THE UNIVERSITY OF MICHIGAN
COLLEGE OF ENGINEERING
Department of Chemical Engineering

Final Report

INVESTIGATION OF RELATIVE MICROBIOLOGICAL RESPONSE TO VARYING TYPES AND METHODS OF APPLICATION OF IONIZING RADIATION

1 December 1959, to 30 November 1961

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OBJECTIVE

Commercial application of radiation sterilization of foods would necessarily involve larger radiation sources than those which have been used to collect research information on the process. Thus, for the design and operation of these larger sources, data would be needed concerning the effects of different field strengths, different beam (radiation) energies, and interruption of irradiation on the lethality of gamma radiations for the spores of Clostridium botulinum. Data are needed concerning the fate of spores of this bacterium because they must be killed in any food "sterilization" processes. The present study has been directed toward these objectives.

The contract stipulated that "should time and funds be available, studies will be pursued on the effect of intermittent irradiation on the lethality of electrons for spores of C. botulinum." A few exploratory runs were made in this connection.

CONTRACT RESEARCH PROJECT REPORT
QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES, CHICAGO

Hq, Qm Research and Development Command
QM Research and Development Center, Natick, Massachusetts

The University of Michigan
Office of Research Administration
Ann Arbor, Michigan

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SUMMARY

The influences of field strength, radiation energy, and intermittent irradiation on the lethality of gamma rays for Clostridium botulinum 62A and 213B spores have been evaluated. Three cobalt-60 and one cesium-137 radiation sources were used for this purpose. Variation in field strengths from 0.002 to 2.31 megarad per hour were utilized in the cobalt-60 sources. None of these variables was found to affect significantly the lethality of gamma rays for C. botulinum spores.

Therefore, insofar as the sporicidal effect of gamma radiations are concerned, the results show that data obtained on moderately weak radiation sources can be extrapolated for design purposes to field strengths of at least 2.31 megarad per hour; that interruption of irradiation for short intervals has little effect; and that cesium-137 should be essentially as effective as cobalt-60 sources for food-sterilization purposes.

INTRODUCTION

As the practical use of ionizing radiation for the sterilization of foods has become increasingly likely, some important questions have arisen about the implementation of this process on a large scale. Three such questions were investigated in detail in the present study; another was only briefly considered.

All pertain to the manner of application of the radiations.

(1) Most of the data upon which gamma-radiation sterilization dosages are based have been obtained with small radiation sources. It is therefore necessary to know whether these results can be applied directly to the much more intense radiation fields that would be used in large-scale operations.

(2) Much of the information available in the literature concerning the lethality of gamma radiation has been obtained with gamma rays from cobalt-60. Other sources could be used which have different gamma-ray energies; among the most likely of these is cesium-137. Here again a question must be asked: Do gamma rays from cesium-137 have a lethal effect on spores of C. botulinum different from that of similar rays from cobalt-60?

(3) Generally there is no conscious attempt to provide continuous irradiation when gamma radiation is used in research. It has been presumed that minor interruptions would not affect radiation sterilization dosages. Other investigators[1] have questioned this, however, and have suggested on increased lethal effect when the radiations are delivered intermittently.

(4) Finally, it is possible that high-energy electrons will also be used for food sterilization. The question of possible differences in the lethality of intermittent as contrasted with continuous irradiation becomes even more pertinent with this kind of radiation field because here pulsed radiations can be produced having pulse intervals in fractions of microseconds.[1] Hence, if an effect truly exists, the pulsations might enhance the lethal effects of such radiations for microorganisms.

These variables were all tested using spores of Clostridium botulinum because any food-sterilization process must, at the minimum, insure that spores of this microorganism be killed. Also, the contract was specifically limited to studies using these spores.

The C. botulinum spores were usually suspended in M/15 phosphate buffer at pH 7.0 during irradiation; however, some studies were included in which the spores were suspended in nutrient broth as well as some in which they were inoculated into canned ground beef.

During the course of the work, and in accordance with a discussion with a representative of the Quartermaster Corps, studies were made to observe effects of irradiation at room temperature on the survival of C. botulinum spores. These experiments were considered useful for comparison purposes because it may be desirable to irradiate food at room temperatures rather than under refrigeration in large-scale operations. This was a special group of experiments, however, the spores being refrigerated during irradiation in most of the work reported herein.

Four different sources of gamma radiation were used. These included three made of cobalt-60 and one of cesium-137. In addition, some exploratory runs were carried out in a high-energy electron source. Two of the cobalt-60 sources are located here at The University of Michigan: the other is an Atomic Energy Commission installation at Oak Ridge, Tennessee. The cesium-137 source is situated in Atlanta, Georgia, at the Georgia Institute of Technology, and the high-energy electron machine is located in the Midwest Irradiation Center at Rockford, Illinois, and is operated by Barnes and Company.

All the irradiations involved many variables requiring either intelligent control or quantitative knowledge of their existence when control was impractical or unnecessary. Every irradiation was, therefore, either done by, or carried out in the presence of a member of this research group. Similarly, the spores were grown, prepared, carried to and from irradiation sites, and counted by us.

Toward the termination of our studies for the Quartermaster Corps, we disposed of many cans of irradiated meat that had resulted from early inoculated pack studies and had been kept in storage for a number of years. Some of the cans were opened and tested for the presence of botulinium toxin.

MATERIALS AND METHODS

General

SPORES

Clostridium botulinum 62A and 213B spores were grown in liver extract and casein media, respectively. The methods described by Reed *et al.*[2] were used except that for growing the 213B spores, Difco BactoCasitone (10%) was substituted for the casein digest specified by Reed. The spores were harvested, washed by centrifugation, frozen in distilled water and stored at -40°C. For irradiation, the frozen suspensions were melted, heat-shocked at 85°C for 15 minutes, diluted into cold phosphate buffer or nutrient broth to a concentration of approximately 5,000,000 spores per ml, and then dispensed into 5-ml glass vials. The necks of the vials were sealed in a flame and the vials then packed in crushed ice for immediate irradiation or transportation to the irradiation site.

Suspending Media

The spores were suspended in M/15 phosphate buffer (pH, 7.0) or in nutrient broth of the following composition:

3-gm beef extract
5-gm NaCl
10-gm peptone
1000-ml distilled water

Counting

The spores were counted by dilution into Prickett tubes containing pork extract agar, followed by incubation at 30°C for five days and enumeration of the number of colonies developed.

COBALT-60

The sources at the Fission Products and Phoenix Laboratories of The University of Michigan are similar in design and operation. They differ only in minor details and in the strength of the radiation fields provided; because of a somewhat more compact spacing of the cobalt-60 rods, the Phoenix source provides a stronger field in a smaller zone. The maximum field strength used in the Fission Products Laboratory source was 0.160 megarad per hour; in the Phoenix Laboratory source, it was 0.591 megarad per hour. The source in the Fission Products Laboratory has been completely described in the literature,[4] a description that suffices, except in minor detail, for the Phoenix source. In both sources, irradiation was conducted in the "center-well," and also at various distances from the outside of the cylindrical carrier in which the cobalt-60 rods are contained. This provided fields whose intensity could be varied at will for essentially any strength below the maximum.

At the Oak Ridge National Laboratories, a large number of cobalt-60 rods were made available as a radiation source. Because this is really a storage accumulation of cobalt-60 and not primarily an irradiation source, the strength varied from time to time due to addition or removal of cobalt-60: the maximum intensity used here was 2.310 megarad per hour. When this source was used, the vials were packed in crushed ice in a Dewar flask, lowered into the radiation zone for the required time and then raised out of this zone to stop the irradiation. The radiation zone consisted of a cubical enclosure surrounded by cobalt-60 rods which in turn were enclosed by an underground, concrete vault.

Availability of the cesium-137 source at the Georgia Institute of Technology in Atlanta, Georgia provided an opportunity to use gamma radiation having an energy of 0.662 mev rather than the 1.25-mev average energy of the cobalt-60 gamma rays. The field strength of this cesium-137 source was 1.42 megarad per hour at the time our experiments were conducted. Fortunately, this field strength lies in between those of the cobalt-60 sources at the Phoenix and Oak Ridge National Laboratories, which allowed an additional opportunity for correlation of the field strength on the lethality of gamma rays for spores of C. botulinum. A detailed description of this source is available in a report of the Georgia Institute of Technology.[5]

A few exploratory experiments were conducted on a linear accelerator located at the Midwest Radiation Center and operated by Barnes and Company. In these studies, we were chiefly involved with conducting the bacteriological work, the details of the source design and operation were matters of concern to the Quartermaster Corps representatives, who helpfully arranged for use of the source and participated in its operation. Therefore further discussion of the source is omitted.

Dosimetry

Dosimetry was needed for two interrelated but different purposes. It was necessary to be able to relate all the gamma-ray sources to a reference standard and also to measure precisely various radiation field strengths within the individual sources. Fricke dosimetry[6] was used for both purposes. However, due to the recognized difficulties with Fricke dosimetry at high dose rates and also to the convenience of Hoecker dosimeters,[7] the latter were also used to relate dosage levels between sources. This procedure also had the desirable quality of using two different chemical principles with which to verify dosage rates.

Fricke dosimetry depends upon the oxidation of ferrous to ferric ions. The yield of the oxidation reaction was calculated according to standards established by the Quartermaster Food and Container Institute, one of whose representatives visited Ann Arbor during July 1960, to check our dosimetry. Their results agreed almost exactly with ours on parallel runs conducted on the Fission Products Laboratory source. Specifically, our calculations are based upon a rep as being that dose of ionizing radiation capable of producing energy absorption of 93 ergs per gram tissue and a rad as that dose producing energy absorption of 100 ergs per gram of substance irradiated. In a like manner the Fricke, or ferrous-ferric, dosimetry was based upon the oxidation of 15.4 micromoles of ferrous ions per liter per 1000 rep.[8]

Dosimetry in the linear accelerator was carried out by representatives of the Quartermaster Corps and the operating personnel of the accelerator. Their data for dose rates were accepted by us for the few exploratory runs that were undertaken.

Toxicity Determinations in Stored, Irradiated Meat

A number of 202 x 202 tin cans containing ground beef that had been inoculated with C. botulinum 62A spores and then stored in our laboratory were examined for the presence of toxin. For this purpose, all the ground meat in the cans was extracted with a cold gelatin-phosphate buffer (pH 7.0). The extract was filtered through a pad of glass wool and 0.25 ml was then injected into 10- 15-gm albino mice. When positive results occurred, the identity of the toxin was checked with specific type A antitoxin.

SURVIVOR CURVE STUDIES

I. Field Strength

Various gamma radiation field strengths were obtained by use of different sources and where feasible by utilizing the decreasing field strengths available at increasing distances from a source. Three cobalt-60 and one cesium-137 sources were used. The design of the two cobalt-60 sources at The University of Michigan allows the selection of an almost infinite number of field strengths below the maximum by merely assigning a suitable spatial location within the irradiation room and then raising the source into the room. This procedure was used at the Phoenix and Fission Products Laboratories. Furthermore, at these sources, as well as at the Oak Ridge National Laboratory and in the cesium-137 source in the Georgia Institute of Technology, the more intense fields, inside these essentially cylindrical sources, were also used.

In all these studies the vials containing spore suspensions were packed in crushed ice and irradiated at constant field strengths, the desired total dosages being obtained by utilizing different irradiation times. Some additional studies were conducted with The University of Michigan cobalt-60 sources at the ambient temperatures of the irradiation rooms rather than at the temperature of ice baths.

II. Beam Energy

Two gamma radiation energies were compared for their lethal effect on C. botulinum spores. These energies were the average 1.25 mev of cobalt-60 and the 0.662 mev of cesium-137. Spore suspensions were prepared in phosphate buffer or in nutrient broth as previously described.

Both strains 62A and 213B were used. Approximately 4 ml of a suspension were transferred into 5-ml vials which were then sealed in a flame. For irradiation in the cobalt-60 source in the Fission Products Laboratory, the vials were packed, along with crushed ice, in a Dewar flask which was then positioned in the center-well of the source. The ice did not melt appreciably during a run, so the irradiations were carried out at temperatures between 0° and 4°C. Vials were removed at desired time intervals and taken to the laboratory for counting. The spores were counted by dilution into Prickett tubes containing pork extract agar, followed by incubation at 30°C for five days and enumeration of the number of colonies developed.

This run was then duplicated using the 12,000-curie Cs-137 source of gamma radiation at the Georgia Institute of Technology in Atlanta, Georgia. Samples were transported in a portable ice box.

For irradiation purposes in this source, three sample vials were locked into position in a test tube by means of a special positioning device. Crushed ice was packed around the vials and the test tube and then placed in the source. The dose rate was 23,700 rads per minute in these vials based upon ferrous sulfate dosimetry at Georgia Tech. This dosimetry, carried out by Georgia Tech personnel checked exactly with the Hoecker dosimeters; the FPL source dosimetry differed slightly. Specifically, at the Fission Products Laboratory we found a gamma-ray field strength of 95,000 rad per hour by Fricke dosimetry and 111,000 rad per hour by the Hoecker dosimeter. In the Georgia Tech source, both their ferrous sulfate and Hoecker dosimetry showed a field strength of 1.42×10^6 rad per hour where our vials were irradiated.

To compare the Georgia Tech and FPL in sources on a uniform basis, the Hoecker dosimetry used by us in both places was accepted as a standard for plotting our results. This involved increasing the dosages obtained with our Fricke-type dosimetry at FPL by a factor of 111,000/95,000 for this purpose since one hour of irradiation in the center-well of this source delivered 95,000 rads by Fricke dosimetry.

IIIA. Intermittent vs. Continuous Irradiation

The lethal effects of gamma radiations from cobalt-60 delivered continuously and intermittently were compared on both the Fission Products and Phoenix Laboratory sources to include the additional factor of the different field intensities of these two sources in this comparison. The irradiation techniques were essentially similar in the two sources. Most of the work was done at ice-bath temperatures of 0°-4°C, with the spores suspended in phosphate buffer as described in detail hereafter. However, some studies were also carried out at 25°C, and also in nutrient broth and in trypticase-yeast-extract broth for comparison purposes. At the Fission Products Laboratory the spores of three strains of C. botulinum were tested, namely, 62A, 213B, and 457A.

The spores were diluted in M/15 phosphate buffer at pH 7.0 to a concentration of approximately 5,000,000 cells per ml and then pipetted into 5-ml glass vials. These vials were sealed in an oxygen-acetylene flame, and placed in a rack. The rack constitutes part of an apparatus that fits into the center-well of the cobalt-60 radiation source in our Fission Products Laboratory. It allows the temperature of the spores to be controlled ±1°C during irradiation over a rather wide temperature range. The spores were then irradiated while packed in crushed ice and suspended in the phosphate

buffer. Vials were removed at desired time intervals and taken to the laboratory for counting. The intensity of the irradiation field was approximately 0.158 megarad per hour during most of these experiments. The spores were counted by dilution into Prickett tubes containing pork extract agar, followed by incubation at 30°C for five days and enumeration of the number of colonies developed.

Specifically, an experimental group consists of three different runs. These were designated UIA, HIA, and NIA; the first two letters indicate two minutes, thirty minutes, or no interruption, respectively; the A stands for C. botulinum 62A spores. For strain 213B spores UIB, HIB, and NIB designations were used.

The two-minute and half-hour-interruption runs were conducted by placing all the vials in the rack, setting the rack in place, raising the source into position, and allowing irradiation to proceed for 2.5 hours. Then the source was lowered into the well. After a two- or thirty-minute period, a vial was removed; then the source was again raised into radiation position. After 45 minutes, the process was repeated and this procedure was continued until approximately one megarep of radiation had been delivered. Thus each sample removed, after the first 2.5-hr sample, had progressively one more interruption than the preceding sample.

For the "uninterrupted" run, a vial containing the spore suspension was placed in the rack, irradiated for a 2.5-hr interval and removed. Then another vial was placed in position, irradiated for 3.25 hr, and removed, etc. Hence each successive sample received the desired amount of irradiation without interruption.

At the Phoenix Laboratory, for the continuous irradiation studies, the vials received gamma rays at a rate of 0.490 megarad per hour, with no interruption. In the intermittent type of run, the vials were irradiated for 0.5 hour; then the source was lowered into the well and two vials were removed during the two-minute interval that the source was "down." These vials were then taken to the laboratory where the spores were counted. The source was next returned to irradiating position for 0.5 hour. This procedure was continued at one-half-hour intervals until approximately 1.5 megarad of gamma radiation were delivered.

IRRADIATION AT ROOM TEMPERATURE

The spores were heat-shocked as usual for 15 minutes at 85°C, diluted into cold M/15 phosphate buffer (pH 7.0), nutrient broth, or trypticase-yeast-extract broth, and transferred into 5-ml vials. The vials were then sealed in a flame, and irradiated in the center-well of the Fission Products Laboratory's cobalt-60 source, with the spore suspensions at the ambient temperature in the source. This temperature was near 25°C during these experi-

ments.

Both continuous and intermittent irradiation was applied to further evaluate the effect of this variable on the lethality of gamma radiation. For intermittent irradiation, all the samples were placed in the center-well initially; then vials were taken at increments of about 200,000 rad to count the remaining viable spores. For continuous irradiation experiments, vials were placed in the center-well as before; then all vials were removed after the desired amount of irradiation had been applied. This process was repeated for the next level of irradiation. No interruption of irradiation of any kind was allowed during each interval.

The surviving spores were counted as usual, using growth in pork infusion agar in Prickett tubes at 30°C as the criterion of viability. Other tests included:

(1) Evaluation of possible germination and growth, by holding some vials at the temperature of irradiation but not in the irradiation chamber. Some of these spores were then counted in the same way as the irradiated spores to observe any increase in numbers which could develop due to growth. Another portion of this suspension was heated to 90°C for 15 minutes and then counted. A reduction in count here would indicate germination. Also, crystal violet stains were examined to see if any germinated spores could be observed.

(2) Check of possible development of toxin in the spore suspension held at room temperature for the period of time that equalled the longest irradiation interval for spores in the center well of the cobalt-60 source was accomplished by inoculating 0.25 ml of this suspension intraperitoneally into 10—15-gm albino mice.

IIIB. Inoculated Pack Studies

In addition to irradiating C. botulinum spores in phosphate buffer and in broth, inoculated pack experiments were carried out at the Fission Products Laboratory to further test possible differences in the lethality of gamma radiation when delivered continuously or intermittently. For this purpose lean beef was ground in a clean, mechanical grinder, packed into 204 x 214 tin cans, inoculated with approximately 10,000 C. botulinum 62A spores per can, sealed in a vacuum closing machine, and then irradiated. The cans were kept refrigerated during irradiation either by dry ice or by the ambient low temperature of the irradiation "cave" during the winter. Cans of meat awaiting irradiation were placed in a refrigerator.

Here, continuous irradiation means that the gamma-ray field remained at the same intensity for the entire irradiation period. Interrupted irradiation involved five intervals of at least 1/2 hour each during which no gamma

radiation passed through the meat.

HIGH-ENERGY ELECTRON STUDIES

Exploratory experiments were made on two occasions using the Mark I linear accelerator at the Midwest Research Institute in Rockford, Illinois. This accelerator is operated by Barnes and Company. These experiments were conducted to learn whether the lethality of the high-energy electrons from the accelerator was affected by changes in the dose rate or by delivery of the electrons in a continuous or intermittent manner.

The C. botulinum 62A spores used in these experiments were the same as those used for the studies with gamma rays. These spores were suspended in phosphate buffer or nutrient buffer, 5 ml were dispensed into 30 x 60-mm flat-top weighing bottles to a depth of approximately 14 mm and the bottles packed in crushed ice, transported from Ann Arbor, Michigan, to Rockford, Illinois, irradiated, and returned to Ann Arbor for counting. The weighing bottles, containing the spores, were irradiated individually. They were packed in crushed ice and covered with a few mm of ice water.

Dosimetry was kindly carried out for us by representatives of the QMC; cobalt glass was used in both experiments; in the second experiment, ceric sulfate dosimetry was used also. The cobalt glass dosimeters were irradiated in the same position in which the spores were to be irradiated. The dose rate was redetermined each time a change was made in the setting of the accelerator.

Changes in dose rate were produced by varying the distance between the portal and the weighing bottle and also by varying the pulse rate. For the intermittent run in the second experiment, the same total dose was applied in both a continuous and in an intermittent manner using different spore suspensions in each case. The intermittent irradiations involved a "fractionation" procedure in which two intermissions of thirty seconds each were provided between the three equal irradiation periods and in which no radiations passed through the spore suspensions.

The radiation resistance of the C. botulinum spores was also determined, as a control, by running a survivor curve on them with the cobalt-60 source in the Fission Products Laboratory.

RESULTS AND DISCUSSION

I. Field Strength

The data plotted in Fig. 1a have been consolidated to include most of the survivor curves we have run during the past three years on C. botulinum 62A spores in phosphate buffer at ice-bath (0-4°C) temperatures. It will be noted that data are presented from all the gamma-ray sources used by us including the cesium-137 source. These latter data were included for two reasons: (1) the strength of the source is at a fortuituous value insofar as the distribution of field strengths is concerned; (2) their inclusion reinforces the observation that no significant difference exists between the lethality of gamma rays from cobalt-60 and cesium-137 for spores of C. botulinum 62A under the experimental conditions used.

