responsible for the production of the zones of inhibition. Experiments were designed to determine the sensitivity of a number of organisms to the growth inhibitors produced by aspen tissue. In each experiment three pieces of tissue each weighing approximately 6 mg, were placed on the surface of the medium and allowed to grow for a period of 3 weeks in the dark at 27° to 29°C. After this growth period the tissue was removed and weighed; the surface of the agar was flooded with a suspension of the test organism in nutrient broth (Difco) or *Staphylococcus* broth (Difco). The excess inoculum was removed and the cultures were incubated until sufficient growth of the test organism was evident.

Data from cultures in which all three pieces of tissue gave consistent results are shown in Table 1. Occasionally, the degree of inhibition was difficult to determine because of poor growth of the test organism or the tissue and in some cases not all three pieces of tissue produced an inhibitory zone even though the test organism and the tissue grew well (footnote to Table 1). As a result of this variability, all experiments were performed at least three times. In most cases the inhibitory zones were quite extensive and clearly defined. The diameter of the inhibitory zones is an indication of the magnitude of inhibition. A quantitative relationship between the diameter of the inhibitory zones and the amount of tissue growth was not observed. This aspect, however, was not extensively investigated.

The best test organisms selected for possible use in further investigation are *Fusarium roseum, Bacillus subtilis, Sarcina lutea,* and *Pullularia pullulans* because of uniform results and the production of clearly defined inhibitory zones (*17*).

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References and Notes

1. E. M. Osborn, *Brit. J. Exptl. Pathol.* **24**, 227 (1943).
2. L. G. Nickell, *Econ. Botany* **13**, 281 (1960); F. A. Skinner, *in Modern Methods of Plant Analysis,* K. Paech and M. W. Tracey, Eds. (Springer Verlag, Berlin, 1955), vol. 3, p. 626.
3. J. Grosjean, *Nature* **165**, 853 (1950).
4. H. L. Klöpping and G. J. M. van der Kerk, *Nature* **167**, 996 (1951).
5. C. J. Bishop and R. E. MacDonald, *Can. J. Botany* **29**, 260 (1951).
6. E. N. Azarowicz, J. E. Hughes, C. L. Perkins, *Antibiot. Chemotherapy* **2**, 532 (1952).
7. G. G. Dull, J. L. Fairley, R. Y. Gottshall, E. H. Lucas, *Antibiot. Ann.* 1956–57, 682 (1957).
8. E. H. Lucas, A. Frisby, R. Y. Gottshall, J. C. Jennings, *Mich. State Univ. Agri. Exptl. Sta. Quart. Bull.* **37**, 425 (1955).
9. A. Frisby, J. M. Roberts, J. C. Jennings, R. Y. Gottshall, E. H. Lucas, *Mich. State Univ. Agr. Exptl. Sta. Quart. Bull.* **35**, 392 (1953).
10. J. E. Bier, *Forest Chron.* **38**, 363 (1962); H. Butin and V. Loeschke, *Naturwiss.* **47**, 451 (1960).
11. M. H. Hubbes, *Science* **136**, 156 (1962).
12. I. M. Sussex, Mary E. Clutter, J. B. Lutinski, L. J. Dilks, *Botan. Gaz.* **121**, 171 (1960).
13. W. Tulecke and L. G. Nickell, *Science* **130**, 863 (1959).
14. W. Tulecke, L. H. Weinstein, A. Rutner, H. J. Laurencot, Jr., *Contrib. Boyce Thompson Inst.* **21**, 291 (1962).
15. P. R. White, *Ann. Rev. Biochem.* **11**, 615 (1942).
16. J. P. Nitsch, *Am. J. Botany* **38**, 566 (1951).
17. Supported in part by the Pioneering Research Program of the Board of Trustees of the Institute of Paper Chemistry, acting on behalf of a group of sponsoring pulp and paper companies. I thank Mrs. G. Pellett and Dorothy McKeever for assistance in the laboratory. The microorganisms used in this study were provided by Olga Smith, J. Conkey, and Dr. R. Anderson.

