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● *Original Contribution*

RADIOBIOLOGICAL INTERCOMPARISONS OF FAST NEUTRON BEAMS USED FOR THERAPY IN JAPAN AND THE UNITED STATES†

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A variety of portable biological systems have been used to intercompare the neutron beams used for radiotherapy in Japan and in the United States. The two neutron centers in Japan have been compared with the four in the United States; all of the machines differ in energy and consequently the biological effectiveness varies from one to another. The biological systems used included survival in three lines of mammalian cells cultured *in vitro*, the response of mouse skin, the survival of crypt cells in the mouse jejunum, and the loss of weight or DNA in the mouse testes. Based on the biological data, estimates have been made of the relative potency of the various neutron beams that will be invaluable when the time comes to evaluate clinical results.

Neutrons, Intercomparison, Relative biological effectiveness.

BACKGROUND AND RATIONALE

The trial use of neutrons as an alternative to X-rays or γ-rays for the radiotherapy of human cancer is actively underway in a number of centers in Great Britain, continental Europe, the United States and Japan. Because of the substantial cost and investment of effort involved in the implementation of clinical neutron trials, the value of full cooperation between the few centers using these particles has been recognized from the outset.

Because of the close ties between the United States and Japan, a special effort has been made to intercompare neutron beams in these two countries. At the time of this study, there were four centers in the United States and two in Japan at which patients have been treated with neutrons. Table 1 summarizes the characteristics of the neutron beams at the various centers.

A substantial effort to achieve compatible dosimetry was mounted by the physicists at the various installations engaged in neutron therapy. As a consequence there is agreement to with ±1.5% for dose measurements in air. The agreement is almost certainly not as good for dose measurements in a

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Table 1. Clinical neutron facilities in the U.S. and Japan involved in the intercomparisons

Facility	Location	Accelerated particle	Energy (MeV)	ssd† (cm)	Dose-rate (rad/min)
University of Washington Cyclotron	Seattle, U.S.A.	d^+	22	125	20–50
Naval Research Laboratory Cyclotron (NRL)	Washington, D.C. U.S.A.	d^+	35	125	30–55
Texas A&M Variable Energy Cyclotron (TAMVEC)	College Station, Texas, U.S.A.	d^+	50‡	140	50–80
Fermilab Linear Accelerator	Batavia, Illinois U.S.A.	p^+	66	150	11–15
National Institute of Radiological Sciences Cyclotron (NIRS)	Chiba, Japan	d^+	30	200	30–60
Institute of Medical Sciences (I.M.S.)	Tokyo	d^+	16	100	20

†Source-Surface Distance.
‡This facility can also be operated at 16 and 35 MeV to simulate the neutron beams at IMS and NRL respectively.

phantom, especially for the various set-ups used for the irradiation of biological specimens. While agreement in the measurement of physical dose is obviously important, compatible dosimetry does not of itself allow radiotherapists to compare dosage schedules, since each neutron facility operates at a different energy and is characterized by a different relative biological effectiveness (RBE). In order to allow a pooling of experience and to form a basis for the subsequent comparison of clinical results, a series of biological intercomparisons was arranged under the sponsorship of the U.S.–Japan Cooperative Cancer Research Program. An extensive series of experiments has been performed in Japan and at one or more of the U.S. neutron facilities, using the various biological test systems listed in Table 2.

EXPERIMENTS PERFORMED

Dr. Joseph Geraci of the University of Washington, Seattle, uses DNA loss and weight loss of mouse testes as an indicator of biological effect. Methods have been described previously.[2] Dose response curves obtained with the National Institute of Radiological Sciences (NIRS) cyclotron at Chiba and the University of Washington Cyclotron, Seattle, appear in Fig. 1.