Run OR-2 from the Oak Ridge National Laboratory is plotted with a dotted square symbol because the dosimetry on that date was not as well defined as it should have been. On the previous occasion the field was essentially of homogenous intensity; in Run OR-2 it was found to be 1.88 to 2.2 megarad per hour in the two flasks in which Hoecker dosimeters were placed. No dosimeter was used in the third flask. Since the vials were not designated according to flask, the resulting datum point is plotted, with reservations, at a field strength of 2.0 megarad with the realization that if the field strength really was at 2.2 megarad the point would have shifted to coincide with that of OR-1.

The data in Table I and Fig. 1a indicate that, within the range of field strengths from 0.002 to 2.42 megarad per hour, there is no significant difference in the lethality of gamma rays from cobalt-60 for the spores of C. botulinum 62A suspended in phosphate buffer at ice bath (0-4°C) temperatures. Data recorded in Table I were calculated from survivor curves given in the final report of our previous QMC project[9] and from similar survivor curves shown in Figs. 2a, 3a, 4a, 5a, 6a, and 20c. A review of the calculations resulted in slight modifications of the previously reported percentage survivors at 0.8 megarad for runs I-R7 and I-R10 from the previous report.[9]

TABLE I

EFFECT OF INTENSITY OF IRRADIATION ON THE LETHALITY OF GAMMA RADIATION FROM *COBALT-60 FOR THE SPORES OF CLOSTRIDIUM BOTULINUM 62A WHEN THEY ARE SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0 AND ARE IRRADIATED AT 0°C

Run No.	Source**	Intensity, Megarad per hr	Initial Spore Inoculum, Millions per ml	% Survivors at 0.8 Megarad	Log % Survivors at 0.8 Megarad
I- 4	FPL	0.002	1.300	0.08	-1.1
I- 6	FPL	0.039	1.700	0.08	-1.1
I- 8	FPL	0.039	1.900	0.04	-1.4
I- 2	FPL	0.193	1.900	0.12	-0.9
T- 5	FPL	0.203	7.150	0.20	-0.7
I- 9	FPL	0.193	1.580	0.22	-0.65
T- 1	FPL	0.203	1.400	0.10	-1.0
I-10	P	0.591	0.990	0.50	-0.3
I-11	P	0.591	1.100	0.63	-0.2
I- 7	P	0.591	4.650	0.04	-1.4
DR- 7	P	0.013	4.700	0.01	-2.0
OR- 1	OR	2.310	4.600	0.10	-1.0
CA- 4	P	0.490	1.260	0.316	-0.50
IA- 4	P	0.490	5.300	0.398	-0.40
IA- 6	P	0.490	2.600	0.64	-0.2
IA- 9	FPL	0.095	2.900	0.12	-0.9
GT- 1	GT	1.42	1.390	0.12	-0.9
GT- 2	GT	1.42	0.765	0.10	-1.0
IA-13	FPL	0.095	2.000	0.10	-1.0
IA-14	FPL	0.095	24.000	0.12	-0.9
DR-7a	P	0.1296	23.000	0.25	-0.6
DR-7b	P	0.0353	23.000	0.09	-1.05
DR-7c	P	0.0133	23.000	0.032	-1.5
DR-2	P	0.438	11.400	0.4	-0.4
DR-7d	P	0.0867	11.400	2.0	0.3
OR-2	OR	2.0	10.700	0.01	-2.0

* Runs GT-1 and GT-2 were made in a cesium-137 source

** FPL Fission Products Laboratory - cobalt-60
P Phoenix Laboratory - cobalt-60
OR Oak Ridge National Laboratory - cobalt-60
GT Ga. Inst. Tech - cesium-137

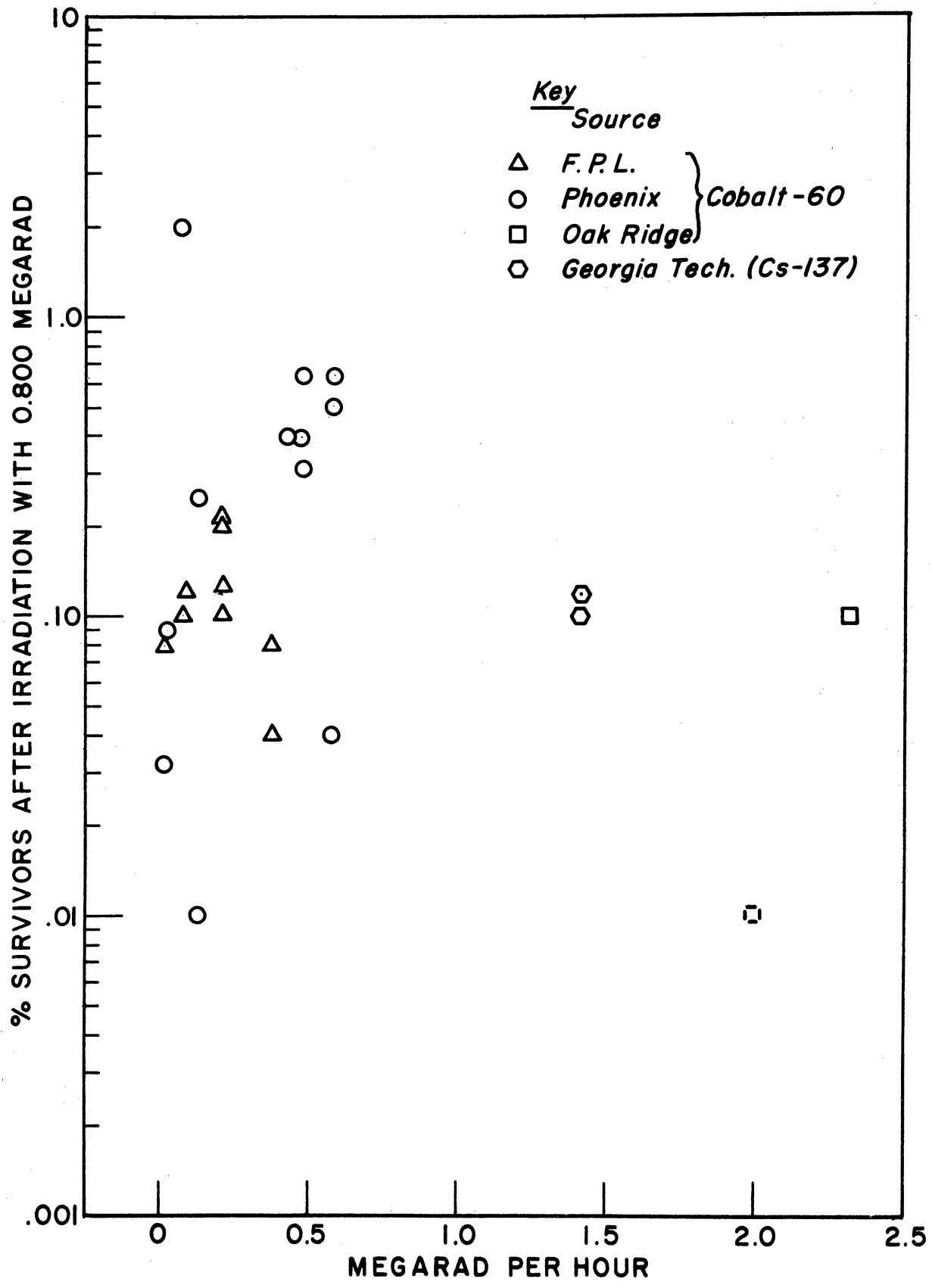


Fig. 1a. Effect of intensity of irradiation on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 62A when they are suspended in M/15 phosphate buffer at pH 7.0 and are irradiated at 4°C. This plot includes two runs made on a cesium-137 source.

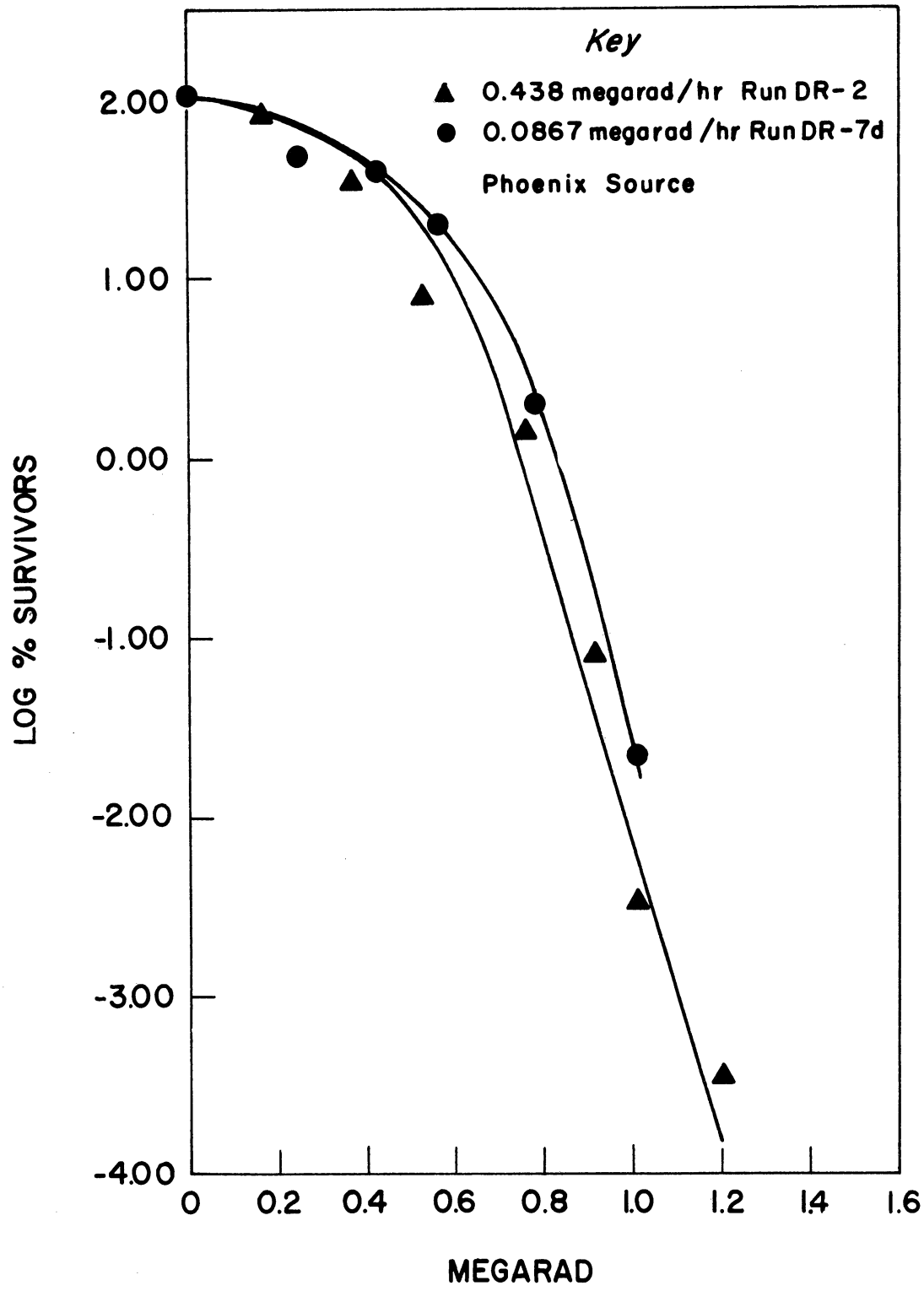


Fig. 2a. Effect of variable radiation field intensity on the lethality of gamma radiation from cobalt-60 for Clostridium botulinum 62A spores suspended in M/15 phosphate buffer and irradiated at 0°C.

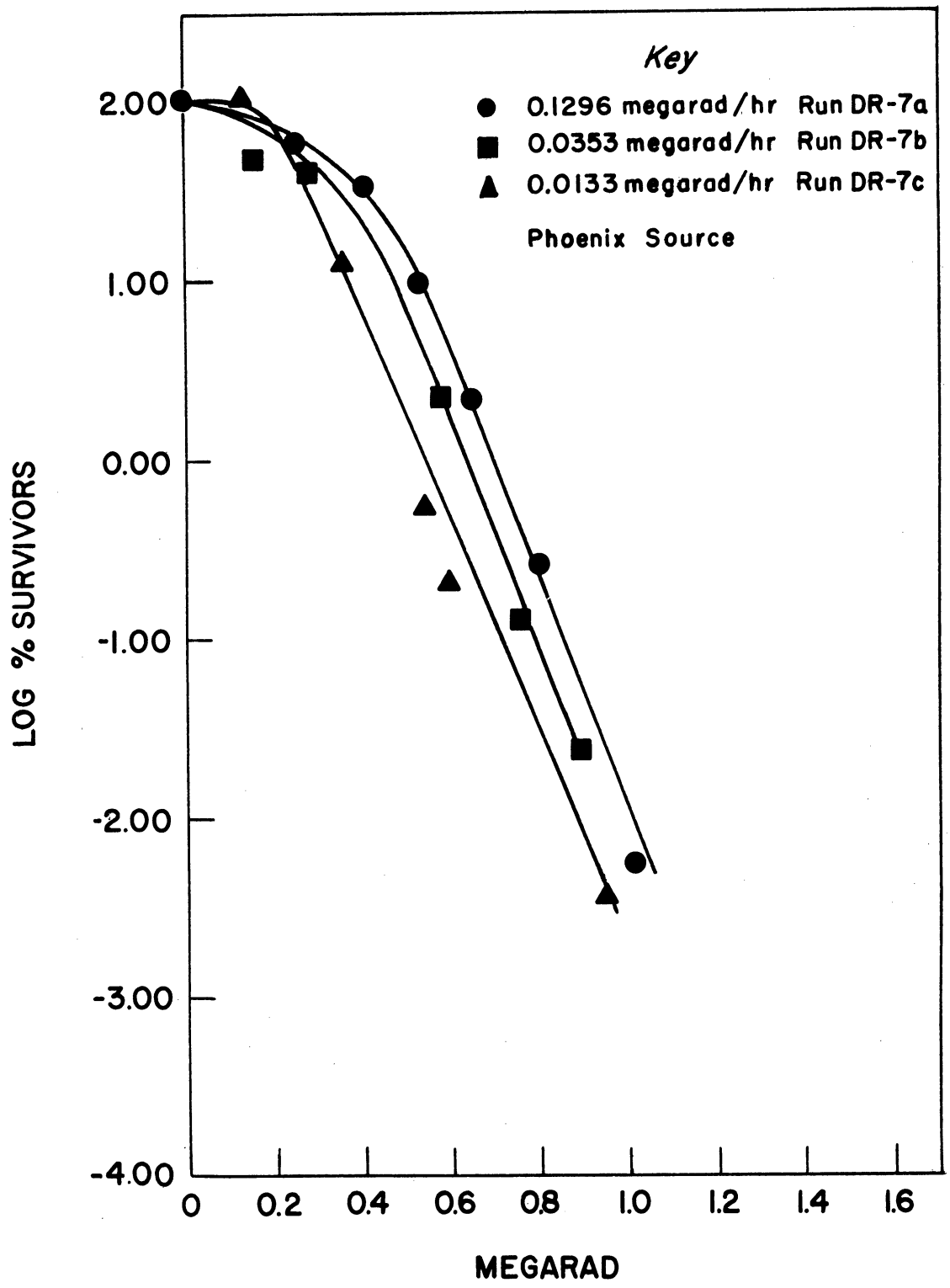
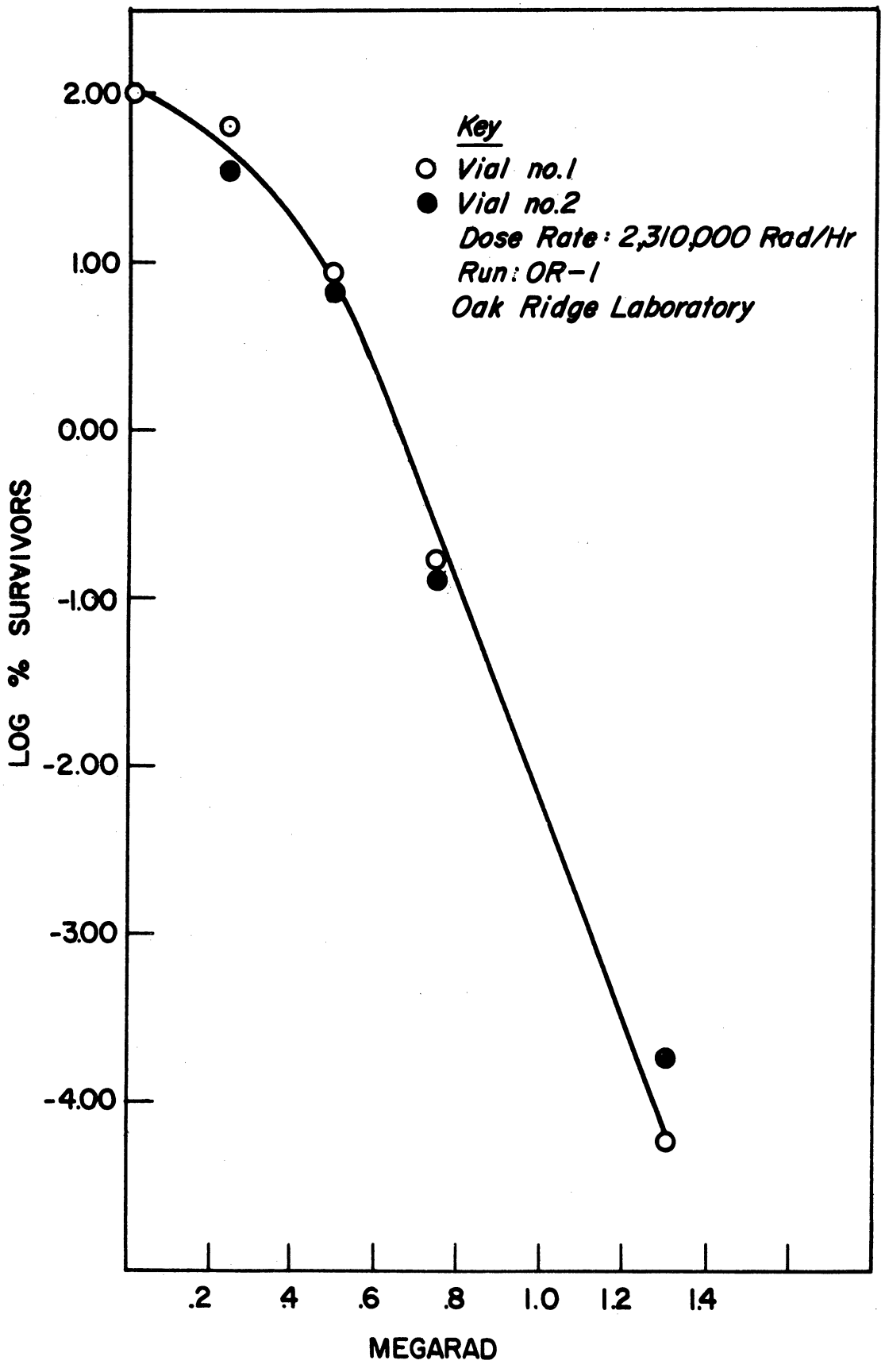


Fig. 3a. Effect of variable radiation field intensity on the lethality of gamma radiation from cobalt-60 for Clostridium botulinum 62A spores suspended in M/15 phosphate buffer and irradiated at 0°C.



Lethality of gamma rays from cobalt-60 for Clostridium botulinum 62A spores suspended in M/15 phosphate buffer at pH 7.0.

