THE INCIDENCE, NUTRITION AND SOME METABOLIC STUDIES OF A RADIATION RESISTANT MICROORGANISM

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INTRODUCTION

Although heat processing protects food against deterioration due to bacterial action, chemical changes may take place during and after heat processing which result in a considerable loss in quality of the product. It would be desirable to have a method of destroying microorganisms in food products which would not involve the application of heat and which might result in less change in the product - the ultimate is a sterile product in the fresh state.

Investigators in their search for new, improved methods of food preservation have paid special attention to the possible use of radiations of various frequencies ranging from the low-frequency electric current to the high-frequency gamma and X-rays (11, p. 144). Much of this work, aimed at obtaining a sterile fresh product, has not advanced beyond the experimental stage.

The ionizing electromagnetic and particle radiations have been receiving the most attention. These include alpha, beta, and gamma radiation from radioactive elements and X-rays, cathode rays and neutrons which sterilize with little or no rise in temperature. This type of food processing has been designated as "cold sterilization."

Ionizing radiation may occur in the form of high-energy particles, as the electrons in the form of beta rays, protons, neutrons and alpha particles; or in the form of electromagnetic waves, as X-rays, gamma rays and ultraviolet rays.

Neutrons cause radioactivity, and protons and alpha particles have little penetration, therefore these ionizing radiations are not suitable for use in food preservation. Ultraviolet rays, which are just beyond the blue of the visible light spectrum, have little penetrating power, (penetrating power is reduced even by clear water) and are suitable only for surface sterilization. X-rays are penetrating electromagnetic waves; gamma rays are similar but are usually emitted from by-products of atomic fission or other radiation materials--Cobalt 60 has been extensively used. Beta rays are streams of electrons emitted from a radioactive material, which are dependent on their energy for depth of penetration. Other beta rays are streams of electrons from the cathode of an evacuated tube--their speed usually accelerated by artificial means.

X-rays, gamma rays and beta rays are equally effective in sterilization for equal quantities of energy absorbed, but while X-rays and gamma rays have good penetration (the pentration decreases exponentially with

depth), beta rays have poor penetration. At present the use of X-rays in food preservation is limited owing to the low efficiency of their production. Most studies on "cold sterilization" of foods then deals with gamma rays.

HISTORICAL

Since the early 1940's there has been considerable interest in the possibilities of food sterilization by ionizing radiation. This research has been stimulated to a great extent by several advantages which this type of sterilization would offer over heat sterilization methods. Damage or destruction caused by heating the product would be eliminated in radiation sterilization because the dosage necessary for complete sterilization of a product rarely causes a temperature rise over 10°F. Packages used in heat sterilization must be constructed of material capable of withstanding high temperatures and pressures, whereas packaging used in radiation sterilization could be much simpler, the only necessity being that they maintain sterility of the processed material.

It is possible that radiation sterilization could be maintained on a production line basis, which is preferable to batch processes. It has been estimated that both space and labor requirements would be less with radiation sterilization than is necessary with heat sterilization. Studies seem to indicate, too, that the cost of radiation sterilization might compare favorably with that of heat sterilization for certain products (22, pp. 191-196).

Although there are definite advantages in subjecting foods to sterilizing radiation, studies of these irradiated foods show that there are definite problems or objections. Briefly, these are 1) certain chemical changes may be produced in foods by ionizing radiation; 2) certain side effects can occur as a result of irradiation which causes changes in the color, flavor and sometimes the texture of the food; 3) other problems concern the packaging of foods, the storage life of irradiation-treated food products, and the possible toxicity of cold-sterilized products.

These problems encountered in the radiation sterilization of food are not specific for a given type of irradiation, for there is great similarity in the ionizing effects of X-rays, electrons and radiation from various radioactive substances. Various types of ionizing irradiations have in the past been considered suitable for sterilizing materials, but recent research has been concentrated on three types of irradiation: X-rays, high voltage electron or beta rays, and radiation from fission waste products made up of alpha, beta, and gamma rays.

As stated in the introduction, X-rays, gamma rays and beta rays are equally effective in sterilization for equal quantities of energy absorbed; while gamma

and X-rays have good penetration of the product, beta rays have poor penetration.

The bactericidal efficacy of a given dose of radiation is dependent upon the following:

1. The kind and species of organism.
2. The number of organisms or spores originally present--the more organisms there are, the less effective is a given dose.
3. The composition of the food. Some constituents as proteins or reducing substances may be protective, while products of ionization may be harmful.
4. The presence or absence of free oxygen--the effect varies from no effect to sensitization.
5. The physical state of the food during irradiation --both moisture content and temperature affect different organisms in different ways.
6. The condition of the organisms--their age, temperature of growth and sporulation and their state--vegetative or spore.

Other factors to consider are: 1) bacterial spores are considerably more resistant than are vegetative cells; 2) in general, gram-negative bacteria are less resistant than are gram-positive bacteria; 3) yeasts and molds vary considerably in their sensitivity to radiation,

some approaching the resistance of some of the bacteria.

Minch in 1896 appears to have been the first to study the effect of X-rays on bacterial populations, especially in reference to bactericidal effects (14, p. 367). His results were essentially negative, as were those of other investigators during the next thirty years. This was probably due to the low intensities of the X-rays and the lack of specialized bacteriological techniques required.

In spite of negative or conflicting results it had been convincingly shown that ionizing radiations do have a marked bactericidal effect. Green (1904) using semi-quantitative bacteriological methods studied the bactericidal effect of the X-rays emitted from radium on 23 species of bacteria including five spore formers. All species were killed and the spore formers were found definitely more resistant than the vegetative forms. Chambers and Russ (1912) using distilled water suspensions of Micrococcus pyogenes var. aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus anthracis, found a decided bactericidal effect for all species, the anthrax spores being the most resistant to radiation. Plate counts for the quantitative estimation of the surviving organisms of an irradiated suspension of M. pyogenes var aureus were made with results plotted

semi-logarithmically. This is the first known exponential survival curve for bacteria subjected to radiation. Some investigators, Holweck (1929) and Lacassagne (1929), however, demonstrated sigmoidal curves in the presence of heavy metal ions, the results possibly being due to the short wave lengths of secondary radiation thought to be the main cause of inactivation.

Stapleton (1952) made the observations that the form of the survival curve is dependent on the stage of the growth cycle of the culture. E. coli B/r cells from fully grown cultures in the stationary phase yield exponential survival curves; cells in the lag phase yield sigmoidal curves, the deviation from exponential killing increasing to a maximum at the end of the lag phase. This agrees with the "target theory" favored by Lea (1947).

There are several factors influencing the sensitivity of microorganisms to ionizing radiation. One suggested factor for the difference in sensitivity between spores and the parent vegetative form is water content. The spores contain less water than the vegetative forms, which may reduce the possibility of radiolysis.

The work of Thoday and Read (1947) was based on the relation of oxygen concentration to the effect of X-rays on living cells. Cells in oxygen-saturated suspensions were more sensitive to radiation. Cells in an anaerobic environment with available soluble oxygen were likewise radiation sensitive.

Certain chemical compounds appear to give radiation protection. These are classified as:

1. Sulfhydryl compounds - cysteine, mercaptosuccinate, 2, 3 mercaptopropanol (BAL), and 2-(2-mercaptoethoxy ethanol).
2. Sodium hydrosulfite ($Na_2S_2O_4$).
3. Alcohols and glycols - methanol, ethanol, isopropanol, propanediol, glycerol, triethylene glycol, and propylene glycol.
4. Metabolic intermediates and products - formate, succinate, pyruvate, fumarate, lactate, malate, and β -hydroxy butyric acid.

The protective action of the chemicals with a few exceptions - notably the sulfhydryl compounds - appear to be explained on their ability to lower the oxygen concentration in the bacterial suspensions during irradiation. Among the better chemical protective agents are sodium hydrosulfite and BAL, compounds known to react with molecular oxygen in solution (Kelner 15, p. 28).

The results of the study of ionizing radiations on the survival of bacteria prompted investigation of the feasibility of using ionizing radiations as a means of food preservation. Intensive study has been sponsored by the Armed Forces. The ultimate objective of the project is to develop a method of treating foodstuffs with ionizing radiations which will provide an acceptable and adequately preserved fresh food for feeding of the military personnel.