18 March 1963

Cancer: Relation of Prenatal Radiation to Development of the Disease in Childhood

Abstract. *Experimental evidence indicating a linear response and the absence of a threshold for the development of childhood cancer and leukemia at total doses below 1 roentgen is contained in recent studies of prenatal diagnostic x-ray exposure. Implications for the nature of the carcinogenic mechanism in the human organism are discussed, with emphasis on the possible effects of the ionizing radiation from iodine-131 and other short-lived isotopes.*

One of the most difficult problems in predicting the effects of low-level radiation on the development of cancer in man has been the lack of data on the dose-response relationships of the human organism at levels approaching those produced by natural background radiation (0.1 to 0.2 roentgen per year). Experimental evidence has recently become available on whole-body doses well below 1 r to the infant *in utero.*

This evidence is contained in the recent study by MacMahon (*1*) of the effect of prenatal x-ray exposure on the mortality of children from neoplastic diseases, when it is combined with the earlier results of Stewart and her coworkers in England which MacMahon's investigation was designed to test (*2*). MacMahon's study was planned to overcome the principal criticisms of the earlier work with objective evidence from hospital records of intra-uterine x-ray exposures and with accurately controlled estimates of mortality rates for malignant diseases in both exposed and unexposed children.

The population studied by MacMahon consisted of all children born in 37 large maternity hospitals in the New York–New England area during the years 1947–54, a total of 734,243 children. Among 584 children who had died of cancer by 1959, sufficiently complete records of 556 single births were located. After correction for birth order and other variables, the average cancer mortality was about 40 percent higher for children who had been x-rayed *in utero* than for those who had not, and the rate was also higher for those irradiated during the first 6 months than the last 3 months. This finding is in qualitative agreement with the earlier result of Stewart (*2*), who had found an increase of about 90 percent for children in England and Wales, as well as increased sensitivity during early pregnancy.

The evidence for the dose-response relationship is in Stewart's and MacMahon's data for cancer mortality as a function of the number of x-ray films taken during a given examination. MacMahon's data have been plotted in Fig. 1, where the length of the vertical bars is a measure of the probable error arising from the size of the sample in each category, and the length of the horizontal bars represents the grouping used. These data show that there is no evidence for a threshold greater than the dose corresponding to one x-ray picture. Furthermore, as the number of films increases by a factor of 4 to 5, the increase in cancer mortality is best fitted by a linear law, although a quadratic law cannot be definitely excluded on the basis of these data alone (dashed line) (*3*).

To distinguish between a linear and quadratic relationship, it is necessary to compare Stewart's earlier data with MacMahon's more recent results grouped in a comparable manner (Fig. 2). Stewart's data also are fitted best by a straight line through the point of zero exposure, but with a slope almost twice that indicated by MacMahon's results. Such a trend is in fact to be expected from the improvement in x-ray techniques starting during the postwar period, when the bulk of Stewart's cases received their irradiation (1944–51), and continuing more rapidly through the later period covered by MacMahon's sample (1947–54).

As shown by detailed comparisons

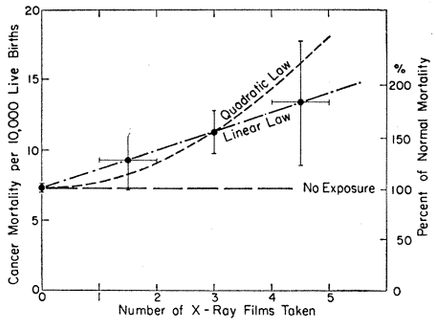

Fig. 1. Cancer and leukemia mortality rates of children irradiated *in utero* as a function of the number of x-ray films taken (MacMahon, *1*). Vertical bars represent probable errors due to sample size; horizontal bars represent the grouping into four exposure categories used by MacMahon. Dashed curve represents a quadratic law fitted to MacMahon's data at 0 and 3 exposures.

of typical doses during diagnostic x-ray procedures before and after the introduction of new techniques (1949–53), dose reductions by factors of four to eight had become possible (*4*). These resulted from a combination of improved films (approximately a factor of 2), development of better intensifying screens (factor of 1.4), and higher voltages accompanied by a change from 1 to 3 mm aluminum filtration of the primary beam (factor of 2). The latter practice especially became rapidly accepted as the result of the work of Trout, Kelly, and Cathay in the United States (*5*), and Ardran and Crooks in Britain (*4*).