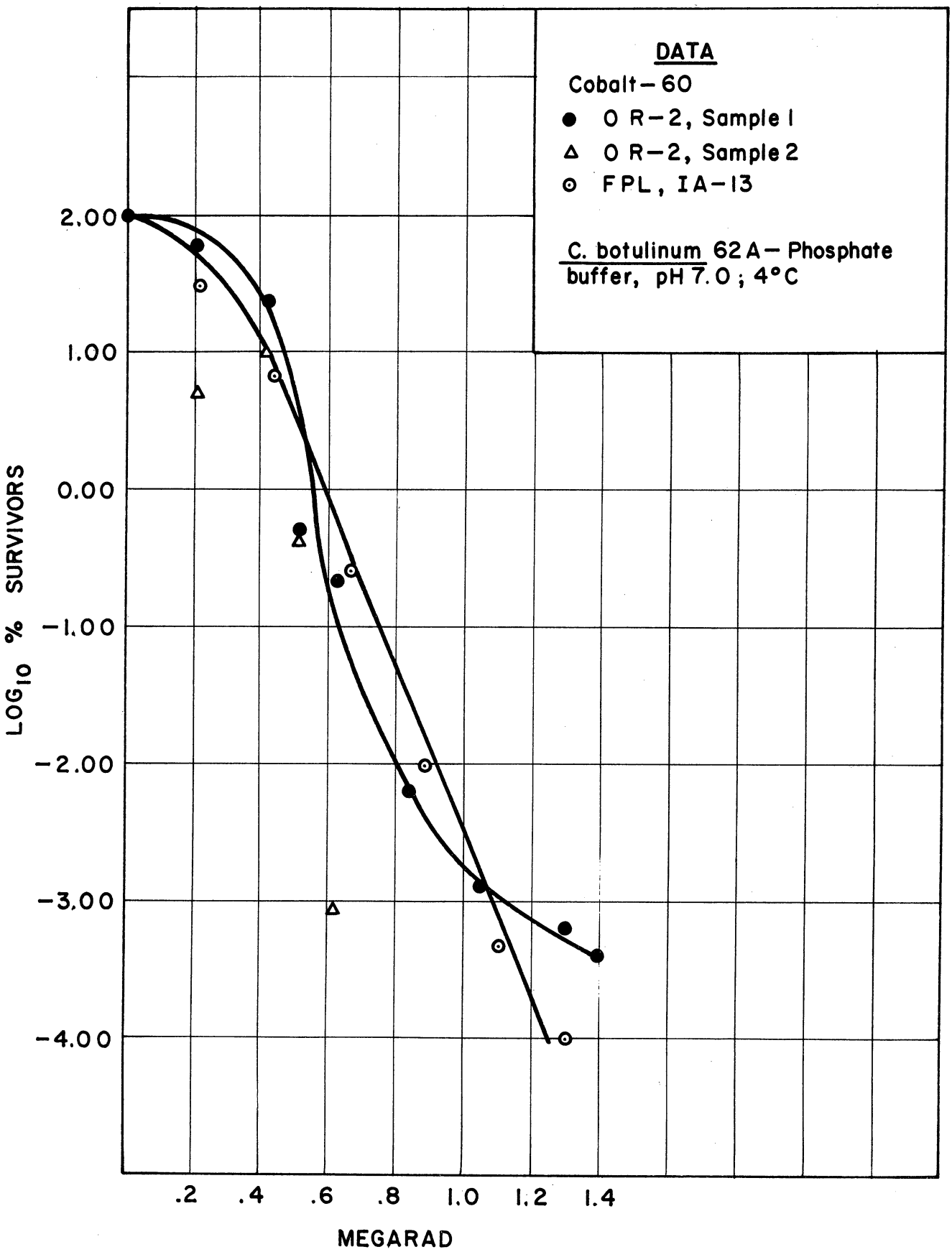


Fig. 5a. Effect of radiation field intensity on the lethality of ga rays from cobalt-60 on C. botulinum 62A spores suspended in M/15 pho phate buffer at pH 7.0 and irradiated at 4°C.

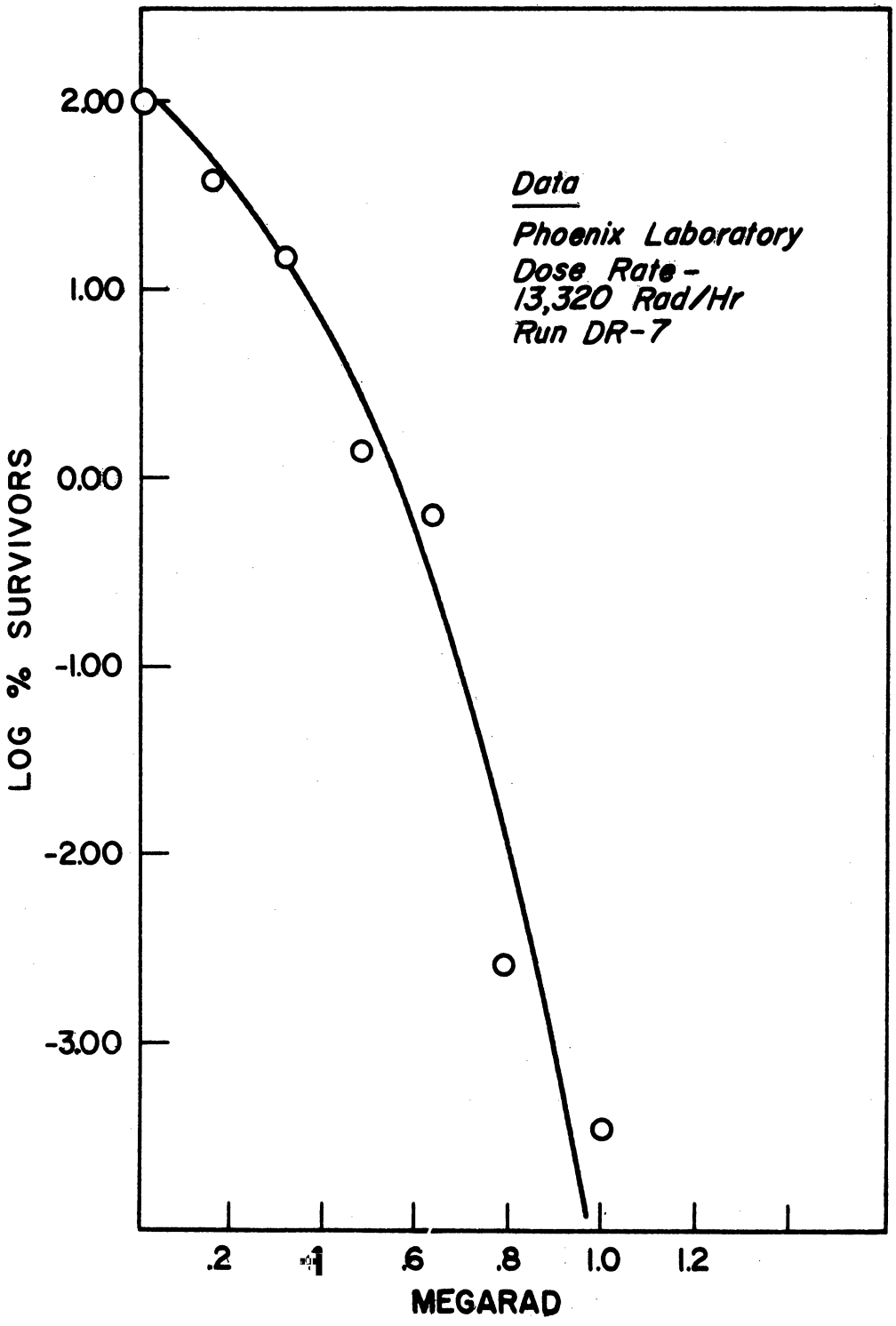


Fig. 6a. Lethality of gamma rays from cobalt-60 for Clostridium botulinum 62A spores suspended in M/15 phosphate buffer at pH 7.0.

II. Beam Energy

The data plotted in Figs. 1b, 2b, and 3b allow comparison of the lethality of the 1.25 mev gamma rays from cobalt-60 with those of 0.662 mev from cesium-137 for spores of C. botulinum 62A and 213B. The data are so closely alike for these two sources that in each of Figs. 1b and 2b, only one curve is plotted. So, for the conditions of these experiments, there is no distinguishable effect of beam energy on the lethality of gamma rays from cobalt-60 and cesium-137 for the spores of C. botulinum strains 62A and 213B.

III. Intermittent vs. Continuous Irradiation

Two types of experiments were used to evaluate this variable, namely, survivor curves and inoculated packs of ground beef. Furthermore, two kinds of survivor-curve experiments were used: in the first, the entire survivor curves were obtained and studied; in the second, a number of vials of spores were irradiated to 0.632 megarad and the number of remaining viable spores at this level of irradiation were evaluated.

SURVIVOR CURVES

The data in Figs. 1c and 2c offer a comparison of the relative lethality of continuous and intermittent gamma irradiation on spores of C. botulinum 62A. These spores were suspended in M/15 phosphate buffer at pH 7.0 and irradiated in an ice bath (0-4°C) at the Fission Products Laboratory. The resulting curves reveal no important difference in the lethality of these rays for C. botulinum spores that can be ascribed to irradiation in a continuous or intermittent manner. Although a comparable percentage of survivors was recorded for the intermittent irradiation in each instance with between 5 and 10% less radiation, differences of this magnitude probably do not have much significance in these experiments.

Furthermore, due to the manner in which these experiments were conducted, there should be an accumulation of an effect of the "rest" periods involved in the intermittent runs. Such an effect would cause the survivor curves to diverge as the number of interruptions increased: this was not evident.

Further comparisons of survivor curves for C. botulinum 62A spores suspended in phosphate buffer are given in Figs. 3c and 4c. These curves are based on data taken with the cobalt-60 source in the Phoenix Laboratory. The data could all be fitted to one curve. If any distinction is to be

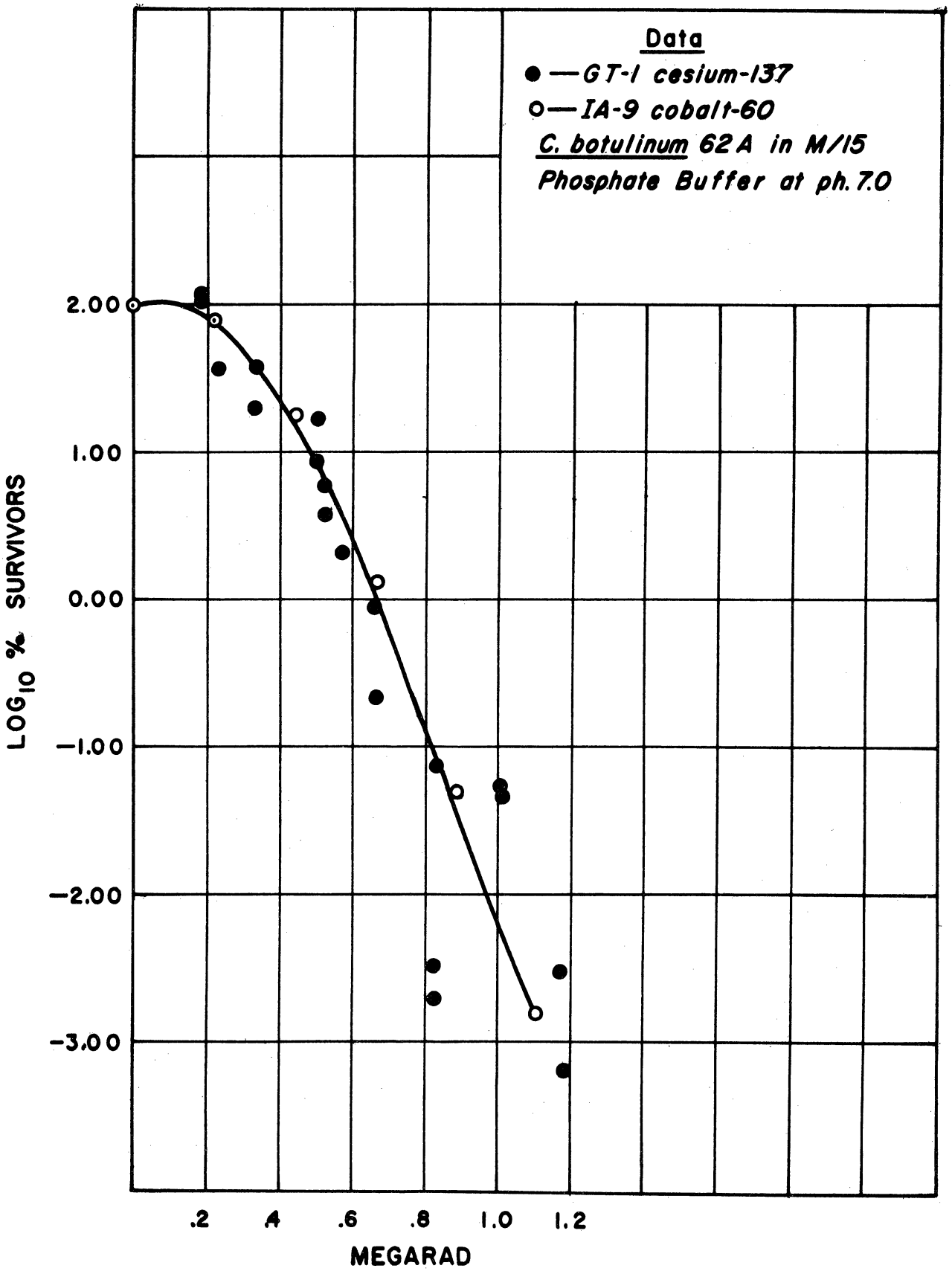


Fig. 1b. Effect of beam-energy on the lethality of gamma radiation for spores of Clostridium botulinum 62A suspended in M/15 phosphate buffer at pH 7.0.

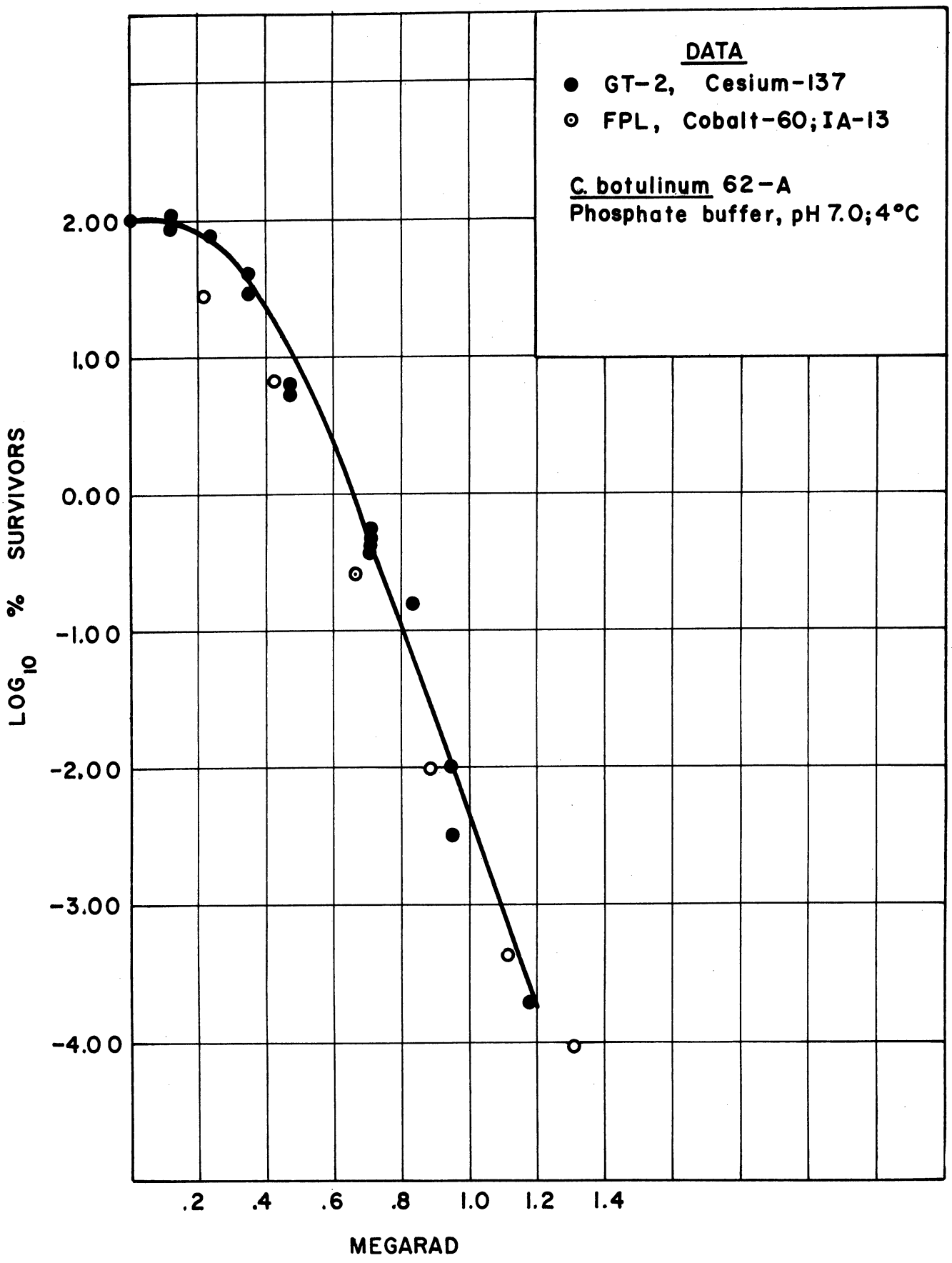


Fig. 2b. Comparison of the lethality of gamma rays from cobalt-60 and cesium-137 for C. botulinum 62A spores suspended in M/15 phosphate buffer at pH 7.0 and irradiated at 4°C.

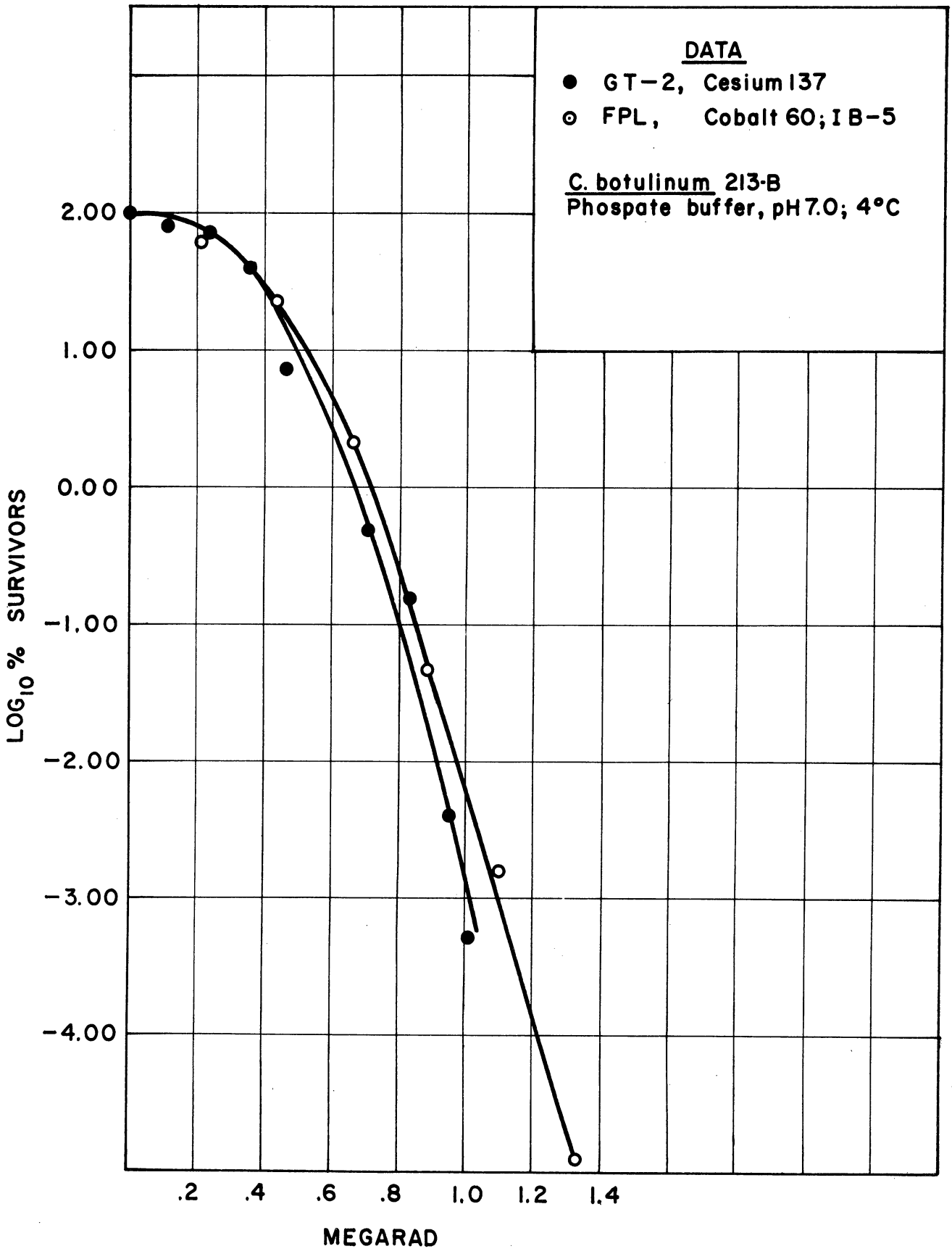


Fig. 3b. Comparison of the lethality of gamma rays from cobalt-60 and cesium-137 for C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0 and irradiated at 4°C.

