Submitted to British Journal of Radiology



UCRL-20669
Preprint

c.2

# RBE AND OER OF $\pi^-$ MESONS FOR DAMAGE TO CULTURED T-1 CELLS OF HUMAN KIDNEY ORIGIN

M. R. Raju, M. Gnanapurani, C. Richman
B. I. Martins, and G. W. Barendsen
DONNER LABORATORY

April 1971

AEC Contract No. W-7405-eng-48



LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

## DISCLAIMER

# RBE and OER of $\pi^-$ mesons for damage to cultured T-1 cells of human kidney origin

by M. R. Raju, D. Sc., M. Gnanapurani, M. S., and C. Richman, Ph. D.

Lawrence Radiation Laboratory and Division of Medical Physics, University of California, Berkeley, California 94720 and University of Texas, Dallas, Texas, 75230.

B. I. Martins, M. Sc., and G. W. Barendsen,* Ph. D.

Lawrence Radiation Laboratory and Division of Medical Physics, University of California, Berkeley, California 94720.

April 1971

Measurements of the relative biological effectiveness (RBE) and of the oxygen enhancement ratio (OER) of $\pi^-$ mesons have in the past been restricted to only a few radiosensitive systems because of the limited intensity available. Reduction of root growth in Vicia faba (Raju et al., 1970) and induction of heteroallelic reversions in diploid yeast (Raju et al., 1971) were studied earlier to obtain the RBE and OER of $\pi^-$ mesons at the peak of the depth-dose distribution, although measurements using mammalian cell systems would have been more relevant for an evaluation of possible advantages of $\pi^-$ mesons in the radiotherapy of cancer.

The use of mammalian cell systems, when long hours of exposures are required, is beset with the problem of rendering the cells sufficiently hypoxic without depriving the cells of the required amount of moisture. With high intensities of radiation, the exposure times are short and the cells can be made severely hypoxic by completely removing the growth medium and passing nitrogen over the cells for 5 to 10 minutes. The present communication describes experiments using impairment of the capacity for clone formation by

*On leave of absence from the Radiobiological Institute of the Health Research Organisation TNO, Langekleineg 151, Rijswijk (Z. H.), The Netherlands.

cultured cells of human kidney origin (T-1 cells) to measure the RBE of $\pi^-$ mesons relative to $^{60}$Co $\gamma$ rays and the OER of $\pi^-$ mesons and of $^{60}$Co $\gamma$ rays, at relatively low dose rates necessitated by the limited yield of $\pi^-$ mesons from the cyclotron used.

## Material and Methods

Primarily cells of human kidney origin (T-1) were used in this investigation. The culture media, the preparation of cell suspensions, and the growth conditions have been described previously (Barendsen, 1960; Todd, 1964). Details of the $\pi^-$ meson irradiation facility at the 184-inch synchrocyclotron and the dosimetric details have also been described earlier (Raju et al., 1970).

A first series of preliminary measurements was made during a 4-month stay by one of us (G. W. B.) at Berkeley. Cells from an overnight culture were plated onto 35-mm Falcon plastic dishes, 2 days prior to exposure. The cells were made hypoxic by circulating oxygen-free growth medium over the cells attached to the dishes during $\pi^-$-meson exposures. The dose rate of $\pi^-$ mesons at the peak of the depth-dose distribution was 0.5 rad/minute.

In the second series of measurements, the dose rate of $\pi^-$ mesons was increased to 1 rad/minute by focusing the beam to the smallest area (2×3 cm) possible with the existing magnets. The cells were plated onto 35-mm plastic dishes from an overnight culture. While plating, care was taken to confine the cells to about 1 cm in the central part of the dish. After plating, the cells were incubated at 37°C for a period of about 24 hours. Just prior to $\pi^-$-meson exposures the growth medium was removed from the dishes and two layers of thin sterile gauze were placed over the cells. A drop of phosphate-buffered medium (L-15) supplemented with fetal calf serum, L-glutamine, and antibiotics was added to the gauze, thus leaving a thin layer

of growth medium in contact with the cells in the dish. The dishes were fitted in Lucite boxes, through which moist air or nitrogen at 30°C was passed. This technique kept the cells sufficiently moist for at least 8 hours. The plating efficiency of unirradiated cells was found to be 60% and did not differ significantly from that of cells kept in standard conditions.