Following suggestions of Hannan (1955) it was deemed desirable by Cain (7, p. 537) to determine: 1) the influence of intermittent gamma irradiation and 2) the influence of gamma irradiation while the product was at a specific temperature, on its acceptability. This acceptability was based on the survival of the bacterial population, peroxide formation, thiobarbituric acid formation, fluorescence value and flavor of ground beef. The results of bacteriological studies demonstrated: 1) the increase in the number of exposures resulted in the decrease in survivors of a radiation resistant micrococcus and 2) the bacterial survival was dependent on both radiation dosage and temperature.

Cain (7, p. 539) found that increases in temperature up to 100°F in conjunction with irradiation decreased the survival of microorganisms. Nickerson's studies

(19, p. 311) indicated that the radiation resistance of Clostridium sporogenes (as compared with air-packed samples of the foods being tested and irradiated at room temperature) was increased by irradiating under the following conditions: nitrogen packing, vacuum packing, freezing and vacuum packing, and freezing.

Batzer (4, p. 64) and Marbach (17, P. 881) have shown that odors develop during the irradiation of fresh meats. This has been attributed to volatile sulfur compounds resulting from the decomposition of glutathione and possibly of proteins containing sulfhydryl groups. Cain (7, p. 539) noted that with increase in irradiation there was a concomitant increase in the amount of peroxides formed. Matsuo (18, p. 1021) found that peroxides degraded methionine and other sulfhydryl compounds to yield free sulfhydryl groups and volatile sulfur compounds. The addition of methionine in minute amounts to irradiated milk (1, p. 242) increased the "off" flavor while the addition of ascorbic acid did not significantly reduce it. Ascorbic acid has been investigated for its protective effect on oxidative changes in irradiated meats (12, p. 885 and 21, p. 237). Conflicting reports have been obtained with its used in fresh meats, but both color and odor of irradiated cured meats may be improved by the addition of ascorbic acid.

Most of the published material on the radiation preservation of meat has dealt with fresh meats. In studies with cured meats, Ercman (9, p. 350) found that irradiation did not delay the onset of bacterial spoilage in cured meat but that it did have less of an "off" odor than irradiated fresh meats.

During studies at the Oregon Agricultural Experiment Station on the effect of gamma radiation on sterilization of meat, a bacterial species was isolated from meats exposed to dosages of 2 and 3 million roentgen equivalent physical (2, p. 575). This organism was a micrococcus resembling Micrococcus roseus and Micrococcus rubens tetragenus. An organism morphologically and physically resembling the radioresistant species was isolated from unirradiated meat. This organism had the same resistance to the effects of gamma radiation as did the originally isolated species, indicating that this degree of resistance is not due to mutation. Studies are under way to further characterize the radiation-resistant microorganism, establish its source and habitat, and to study its metabolism and related functions so as to determine the mechanism of resistance.

It has not been established definitely yet that the irradiation of fresh packaged meat at radiation doses sufficient for sterility can be achieved, and yet maintain

a pleasing esthetic appearance and organoleptic properties.

EXPERIMENTAL STUDIES

INCIDENCE

The most notable effect on bacteria exposed to radiation, either high-energy ionizing radiation or ultra-violet radiation, is the apparent killing of a percentage of the cells, the portion being killed or inactivated being a function of the energy absorbed, the criterion of survival being the ability of the bacteria to form colonies visible to the eye when incubated following plating on special culture media.

With this view in mind, the effect of ionizing radiation using gamma rays, as an effective means of meat preservation, was studied. Fresh meats used in this study were in the following catagories: 1) hamburger; 2) bulk pork sausage; 3) chicken thighs and wings; 4) chicken giblets. Portions of each type of meat sample were placed in No 1½ flat tins and hermetically sealed in the Food Technology laboratories at Oregon State College. These meat samples hereafter designated as "canned" were shipped under refrigeration to the Materials Testing Reactor, Arco, Idaho, where they were irradiated at the gamma test facilities. In the first series, samples of meat were irradiated at 2 and 3

million roentgen equivalent physical (2 and 3 megarep) respectively; in a subsequent series, the meats were irradiated at 3 and 5 megarep respectively. In all, 321 "canned" meat samples were tested.

Experimental Methods:

Samples of the irradiated "canned" meat specimens were handled in the following manner for the assay of the effectiveness of gamma radiation as a means of sterilizing "canned" fresh meats.

1. Cans of meat samples to be tested were immersed in a strong solution of a chlorinated compound (in excess of 500 ppm) for five minutes, drained of excess solution and placed on a disinfected working table.
2. The cans were observed for swelling, buckling, leaking, or other outward signs of spoilage.
3. The indented top of each can was flooded with ethyl alcohol, 95%, and flamed almost to dryness.
4. As the flaming subsided, sterile petri dish halves were placed over the cans to prevent airborne contamination.
5. Using alcohol - flame sterilized can openers, the cans were aseptically opened.
6. The specimens were observed for color, odor, and appearance.

7. Approximately 10 gram samples were taken from each can and placed in correspondingly numbered metal Waring blenders. The Waring blender had been previously sterilized at 15 pounds pressure, 250°F for 45 minutes, with a covering of a double sheet of aluminum foil, as the customary plastic or metal lids were not found satisfactory for ease in pipetting samples.
8. 99 ml of sterile phosphate buffer was added aseptically to the contents of each Waring blender.
9. The meat samples were blended 40 seconds at 4000 RPM

This blended meat suspension (1:10) was cultured in the following manner:

1. Pour plates for plate counting.
   Pour plates 1:2 dilution (5 ml of 1:10 meat suspension) and 1:5 dilution (2 ml of 1:10 meat suspension) were made in duplicate for each specimen; after the agar had solidified the plates were counted for bacterial colonies after 48 and 72 hours incubation. Colonies were subcultured by means of streak plates for further testing.

2. Shake culture flasks.

Shake cultures were set up in duplicate. 10 ml of 1:10 meat suspension was pipetted into each shake flask containing 50 ml of fluid media. These flasks were incubated at 28°C on a rotary shaker for 48 hours, at which time they were observed for growth, smears made of the broth media and stained by the gram-method. Streak plates were also made from each flask to isolate any organisms present for identification and further study.

3. Anaerobic cultures.

Into each of three tubes of melted anaerobic agar was pipetted 1 ml of 1:10 meat suspension. One tube culture of each specimen was incubated respectively, at room temperature (±22°C), 37°C and 55°C. The tubes were observed for growth at one, two and three weeks. If present, colonies were isolated for further study.

Media used for the above cultural procedures was as follows:

1. Pour Plates and Streak Plates - TGY agar

Tryptone (Difco)	5 g
Yeast Extract (Difco)	1 g
Dextrose (Anhydrous)	1 g
Agar (Difco)	15 g

Dilute to 1000 ml with distilled water.

Adjust pH to 7.0 before autoclaving at

15 pounds, 250°F for 20 minutes.

2. Shake Flasks - 50 ml TGY broth/250 ml Erlenmeyer flask.

Tryptone (Difco)	5 g
Yeast Extract (Difco)	1 g
Dextrose (Anhydrous)	1 g
NZ-Amine (Sheffield Biological Company)	1 g

Dilute to 1000 ml with distilled water.

Adjust pH to 7.0 before autoclaving at

15 pounds for 20 minutes.

3. Anaerobic Tubes - Brewer's anaerobic agar

Tryptone (Difco)	5 g
Proteose Peptone #3 (Difco)	10 g
Yeast Extract (Difco)	5 g
Dextrose	10 g
Sodium Chloride	5 g
Agar (Difco)	20 g
Sodium Thioglycollate (Difco)	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Resazurin, certified	2 mg

Dilute to 1000 ml with distilled water.

Results:

A total of 321 cans of meat stored at varying temperatures (72°C - 100°F) for varying periods of time (1-3 weeks) before culturing were tested with the average results of culture listed, tables I to V.

