Reports

Population Size Required for Investigating Threshold Dose in Radiation-Induced Leukemia

Abstract. Studies of leukemia in populations receiving small amounts of radiation are needed to investigate the question of a threshold dose. Estimates have been made of the population sizes required to detect a statistically significant increment of leukemia at specified low exposures, by means of the dose-response relation observed by Court-Brown and Doll at high doses.

The leukemogenic effect of large doses of ionizing radiation is now well established. A problem which remains unsolved is whether there exists a threshold dose of radiation below which leukemia is not induced.

Various studies of populations exposed to low doses of radiation have been proposed to settle this question, including a comparison of populations living at high and low altitudes. At an altitude of 6000 feet, the annual excess of cosmic radiation over that received at sea level is approximately 23 mr, or 1.5 r by age 65. I attempted to examine leukemia death rates by altitude in the United States, only to realize that it was extremely unlikely that the effect of such a small dose, even if it existed, could be demonstrated as statistically significant with the sizes of populations available. This realization led to an attempt to estimate what population sizes would be required to demonstrate significant leukemogenic effects of small doses of radiation.

For this purpose, the dose-response data of Court-Brown and Doll (*1*) were used. Over a dose range of approximately 500 to 2000 r, Court-Brown and Doll observed a linear relationship such that the annual incidence of leukemia per 100,000 population is increased by 4.9 cases per dose of 94 r to the spinal marrow. The equivalent whole-body dose is estimated by them to be between 30 and 50 r. By taking their upper estimate, this dose-response relationship can be extrapolated and expressed as follows: for each roentgen unit received as a whole-body dose, the annual death rate from leukemia per 100,000 population would be increased by 0.10 (for leukemia, death rate and case rate can be equated).

This relationship was then applied to hypothetical populations receiving specified whole-body doses of radiation from birth to age 34, and followed for 10 years from age 35 to 44 years. In making the calculations, two assumptions were invoked: i) that if deaths from radiation-induced disease resulted from that part of the dose received before age 35, such deaths would not occur selectively, and thus the population remaining at age 35 would have the same dose-response rate as the original cohort; ii) that, for ease of calculation, general mortality between ages 35 and 44 could be neglected. This is a reasonable assumption since mortality loss over this 10-year age span is only about 3 percent (*2*).

For each dose of radiation, the leukemia deaths predicted from Court-Brown and Doll's dose-response rate were taken for populations of decreasing size, until the difference between this hypothetical "observed" number of deaths and the number expected from general population rates failed to reach statistical significance at the 5-percent level. The general population rate used was that given by MacMahon (*3*) for the United States population aged 35 to 44 from 1949 to 1953 (3.32 per 100,000). Statistical significance of the difference between an "observed" and expected number of deaths was based on confidence limits for the expectation of a Poisson variable (*4*).

Table 1 gives, for doses ranging from 5 to 200 r, the minimum population size which *on the average* would reveal a significant effect. Population size is expressed in person-years—for example, for 5 r, 6 million persons followed for 1 year or 600,000 followed for 10 years are required.

It is now of interest to relate these estimates to completed and proposed studies of the threshold question. The unique exposure of a population of 100,000 persons living in a radioactive monazite sand area in the state of Travancore, India, is a case in point. The dose received over a 30-year period is said to lie between 10 and 30 r (*5*). The estimates shown in Table 1 indicate that it should be possible with a population of this size to demonstrate, statistically, a leukemogenic effect of 30 r, if such exists. However, these estimates can be applied to the Travancore situation only if age-specific leukemia mortality rates of the Indian general population are comparable to those in the United States and if leukemia is diagnosed as adequately there as it is in the United States. The latter requirement presumably could be met if a special study were made of the Travancore inhabitants and of a sizable Indian control population.

Court-Brown and Doll (*6*) have published a study of the mortality of 1377 British radiologists from 1897 to 1957. For the 10,279 person-years of risk among radiologists who began practice before 1921, when less care was taken to prevent exposure to x-rays, the excess of observed leukemia deaths over the expected number did not reach statistical significance. The average total dose received by the group obviously cannot be determined, but if it were just short of 200 r, the available population size would not be quite sufficient to permit detection of a statistically significant excess of leukemia deaths. Moreover, the estimates given in Table 1 refer to exposures from birth to age 34, which is not the situation among radiologists. If dose-response rate does not rise with age, the sizes of the required populations would be greater for exposures occurring at an older age, since leukemia mortality among adults increases with age.

The possibility that dose-response rate may actually fall with age is suggested by the observation of Stewart *et al.* (7)

Table 1. Minimum population sizes for specified doses of radiation.

Dose from birth to age 34 (r)	Minimum person-years at ages 35 to 44
5	6,000,000
10	1,600,000
15	750,000
20	500,000
50	100,000
100	30,000
200	10,000

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

that mothers of children dying from leukemia had a significantly greater frequency of antenatal abdominal x-ray examinations than mothers of matched control children (13 percent compared with 7 percent). The x-ray dosage in this type of examination would not exceed 5 r. It is apparent that such a large difference between the two groups of children would not be found if the dose-response relationship in the fetus were that estimated for adults by Court-Brown and Doll. This leads one to estimate from Stewart's data the fetal dose-response which would produce such a difference. This was done as follows:

1) The estimated population under age 10 in England and Wales in 1951 was 6 million. If the percentage of mothers of control children reporting an antenatal x-ray (7 percent) is applied to this population, there would be an estimated 420,000 children exposed in utero to a dose of approximately 5 r and 5.58 million children not so exposed.

