kraybill	1
University of Chicago, Food Research Institute	G. M. Dack	1
University of Michigan Memorial-Phoenix Project	R. A. Sawyer; H. G Gomberg; G. G. Brown; H. B. Lewis; C. A. Lawrence; L. Kempe; H. C. Eckstein; S. A. Gould; L. M. Hobbs; D. J. Merchant	10
University of Minnesota	R. C. Jordan	1
University of Notre Dame	M. Burton	1
University of Notre Dame, Lobund Institute	T. D. Luckey	1
University of Southern California	M. D. Appleman	1
The Upjohn Company	O. R. Woods; G. C Bond	2
Victoreen Instrument Company	W. W. Managan	1
Wright Aeronautical Corporation	K. Campbell	1
Wright Air Development Center	O. P. Morgensen, Jr.	1
Yale University	R. H. Bretton	1
Yale University, Sloan Physics Institute	E. C. Pollard	1