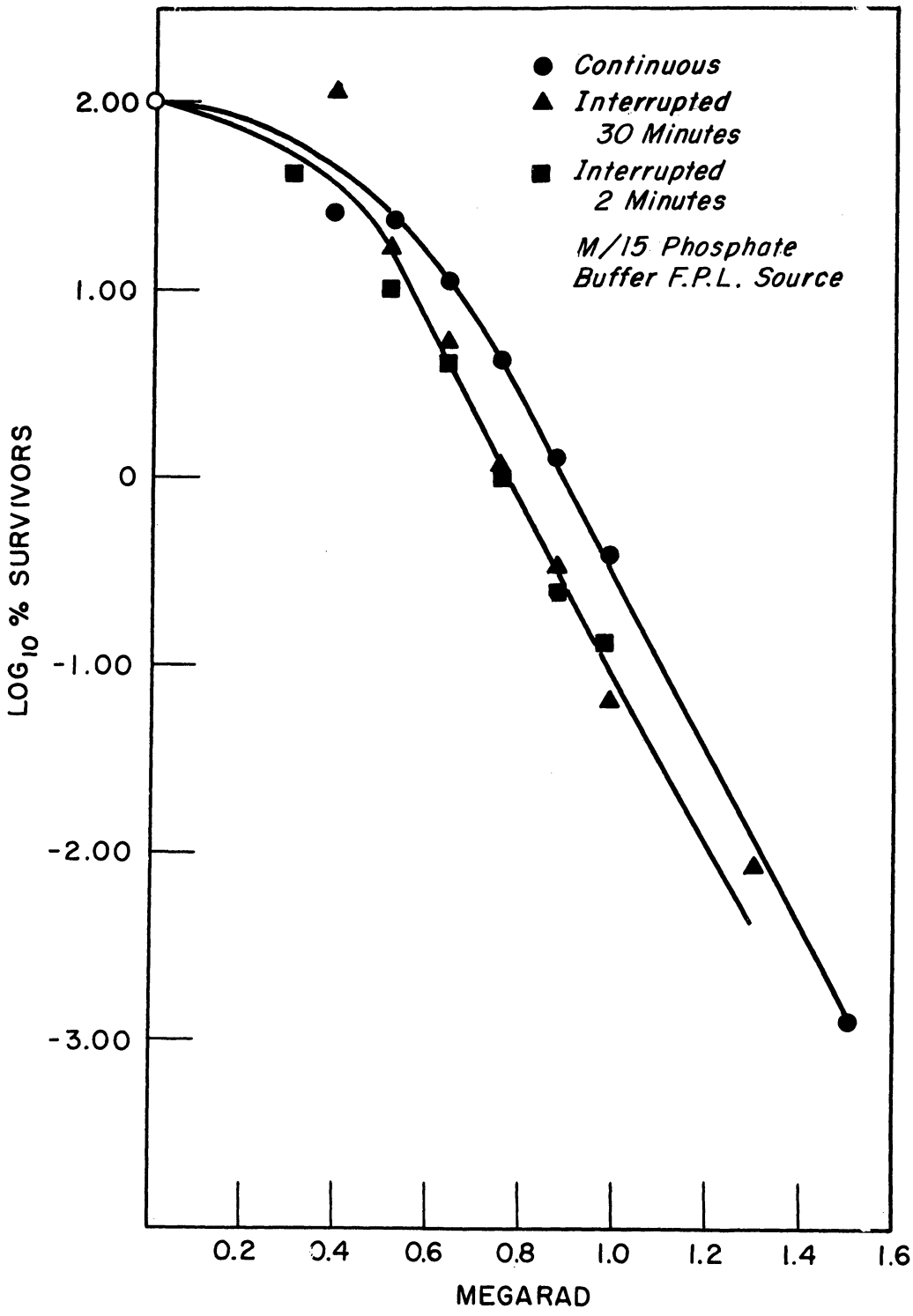


Fig. 1c. Effect of continuous vs. interrupted irradiation on the lethality of gamma radiation from cobalt-60 for spores of Clostridium botulinum 62A.

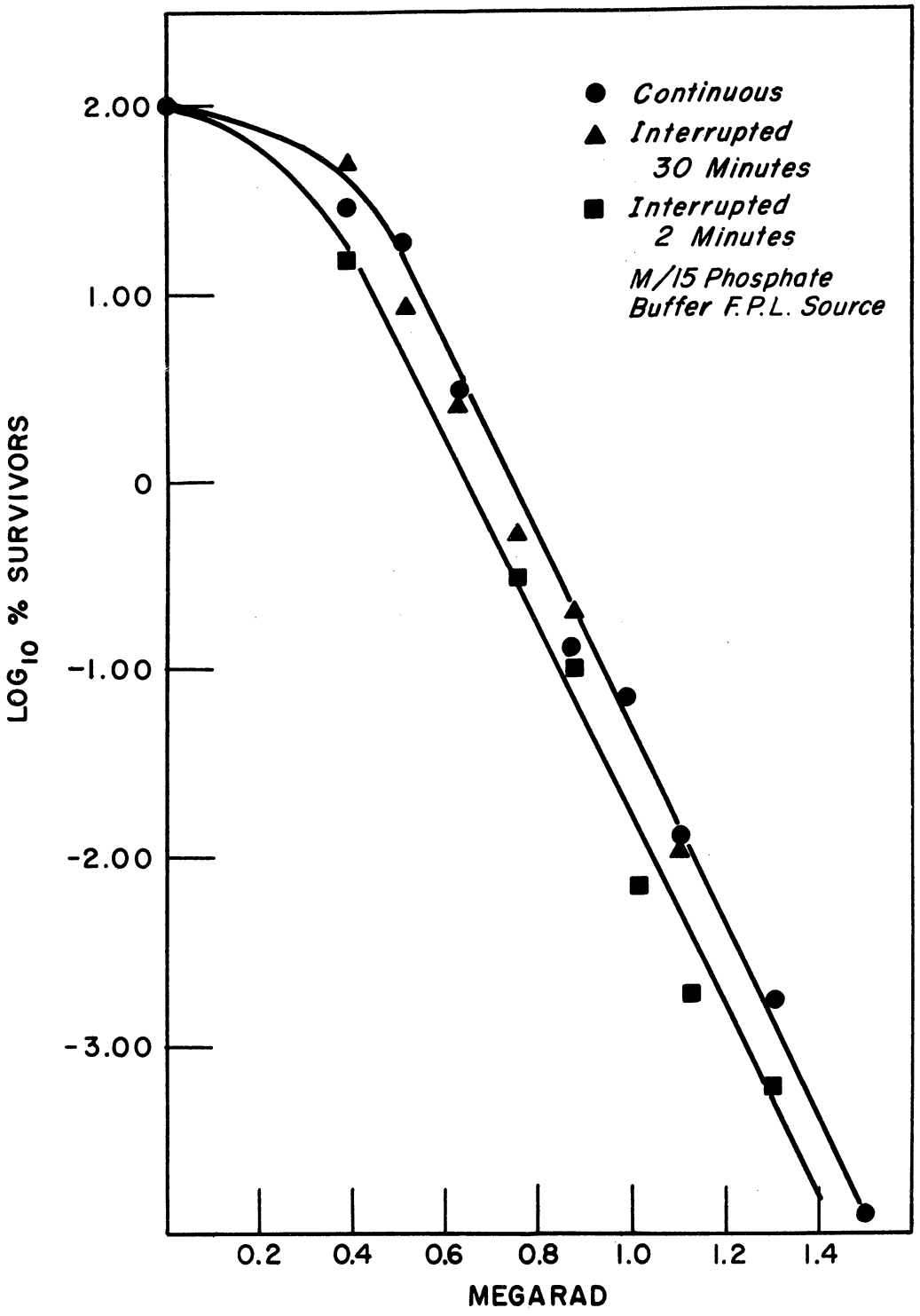


Fig. 2c. Effect of continuous vs. interrupted irradiation on the lethality of gamma radiation from cobalt-60 for spores of Clostridium botulinum 62A.

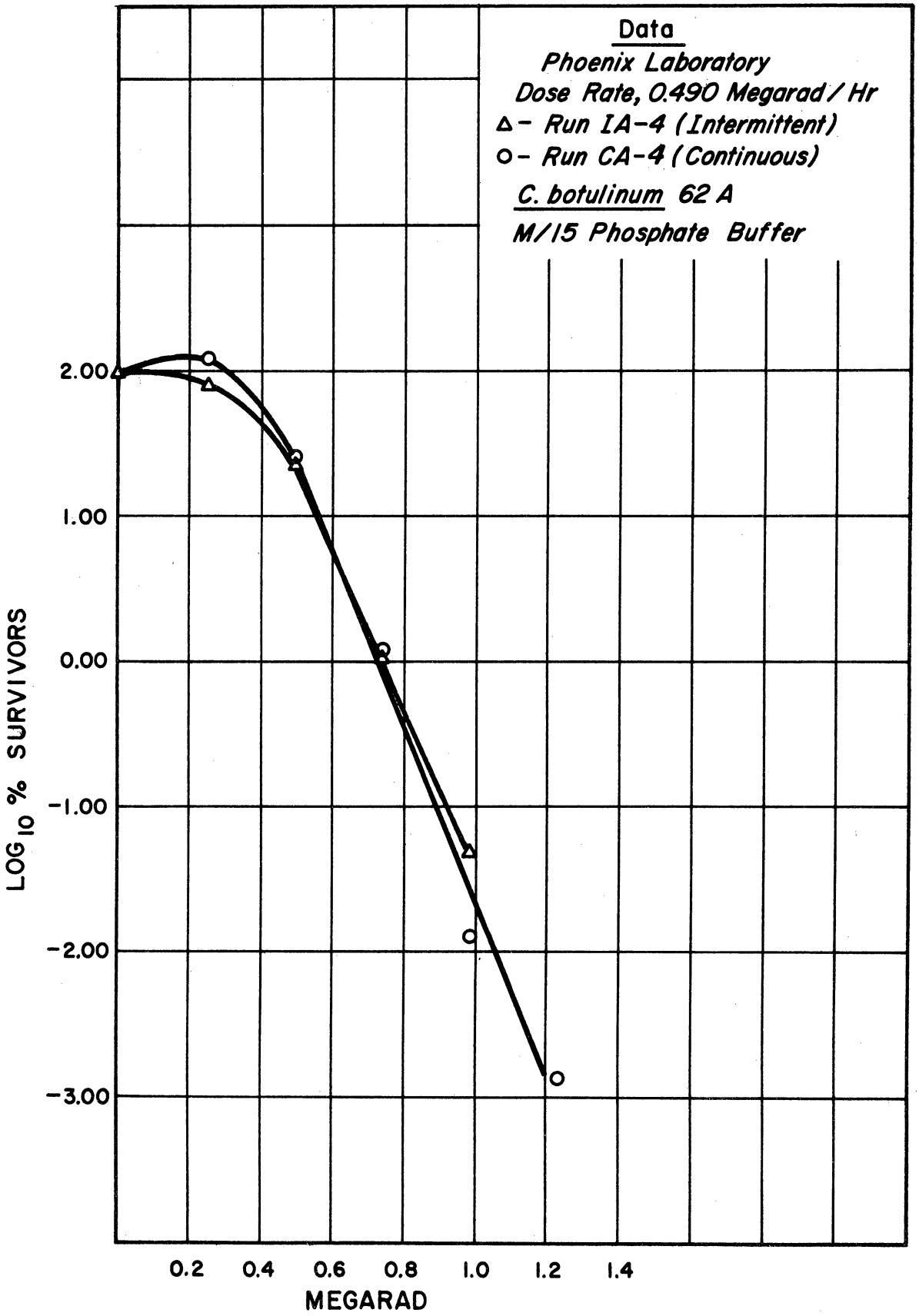


Fig. 3c. Comparison of continuous vs. intermittent radiation on the lethality of gamma rays from cobalt-60 for C. botulinum 62A spores suspended at pH 7.0 in M/15 phosphate buffer.

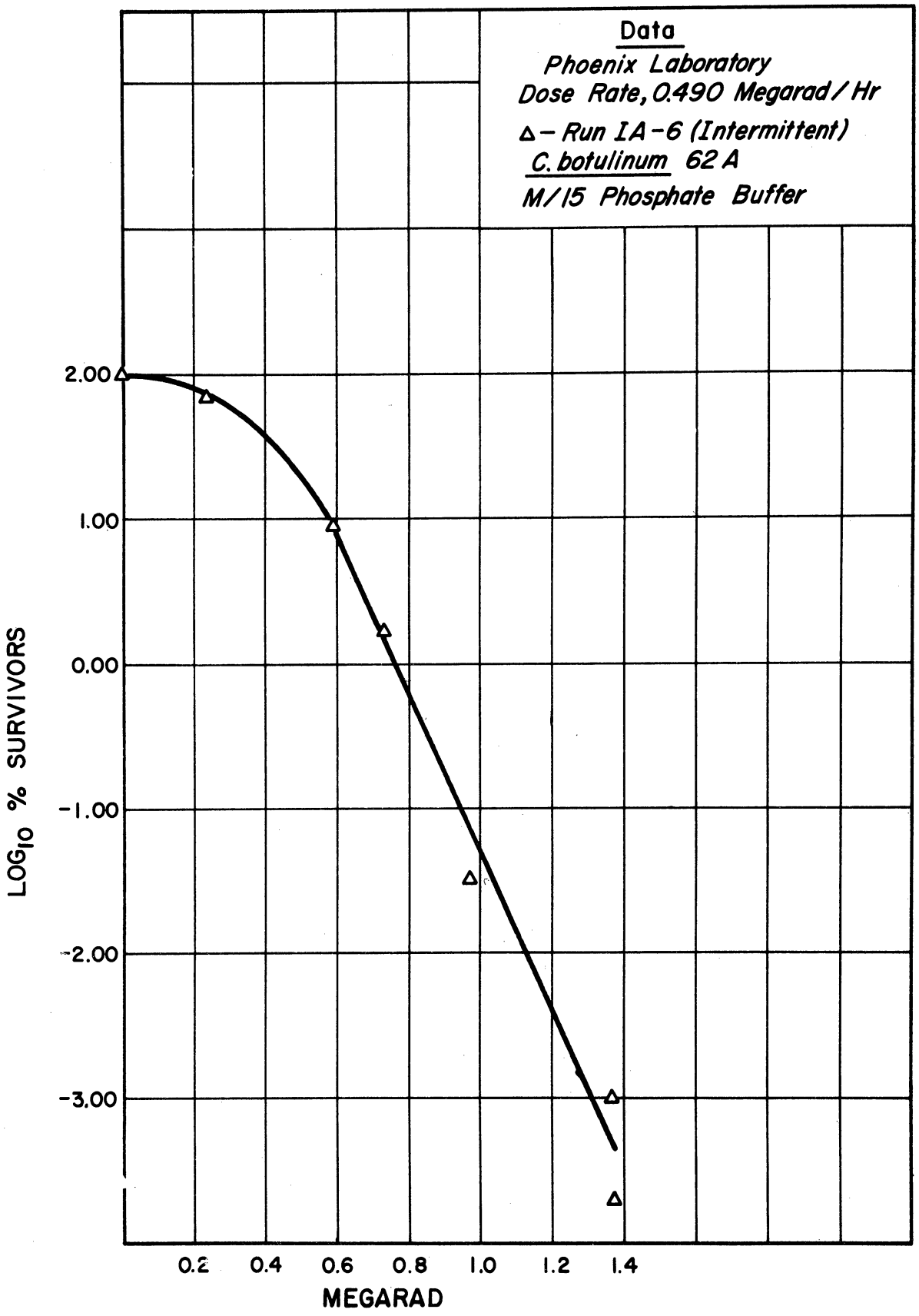


Fig. 4c. Comparison of continuous vs. intermittent radiation on the lethality of gamma rays from cobalt-60 for C. botulinum 62A spores suspended at pH 7.0 in M/15 phosphate buffer.

made in this case, the opposite effect would be noted, i.e., the points for this continuous run fall very slightly below the intermittent ones. This further emphasizes a lack of a significant difference in the lethality of continuous and intermittent irradiation for C. botulinum spores in these experiments.

The data for continuous and intermittent irradiation of C. botulinum 457A are given in Figs. 5c and 6c. Here again the survivor curves for these two methods of irradiation are indistinguishable. Similar data for C. botulinum 213B spores are shown in Fig. 7c. A slightly increased lethality for interrupted over continuous irradiation is suggested by these curves. The difference in dosage required to produce a given reduction in viable spores appears, from the graph, to lie between 5 and 10%; the difference decreases, however, as the number of interruptions increases. This would suggest a negative effect of interruptions for C. botulinum 213B, a condition which seems highly improbable.

Because phosphate buffer is not a food menstruum, possible differences between the lethality of gamma rays from cobalt-60 for spores of C. botulinum, based upon delivery of the radiation in continuous or intermittent fashion, were further tested with such spores suspended in nutrient broth. Figures 8c through 14c show the results of these irradiations, which were carried out at ice-bath temperatures.

In this connection, it was also considered desirable to evaluate these effects in nutrient broth at room temperature since irradiation of foods could be done without refrigeration. It should be pointed out that nutrient broth is essentially a food, a meat broth. Data for irradiations in nutrient broth at room temperature are shown in Figs. 15c and 16c; control experiments in phosphate buffer are given in Figs. 17c and 18c. Obviously, it is possible that the spores could germinate and develop toxin during the experiments under these latter circumstances. Details of the techniques used to evaluate these potentialities were previously described in the Materials and Methods section of this report.

Taken as a group, the curves in Figs. 8c to 14c indicate that gamma radiation from cobalt-60 is essentially as lethal for spores of C. botulinum suspended in nutrient broth and irradiated at ice-bath (0-4°C) temperatures whether the radiations are delivered continuously or intermittently. Figs. 8c, 9c, 10c, and 11c could be interpreted as suggesting a slightly increased killing rate for intermittent irradiation; Figs. 12c, 13c and 14c indicate no advantage for either. It should be noted that the data shown in Figs. 8c through 14c are taken with C. botulinum strain 62A spores, while Figs. 13c and 14c refer to spores of strain 213B.

Data on irradiation of C. botulinum 62A spores at room temperature in nutrient broth are given in Figs. 15c and 16c. The controls in phosphate buffer are shown in Figs. 17c and 18c. No difference whatsoever is noted

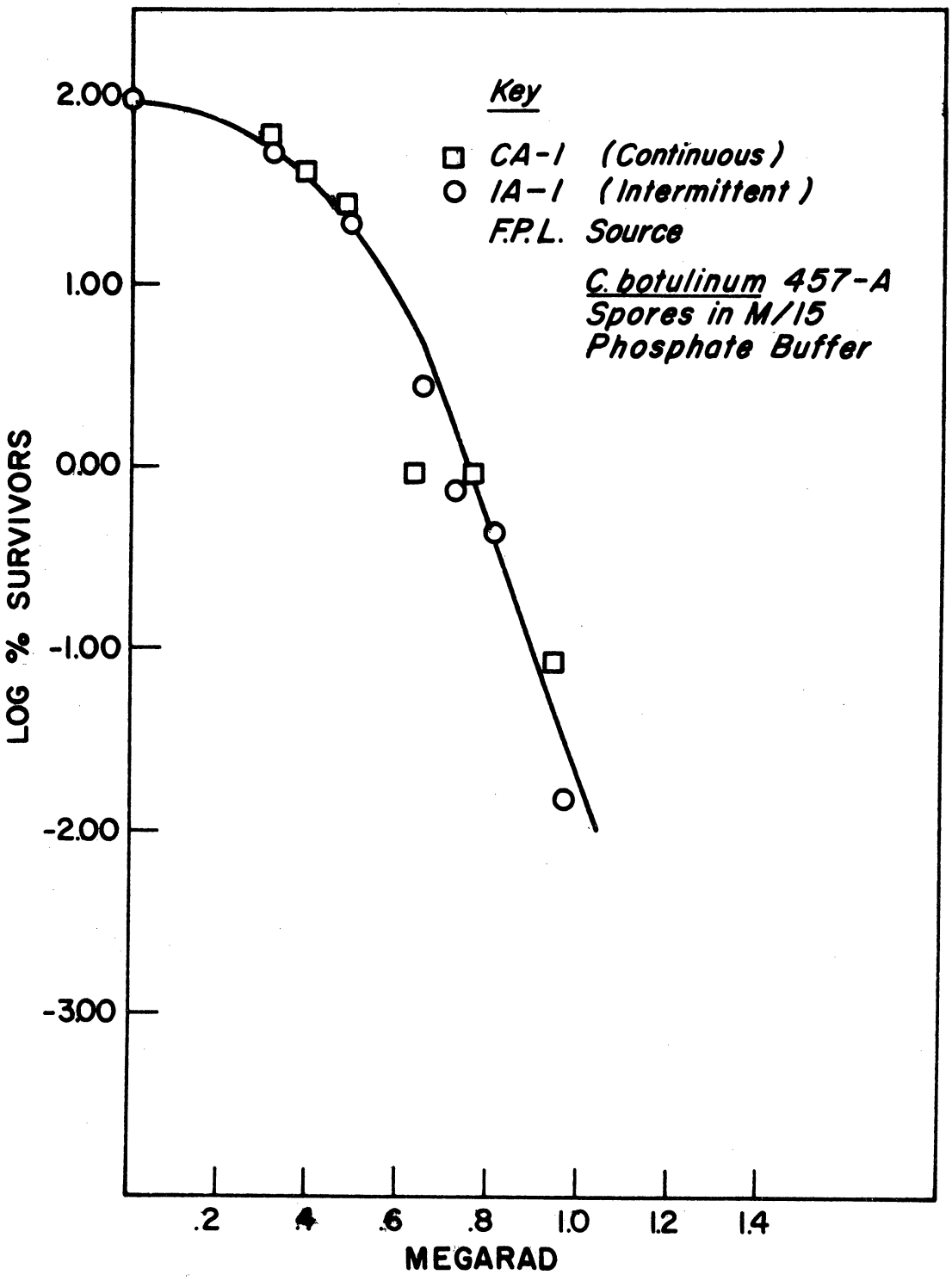


Fig. 5c. Effect of continuous vs. intermittent irradiation on the lethality of gamma rays from cobalt-60 for the spores of Clostridium botulinum 457A suspended in M/15 phosphate buffer at pH 7.0.