Dr. Eric Hall, using Chinese hamster V79 cells cultured *in vitro*, has performed experiments at Chiba and at all of the U.S. facilities. In order to fully exploit the precision of which the *in vitro* system is capable, cyclotrons were intercompared in pairs, within a given experiment on the same day. For cells in culture, variations *within* an experiment are much

Table 2. Investigators and biological test systems used for U.S.–Japan neutron intercomparisons

Investigator	Biological test systems	Facilities visited†	Level at which RBE computed	Dose level at Chiba (rad)
J. Geraci	Mouse testes weight loss	S, C, T	50%	40
Univ. of Washington	DNA content		50%	65
E. J. Hall	Chinese hamster	S, N, T, F, C	Overall curve shape	50–800
Columbia University	V79 cells in culture		(see text)	
R. Meyn	Chinese hamster	S, N, T, C, I	0.3 survival	150
M.D. Anderson Hospital	CHO cells in culture			
J. Rasey	Mouse skin	I, S, C, T, F	Ave. skin	1900
Univ. of Washington			reaction of 1.25	
P. Todd	*T*1 human cells in culture	I, S, C, N	0.3 survival	160 ± 16
Pennsylvania State Univ.				
H.R. Withers	Jejunal crypt cells	S, N, T, F, C	10 surviving crypts	850
M.D. Anderson Hospital	in the mouse		per circumference	

†Key to facility at which intercomparison experiments were performed. S: Univ. of Washington, Seattle, N: Naval Research Laboratory, Washington, D.C., T: TAMVEC, Texas, F: Fermilab, Batavia, Illinois, C: Chiba, Japan, I: IMS, Tokyo.

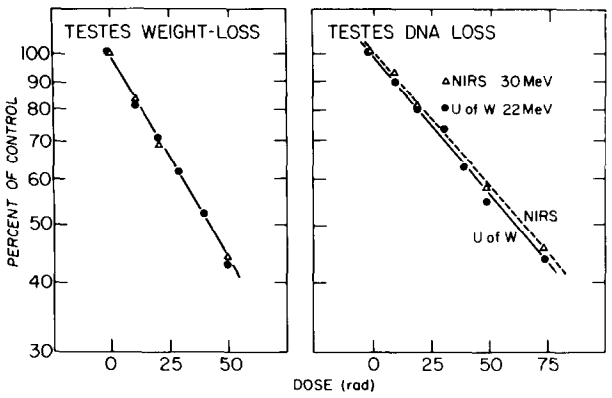

Fig. 1. Dose response relationships for the weight loss and DNA loss of the mouse testes irradiated with neutrons from the cyclotrons at National Institute of Radiological Sciences (NIRS), Chiba and the University of Washington (U. of W.) at Seattle.

smaller than *between* experiments. Within a given experiment, the repeatability between replicate flasks is limited only by the counting statistics, and since large numbers of colonies can be used, the accuracy is of the order of a few per cent.

By contrast, when experiments are repeated on separate occasions, it is not unusual for the cell surviving fraction at a given dose level to differ by a factor of two. For this reason, the system was adopted of intercomparing facilities within a given experiment. The details have been published previously;[4] briefly, appropriate numbers of cells were plated into Falcon tissue culture flasks in New York, and allowed to attach by overnight incubation at 37.5°C. Half the flasks were flown to the Naval Research Laboratory (NRL) cyclotron in Washington, D.C., which always served as one arm of each experiment, and the other half to the facility to be compared. In this way NRL was compared in successive experiments with each of the facilities listed in Table 1. The cells were transported in insulated water jacketed carriers with the temperature maintained at 17°C to prevent cell division and progression through the cycle, while preserving a high plating efficiency. Irradiations were performed simultaneously, at the same real time, at the two neutron facilities to be compared, and the cells returned to an incubator at 37°C for 8 days. This procedure was possible in the case of the Japanese intercomparisons by making use of the non-stop polar flights.

Figure 2 shows dose-response data from simultaneous irradiations at NRL and Chiba, while Fig. 3 refers to NRL and Fermilab. These data were analysed by a new non-parametric method that evokes no form for the dose-response relationship. A computer program fits curves of the same shape simultaneously to the survival data for the two neu-