Discussion:

From the results in these tables the following conclusions could be drawn:

1. Regardless of temperature and length of storage time, the over-all plate counts of the irradiated meats were not high.
2. The fact that many shake flasks were positive for growth when pour plates were negative, or had a very low count could indicate that neither method was strictly satisfactory to determine the extent of contamination because:
   a) In pour plates under optimum conditions one bacterium can produce one bacterial colony.
   b) In broth cultures under stimulatory conditions (shaking) one bacterium may reproduce easily.
   c) An apparently spoiled appearance of the meat, or "off" or offensive odor did not

Table I

Survival of microorganisms in meats irradiated at 2 megarep and stored at 72°F

Group number	Total number organisms/gram	Shake flask	Streak plate	Anaerobic RT	Anaerobic 37°C	Anaerobic 55°C
1-2	2	+	+	+	+	+
3	2	-	-	-	-	-
4-8	0	-	-	-	-	-
9-11,13	<1	-	-	-	-	-
14-16	0	-	-	-	-	-
17	1	+	+	-	-	-
18	1	-	-	-	-	-
19	2	+	+	+	+	-
20	1	+	+	-	-	-
21	0	+	+	-	-	-
22-23	<1	+	+	-	-	-
24	0	-	-	-	-	-
25	<1	+	+	-	-	-
26-28	0	-	-	-	-	-
29	<1	+	+	-	+	-
30	<1	+	+	-	-	-
31	0	+	+	+	-	-
32	0	-	-	-	-	-
33	<1	+	+	-	-	-
34	<1	-	-	-	-	-
35	1	+	+	+	-	-
36-39	<1	+	+	-	-	-
40	<1	-	-	-	-	-

Table II

Survival of microorganisms in meats irradiated at 2 megarep and stored at 100°F

Group number	Total number organisms/gram	Shake flask	Streak plate	Anaerobic RT	Anaerobic 37°C	Anaerobic 55°C
1	2	+	+	-	-	-
2	<1	-	-	-	-	-
3	2	+	+	-	+	-
4	0	-	-	-	-	-
5	0	+	+	-	-	-
6	<1	-	-	-	-	-
7-8	0	-	-	-	-	-
10-11	<1	-	-	-	-	-
13	TMTC	+	+	+	+	+
14	<1	-	-	-	-	-
15	0	-	-	-	-	-
16	<1	-	-	-	-	-
17-18	0	+	+	-	-	-
19	<1	-	-	-	-	-
20	0	-	-	-	-	-
21	<1	-	-	-	-	-
22	0	+	+	-	-	-
23	<1	-	-	-	-	-
24	0	+	+	-	-	-
25	TMTC	+	+	+	+	+
26	<1	-	-	-	-	-
27-30	0	-	-	-	-	-
31	0	+	+	-	-	-
32	0	-	-	-	-	-
33	<1	-	-	-	-	-
34	0	-	-	-	-	-

Table III

Survival of microorganisms in meats irradiated at 3 megarep and stored at 72°F

Group number	Total number organisms/gram	Shake flask	Streak plate	Anaerobic RT	Anaerobic 37°C	Anaerobic 55°C
1	<1	+	+	-	-	-
2	TMTC	+	+	+	+	+
3	0	+	+	-	-	-
4-5	<1	-	-	-	-	-
6	0	-	-	-	-	-
7	0	+	-	-	-	-
8,10	<1	-	-	-	-	-
11	0	-	-	-	-	-
13	0	+	-	-	-	-
14	0	-	-	-	-	-
16	TMTC	+	+	-	-	-
17	0	-	-	-	-	-
18	0	+	+	-	-	-
19	0	+	-	-	-	-
20	0	-	-	-	-	-
21	<1	+	+	-	+	-
22-24	0	+	+	-	-	-
25	<1	-	-	-	-	-
26	0	-	-	-	-	-
27-28	0	+	+	-	-	-
29	<1	-	-	-	+	-
30	<1	+	+	+	+	-
31	<1	-	-	-	-	-
32	0	-	-	-	-	-
33	<1	-	-	-	-	-
34	<1	+	+	-	-	-
35	<1	+	+	-	+	-
36	<1	+	+	-	-	-
37	<1	-	-	-	-	-
38	<1	+	+	+	+	-
39	<1	+	+	-	-	-
40	<1	-	-	-	-	-
41	0	-	-	-	-	-
42	<1	+	+	-	-	-

Table III - Continued

43	<1	-	-	-	-	-
44	0	-	-	-	-	-
45	<1	+	+	+	-	-
46	0	-	-	-	-	-
47	1	+	-	-	-	-
48-49	0	+	+	-	-	-
50	1	-	-	-	-	-
51	1	+	+	-	-	-

Table IV

Survival of microorganisms in meats irradiated at 3 megarep and stored at 100°F

Group number	Total number organisms/gram	Shake flask	Streak plate	Anaerobic RT	Anaerobic 37°C	Anaerobic 55°C
1-2	<1	+	+	-	-	-
3	2	-	-	-	-	-
4	<1	-	-	-	-	-
5	0	+	+	-	-	-
6	0	-	-	-	+	-
7	<1	+	-	-	-	-
8	0	-	-	-	-	-
10-11	<1	-	-	-	-	-
13	0	+	-	-	-	-
14	TMTC	+	+	+	+	+
16	0	-	-	-	-	-
17	0	+	-	-	-	-
18	0	+	+	-	-	-
19	<1	+	+	-	-	+
20	0	-	-	-	-	-
21	<1	+	+	+	+	-
22-24	<1	+	+	-	-	-
25	TMTC	+	+	+	+	+
26	<1	-	-	+	-	+
27-28	0	-	-	-	-	-
29	0	-	-	-	+	-
30-31	0	+	+	-	-	-
32-33	<1	-	-	-	-	-
34	<1	+	+	-	-	-

Table V

Survival of microorganisms in meats irradiated at 5 megarep and stored at 72°F

Group number	Total number organisms/gram	Shake flask	Streak plate	Anaerobic RT	Anaerobic 37°C	Anaerobic 55°C
40	<1	-	-	-	-	-
41	0	-	-	-	-	-
42	<1	-	-	-	-	-
43	<1	-	-	-	+	-
44	<1	+	+	+	-	-
45	2	-	-	+	-	-
46	0	-	-	+	+	-
47	1	-	-	-	-	-
48	0	+	+	-	-	-
49	<1	-	-	+	+	-
50	<1	-	-	-	-	-
51	0	-	-	-	-	-

necessarily indicate bacterial contamination but possibly protein decomposition due to enzymatic reaction and irradiation - although the extent was not necessarily correlated with the amount of irradiation.

d) The growth of some anaerobes or microaerophiles at room temperature and at 37°C and little at 55°C would indicate that ordinary canning procedures would eliminate the contamination.

e) The degree of bacterial contamination of meat irradiated at two and three megarep and relatively little contamination at five megarep would indicate that an irradiation level of five megarep was considered minimum for meat sterilization.

Organisms isolated from cultures of the irradiated meat samples were selected for re-irradiation and determination made of survival. One group of organisms persisted in spite of repeated irradiation and of increases in radiation dose. An outstanding characteristic of this group was an almost universal salmon pink color of varying intensities. One organism of this group was a micrococcus which occurred in packets of four, had a characteristic salmon-pink color, and survived repeated irradiation and

increases in radiation dose - up to 6,000,000 rep (roentgen equivalent physical) whereas the average microorganism can tolerate a radiation dose up to a maximum of 500,000 rep. This organism was designated as "R_1" to distinguish it from other members of this group surviving radiation or having cultural similarity - designated variously as U_1, R_4R, M_1, M_1R. Preliminary work on this microorganism has indicated it may be a new species and has been tentatively named Micrococcus radiodurans nov. sp. Studies were undertaken to determine the nutritional requirements of the cell, and its cellular metabolism.

NUTRITION

The studies in nutrition were necessary in order that a synthetic media could be developed to facilitate carbohydrate metabolic studies using the Warburg respirometer and labeled carbon compounds. Using these methods it can be determined if a definite metabolic pathway is involved in radiation resistance. The determination of vitamins essential to bacterial growth was necessary also in the development of a synthetic medium.