From experiments done with $^{60}$Co $\gamma$ rays, it was found that a nitrogen pregassing time of 1.5 hours was adequate for making the cells sufficiently hypoxic to obtain the maximum OER obtainable with this system. With $\pi^-$ mesons, irradiation times were 1 to 4.5 hours in air and 2 to 6.5 hours in nitrogen. A set of controls, which were kept in identical boxes and through which air or nitrogen was passed for the same duration as above, was used for comparison.

After exposure, the cells were removed from the dishes by trypsinization, and a cell count was made with a Coulter counter. Suitable dilutions were then made so that there would be about 100 clonogenic cells in each dish. Ten 35-mm dishes were plated for each dose, except for the highest dose, in which case only 5 to 6 dishes could be inoculated. After 12 to 14 days, the cells were stained with 1% methylene blue and dried. All clones containing more than 30 cells were counted and the percent survival was obtained by comparison with the plating efficiency of unirradiated controls. Another human cell line (Chang liver) was also used in a similar way.

By use of the same setup and procedure, T-1 cells were exposed to $^{60}$Co $\gamma$ rays from a 150-Ci source at a dose rate of 240 R/hr such that the exposure times were approximately the same as those of $\pi^-$ mesons.

## Results

Figure 1 shows the survival curves obtained for irradiations of T-1 cells with $^{60}$Co $\gamma$ rays, for aerobic and hypoxic conditions. The OER at the 50% survival level was found to be 2.6 and at the 10% survival level 2.3.

Figure 2 is a plot of the percent survival as a function of the dose obtained with exposures of T-1 cells to $\pi^-$ mesons. The figure contains data obtained from both series of experiments. The value for the RBE for oxygenated cells at 10% survival was found to be 2.4. The OER at 10% survival was 1.5. Figure 3 shows the survival curves for Chang liver cells exposed to $\pi^-$ mesons. It can be seen that there is no significant difference between the survival curves for the two cell lines.

## Discussion

The curves presented in the figures were drawn by eye. It can be seen that, in spite of the significant difference in the techniques employed in the two series of experiments with T-1 cells exposed to $\pi^-$ mesons under hypoxic conditions, the results are in fair agreement.

It can be seen from the figures that the survival curves appear to bend continuously downwards. This might be caused partly by an accumulation of cells in a relatively sensitive phase of the cell cycle and partly by accumulation of damage from low-LET components of the energy deposited. Similar results have been reported by exposing an asynchronous population of HeLa cells to $\gamma$ rays at 30 rads/hr, in which case the curve was best fitted by a two-phase curve (Hall, 1969).

From Fig. 2, the value of the OER appears to be dose dependent. At 50% survival, the value of the OER is 1.9, whereas at 10% survival it is 1.5. The same trend has been observed with the values of OER calculated at various survival levels by using a best-fit computer program to

perform a least-squares analysis on our experimental data (Curtis, 1971).

Hall and Cavanagh (1967) have shown, from measurements of OER made with seedlings of Vicia faba at different dose rates of γ rays, that the OER decreases when the dose rate is reduced. A similar result of lowering of the OER with dose rate has been obtained by Hall and Bedford (1966) and Berry (1968) with mammalian cells in vitro and in vivo. However, the situation with neutrons, was suggested to be different. Exposures at 30 to 50 rads/min of 14-MeV neutrons and at 16 rads/hr of $^{252}Cf$ fission neutrons yielded the same OER value of about 1.66 (Hall, 1969). For $\pi^-$ mesons at the peak of the depth dose distribution, the LET spectrum is very broad, and similar to that of 14-MeV neutrons except that the LET distribution of $\pi^-$ mesons extends to lower values (Curtis and Raju, 1965). The survival curves in Figs. 2 and 3 display a small shoulder, which may be due to accumulation of cells in a sensitive phase or to induction of a small contribution of sub-lethal damage.