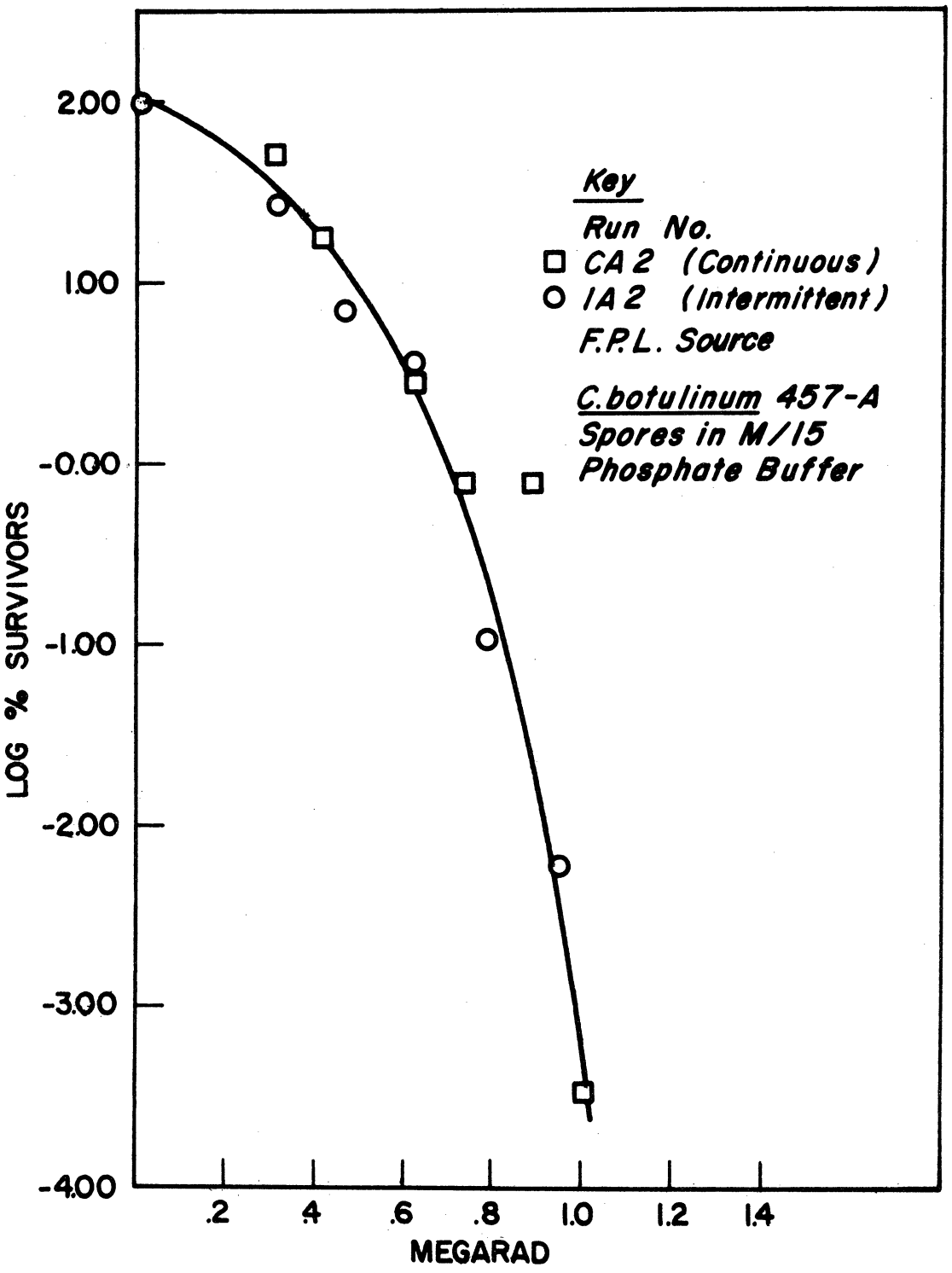


Fig. 6c. Effect of continuous vs. intermittent irradiation on the lethality of gamma rays from cobalt-60 for the spores of Clostridium botulinum 457A suspended in M/15 phosphate buffer at pH 7.0.

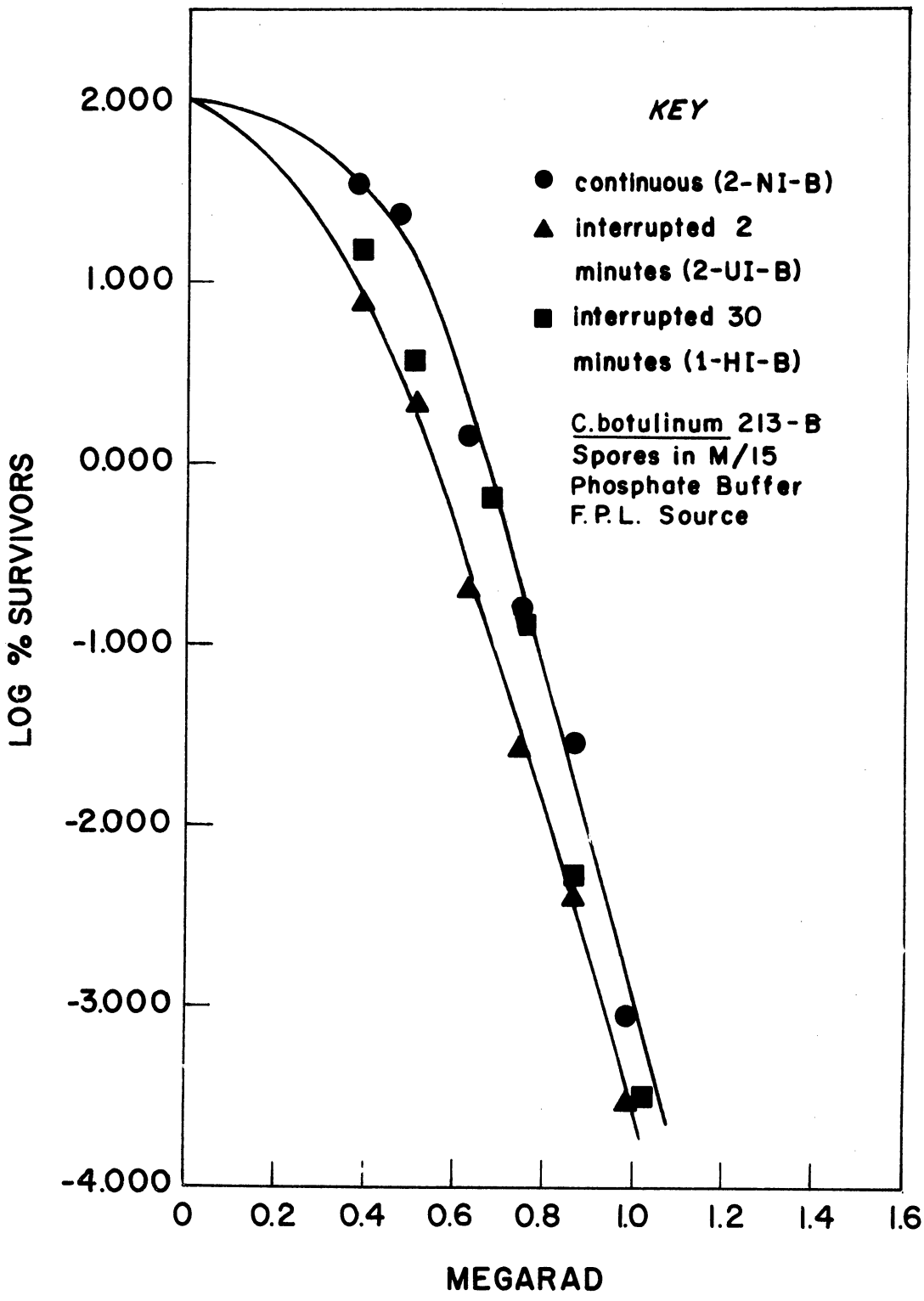


Fig. 7c. Effect of continuous vs. intermittent irradiation on the lethality of gamma rays from cobalt-60 for the spores of Clostridium botulinum 213B.

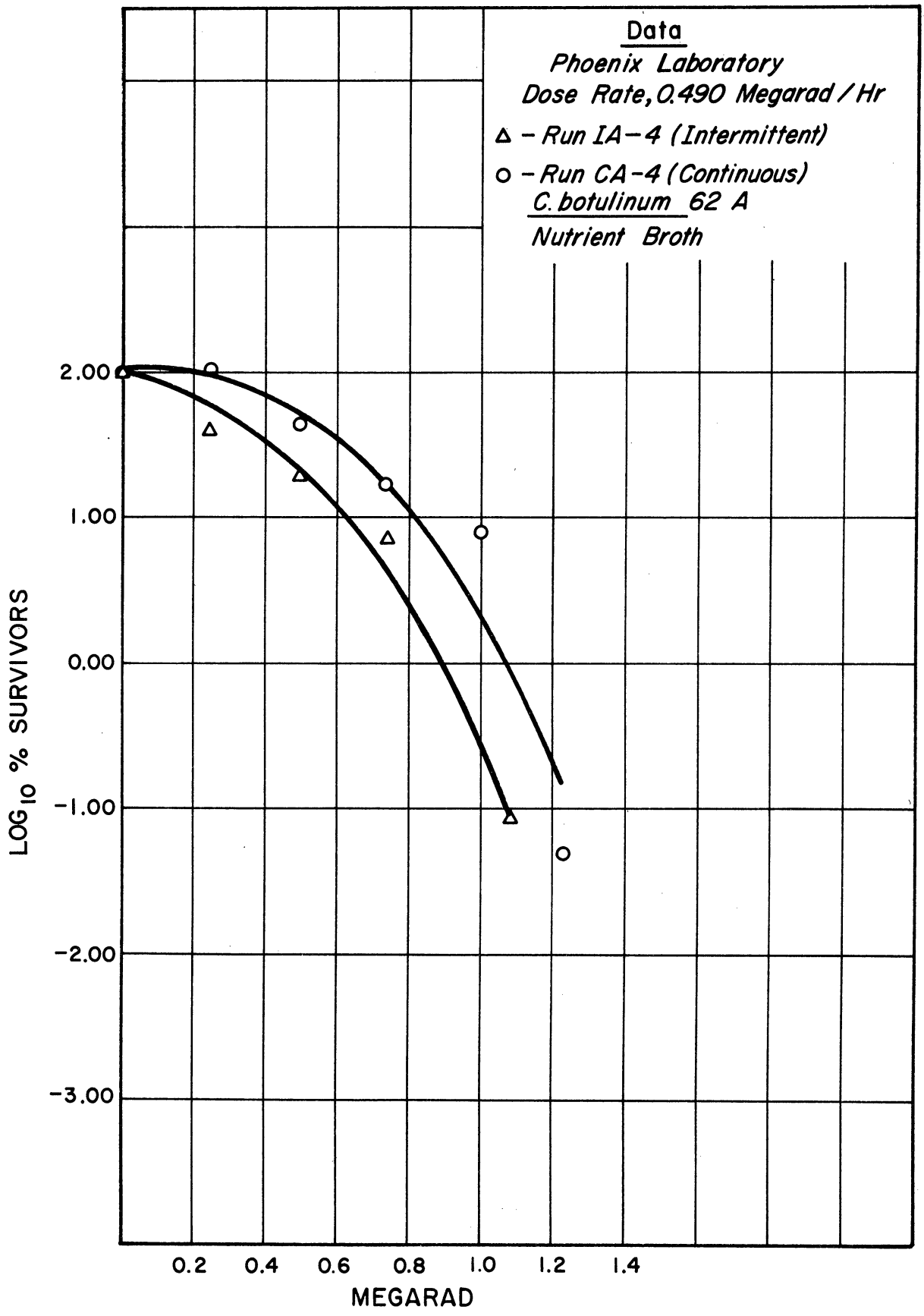


Fig. 8c. Comparison of continuous vs. intermittent radiation on the lethality of gamma rays from cobalt-60 for C. botulinum 62A spores suspended in nutrient broth.

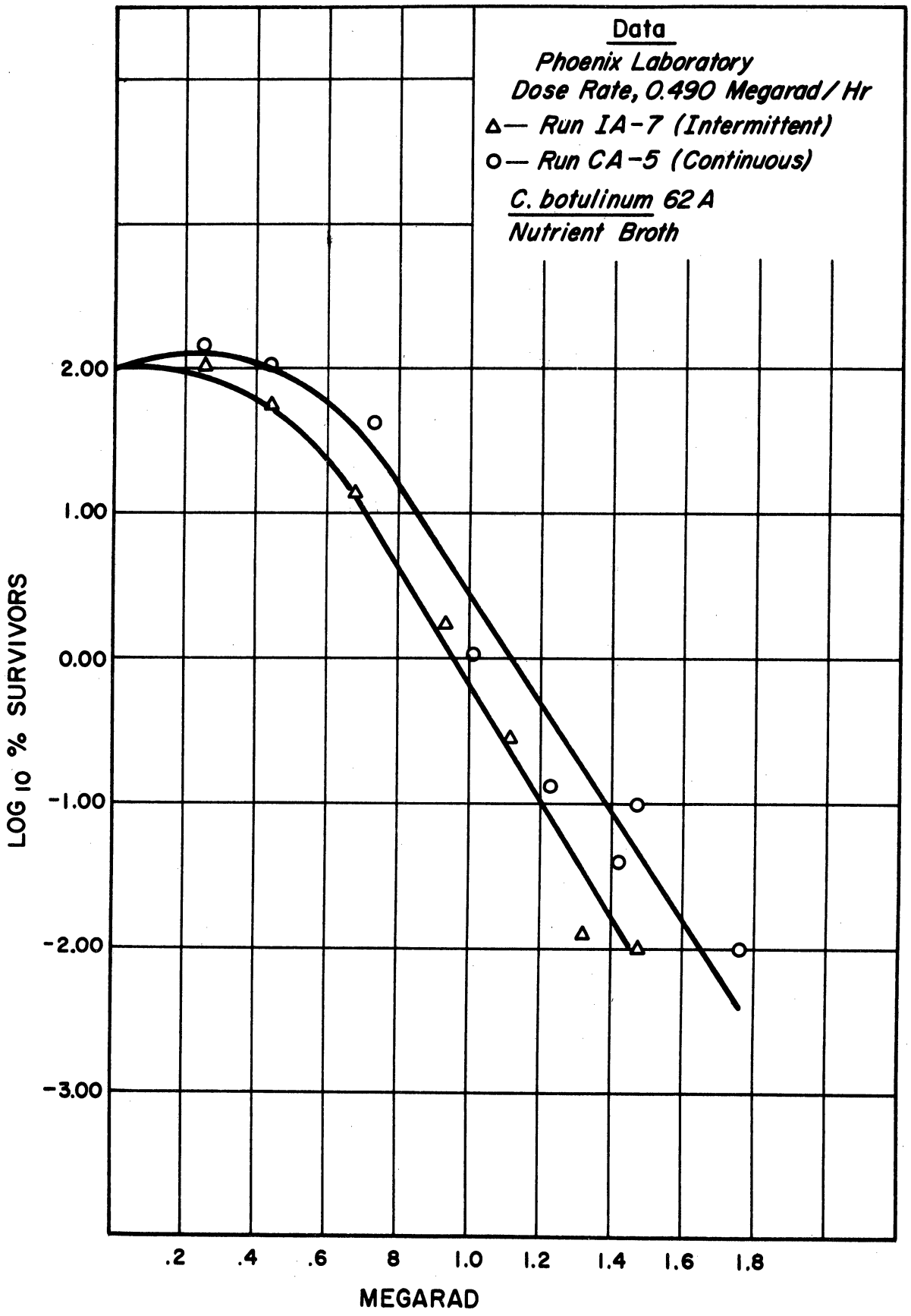


Fig. 9c. Comparison of continuous vs. intermittent radiation on the lethality of gamma rays from cobalt-60 for C. botulinum 62A spores suspended in nutrient broth.

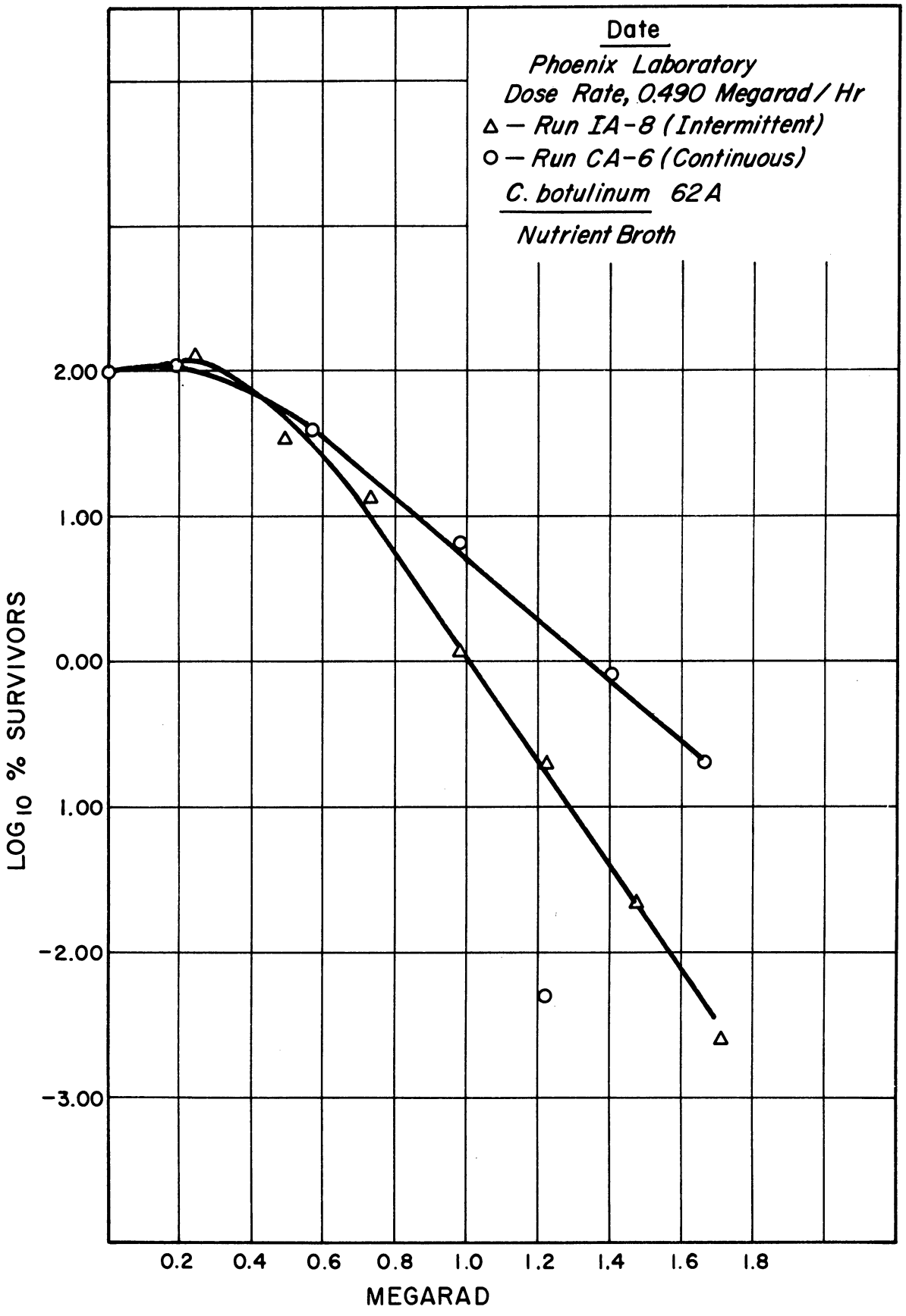


Fig. 10c. Comparison of continuous vs. intermittent radiation on the lethality of gamma rays from cobalt-60 for C. botulinum 62A spores suspended in nutrient broth.