The determination of the essential amino acids was necessary for several reasons. Amino acids are usually thought to arise as the hydrolytic products of proteins. There have been about twenty-five isolated and purified

from proteinaceous sources. About fifteen others have been identified but these occur in special tissues and are not commonly considered in microbial metabolism. Amino acids contain at least one carboxyl group and one amino group. A variety of other functional groups may be included as hydroxy, phenyl, phenol, imidazole, guanido, indole and sulfhydryl. Most of the naturally occurring amino acids are of the "levo" configuration but small amounts of certain dextro-acids are known to occur, e.g. D-glutamic acid, in the capsular material of several of the Bacillus sp.

"Essential" amino acids may vary widely for different microorganisms. All amino acids are essential from the viewpoint of the organism. However, here in the use of the term "essential" is meant amino acids that the organism is incapable of synthesizing itself, or of synthesizing in quantities sufficient for normal growth rates.

The biogenesis of amino acids is a very complex series of reactions, much of the pattern depending on the microorganisms concerned, conditions of growth, and substrates available. The main types of reactions involved are:

1. amination and deamination
2. transamination

3. carboxylation and decarboxylation
4. oxidation and reduction
5. hydrolysis and condensation
6. miscellaneous reactions such as carbon dioxide fixation or nitrogen fixation.

Amino acids produced by, or available to, microorganisms may be a source of energy, for the synthesis of proteins in the cells, for the production of intermediates of other synthetic reactions, or for apoenzymes.

Experimental Methods:

A standard basal test assay medium was first utilized for nutrition studies. This medium consisted of:

casamino acids	10 g
glucose	10 g
D-L tryptophan	1 g
sodium acetate	6 g
salts A and B (Snell)	10 ml

dilute to 1000 ml with deionized water

pH 6.8 - 7.0

Prior investigation had shown that this microorganism was highly aerobic. It was found that the tube assay method was unsatisfactory even though the tubes were placed on a rotary shaker to increase the surface being

aerated. Even this proved insufficient so all further studies were done using 250 ml Pyrex Erlenmeyer flasks containing 50 ml of the media being tested. This standard basal test assay medium was found unsatisfactory for studying this microorganism even in flask cultures.

Since previous work had shown that a "salt-effect" was inhibitory to the organism, possibly the sodium ion or the chloride ion, or both, was responsible. To dilute the sodium and chlorine concentrations, a four-fold dilution of the medium was made using deionized water, and sterilized as before. This adapted media was not found satisfactory, either.

The following enriched synthetic media was then tried:

Ammonium acetate	3 g*
Dipotassium phosphate	5 g
Salts A - Snell	5 ml stock solution
Salts B - Snell	5 ml stock solution

*The solution was made double strength so that test solutions could be added, and the media then diluted to volume.

**Fructose	5 g
**Glucose	5 g
Adenine, guanine, uracil	5 mg each
L-aspartic acid	1 g
L-glutamic acid	1 g
Alanine, arginine, lysine	200.0 ml stock solution
Casein, hydrolyzed	10.0 g
Amino Acids	200.0 ml stock solution
Vitamins	10.0 ml of each used in the assay

dilute to 1000 ml with deionized water

The media was made by using deionized water and dispensed in 50 ml aliquots into acid-washed chemically clean Pyrex 250 ml Erlenmeyer flasks which were stoppered with gauze-wrapped cotton plugs. The media was then sterilized in the autoclave for 5 minutes at 15 lbs. pressure and 250°C.

Salts A

KH_2PO_4	25 g
K_2HPO_4	25 g
HOH dist. q.s. ad 250 ml	

**Both glucose and fructose were used because some preliminary work seemed to indicate a preference for L-fructose.

Salts B

$MgSO_4.7H_2O$	10.0 g
NaCl	0.5 g
$Fe_2SO_4.H_2O$	0.5 g
$MnSO_4.4H_2O$	0.5 g

HOH dist. q.s. ad 250.0 ml

Stock amino acid solution

A 0.5% concentration of each of the following was prepared in deionized water - histidine, isoleucine, leucine, methionine, phenylalanine, proline, valine, tryptophan, serine, glycine.

Vitamin solutions

100 mcg/ml - thiamine, riboflavin, niacin, pyridoxal, pyridoxamine

20 mcg/ml - paraaminobenzoic acid

10 mcg/ml - biotin, folic acid

Results:

The medium as given was able to support some growth, although delayed. For assay purposes the medium was made minus all the amino acids. The amino acids were added back singly until growth occurred. The same practice was followed with the vitamins assayed. It was determined that although glutamic acid stimulated growth, of all the amino acids added only methionine was

required, and of all the vitamins added only biotin and niacin were absolutely essential for growth. However cell growth was minimal, and as such the yield was not sufficient for Warburg studies, or mutation studies. Other substances such as oleates and Tween 80 were then used to see if a stimulatory effect were produced, or if they could be substituted for known essential nutrients. Only yeast extract in concentrations so minute as to act only as a growth factor (0.5 ml of 0.1% yeast extract per 100 ml of medium) proved to be stimulatory, however it did not replace any of the known essential requirements.

The Tween 80 was present in 5 mcg per ml concentration; the sodium oleate was present in 0.1% concentration; the yeast extract was present in 0.05% concentration.

Growth in any of the combinations lacking any of the essential nutrients was minimal. The conclusions from this supplementary nutritional assay would indicate that although yeast extract, Tween 80, and sodium oleate did not replace any of the essential constituents, the yeast extract greatly stimulated the growth in excess of that in the basal medium plus the essential constituents, so that it was then necessary to test different components of yeast extract.

Different constituents known to be present in yeast extract were added to the synthetic medium singly and in combination. At the same time work was done independently by other workers to find a growth stimulus other than yeast extract. Several other constituents such as folic acid, paraaminobenzoic acid, 20 nucleic acids, etc. were added to the medium with no apparent increase in growth. Finally thiamine hydrochloride (1 mcg) was used and a luxuriant growth was obtained in 24 hours after incubation at 30°C on a rotary shaker. The final satisfactory synthetic medium now used is as follows (quantities given are for 1000 ml of medium):

SYNTHETIC MEDIUM

Ammonium phosphate (dibasic)	500 mg
L-methionine	10 mg
Thiamine hydrochloride	10 mcg
L-glutamic acid	500 mg
Biotin	10 mcg
Pyridoxine	200 mcg
Niacin	250 mcg
Glucose	5 g
Salts A	5.0 ml
Salts B	5.0 ml

All constituents are combined except Salts B, and

and diluted to 800 ml; Salts B are added and the media is diluted to 1000 ml. The pH is adjusted to 6.8 - 7.0 before sterilizing for 15 minutes at 15 lbs. pressure (250°C). Salts B were altered as follows because of the salt effect:

Salts B

$MgSO_4.7H_2O$	10 g
$FeSO_4.7H_2O$	0.5 g
$MnSO_4.4H_2O$	0.5 g
HOH dist., q.s. ad 250 ml	

The media was dispensed in 50 ml aliquots into 250 ml capacity Pyrex Erlenmeyer flasks, and stoppered with cotton plugs.

Discussion:

It was determined that the tube assay method is not entirely satisfactory for this organism. The organism is highly aerobic and requires aeration for efficient growth. Aeration is increased if the tubes are placed on a rotary shaker, but it was felt that flask cultures with aeration was more efficient.

It was interesting to note that of the essential and growth stimulating amino acids and vitamins, methionine, niacin, biotin and thiamine, only niacin was a non-sulfur containing compound. Sulfhydryl groups are known to have a certain radiation protective effect,

and perhaps the requirement of this radiation resistant microorganism for this type of compound is related to its radiation resistance. Cystine and cysteine are sulfur amino acids which are known to have a "sparing" effect on methionine usage and also give radiation protection. However, the addition of these compounds to the medium singly or in combination, did not increase growth nor did they serve as a substitute for the required methionine.

The incorporation of L-glutamic acid and pyridoxine into the medium was based on Warburg studies which showed active utilization of glutamic acid, and evidence for tricarboxylic acid cycle activity.