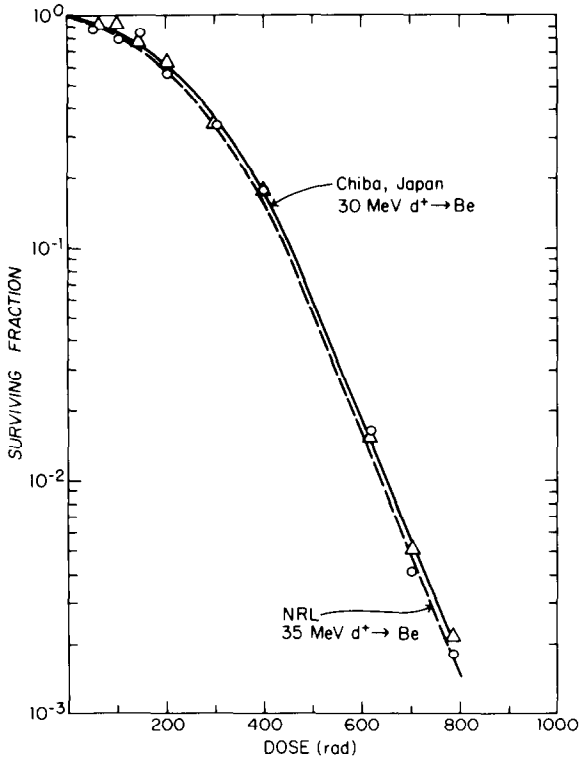

Fig. 2. Survival curves for V79 Chinese hamster cells irradiated at Chiba or at the Naval Research Laboratory (NRL), Washington, D.C.

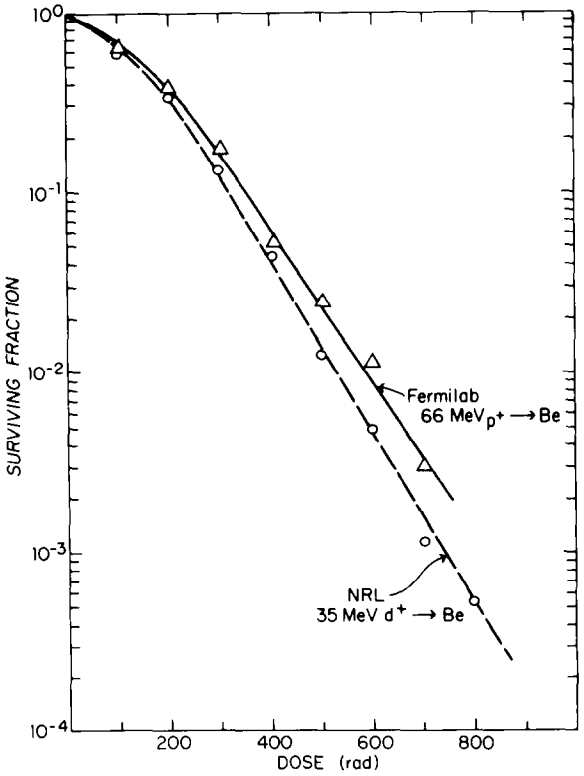

Fig. 3. Survival curves for V79 Chinese hamster cells irradiated at the Fermilab or at the Naval Research Laboratory (NRL), Washington, D.C.

tron energies to be intercompared, the only constraint being that the curves must be convex upwards. The single dose factor between the two curves is then a measure of the RBE difference, or relative potency, between the two neutron beams, based on the data accumulated over the entire dose range.

Dr. Raymond Meyn of the M.D. Anderson Hospital in Texas has performed experiments at both Chiba and IMS in Japan and also at the Texas A&M Variable Energy Cyclotron (TAMVEC), NRL and Seattle in the U.S., using Chinese hamster ovary (CHO) cells cultured *in vitro*. A detailed description of the experimental methods has been published.[3] Data from experiments performed in the U.S. and Japan appear in Fig. 4.

Dr. Janet Rasey of the University of Washington, Seattle, uses the skin reactions in the feet of mice as an index of radiation effect. *In vivo* systems tend to be more variable than techniques involving cells in culture and in addition the skin scoring system is subjective. The subjective scoring scale used was a modification of that described by Fowler *et al.*[1] Each intercomparison experiment included a full control neutron dose-response relationship determined at the University of Washington cyclotron. Experiments were performed at both facilities in Japan and at several U.S. centers. Data from some of these experiments are shown in Fig. 5.

Dr. Paul Todd, of the Pennsylvania State University, with the collaboration of Dr. Geraci, used *T*1 kidney cells of human origin to make RBE determinations at both cyclotrons in Japan, as well as NRL and Seattle in the United States. Techniques and detailed data analysis have been described previously.[5] A representative selection from the more extensive data set is shown in Figs. 6 and 7, which illustrate results of experiments in which two cyclotrons were compared on the same day using a single population of cells.