Root-growth sutdies by Raju et al. (1970) on Vicia faba gave a value of 1.5 for the OER of $\pi^-$ mesons at a temperature at which the value is considered to be relatively independent of dose rate (Hall and Cavanagh, 1967). Strict comparison between the results obtained with the bean root system and those obtained with the mammalian cell system is not possible, since in the root-growth studies the OER was relatively independent of the dose level, whereas for T-1 cells the value of OER depends on the dose level at which it is considered. However, both the systems display a similar trend in reduction in the OER below that for $^{60}Co$ γ rays.

## Summary

Cultured cells of human kidney origin (T-1 cells) have been used to measure the oxygen-enhancement ratio of $\pi^-$ mesons at the peak of the depth-dose

distribution, and the biological effectiveness of $\pi^-$ mesons was compared with that of $^{60}$Co $\gamma$ rays. The OER appears to be dose dependent, and has values of 1.9 and 1.5 at survival levels of 50% and 10% respectively. The relative biological effectiveness of $\pi^-$ mesons was found to be between 2.2 and 2.4 for oxygenated cells. The OER of $^{60}$Co $\gamma$ rays (240 R/hr) was 2.6 and 2.3 measured at 50% and 10% survival respectively. Similar measurements with Chang liver cells yielded identical values for the OER of $\pi^-$ mesons.

Acknowledgement

We are indebted to Dr. John H. Lawrence and Dr. C. A. Tobias for their continued interest and encouragement. We thank Mr. U. Madhvanath and Mr. B. Stackler for their help during radiation exposures. We have profited considerably from discussions with Dr. P. W. Todd. This work was done under auspices of the U. S. Atomic Energy Commission and the American Cancer Society.

REFERENCES

BARENDSEN, G. W., BEUSKER, T. L. J., VERGROESEN, A. J., and BUDKE, L., 1960, Radiat. Res. 13, 841-849.

BERRY, R. J., 1968, Brit. J. Radiol., 41, 921-926.

CURTIS, S. B., 1971, private communication.

CURTIS, S. B., and RAJU, M. R., 1965, Radiat. Res. 34, 239-255.

HALL, E. J., 1969, In Time and Dose Relationships in Radiation Biology as Applied to Radiotherapy, BNL 50203 (C-57), 308-317.

HALL, E. J., BEDFORD, J. S., and OLIVER, R., 1966, Brit. J. Radiol., 39, 302-307.

HALL, E. J., and CAVANAGH, J., 1967, Brit. J. Radiol., 40, 128-133.

RAJU, M. R., AMER, N. M., GNANAPURANI, M., and RICHMAN, C., 1970, Radiat. Res., 41, 135-144.

RAJU, M. R., GNANAPURANI, M., STACKLER, B., MARTINS, B. I., MADHVANATH, U., HOWARD, J., LYMAN, J. T., and MORTIMER, R. K., Radiat. Res. (to be published).

TODD, P. W., 1964, Ph. D. Thesis, UCRL-11614.

## FIGURE CAPTIONS

Fig. 1. Survival curves for T-1 cells exposed to $^{60}Co$ $\gamma$ rays under aerobic and hypoxic conditions.

Fig. 2. Survival curves for T-1 cells exposed to $\pi^-$ mesons at the peak of the depth-dose distribution under aerobic and hypoxic conditions. The figure also shows the data obtained by circulating medium over the cells.

Fig. 3. Survival curves for Chang liver cells exposed to $\pi^-$ mesons at the peak of the depth-dose distribution under aerobic and hypoxic conditions.