METABOLISM

1. Effect of Metal Ions

Some of the copper compounds have been cited in the literature as giving a degree of radiation protection. This fact, coupled with the observation that the pigment of cultures of the microorganism, especially old cultures which had diffused pigment into broth or agar cultures, was somewhat characteristic of metallic copper, was the basis for an assay made to determine the reaction of the organism to copper, as $CuSO_4$. Copper sulfate was used because: 1) Bollen (5) had

reported a "copper bacteria" with the same characteristic red-brown pigment which would tolerate 500 ppm copper sulfate in soil (this culture has been lost so no parallels could be drawnwith the organism being studied); and 2) the organism appeared to favor sulfur compounds.

This line of study was felt valid in considering the metabolism of the microorganism. Saklewska-Szymonawa (23, pp. 299-310) in studying the effect of copper on the growth and glucose metabolism of Escherichia coli had noted; 1) the toxic influence of copper could be reversed or prevented by cysteine, dithiopropanol and diethyl thiocarbamate. These results were interpreted to suggest that the sulfhydryl groups are important in the mechanism of the toxic action of copper ions. Weed and Longfellow (27, pp. 27-33) in studies with E. coli showed that the presence of small amounts of copper ions in a liquid synthetic media resulted in the appearance of a small colony variant which then persisted in the absence of copper. This variant possessed certain significant differences including increased resistance to ultraviolet light when compared to normal E. coli. It is believed that the cytochromes, including copper cytochrome and the ferric enzyme, catalase, increase resistance to ultraviolet light.

A copper protein, dopa-oxidase, is known to oxidize tyrosine and dihydroxyphenylalanine ("dopa") to melanin (29, p. 373). This microorganism being studied produces, at times, a melanin-like pigment on agar slants. Copper, as the cuprous ion (0.001M) is known to decrease the activity of thiaminase I in a thiamine-decomposing bacterium, Bacillus aneurinolyticus (3 pp. 239-244). The inactivation of the enzyme would decrease the amount of thiamine required for growth.

Experimental Methods:

Having in mind a radiation protective effect of copper compounds, the metabolic effect of metals on cultures of R_1 was studied, using copper sulfate as the metal ion source. The paper disc method of Feeney (10, pp. 284-290) was used in studying the effect of metals in the presence of chelating agents, Versene and sodium citrate.

Tryptone-glucose-yeast extract agar was used as the plating medium. This was prepared with deionized water to 75% of its final concentration and dispensed in 50 ml quantities into acid-washed French squares and sterilized. Noble's agar was used in order that trace elements might be reduced to a minimum. Acid-washed petri dishes were prepared, rinsed in deionized

water, and sterilized by dry heat. A heavy suspension of a twelve hour culture of the microorganism was washed three times in phosphate buffer 0.0666M at pH 7.0, prepared with deionized water. 1 ml aliquot of a heavy suspension of the washed cells were dispensed aseptically into the melted agar medium.

Previously prepared and sterilized were the chelating agents Versene and citrate. These were prepared to give a final concentration in agar of 1.0 M, 0.1 M, 0.01 M to 0.00001 M. These were aseptically distributed into the bottles of melted agar, the water content of the agar was brought up to 100% volume by aseptically adding the required amounts of sterile deionized water. The cells were then introduced.

The copper sulfate, $CuSO_4.5H_2O$ was prepared so that the concentration of copper sulfate with which the discs were saturated would be 1.0 M, 0.1 M to 0.00001 M. The paper discs in their respective copper sulfate solutions were sterilized in the autoclave at 15 pounds pressure, 250°F, for 15 minutes.

The melted agar containing the chelating agents and the 1 ml aliquots of cell suspension were poured into the sterile petri dishes. When the agar had solidified, the saturated discs were placed on the plates. The plate had a primary incubation period of 24 hours at 4°C

to permit the copper sulfate to diffuse into the medium prior to growth of the organism. The plates then had a secondary incubation period of 24 hours at 30°C, at which time the first observation was made. All plates were set up in triplicate with standard controls for the chelating agents and for the paper discs.

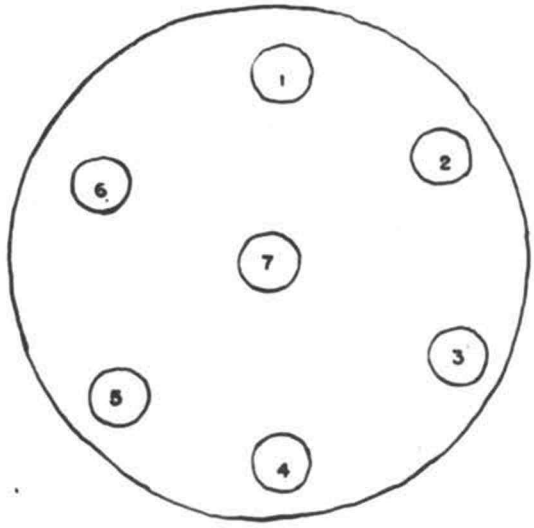
Results:

The observations after 24 hours incubation at 30°C are given in Table VI.

The plates were reincubated at 30°C and observed again at 48 hours. Inhibition previously noted at 0.1M $CuSO_4$ was no longer evident. Although the zone of inhibition around the 1.0M $CuSO_4$ was still present, at the edge of this zone of inhibition there was a zone of greatly intensified growth. This is unusual since the concentration of 1.0M $CuSO_4$ was 15.63 g per 100 ml. This would be equivalent to 156,300 ppm with the tolerated dose of the organism being 15,630 ppm $CuSO_4$. So far no organisms have been reported with this high a $CuSO_4$ tolerance. The highest $CuSO_4$ tolerances reported to date are those of three new species of marine bacteria. These three species, Pseudomonas turbinellae, Flavobacterium pinctadum and Achromobacter viscosus tolerate 1000, 750, and 500-750 ppm $CuSO_4$ respectively (24, pp. 92-93).

FIGURE I

PAPER DISC ASSAY METHOD FOR EFFECT OF METAL IONS



1. 1.0 M $CuSO_4$
2. 0.1 M
3. 0.01 M
4. 0.001 M
5. 0.0001 M
6. 0.00001 M
7. CONTROL

Table VI

Copper tolerance in the presence of a chelating agent

Chelating Agent	Copper sulfate - Molar concentration						
	1.0M	0.1M	0.01M	0.001M	0.0001M	0.00001M	Control
Versene							
1.0M	-	-	-	-	-	-	-
0.1M	-	-	-	-	-	-	-
0.01M	-	±	+	+	+	+	+
0.001M	-	±	+	+	+	+	+
0.0001M	-	±	+	+	+	+	+
0.00001M	-	±	+	+	+	+	+
Citrate							
1.0M	-	-	-	-	-	-	-
0.1M	-	±	+	+	+	+	+
0.01M	-	±	+	+	+	+	+
0.001M	-	±	+	+	+	+	+
0.0001M	-	±	+	+	+	+	+
0.00001M	-	±	+	+	+	+	+
Control	-	±	+	+	+	+	+

Legend - = inhibition
± = slight inhibition
+ = growth

Discussion:

These results would lead one to think of the possibility of a copper cytochrome mechanism, or that copper is a part of the pigment structure. Again there may be a protective effect or detoxifying effect against the cuprous ion produced by the sulfate radical, or possibly this method of metal toxicity assay is not suitable for all microorganisms.

One point noted in the literature is that in studies of an E. coli mutant requiring methionine, the addition of 0.004% copper inhibits growth. Homocysteine and cysteine gave some protection to the methionine (13, pp. 349-357). Further studies of copper sulfate on the methionine-requiring microorganism should be carried out using an agar of the synthetic media to see if this methionine-effect holds true.

2. Antibiotic Assay

Antibiotics have been used for the inhibition of microbial spoilage of fresh red meats. The antibiotics approved by the Food and Drug Administration are the broad spectrum antibiotics chlortetracycline (aureomycin), oxytetracycline (terramycin), and tetracycline. It had been suggested to combine antibiotics, used at bacteriostatic levels, with ionizing radiations at low

(pasteurization) levels.