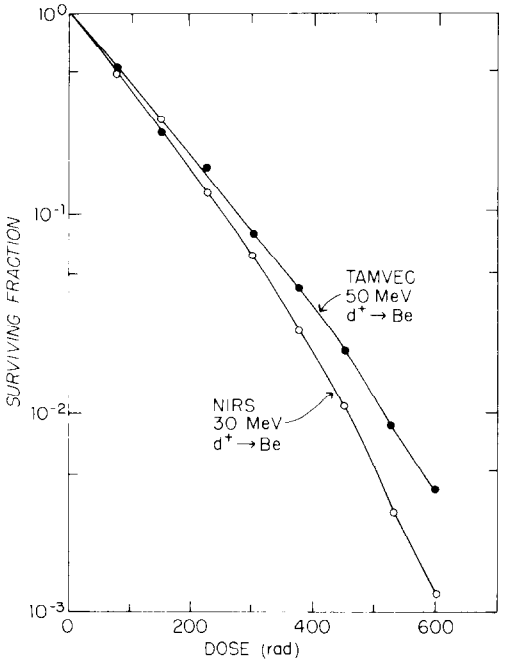

Fig. 4. Survival curves for CHO cells irradiated with fast neutrons at TAMVEC in the United States and at NIRS in Japan.

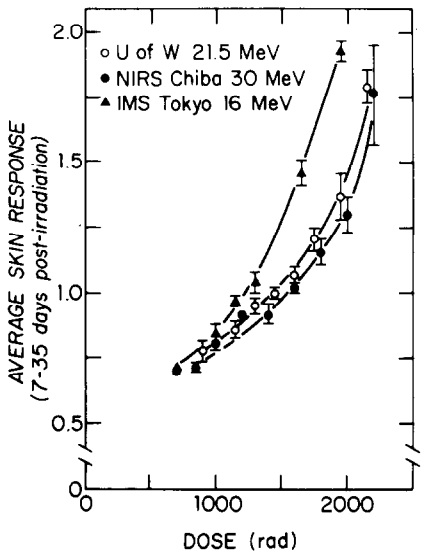

Fig. 5. Dose response relationships for the early skin response in mouse feet irradiated with neutrons at NIRS, Chiba, IMS, Tokyo, and the University of Washington, Seattle. The points represent the average skin reaction for the group of mice treated, and the vertical bars the standard error.

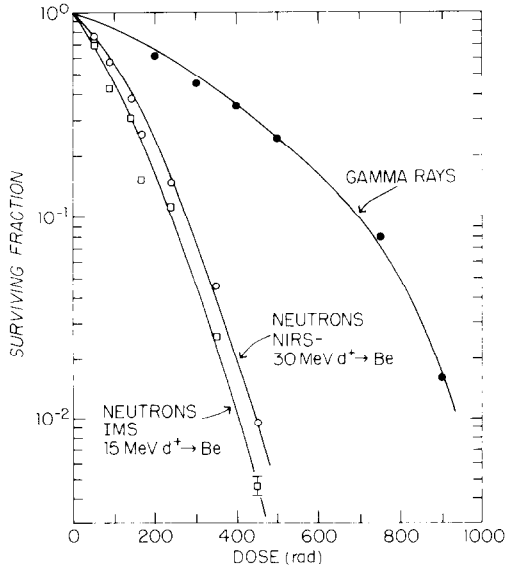

Fig. 6. Survival curves for *T*1 cells exposed to gamma rays or to fast neutrons at the NIRS Chiba, or the IMS Tokyo ted (from Todd, Geraci, Furcinitti, Rossi, Mikage, Theus & Schroy).

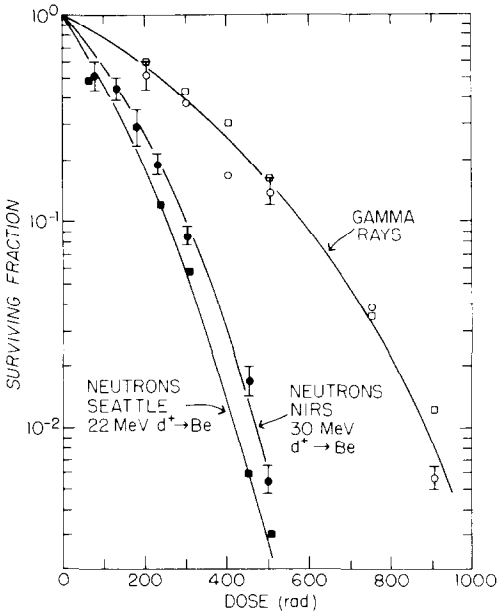

Fig. 7. Survival curves for *T*1 cells exposed to X-rays, γ-rays, or fast neutrons at the NIRS, Chiba or the University of Washington, Seattle cyclotrons. The standard errors are shown where larger than the points plotted.