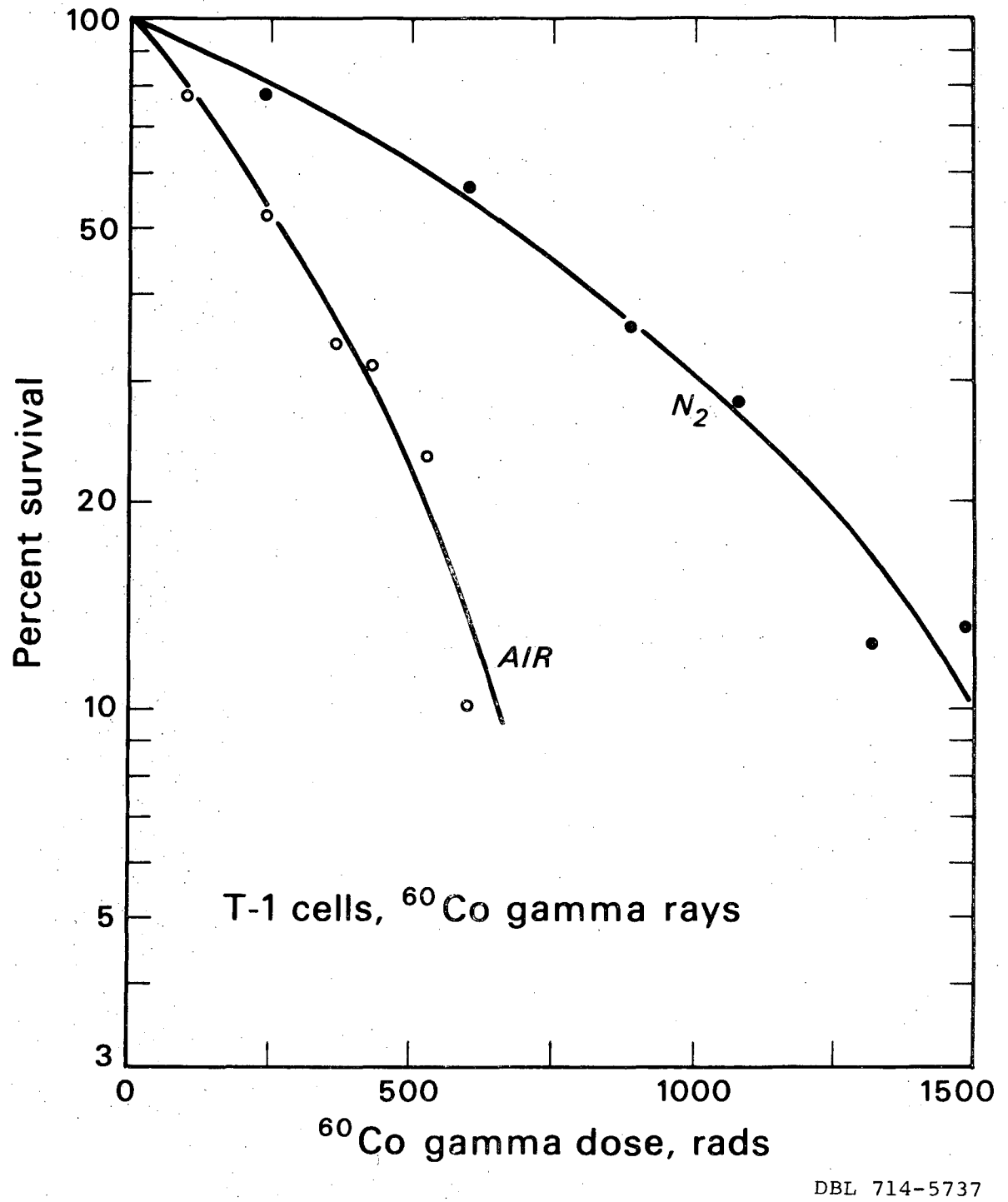
100
50
20
10
5
3
Percent survival
$N_2$
AIR
T-1 cells, $^{60}$Co gamma rays
0
500
1000
1500
$^{60}$Co gamma dose, rads
DBL 714-5737