Niven (20, p. 856) showed through antibiotic sensitivity studies that bacteria resistant to irradiation were sensitive to the tetracylines. Cain (6, P. 852) investigated the stability of the tetracyclines at different levels of irradiation to see if appreciable amounts of antibiotics remained in meat after irradiation to offer antibacterial protection during storage. He found that antibiotics when incorporated in meat or water and subjected to ionizing radiations were destroyed as the radiation dosage increased. At sterilization doses of radiation (3 megarep) the antibiotic was completely or almost completely destroyed, while at pasteurization levels of radiation, sufficient antibiotic remained to offer protection during storage.

Methods:

In assaying the sensitivity of the radioresistance microorganisms, R_1, the disc-plate method was used. Agar plates (Tryptone-Glucose-Yeast Extract Agar) were heavily seeded with the organism. The antibiotic impregnated discs (Desi-Discs,® supplied by the Bio-Test Corporation) were distributed over the plate by using a sterile forceps. Antibiotics used in this survey were aureomycin (oxytetracyline)-50 mcg, bacitracin-20 units, carbomycin

(magnamycin-15 mcg, chloromycetin (chloramphenicol) -50 mcg, erythromycin-10 mcg, matromycin-30 mcg, neomycin-30 mcg, penicillin-10 units, polymyxinB-10 units, streptomycin-100 mcg, terramycin (chlortetracycline)-50 mcg, tetracycline-50 mcg, and novobiocin -30 mcg.

Results and Discussion:

The plates were incubated at 30°C for 48 hours and then observed for zones of inhibition. It was noted that the micrococcus was very sensitive to all the antibiotics against which it was tested. This was in agreement with Niven's observations (20, p. 856) that bacteria resistant to irradiation are sensitive to the tetracylines.

3. Effect of Spices

Spices have been used for centuries as a means of preserving foods. Before the use of canning and freezing as methods of food preservation the only methods for storing perishible foods were drying and the addition of spices.

It was decided to determine the effect of spices, at concentrations normally used in foods, plus irradiation on the survival of the radiation-resistant micrococcus in a raw-meat suspension. After the blending of

the meat, spice and microorganism, the sample was placed in 1½ flat tins, hermetically sealed and shipped to the gamma test facilities at Arco, Idaho, where they were irradiated at 1.5 megarep. This dosage was used to reduce the bacterial load to a minimum and because it caused the least change of the original product from its natural raw state.

Method:

The spices tested were fresh oil of pepper, oil of garlic, oil of nutmeg, oil of cinnamon, oil of cloves, oil of mustard, and salt. The spices were diluted with Wesson® oil to that concentration which would give 1.0 ml of the required concentrations of spice per pound. The concentration of spices used was as follows:

Pepper	5 oz per 100 lbs meat
Garlic	1 oz " " " "
Nutmeg	1.5 oz " " " "
Cinnamon	1.5 oz " " " "
Cloves	1.5 oz " " " "
Mustard	1.5 oz " " " "
Salt	1%, 2%, 3%

The fresh ground meat was inoculated with that number of organisms in a suspension calculated to give

100,000,000 organisms per ml, so that the meat would contain 100,000,000 organisms per gram of meat. The spices and organisms were mixed with the meat sample by blending for five minutes in a Buffalo Standard Silent Cutter (model #21). Each No. 1½ flat tin was filled with a portion of meat, and was then hermetically sealed. The inoculated cans were then held at wet ice temperature until irradiated.

A total of 132 cans of meat-organism-spice samples were prepared, in eleven categories - six spices (pepper, garlic, cloves, cinnamon, mustard, nutmeg; three salt concentrations (1%, 2%, 3%) inoculated control, uninoculated control. Half (six) of each category were irradiated at 1.5 megarep and half (six) were unirradiated.

The cans were treated as before (see "incidence"), and 10 gram samples were again used as before for plate counts of surviving organisms, using Tryptone-Glucose Yeast Agar. The plates were incubated at 30°C for 48 hours and then observed for bacterial survival.

Results:

The results are listed in Table VII.

Discussion:

From these results it would appear that garlic, which contains sulfhydryl groups exert a protective

Table VII

Action of spices on radiation resistant microorganism irradiated in meat in the presence of individual spices

Spice additive	Irradiated at 1.5 megarep	Unirradiated	% Survival
	Total count/gram	Total count/gram	
Inoculated control	2,500	205,000	1.2
Pepper	6,600	169,000	3.9
Cinnamon	176	162,000	0.12
Mustard	297	23,800	0.12
Garlic	23,800	171,000	14.00
Nutmeg	4,500	155,000	3.00
Cloves	5,300	159,000	3.3
NaCl 1%	2,140	202,000	1.2
NaCl 2%	1,600	151,000	1.0
NaCl 3%	3,870	155,000	2.5
Uninoculated control	2	70	0.03

effect against ionizing radiation, resulting in a tenfold recovery over the control. Radiation in the presence of cinnamon and mustard oils reduced the surviving bacterial numbers greater than could be attributed to either radiation or the spices alone. Salt up to 3% had no apparent effect on bacterial survival, while all the other spices tested appeared to protect the organism from radiation death.

4. Catalase Production

It is an established fact that peroxides can be used both as a surface disinfectant and as a preservative, usually in conjunction with heat because of the strong oxidizing effect. One method for the pasteurization of milk (11, p. 135) involves the addition of H_2O_2 and the use of a relatively low heating temperature, with excess peroxide decomposed by the addition of catalase. In the processing of sugar thermophiles are destroyed by a combination of heat and H_2O_2.

Cain (7, p. 539) demonstrated that the peroxide number or content of irradiated meat samples increased significantly due to irradiation alone, there being no significant differences between specific radiation levels. The formation of the peroxide is possibly due to the radiolysis of the water molecule of the meat

irradiated, or to the reduction of the oxygen present in the cans.

The formation of peroxides from the radiolysis of water is suggested by the following:

$$HOH \longrightarrow H + OH$$

$$2(OH) \longrightarrow H_2O_2$$

Hollaender (14, p. 381) believes that the reduction of oxygen is more plausible. This would be:

$$O_2 + H \longrightarrow HO_2$$

$$HO_2 + H \longrightarrow H_2O_2$$

The reduction of the oxygen concentration in the cans to be irradiated would then reduce the amount of peroxide and HO_2 radicals formed. However, as stated earlier, the reduction of oxygen content in the medium exerts a protective effect against microorganisms being irradiated.

Two enzymes, peroxidase and catalase, are known to destroy hydrogen peroxide, with peroxidase decomposing the peroxide, while catalase produces oxidation when combined with H_2O_2 (28, p. 340). The over all reaction for both is:

$$2\ H_2O_2 \longrightarrow 2H_2O + O_2$$

Experimental Methods:

It was decided to determine the catalase activity of the radiation-resistant microorganisms to see if this would have some bearing on its radiation resistance. The method to determine catalase activity utilized the Warburg respirometer.

Eight-hour cells were washed three times in 0.066 M phosphate buffer at pH 7.0 then resuspended in 1%, 2%, 3% and 4% suspensions of the nitrogen content of the cells (the cells are known to have 1.02 mg nitrogen per 1 ml of cell suspension). 30% hydrogen peroxide ("superoxyl") was diluted to a calculated amount of 500 microliters of total oxygen. It was necessary to make the dilutions of cells so that one concentration would liberate the total amount of oxygen, and one would not liberate the total amount.

The following procedure was used in the run:

1. 1 ml of cell suspension in Warburg flask.
2. 1 ml of phosphate buffer (0.066M) at pH 7.0.
3. 0.5 ml H_2O_2 dilution in side arm of flask.
4. The flask contents were allowed 10 minutes to equilibrate the temperature.
5. The hydrogen peroxide was then tipped in.
6. 30 minutes were allowed for the run, with first readings at 30 second intervals,

later readings at 5 minute intervals.

7. A blank flask was run for oxygen uptake.

A preliminary run had shown that the maximum catalase production in this radiation-resistant microorganism was found in 6-8 hour cells.

Results:

1. 270 mcl of oxygen was liberated from hydrogen peroxide per mg nitrogen of cells.
2. Pseudomonas leisodikticus liberates up to 500 mcl of oxygen.
3. The average bacterium liberates 80-100 mcl of oxygen.

Discussion:

It would appear that the toxic hydrogen peroxide formed in the radiation sterilization of meats is inactivated by the catalase activity of the radiation resistant microorganism, enabling the organism to survive in irradiated meats, even during storage.