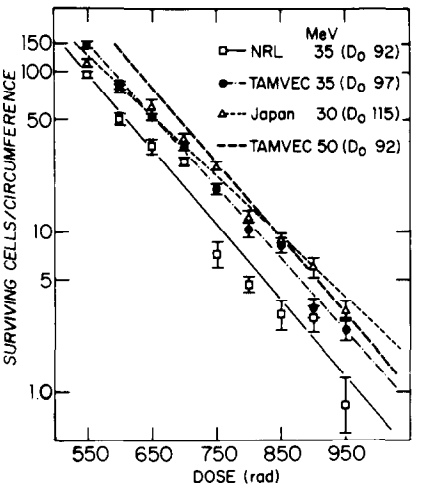

Fig. 8. Dose response relationships from the number of surviving crypt cells in the mouse jejunum irradiated with neutrons from the NRL, TAMVEC or NIRS, Chiba cyclotron. The points plotted are the mean number of cells surviving per circumference, and the vertical bars represent the standard error.

Figure 8 is a collection of data from experiments performed by Dr. H. Rodney Withers, of M.D. Anderson Hospital, Houston, Texas. Dr. Withers uses as endpoint the survival of crypt cells in the mouse jejunum.[6] Experiments were performed at Chiba and at all of the U.S. facilities.

RESULTS

Table 3 summarizes the results and conclusions of all of the intercomparisons performed under the auspices of this program. The relative biological effectiveness of each of the various neutron beams at IMS, Tokyo and at the four U.S. facilities is compared with that of the Chiba neutron beam which is taken to be 1.0. Most of these ratios are calculated from direct experimental comparisons between the Chiba beam and each facility, but in some cases, in order to provide additional figures relevant to more of the U.S. Centers, data reported at the "particles in Radiation Therapy" part II meeting held at Berkeley in September 1976[4] have also been used.

The data contained in Table 3 are reproduced in graphical form in Fig. 9. The effectiveness of the various neutron beams is compared with the Chiba beam and plotted as a function of the energy of the accelerated deuteron or proton used in the neutron production process.

In general, the "potency" of the various neutron beams decreases with increasing energy of the photon or deuteron, as would be expected. There are, however, two important exceptions.

(a) The Chiba neutron beam (30 MeV $d^+ \rightarrow$ Be) is slightly less effective by about 2% than the NRL

Table 3. Relative biological effectiveness of the neutron beams at the various facilities with Chiba, as the reference beam

Facility	Energy (MeV)	Geraci Mouse testes (wt)	Geraci Testes DNA	Hall V79 cells	Meyn CHO cells	Rasey Mouse skin	Todd *T*1 cells	Withers Mouse crypt cells
Chiba	30	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IMS	16	†	†	†	1.05 ± 0.06	1.31	1.22 ± 0.09	†
Seattle	22	1.02 ± 0.10	1.06 ± 0.14	1.10	1.11 ± 0.04	1.07	1.18 ± 0.27	1.15
NRL (TAMVEC 35)	35	†	†	1.02	1.00 ± 0.04	†	1.10 ± 0.08	1.12 (1.02)
TAMVEC	50	0.83 ± 0.11	0.91 ± 0.13	0.89	0.93 ± 0.06	0.97	†	0.97
Fermilab	66	†	†	0.92	†	0.96		†
TAMVEC	15	†	†	†	†	†	1.39 ± 0.14	†

†No comparison was made for that machine with a given biological system.

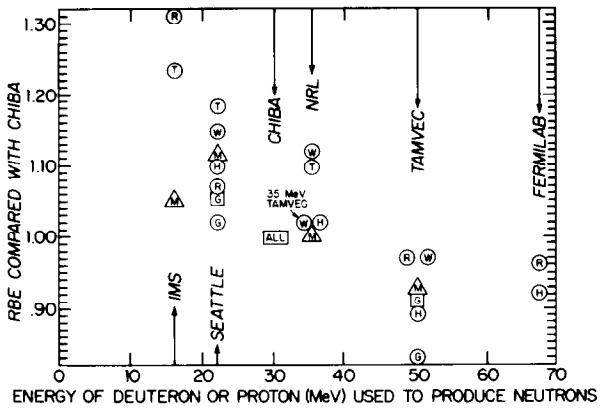

Fig. 9. Relative Biological Effectiveness of the neutron beams at IMS, Tokyo, or at the four U.S. facilities, compared with Chiba which is taken as one. The RBE values are plotted as a function of the energy of the deuteron or proton used in the neutron production process.