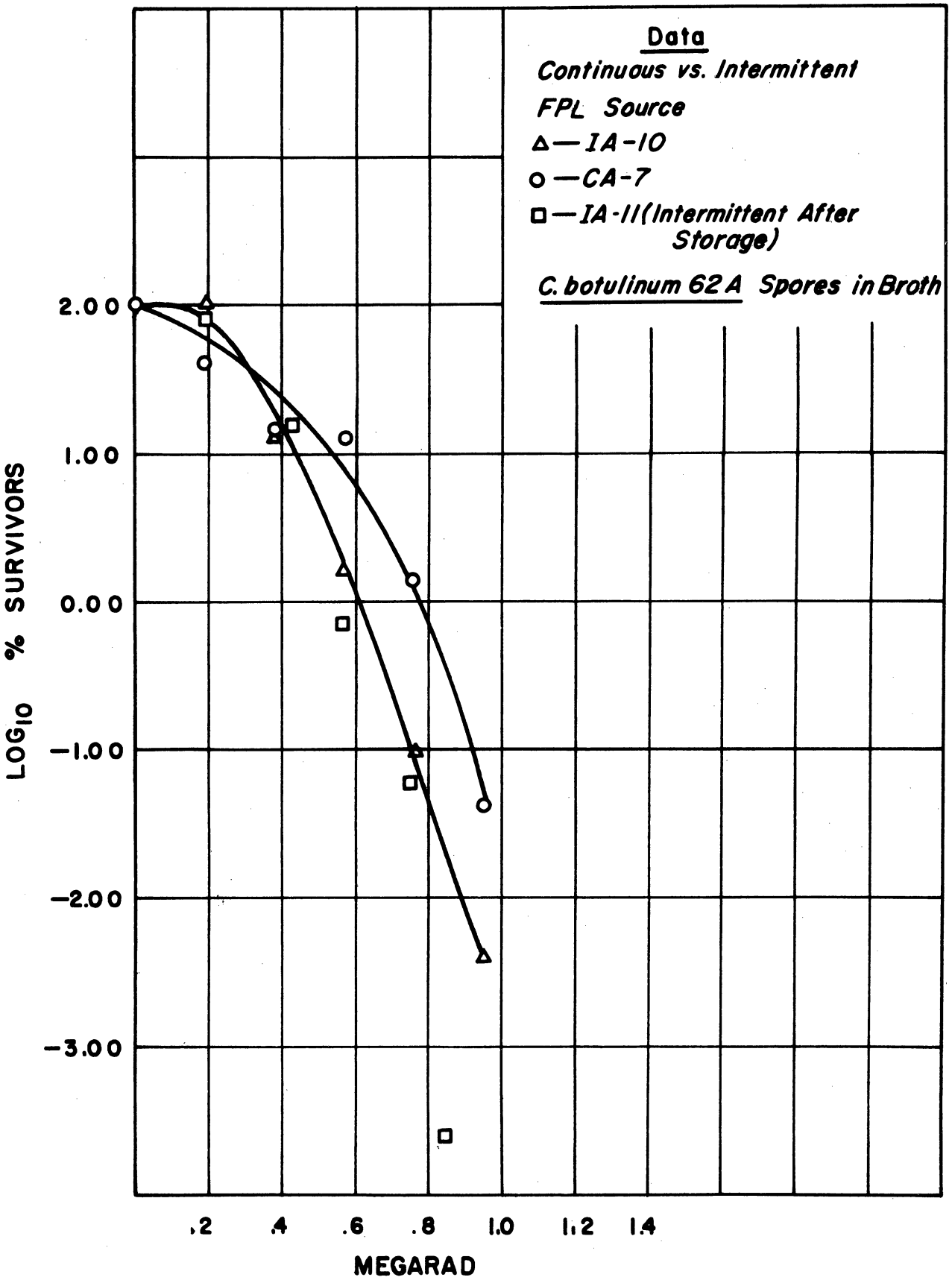


Fig. 11c. Comparison of continuous with intermittent irradiation with gamma rays from cobalt-60 on the lethality of such rays for Clostridium botulinum 62A spores suspended in nutrient broth.

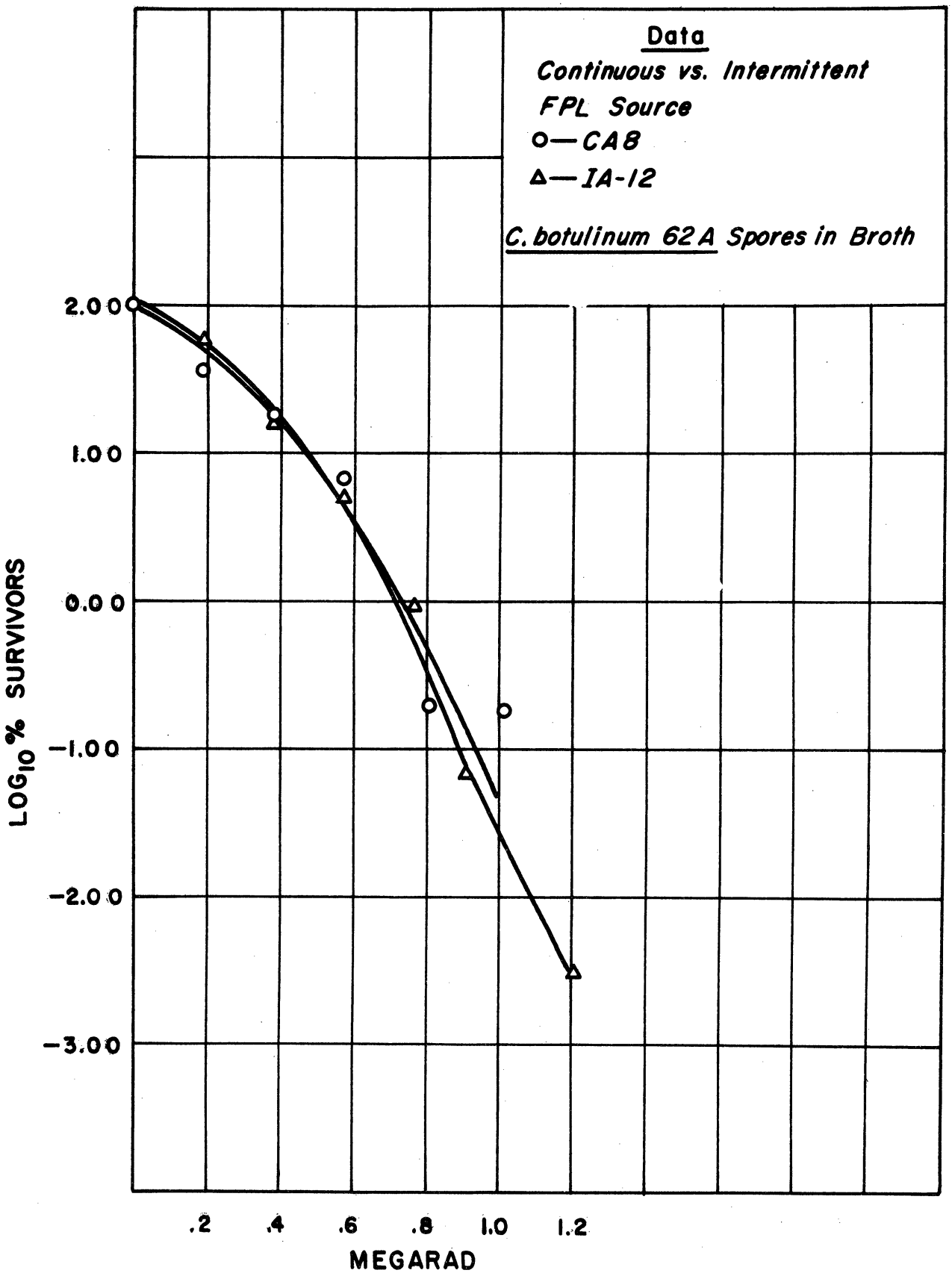


Fig. 12c. Comparison of continuous with intermittent irradiation with gamma rays from cobalt-60 on the lethality of such rays for Clostridium botulinum 62A spores suspended in nutrient broth.

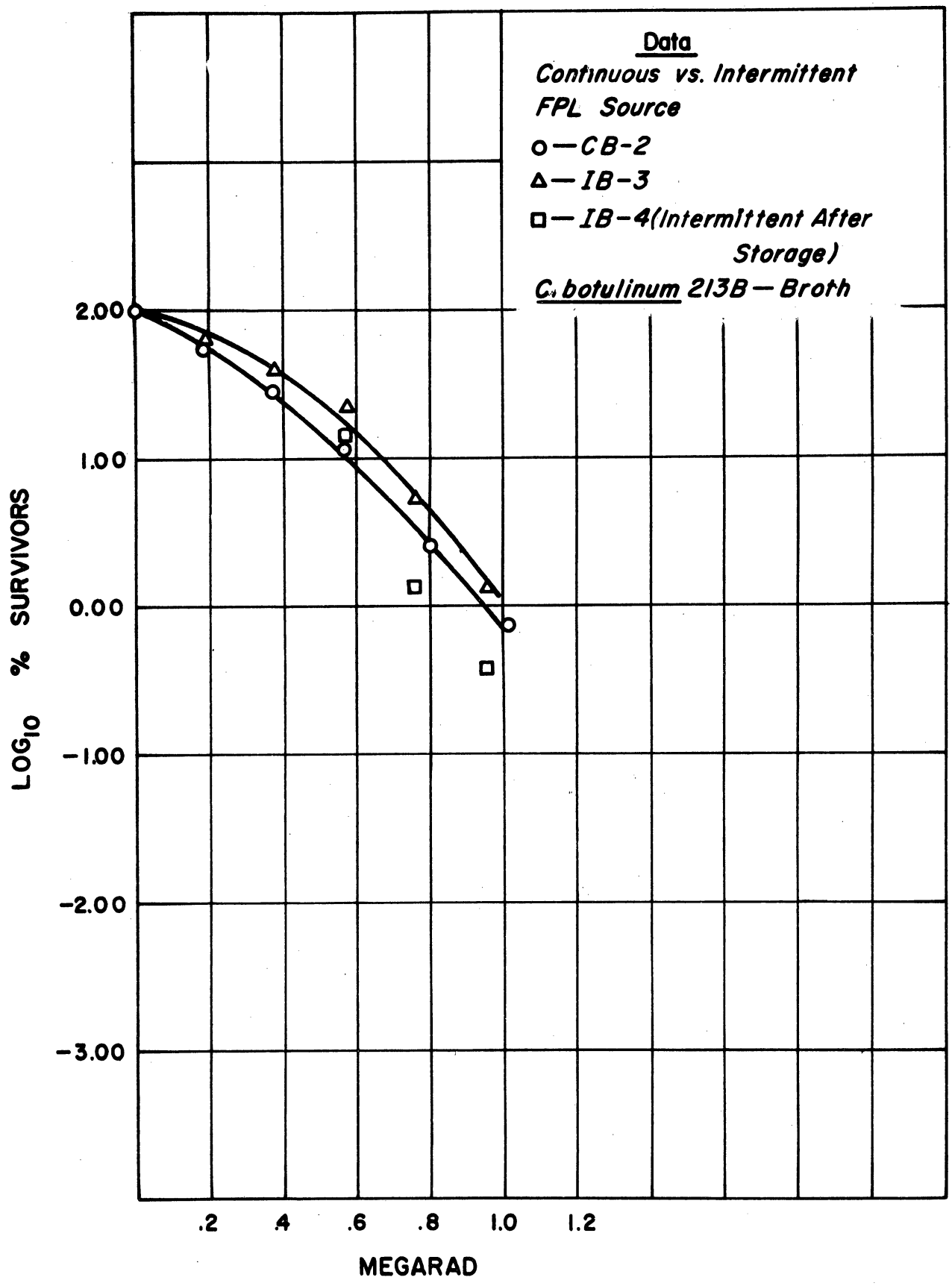


Fig. 13c. Comparison of continuous with intermittent irradiation with gamma rays from cobalt-60 on the lethality of such rays for Clostridium botulinum 213B spores suspended in nutrient broth.

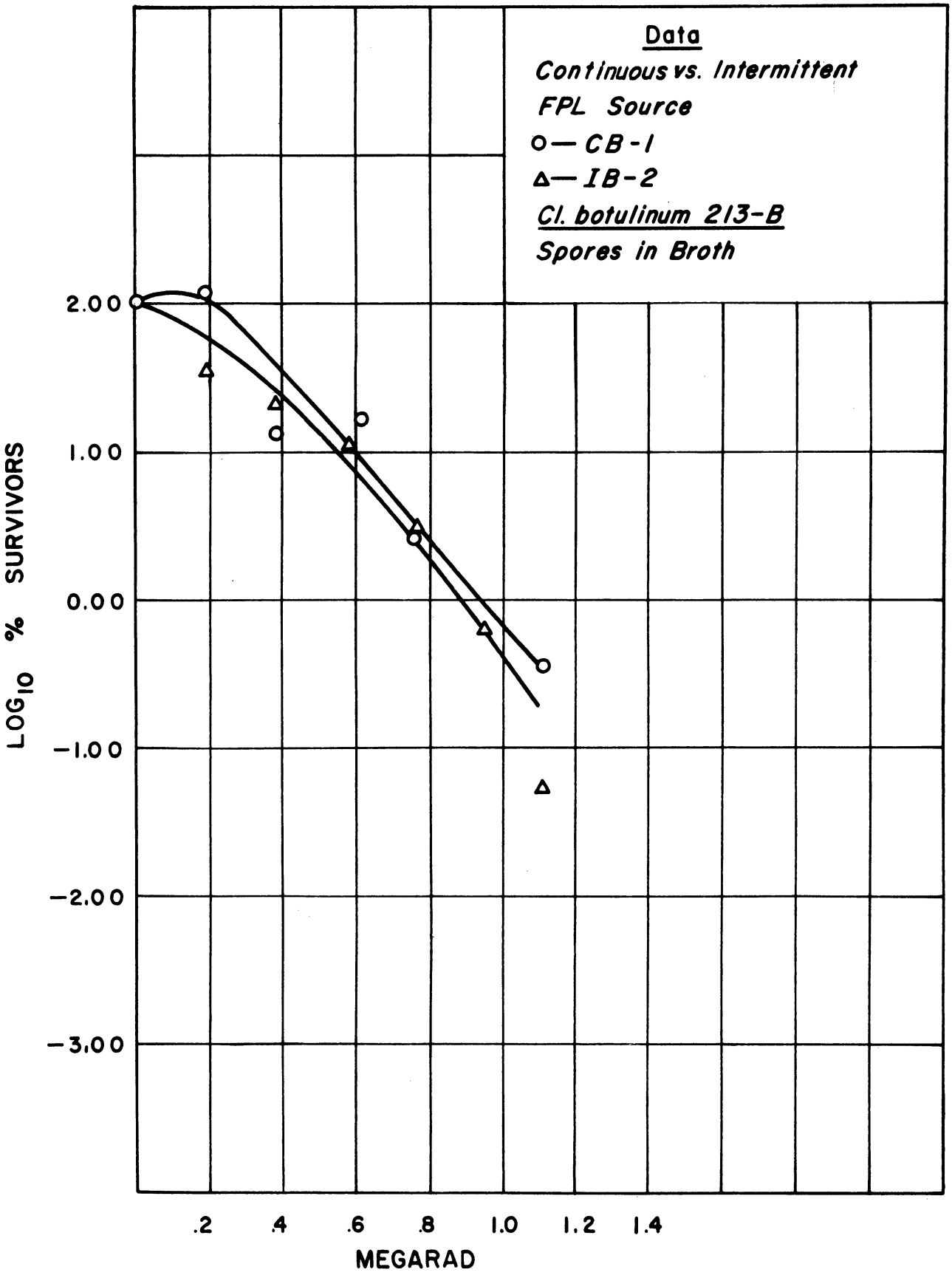


Fig. 14c. Comparison of continuous with intermittent irradiation with gamma rays from cobalt-60 on the lethality of such rays for Clostridium botulinum 213B spores suspended in nutrient broth.

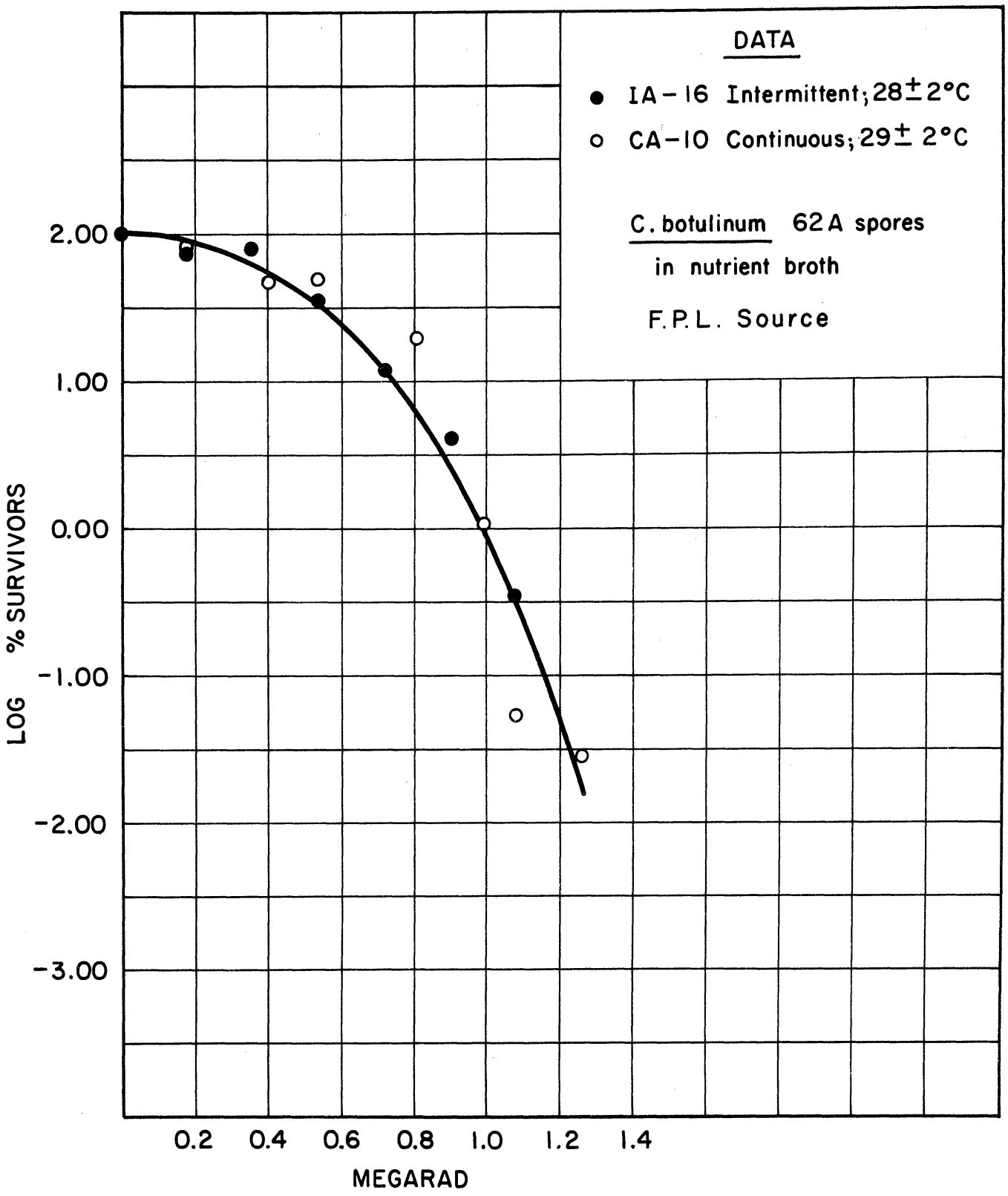


Fig. 15c. Comparison of intermittent and continuous irradiation on the lethality of gamma radiation for spores of C. botulinum 62A suspended in nutrient broth and irradiated at room temperature.

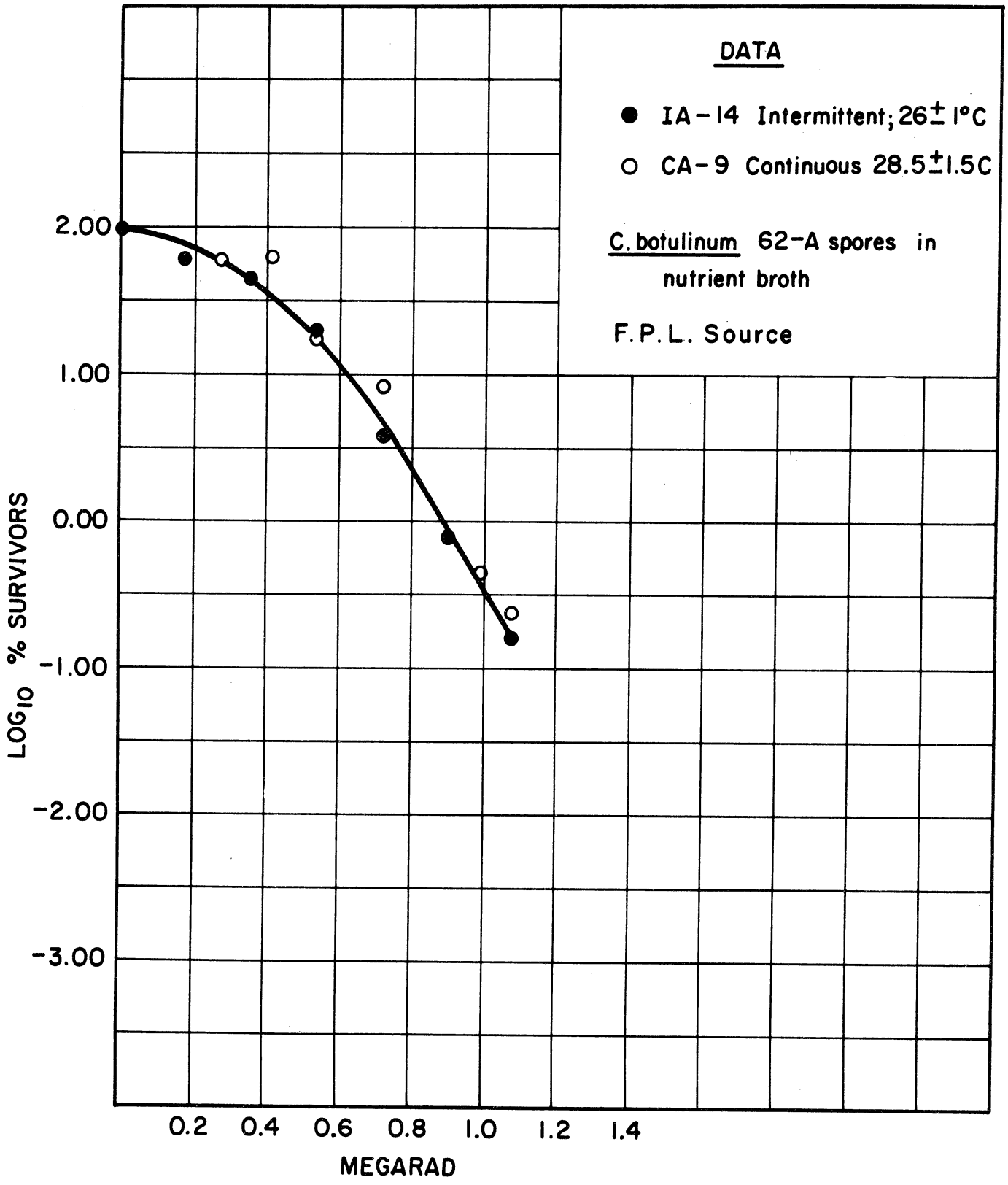


Fig. 16c. Comparison of intermittent and continuous irradiation on the lethality of gamma radiation for spores of C. botulinum 62A suspended in nutrient broth and irradiated at room temperature.