Fig. 1

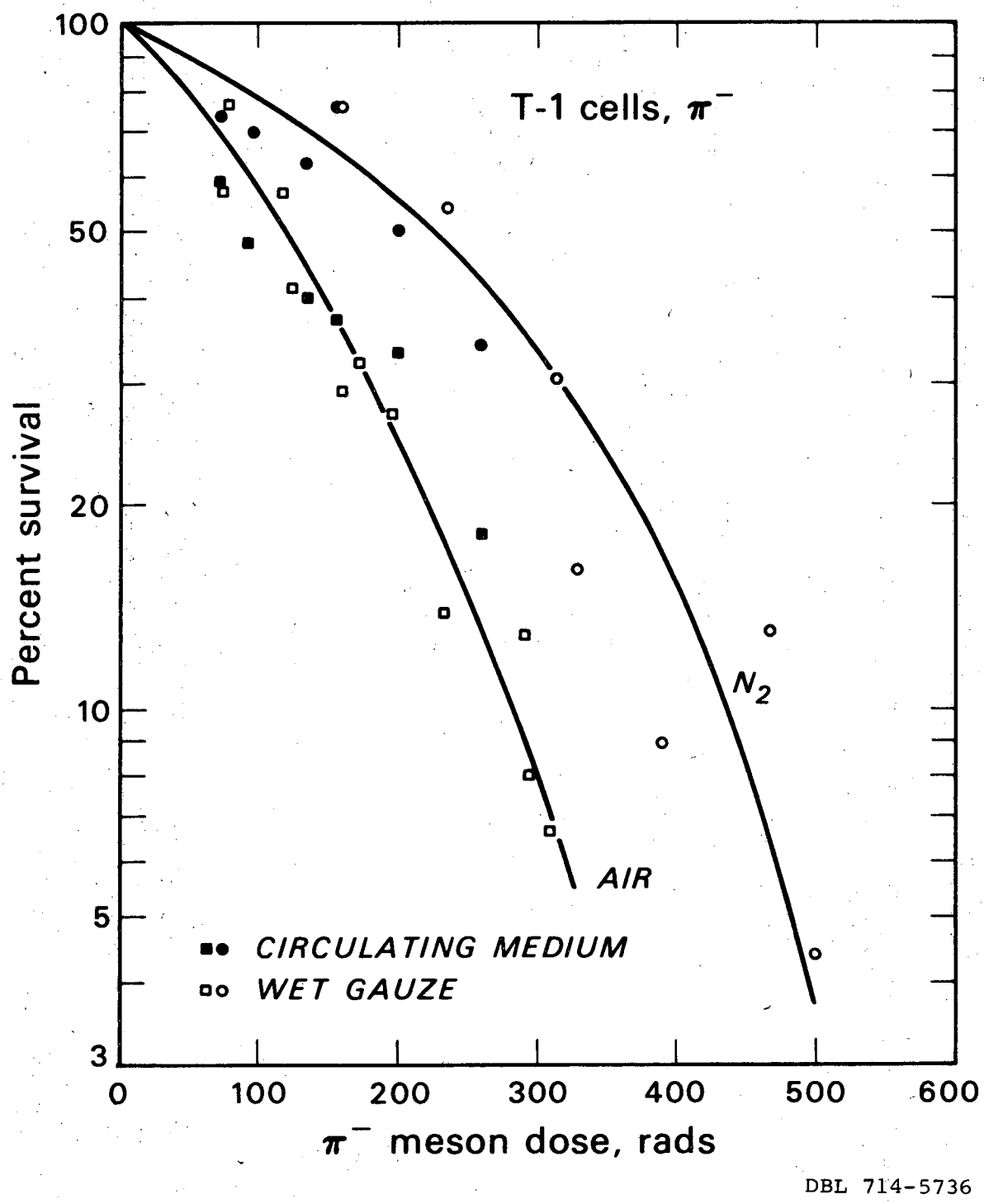
T-1 cells, π⁻
Percent survival
100
50
20
10
5
3
0
100
200
300
400
500
600
π⁻ meson dose, rads
N2
AIR
CIRCULATING MEDIUM
WET GAUZE
DBL 714-5736