beam (35 MeV $d^+ \rightarrow$ Be). There is good agreement in this estimate among three investigators (Todd, Hall and Withers). Hall and Withers made the direct comparison between 30 and 35 MeV $d^+ \rightarrow$ Be neutrons within a given experiment. Withers' data at NRL are in doubt because of technical difficulties encountered in this particular experiment and the point plotted in Fig. 9 is for 35 MeV $d^+ \rightarrow$ Be at TAMVEC. It is not clear why the Chiba beam should be *less* effective than NRL when its energy is lower. It is possible that the neutron spectrum, or the proportion of gamma rays contained in the beam, is altered by the special variable collimator used at Chiba, or influenced by the thickness of the beryllium target.

(b) The Fermilab beam has the same or a slightly higher RBE than TAMVEC, in spite of its higher energy. This was the conclusion of both investigators who made measurements at the two facilities (Rasey and Hall). This is not surprising because at TAMVEC the accelerated particles are deuterons, whereas at the Fermilab protons are used and it is known that the neutron spectrum is different for the two production processes.

DISCUSSION

An overall view of the data leads to certain conclusions.

(1) There is a larger spread in the RBE estimates than might have been desired in a concerted intercomparison effort of this sort. This was noted in the results of the first round of intercomparisons of the U.S. facilities reported previously.[4] There are several possible explanations for this situation. For example, the various biological test systems used vary in sensitivity; consequently the relative RBE's intercompared are computed at different dose levels. In the case of the mouse testes weight loss system, the RBE estimates were calculated for a dose of about 40 rad in the case of neutron beam at Chiba, while the mouse skin reaction system required a dose of 1900 rad. It is well known that the RBE of neutrons relative to X-rays is a decreasing function of increasing dose, and it is probable that the relative potency of two neutron beams also varies with dose, although there are cogent reasons to suppose that the variation is small. With most biological systems the RBE difference between two neutron beams that are close in energy appears to be nearly independent of the dose level when the intercomparison is made within the same experiment. Certainly this is not a dominant factor since the biological systems that require the highest doses (mouse skin or jejunal crypts) give rise to larger RBEs than endpoints requiring the smallest doses (mouses testes weight loss or DNA loss or CHO cells survival). The opposite would be expected if the variation of RBE with dose were an important factor.

Another consideration is the consistency of neutron spectra compared. For the irradiation of cells in culture, for example, the specimens were located at the center of a large neutron field. By contrast, the skin reaction experiments involved irradiating the feet of mice, which of necessity were located at the edge of the field so that the bodies of the animals could be shielded. It is possible that the difference in the neutron spectrum between the center and periphery of the field would be greater for the higher than for the lower energy cyclotrons. It is difficult to know to what extent this factor may influence the biological intercomparisons, since detailed data on neutron spectra are simply not available.

(2) The mouse testes system, used by Geraci *et al.*[2] appears to give lower estimates of the U.S. Machines, relative to Chiba, than the other biological test systems. V79, CHO and *T*-1 cells tend to give RBE estimates closer to those obtained with normal tissue systems such as skin and jejunal crypt cells, which may be a consequence of the fact that all of these systems have in common the capacity to accumulate and repair a substantial amount of sublethal radiation damage.

(3) The results demonstrate the importance of using a variety of biological test objects and endpoints and of performing simultaneous irradiations at sites to be compared when test objects with high inter-experiment variability are used.

By using a sufficiently diverse collection of biological endpoints, it is possible to produce an almost consistent set of relative calibration factors among therapeutic neutron sources. Based on the accumulated data, Chiba neutrons appear to be approximately:

(1) 7–31% less effective than the neutrons at IMS,

with more emphasis to be placed on the higher end of this range, namely 23–31%.

(2) 2–18% less effective than Seattle neutrons, with the most likely range being 8–16%.

(3) 2% less effective than NRL.

(4) 3–17% more effective than TAMVEC, with the most likely range being 3–11%.

(5) 4–8% more effective than Fermilab.

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