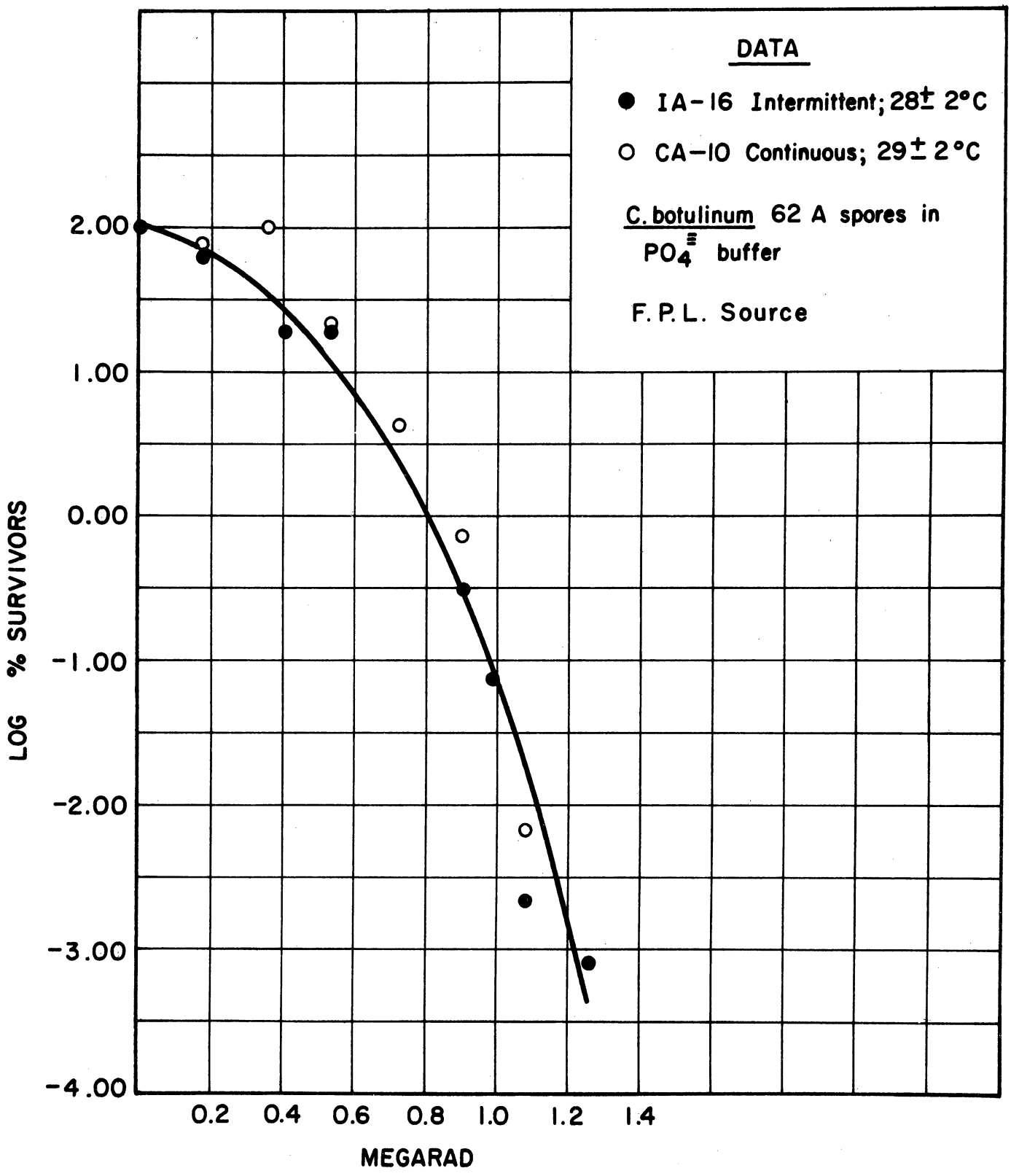


Fig. 17c. Comparison of intermittent vs. continuous irradiation on the lethality of gamma radiation from cobalt-60 on spores of C. botulinum 62A suspended in M/15 phosphate buffer at pH 7.0 and irradiated at room temperature.

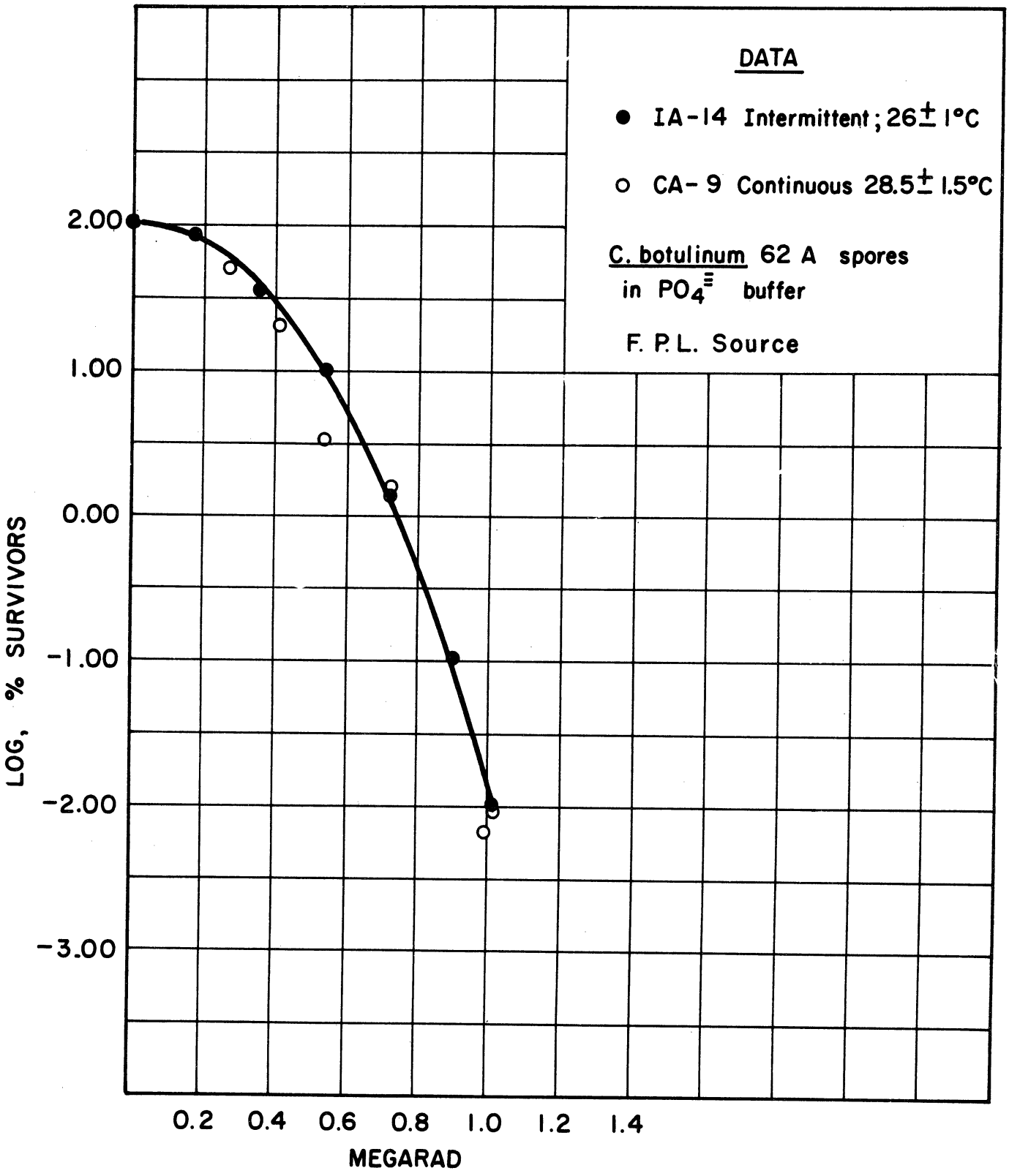


Fig. 18c. Comparison of intermittent and continuous irradiation on the lethality of gamma radiation from cobalt-60 for spores of C. botulinum 62A suspended in M/15 phosphate buffer at pH 7.0 and irradiated at room temperature.

between the lethality of continuous and interrupted irradiations in any of these four experiments; this includes both the experimental runs in nutrient broth and the controls in phosphate buffer. Incidentally, the well-known protective effect of organic matter is evident from a comparison of these data: the gamma radiation is considerably more lethal for spores suspended in phosphate buffer than for those in nutrient broth.

Because of the possibility of germination growth and toxin development by spores of C. botulinum 62A held in nutrient broth at room temperature, control samples of spores were prepared and treated like the experimental samples except that they were not irradiated. Some of the liquids from both irradiated and nonirradiated suspensions were tested for toxin by injection into mice, stains were prepared for microscopic examination, and portions were heated to 90°C for 15 minutes to kill any vegetative cells. This was followed by counting the survivors. The heated controls of spore suspensions held at the irradiation temperature and for the time of the longest irradiation, did not indicate any appreciable germination of the spores in buffer or nutrient broth. No toxin was demonstrated in the spore suspensions in phosphate buffer held at room temperature and irradiated for approximately 12 hours. However, some toxin was demonstrated in the irradiated spore suspensions in nutrient broth, indicating that some germination and toxin release had occurred: crystal violet stains showed that a few spores had taken up the stain (Tables II and III).

To be sure that the spores being used would develop toxin under these conditions, a better germination and growth medium than nutrient broth, trypticase-yeast-extract broth,was used as a control in Run C-11. In this medium C. botulinum 62A spores germinated and developed toxin in the controls as expected and as shown in Tables II and III. However, even in trypticase-yeast extract medium only a slight amount of germination occurred as evidenced by the small decrease in numbers of organisms surviving heating at 90°C for 15 minutes and as shown in Table III for run C-11. A comparison of the survivor curves shown in Fig. 19c again illustrate the observation that radiation sensitivity did not change appreciably during the time of irradiation (approximately 15 hours in this instance) whether the spores were suspended in phosphate buffer, nutrient broth, or a yeast-extract-trypticase broth medium.

Runs IA-14 and IA-15, shown in Fig. 20c, indicate that while a slight degree of protection is produced for C. botulinum 62A spores in phosphate buffer by irradiating at 26°C rather than 4°C, the difference is slight enough to be negligible.

In the second kind of experiments, C. botulinum spores were suspended in phosphate buffer. The suspensions were then distributed into vials which were placed in the irradiation rack, packed in ice, and irradiated at the Fission Products Laboratory. Both continuous and intermittent irradiation were used. Results are given in Table IV.

TABLE II

EFFECT OF INCUBATION OF CONTROL SUSPENSIONS AT ROOM TEMPERATURE ON THE HEAT RESISTANCE OF C. BOTULINUM 62A SPORES

CONTROL

Experiment	Medium	Count of Spore Suspensions	
		Held at Room Temperature	Heated 90°C - 15 min
IA-14	*Broth	2.8×10	
	**Buffer	4.0×10^{7}	
CA-9	Broth	7.9×10^{6}	1.8×10^{6}
	Buffer	5.5×10^{6}	4.7×10^{6}
CA-10	Broth	2.4×10^{7}	1.2×10^{7}
	Buffer	1.6×10^{7}	1.1×10^{7}
C-11	Buffer	2.6×10^{6}	2.2×10^{6}
	Broth	4.7×10^{6}	2.4×10^{6}
	***Trypticase-Yeast	4.0×10^{6}	2.3×10^{5}

* Nutrient broth

** M/15 phosphate buffer, pH 7.0

*** Trypticase-yeast-extract broth

TABLE III

EFFECT OF INCUBATION OF CONTROL SUSPENSIONS AT ROOM TEMPERATURE ON TOXIN PRODUCTION BY C. BOTULINUM 62A SPORES

TOXICITY CHECK

Experiment	Sample	No. Deaths
IA-14	Irradiated Broth, 12 hr	4/4
IA-14	Control - Non Irradiated	0/4
CA-9	*Broth Irradiated	0/4
	Buffer Irradiated	0/4
CA-10	Broth - Room Temperature	0/3
	Broth Irradiated	1/3
	**Buffer - Room Temperature	0/3
	Buffer Irradiated	0/3
C-11	Buffer Irradiated	0/3
	Broth Irradiated	0/3
	***Trypticase-Yeast Irradiated	2/3
	Trypticase-Yeast Irradiated + AT	0/3

* Nutrient broth

** M/15 phosphate buffer at pH 7.0

*** Trypticase-yeast-extract broth

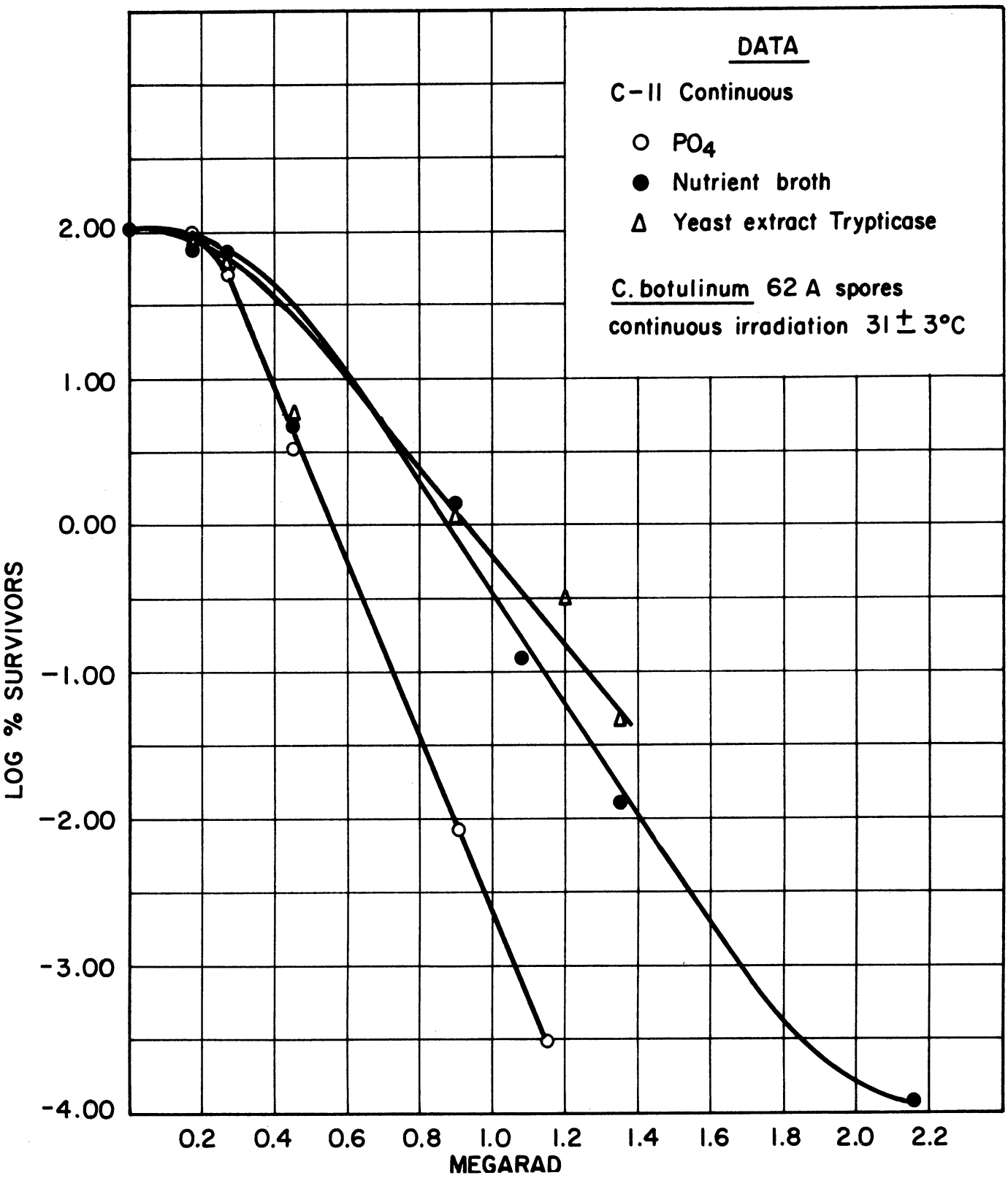


Fig. 19c. Comparison of continuous irradiation on the lethality of gamma radiation for spores of C. botulinum 62A suspended in phosphate buffer nutrient broth and trypticase-yeast extract medium and irradiated at room temperature.

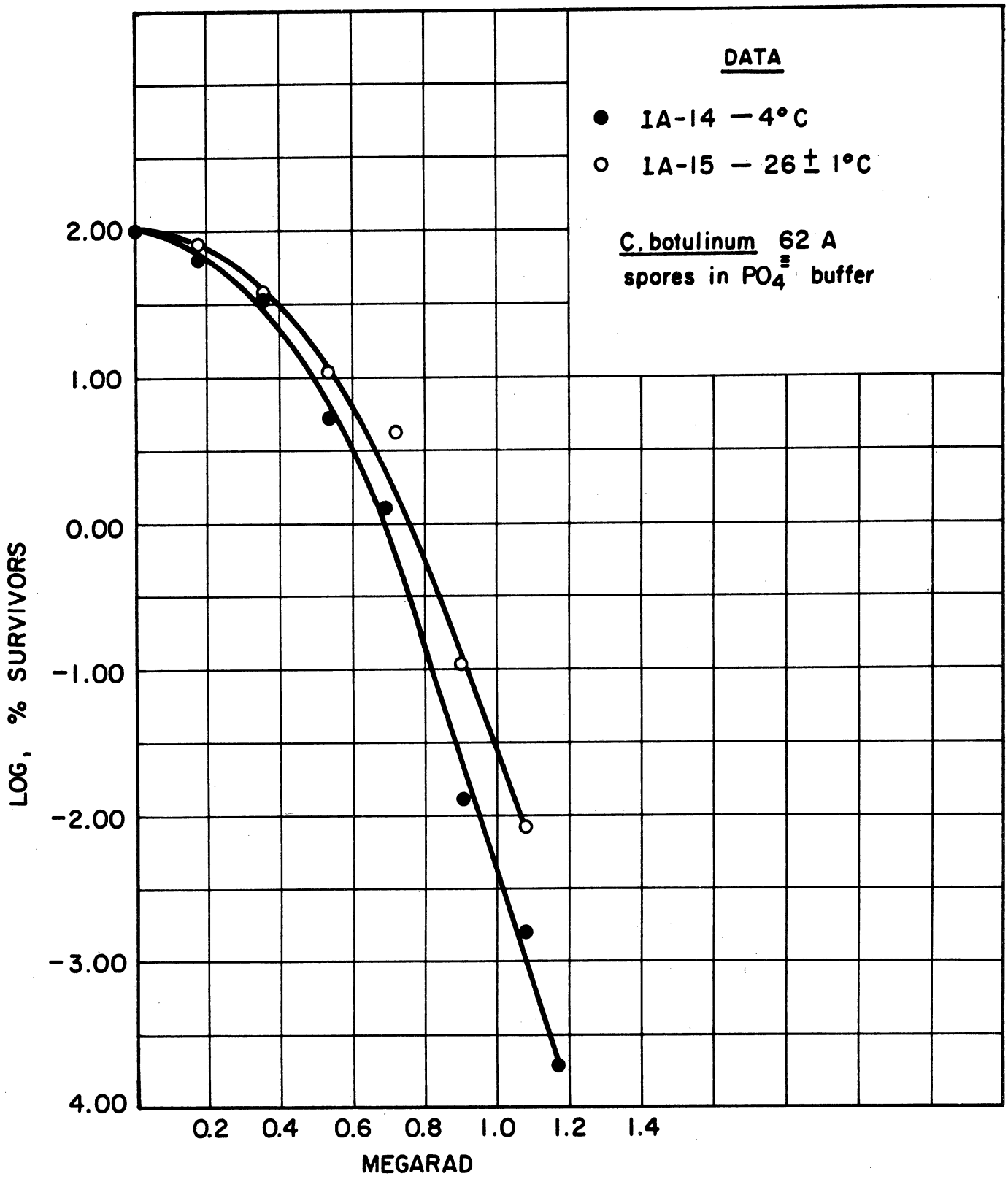


Fig. 20c. Effect of temperature during irradiation on the lethality of gamma radiation from cobalt-60 for C. botulinum 62A spores suspended in M/15 phosphate buffer and irradiated intermittently.

Comparison of the average numbers of spores surviving 0.632 megarad of irradiation in the three experiments shown in Table IV discloses two facts: (1) in runs (a) and (c) intermittent irradiation produced less survivors than continuous irradiation but in (b) the opposite situation occurred; (2) the data overlap at 2σ. Thus this set of data indicates no significant difference in the lethality of gamma rays for spores of cobalt-60, under the conditions of the experiment, whether the radiations are delivered continuously or intermittently.