2) From 1953 to 1955 there were 792 leukemia deaths in this age group, an annual average of 264.

3) Let R_1 and R_2 be the annual leukemia death rates per 100,000 population under age 10, for children with and without antenatal irradiation, respectively.

4) Since 13 percent of the leukemia children had a history of antenatal x-ray, 34 (0.13×264) children dying of leukemia in one year would have such a history. Therefore

$$R_1 = \frac{34 \times 100{,}000}{420{,}000} = 8.1$$

5) Since the remaining 87 percent of the 264 leukemia deaths would come from the nonirradiated group,

$$R_2 = \frac{230 \times 100{,}000}{5{,}580{,}000} = 4.1$$

6) Thus an estimated annual increment of 4.0 deaths per 100,000 population followed a dose of 5 r. The increment expected from Court-Brown and Doll's dose-response data is only 0.5 per 100,000.

Thus it appears that if Stewart's observation does, in fact, reflect an association between antenatal irradiation and leukemia, the response rate of embryonic tissue is approximately 8 times that of adult tissue.

The estimates which form the basis of this report are not intended to be precise figures, but are offered merely to stimulate interest in a very practical aspect of the problem of investigating the existence of a threshold leukemogenic dose of radiation. This aspect of the problem has so far received little attention.

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Nitrogen Partition in Excreta of Three Species of Mosquitoes

Abstract. Adults of three species of mosquitoes, *Aedes aegypti, Anopheles quadrimaculatus* and *Culex pipiens,* showed essentially similar patterns of nitrogen output as judged by their excretion of total nitrogen and by their excretion of nitrogen as uric acid, urea, ammonia, amino acid, and protein. About 80 percent of their total nitrogen has been accounted for. Substances that seem on analysis to be like glycoprotein have been found in the excreta of the three species.

In continuing our previous work on the total nitrogen and uric acid patterns in the excreta and body tissues of adult *Aedes aegypti* (*1*) we have further partitioned the nitrogen excretion and have extended the analysis to two other species of mosquitoes, *Anopheles quadrimaculatus* and *Culex pipiens.*

The methods for the collection and extraction of mosquito excreta and lyophilization of the extracts were essentially similar to those employed in previous work (*1*). All analyses were performed in duplicate on extracts (lyophilized dry solid form) of excreta collected for a period of 2 weeks from insects on a diet of 4 percent sucrose and without any source of nitrogen. Total nitrogen was determined by the Kjeldahl method, and uric acid nitrogen was determined by the method of Kalckar (*2*) as modified by Praetorius (*3*). Urea and ammonia nitrogen were determined by the method of Seligson and Seligson (*4*).

The amino acid nitrogen determination was performed on desalted material as follows. A weighed amount of excreta (20 to 50 mg) was dissolved in 25 to 50 ml of distilled water and passed through a column (1 by 4 cm) of Dowex-50 (X-4, 50 to 100 mesh) in the hydrogen form. The column was washed with distilled water until the washings were neutral. The amino acids were eluted with 14 percent ammonia. The excess ammonia was removed in a vacuum at 25°C, and the solution was lyophilized. Amino acid nitrogen was determined by the Ninhydrin method of Moore and Stein (*5*) on a portion of the lyophilized material after the solids had been dissolved in a measured volume of distilled water.

Protein nitrogen was determined as follows. A weighed amount of the dried excreta extract (30 to 100 mg) was dissolved in a minimum volume of water, placed in a cellophane bag, and dialyzed for 3 days at +2°C against several changes of water. The dialyzed material was evaporated to dryness and hydrolyzed with 6*N* HCl at 110°C for 18 to 24 hours. The hydrolyzate, after removal of the HCl in a vacuum, was diluted to a convenient volume, and the amino acid nitrogen was determined by the Ninhydrin method (*5*).

Table 1 shows that the three species of mosquitoes, *Aedes aegypti, Anopheles quadrimaculatus* and *Culex pipiens* have essentially similar patterns of nitrogen excretion. Uric acid N represents approximately half of the total N, urea N about 10 percent, ammonia N about 10 percent, amino acid N about 5 percent, and protein nitrogen about 10 percent. Thus 80 percent of the total N excreted is accounted for.

Paper chromatography (*6*) was also performed on the protein hydrolyzates. The results showed the presence of tyrosine, phenylalanine, leucine, isoleucine, valine, proline, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine, lysine, cysteic acid, galactosamine, glucosamine, and trace amounts of β-alanine, indicating that the nondialyzable material probably contained a glycoprotein. This material was likewise found in the excreta of *Aedes aegypti* during the third and fourth

Table 1. Nitrogen partition in mosquito excreta collected for 2 weeks from mosquitoes on a diet of 4 percent sucrose.

Species	Nitrogen (%)					Total nitrogen accounted for (%)
	Uric acid	Urea	NH_3	Protein*	Amino acid	
Aedes aegypti	47.30	11.90	6.40	10.82	4.40	80.82
Anopheles quadrimaculatus	42.50	9.50	7.80	9.22	4.70	73.72
Culex pipiens	46.90	7.90	10.00	9.67	5.50	79.97

* Since chromatograms of the protein hydrolyzates indicated the presence of hexoseamines, the protein N values include amine N.