Fig. 2

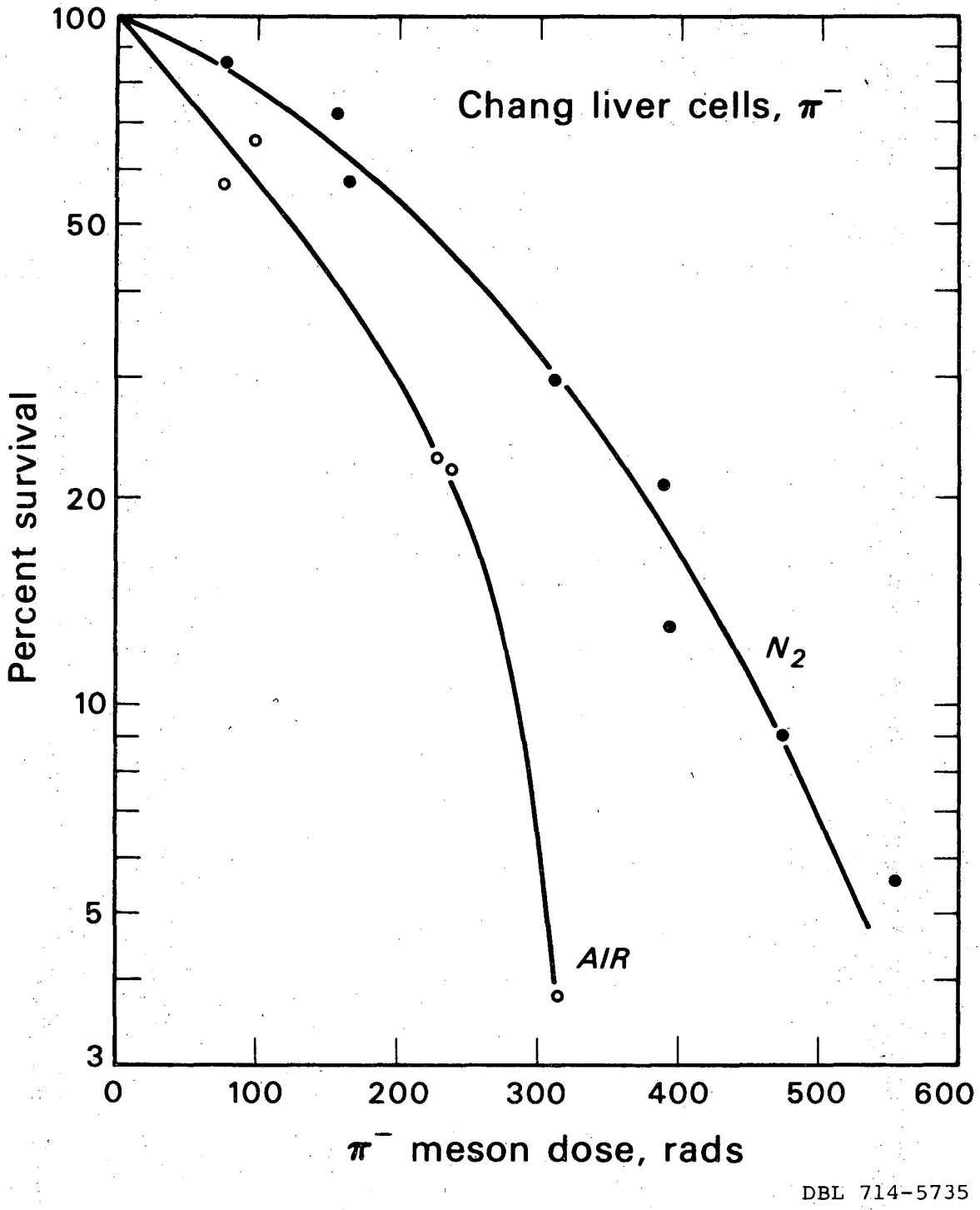
Chang liver cells, π⁻
Percent survival
100
50
20
10
5
3
0
100
200
300
400
500
600
π⁻ meson dose, rads
N2
AIR
DBL 714-5735

Fig. 3

*TECHNICAL INFORMATION DIVISION*
*LAWRENCE RADIATION LABORATORY*
*UNIVERSITY OF CALIFORNIA*
*BERKELEY, CALIFORNIA 94720*