INOCULATED PACKS OF GROUND BEEF

Lean beef was ground in a clean, mechanical grinder, packed into 204 x 214 tin cans, inoculated into the geometrical center with approximately 10,000 C. botulinum 62A spores per can, sealed in vacuum-closing machine, and irradiated. The temperature varied between 4 and 8°C during irradiation, which was carried out in the center-well of the cobalt-60 source in the Fission Products Laboratory.

The cans were divided into two groups for purposes of this experiment. For the continuous runs, no interruptions were permitted; for the interrupted experiments, irradiation was purposely stopped at half the total dose, the cans were refrigerated for 12 to 16 hours, and then the irradiation was completed. Also, normal use of the cobalt-60 source was permitted during the interrupted runs. This generally resulted in unscheduled, short interruptions of a few minutes each. Notations concerning these short, additional interruptions are given with each experiment.

Following irradiation, the cans were incubated at 30°C. When spoilage occurred, verification of the growth of C. botulinum was carried out by bacteriological and mouse inoculation tests as required.

The data in Table V show that continuous or interrupted delivery of ionizing radiations did not alter the lethality of gamma radiations from cobalt-60 for spores of C. botulinum inoculated into raw ground beef. In every run the sterilization dose could be placed somewhere between 1.69 and 1.93 megarad whether the gamma radiation was delivered continuously or intermittently.

TOXICITY DETERMINATIONS IN STORED, IRRADIATED MEAT

The irradiated cans of meat checked for botulinum toxin were from the same experiments conducted approximately two years ago and in which botulinum toxin was found. The present work shows that the toxin persists even after the long incubation period at 30°C. However, the amount present is not great since a 1/2 dilution of the meat extract did not produce death in animals. This is shown in Table VI.

TABLE IV

EFFECT OF CONTINUOUS VS. INTERMITTENT
IRRADIATION ON THE LETHALITY OF GAMMA RAYS FOR
CLOSTRIDIUM BOTULINUM SPORES USING 0.632 MEGARAD IN EACH INSTANCE

General conditions of experiments

Irradiation carried out with:

Ice bath temperature (0-4°C)
Irradiation field intensity, 158,000 rads per hour
Spores suspended in M/15 phosphate buffer at pH 7.0

(a) Strain - Clostridium botulinum 62A

Continuous for four hours and

Intermittent: seven 30-minute irradiation periods with 20-minute intervals between irradiation periods

Sample	Number of Viable Spores per ml	
	Intermittent	Continuous
Control	4,850,000	4,850,000
1	735,000	1,100,000
2	695,000	845,000
3	605,000	905,000
4	550,000	580,000
5	730,000	635,000
Average	663,000	813,000
σ	73,200	189,000
$+2\sigma$	809,400	1,191,000
-2σ	516,600	435,000

TABLE IV (Concluded)

(b) Strain - Clostridium botulinum 62A

Continuous for 4 hours and

Intermittent: thirteen three-minute interruptions between irradiation periods.

Sample	Number of Viable Spores per ml	
	Intermittent	Continuous
Control	18,000,000	18,000,000
1	350,000	310,000
2	730,000	620,000
3	500,000	230,000
4	420,000	330,000
5	370,000	360,000
Average	474,000	370,000
σ	138,000	132,000
$+2\sigma$	750,000	634,000
-2σ	198,000	106,000

(c) Strain - Clostridium botulinum 213B

Continuous, 4 hours and

Intermittent: thirteen three-minute interruptions between irradiation periods.

Sample	Number of Viable Spores per ml	
	Intermittent	Continuous
Control	17,000,000	17,000,000
1	52,000	53,000
2	60,000	55,200
3	79,000	87,000
4	37,000	84,000
5	83,000	84,000
Average	62,200	72,600
σ	17,100	15,300
$+2\sigma$	79,300	103,200
-2σ	45,100	42,000

TABLE V

COMPARISON OF CONTINUOUS AND INTERMITTENT IRRADIATION WITH GAMMA RAYS FROM COBALT-60 ON THE SURVIVAL OF C. BOTULINUM 62A SPORES IN RAW GROUND BEEF

	Radiation Dose, Megarad	Number of Cans Producing Gas	Days-to-Gas Production
Continuous Irradiation			
Run: CM-3			
	1.690	5/6	5
	1.925	0/6	-
	2.110	0/6	-
Remarks: 13,500 spores per can			
Run: CM-4			
	1.690	4/6	4
	1.925	0/6	-
	2.110	0/6	-
Remarks: 9800 spores per can			
Run: CM-5			
	1.463	6/6	3;4
	1.664	3/6	5
	1.724	3/6	5
Remarks: 12,000 spores per can			
Intermittent Irradiation			
Run: IM-1			
	1.690	4/6	4;5
	1.930	0/6	-
	2.110	0/6	-
Remarks: 9800 spores per can. Four 30- to 60-minute interruptions, plus one 12-hour interruption after half the dose was delivered.			
Run: IM-2			
	1.463	6/6	4
	1.664	5/6	5-7
	1.724	2/6	5
Remarks: 12,000 spores per can. Seven 2- to 15-minute interruptions, plus one 16-hour interruption. The latter came after approximately half the dose was delivered.			

TABLE VI

TOXICITY CHECK ON LONG-TERM INCUBATED RAW AND COOKED IRRADIATED MEAT INOCULATED WITH LARGE SPORE LOADS

Sample	Can No.	Date of Experiment	Time and Number of Deaths	
A-2	3	7-7-58	3/3* dil.	24-48 hr**
	7		3/3	24-48 hr
A-3	4	7-21-58	0/3	> 5 days
A-5	8	12-12-58	0/3	> 7 days
	19		0/3	> 7 days
	23		3/3	< 24 hr
	31		3/3	< 24 hr*
	32		3/3	< 24 hr*
	33		3/3	< 24 hr
	34		2/3	24-48 hr
AC-1 (cooked meat)	30	7-11-58	3/3	24-48 hr
AC-2 (cooked meat)	1	7-23-58	3/3	24-48 hr
	9		3/3	24-48 hr

* Check with Antitoxin indicated "A" toxin.

** Dilution of sample to 0, 1/2, 1/4, 1/8 indicated deaths only in zero dilution.

IV. Results and Discussion

The results of the first experiment using high-speed electrons are given in Tables VII and VIII. In both the determination of a dose survival curve and the effect of various dose rates at a constant dosage, unusually heavy killing of the spores occurred for the dosages used; this was unexpected. A dose survival curve using the same lot of C. botulinum 62A spores suspended in broth and phosphate buffer, but irradiated with cobalt-60, indicated a much higher survival ratio. This is shown in Fig. 21c and Table IX. The comparison is based upon the assumption that the cobalt glass and ferrous sulfate dosimetry were equivalent.

In designing the second experiment, it was assumed that the cobalt glass dosimeters yielded a lower value for the dose rate. This was based on a comparison with previous ceric sulfate dosimetry. When this run was made, the cobalt glass was found to actually read 53% of the dose as compared with ceric sulfate dosimetry. Using this correction factor, the dose survival curve was obtained that is given in Table X and Fig. 22c. Four points for a survivor curve in a "fractionated" run are also given in Table XI and Fig. 22c. The experimental procedure for this kind of run was described in the Materials and Methods section of this report. From this curve it appears that the numbers of surviving spores was significantly greater when the fractionation procedure was used.

The results of the experiment in which the dose rate was varied are presented in Table XII. These results are not conclusive since a higher number of spores survived than was expected and no correlation exists in counts between samples. Hence no conclusion can be drawn from these runs concerning the effects of different dose rates and of intermittent irradiation using high-speed electrons. This appeared to be principally caused by difficulties experienced in the irradiation techniques. The errors encountered in these experiments were probably due to unequal dose distribution in the samples; several cobalt glass dosimeters showed variations in color after irradiation. Difficulty in timing at the high dose rates could also have produced errors in delivering a predetermined dose.

These latter experiments must be considered exploratory; more study seems to be indicated.

TABLE VII

SURVIVAL OF *C. BOTULINUM* 62A SPORES SUSPENDED IN PHOSPHATE BUFFER AND IRRADIATED WITH HIGH-SPEED ELECTRONS

Beam Energy - 9.6 mev
Current - 16 microamperes
15 pulses per sec
Location of Sample: 12 in. from window

Sample Code	Time of Irradiation	Dose Rads	No. of Spores Surviving	% Survivors	Log % Survivors
D-0	0	0	28,000,000	100.0	2.000
D-1	30 sec	110,000	6,600,000	23.6	1.3729
D-2	60 "	220,000	50,000	.179	- .7471
D-3	90 "	330,000	1,030	.00368	-2.4342
D-5	150 "	550,000	8	.0000286	-4.5436
D-7	210 "	770,000	0	-	-
D-9	270 "	990,000	0	-	-
D-11	330 "	1,210,000	0	-	-
D-12	360 "	1,320,000	0	-	-

TABLE VIII

SURVIVAL OF C. BOTULINUM SPORES SUSPENDED IN NUTRIENT BROTH AND IRRADIATED WITH HIGH-ENERGY ELECTRONS AT DIFFERENT DOSE RATES

Sample	Distance to Sample	Pulses per Second	Time of Irradiation	Dose Rate Rads/sec	Total Dose Rads	Surviving Spores/ml* No.1	No.2	No.3
R-120-2	4 in.	120	2 sec	700,000	1.4×10^6	0	2	0
R-15-16	4 "	15	16 "	87,500	1.4×10^6	11	21	240
R-9-12	9 "	120	12 "	90,000	1.08×10^6	2	6	28
R-9-96	9 "	15	96 "	11,300	1.08×10^6	0	96	0
D-9	12 "	15	270 "	36,667	$.99 \times 10^6$	0	-	-

* Initial count, 28,000,000 spores/ml

TABLE IX

THE EFFECT OF GAMMA RADIATION FROM COBALT-60 ON C. BOTULINUM 62A SPORES SUSPENDED IN NUTRIENT BROTH AND PHOSPHATE BUFFER, IRRADIATED AT 0°C

Dose Megarad	Number of Spores Surviving: Nutrient Broth	Phosphate Buffer
0	10,000,000	20,000,000
.18	8,700,000	17,000,000
.36	6,100,000	5,900,000
.54	1,650,000	2,100,000
.81	310,000	52,000
.90	TNC	TNC
1.08	500	1,070
1.26	1,700	56

TABLE X

EFFECT OF HIGH-SPEED ELECTRONS ON THE SURVIVAL OF C. BOTULINUM 62A SPORES SUSPENDED IN NUTRIENT BROTH AT 0°C

Data: 9.6 mev
16 microamperes
15 pulses per second
12 inches from window

Sample	Time of Irradiation	Dose Rads	No. of Spores Surviving
D-0	0	0	46,000,000
D-1	9 sec	109,000	40,000,000
D-3	25 "	303,000	22,000,000
D-5	41 "	495,000	4,700,000
D-7	58 "	700,000	470,000
D-9	75 "	906,000	5,700
D-11	91 "	1,100,000	310
D-13	108 "	1,305,000	15

TABLE XI

THE EFFECT OF INTERMITTENT IRRADIATION WITH HIGH-SPEED ELECTRONS ON THE SURVIVAL OF C. BOTULINUM 62A SPORES

Sample	Time* of Irradiation	Dose Rads	No. of Spores Surviving
D-0	0	0	46,000,000
DI-7	7 sec	700,000	250,000
DI-9	9 "	906,000	2,000,000
DI-11	11 "	1,100,000	750,000
DI-13	13 "	1,305,000	2,700

*"Fractioned" in thirds, with a 30-sec waiting interval between irradiations.

TABLE XII

SURVIVAL OF C. BOTULINUM SPORES SUSPENDED IN NUTRIENT BROTH AND IRRADIATED WITH HIGH-ENERGY ELECTRONS AT DIFFERENT DOSE RATES

Sample	Distance Window to Sample	Pluses Per Sec	Dose Rate Rads per Pulses	Irradiation Times Sec	Number of Pulses	Total Dose Rads	No. of Spores** Surviving/ml
A-1	8.5 in.	15	0.00533	11	169	900,000	10,400,000
A-2	8.5 "	15	0.00533	11	167	890,000	> 10,000
B-1	8.5 "	120	0.00533	~ 1.3	158	842,000	> 10,000
B-2	8.5 "	120	0.00533	~ 1.5	182	960,000	> 10,000
C-1	4.0 "	15	0.00866	~ 6.8	103	890,000	30,000
C-2	4.0 "	15	0.00866	~ 6.8	102	883,000	12,000
D-1	4.0 "	120	0.00866	~ 1	126	1,090,000	> 10,000
D-2	4.0 "	120	0.00866	~ 1	120	1,040,000	6,000
E-1	12.0 "	15	*12000	75	1,252	906,000	5,700
E-2	12.0 "	120	*96700	9	1,213	870,000	> 10,000

* Rads per second

** Initial spore count, 46,000,000/ml

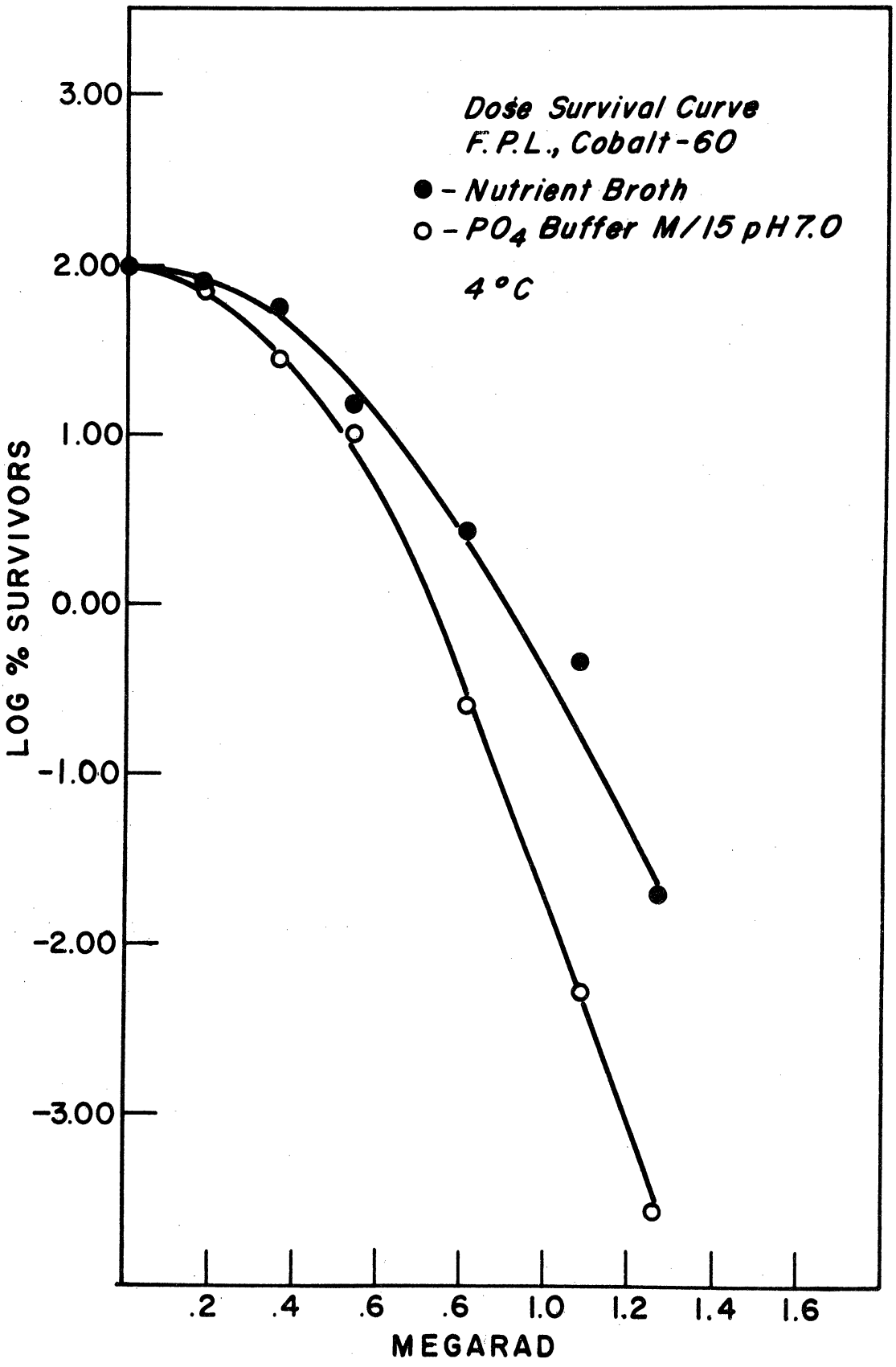


Fig. 21c. The effect of gamma radiation from cobalt-60 on C. botulinum 62A spores suspended in nutrient broth and in phosphate buffer and irradiated at 4°C.

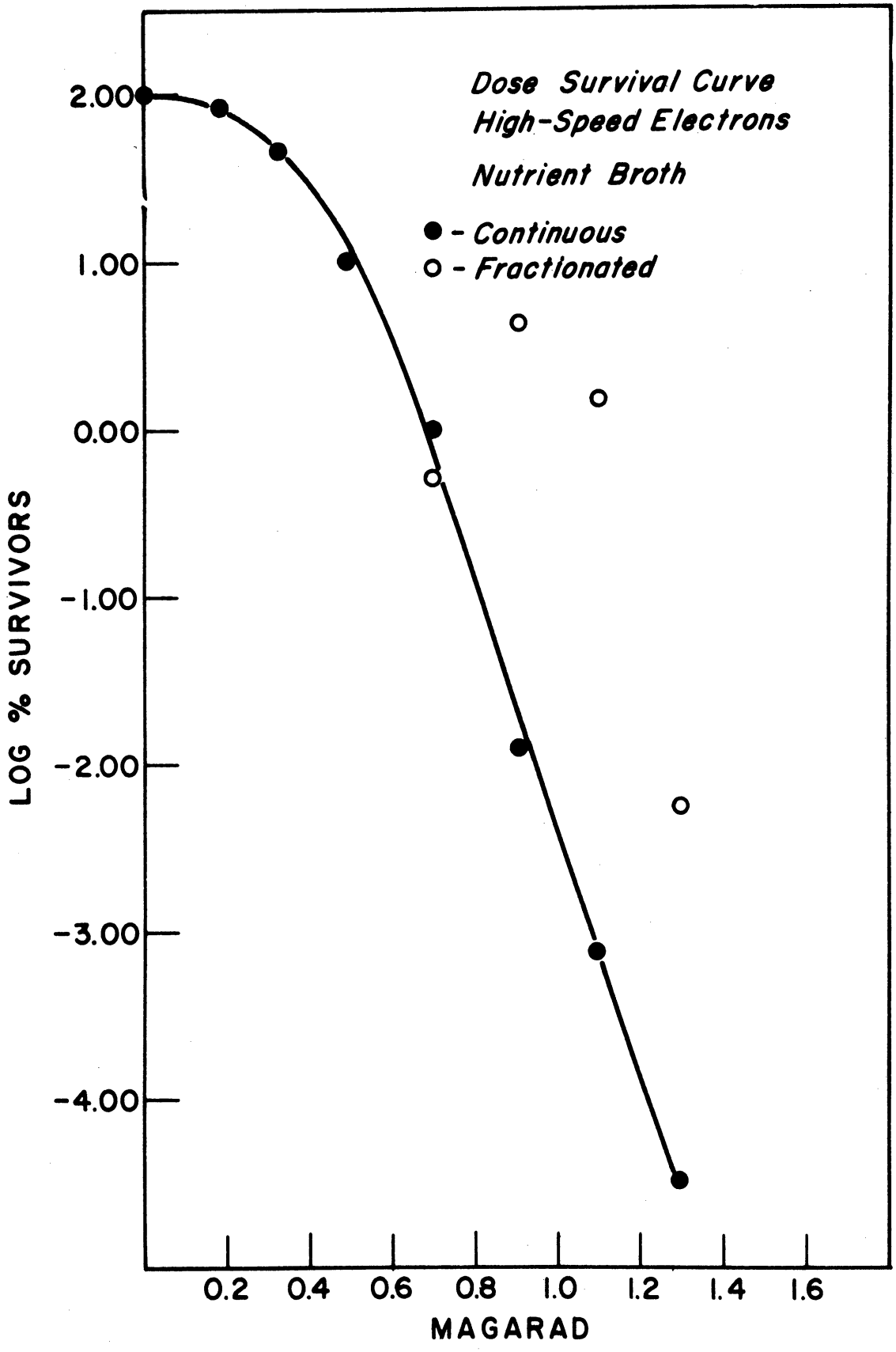


Fig. 22c. The effect of high-speed electrons on the survival of C. botulinum 62A spores suspended in nutrient broth and irradiated at 0°C.

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Therefore, insofar as the sporicidal effect of gamma radiations are concerned, the results show that data obtained on moderately weak radiation sources can be extrapolated for design purposes to field strengths of at least 2.31 megarad per hour; that interruption of irradiation for short intervals has little effect; and that cesium-137 should be essentially as effective as cobalt-60 sources for food-sterilization purposes. It was also considered desirable to know whether the variables tested for gamma radiations affected the lethality of high-energy electrons for spores of C. botulinum. A few exploratory experiments were conducted for this purpose, but no firm conclusions resulted. UNCLASSIFIED

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