5. Substrate Oxidation

The principle of the Warburg respirometer is that a gas volume held at a constant volume changes its pressure in accordance with additional gas evolved or gas absorbed from a constant volume system. When

temperature and pressure are held constant, any change in pressure of the closed system is reflected by a corresponding change in the gas volume enclosed. When a microorganism oxidizes a substrate, gas is evolved and this may be measured in the calibrated manometer arm so that the efficiency and rapidity of oxidation may be determined, using the Warburg technic of Umbreit (26, p. 1-17).

Experimental Methods

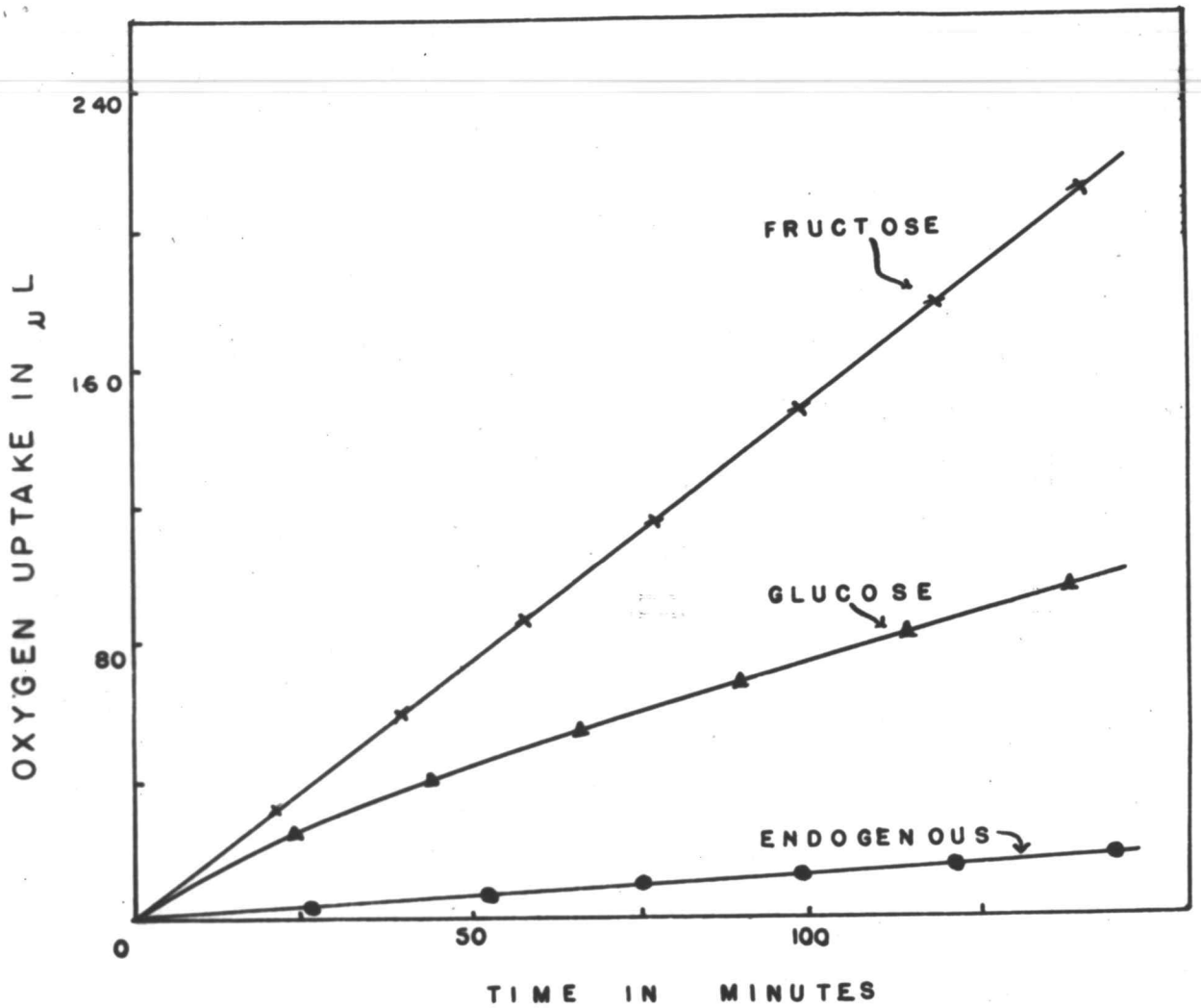
The Warburg respirometer studies were done on a 24 hour culture of the microorganisms, grown in Tryptone-Glucose-Yeast Extract broth (see "incidence") at 30°C. The cells were collected from the medium by centrifugation at 3000 rpm. They were then washed three times with 0.066M phosphate buffer at pH 7.0. The concentrated cells, 11 ml, were resuspended in phosphate buffer to a volume of 100 ml.

The substrates used for oxidation studies were prepared in 0.02M concentrations in 0.066M phosphate buffer-glucose, glutamic acid and fructose. Into each of four Warburg flasks were placed 2.5 ml of cell suspension; into the side arm was placed 0.5 ml of substrate (0.5 ml of 0.066M phosphate buffer in the case of the endogenous).

The conventional Warburg procedures were employed for manometric studies. The reaction vessels were equilibrated for 10 minutes at the water bath temperature of 30° before substrate addition.

Results:

In these substrate oxidation studies it was found that glucose was more effectively metabolized than was fructose, although other workers have found greater utilization of fructose. However, it will be noticed from Figures II and III that while CO_2 production is greater from glucose than from fructose, the substrate oxidation by this organism is greater for fructose than for glucose. Glutamic acid was actively utilized, so that this substrate has been incorporated into the synthetic medium.



SUBSTRATE OXIDATION-OXYGEN UPTAKE

FIGURE II

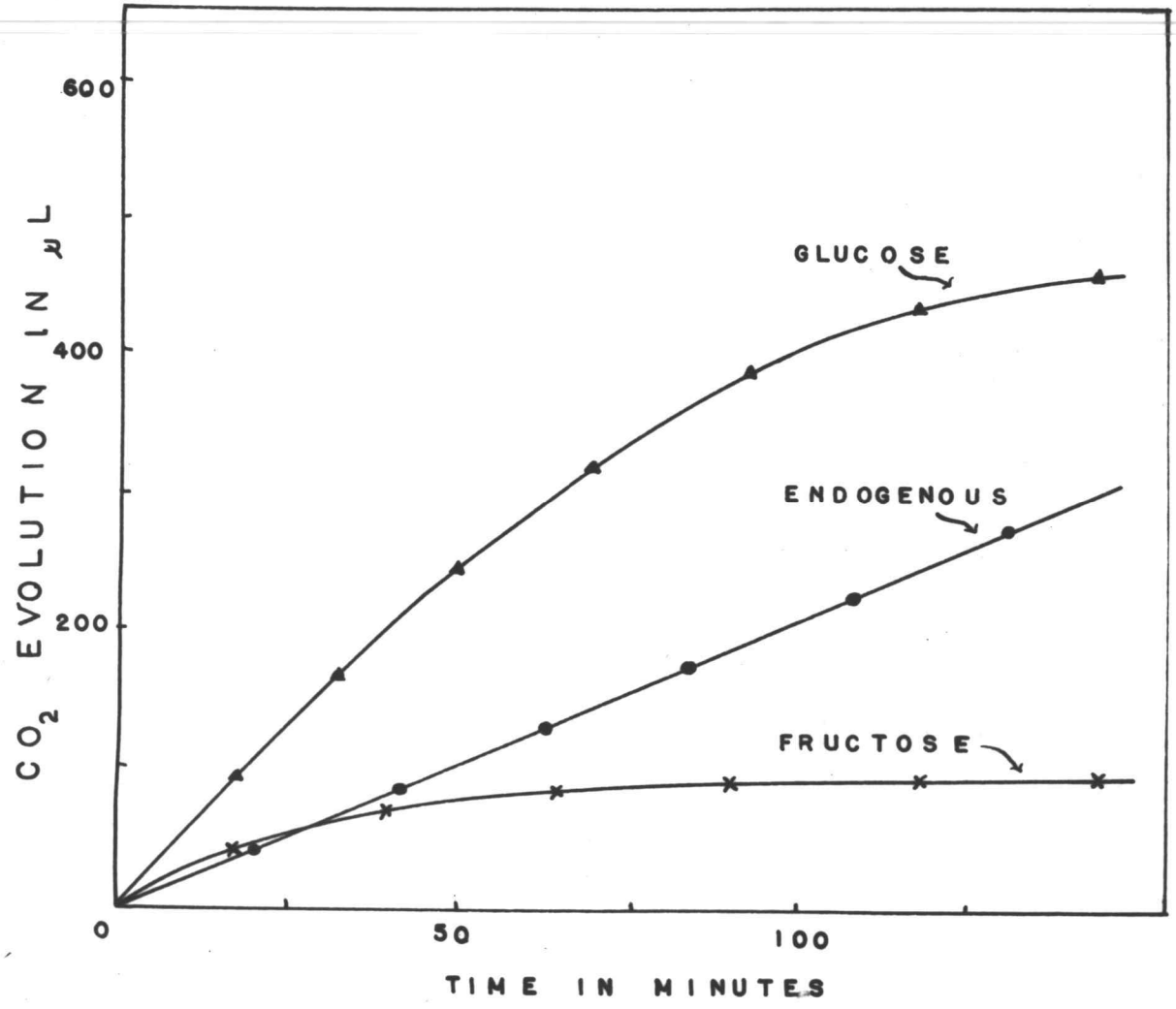
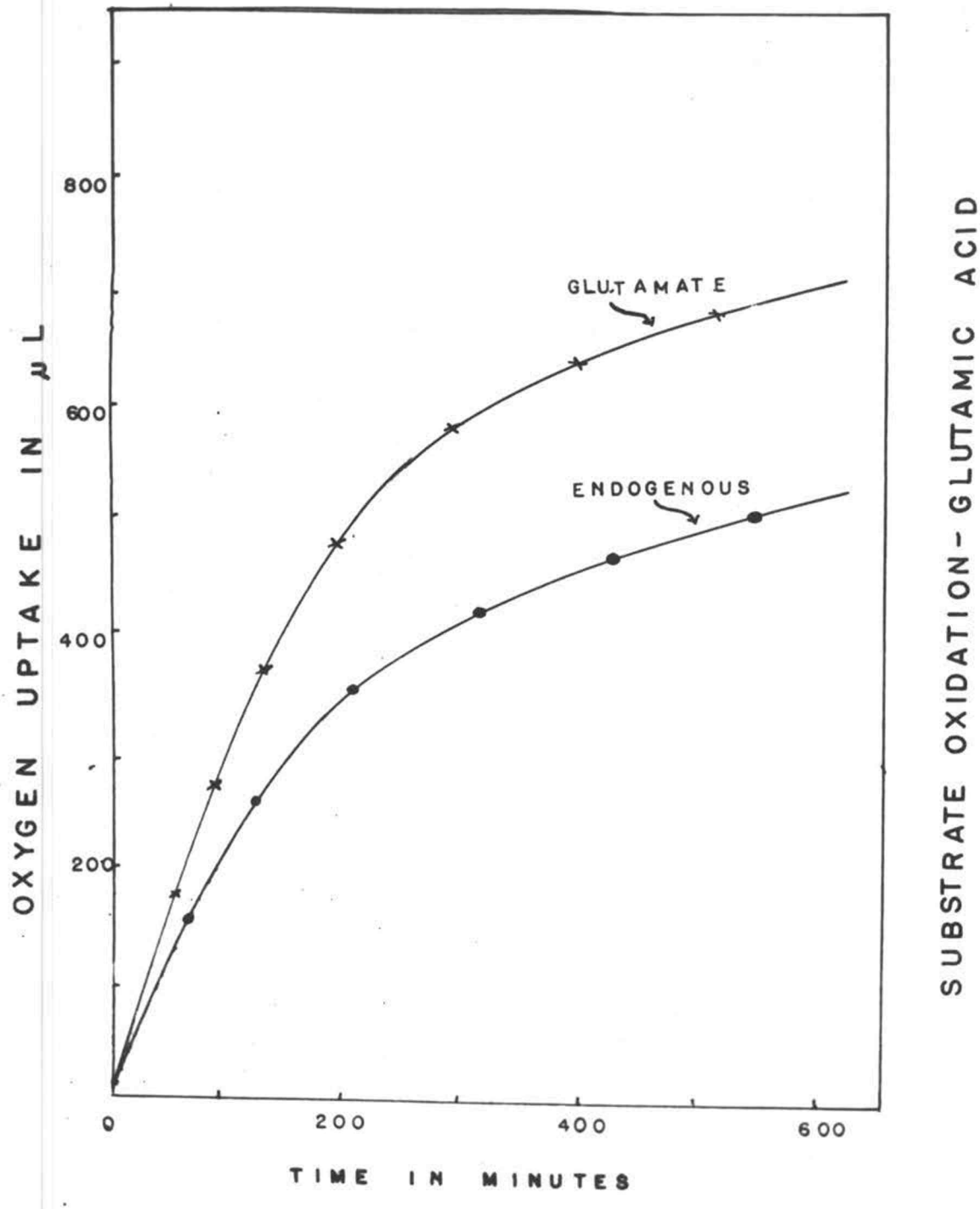


FIGURE III

SUBSTRATE OXIDATION ← CO_2 EVOLUTION

FIGURE IV


OXYGEN UPTAKE IN µL
800
600
400
200
0
200
400
600
TIME IN MINUTES
GLUTAMATE
ENDOGENOUS
SUBSTRATE OXIDATION- GLUTAMIC ACID

SUMMARY

Radiation sterilization studies for the preservation of meats in the fresh state have shown that a minimum of 5 megarep (5 million roentgen equivalent physical) is necessary for sterilization. In spite of the radiation level, a pink micrococcus was capable of survival, even with repeated exposure and increase of radiation up to 6 megarep.

Investigations were undertaken with this microorganism to determine its natural habitat, study its nutrition and metabolism, and attempt to characterize the factor, or factors, that contribute to its radiation resistance.

Studies on the incidence of this organism appear to show that meats are its natural habitat. A morphologically and physiologically similar microorganism was isolated from unirradiated meat samples. This organism possessed the same degree of radiation resistance observed in the species isolated from irradiated meats, indicating that the radiation resistance is not the result of mutation.

Nutrition studies showed this organism to have simple but fastidious requirements. Glucose and glutamic acid are utilized as carbon sources.

Methionine is the only essential amino acid; biotin and niacin are the only essential vitamins; thiamine is not an essential vitamin but it is an effective growth stimulant.

The requirement for methionine and biotin, and the stimulatory effect of thiamine proved of considerable interest. All three are sulfur-containing compounds, and sulfhydryl groups are known to give radiation protection.

The effect of metal ions in the presence of chelating agents was investigated. Copper sulfate, in concentrations ranging from trace amounts to toxic amounts, was studied. The organism was found to tolerate 0.1M copper sulfate (15,630 ppm). This fact could have some bearing both on mechanisms of radiation protection and on sulfur metabolism.

The effect of antibiotics was tested against this organism. All antibiotics tested were without exception effective against the organism, in agreement with the fact that known radiation-resistant microorganisms are sensitive to antibiotics. Studies on antibiotics plus radiation showed that antibiotics are destroyed in proportion to increases in radiation dosage.

The effect of spices plus irradiation on the survival of this microorganism was investigated. Spices

were used in concentrations generally used in foods. The results of this study showed that garlic, which has a sulfhydryl group, protected this microorganism from the effects of irradiation.

The irradiation of meats results in the formation of peroxides. Peroxides are toxic to microorganisms unless the organisms possess effective means of destroying the peroxides, such as peroxidase or catalase activity. Catalase studies showed great activity - 270 mcl oxygen was liberated from hydrogen peroxide per mg N of cells; the average bacterium liberates 80-100 mcl oxygen per mg N of cells.

Warburg respirometer studies were made using fructose, glucose and glutamic acid as substrates. All three substrates were actively utilized by this microorganism.

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