Since the peak in the time-incidence curve for leukemia and childhood cancer is close to the fourth year, it may be assumed that the great majority of the cases in Stewart's sample, consisting of children up to age 10 who died during the years 1953–55, received the higher dose per picture associated with the older techniques. On the other hand, the latter half of MacMahon's test population (born 1951–54) undoubtedly experienced a lower dose per exposure, especially since they were born in some of the largest hospitals in the New York–New England area.

As a result, although dose-reductions by a factor of 4 probably apply for the most recently born cases of MacMahon's sample, as compared to Stewart's, the average reduction for MacMahon's group as a whole may be closer to a factor of two as suggested by the observed ratio of the slopes. To test this hypothesis further, Stewart's data has been plotted together with MacMahon's with a linear change in the scale of the abscissa by a factor of 2 (Fig. 3). A linear transformation of this type should cause the two sets of data to fall along a single straight line if a linear dose-response relationship exists.

With this scale change, Stewart's points do in fact fall on the same straight line as MacMahon's (Fig. 3). Furthermore, because of the greater range of exposures represented by MacMahon's and Stewart's combined data, the quadratic curve fitted to MacMahon's data is clearly ruled out (dashed curve, Fig. 1). Thus the dose-response relationship for childhood cancer appears to show no threshold and to be linear down to below the dose received by the embryo in a single pelvic x-ray picture.

Schubert applied the best available data to Stewart's earlier study and arrived at an estimate of 2 r to the embryo in a typical pelvimetry consisting of three exposures, or about 0.6 r per film (*6*). With heavier filtration, high voltages, and modern films, a mean value of 0.3 r per exposure seems to be a reasonable estimate for MacMahon's cases, with a spread due to variations in technique of about ± 33 percent (*4, 7*). This leads to an estimate of the doubling dose consistent with both Stewart's and MacMahon's data of 1.7 r, or a 1 percent increase in mortality for each 17 mr to the embryo in the range investigated. This value is in good agreement with the calculations of Lejeune and Turpin (*8*) who arrived at a doubling dose of 1 to 3 r for irradiation during the last 3 months of pregnancy. Such a response to ionizing radiation is about 20 to 30 times greater than that obtained from the slope of the response curve for adults as judged by the studies on the leukemia cases among the survivors of Hiroshima (*9*) and individuals treated for ankylosing spondylitis (*10*) at total doses above 100 r.

The evidence for a linear dose-response relationship indicates that the significant dose parameter is the total accumulated dose, essentially independent of the period over which the radiation is administered (*11*). That dose-rate effects are not likely to be very strong at these relatively low total doses is supported by the following consideration: although the x-ray pictures are all taken within approximately the same brief examination period so that the highest exposures of Stewart's sample represent dose-rates in roentgens per hour 10 times as high as for the lowest

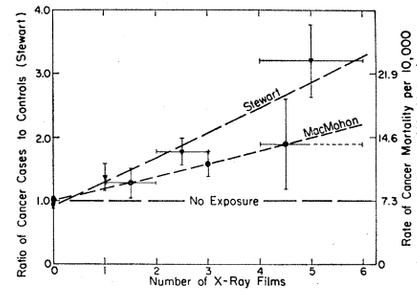

Fig. 2. Comparison of the data obtained by Stewart *et al.* (*2*) and MacMahon (*1*) for cancer mortality of children irradiated *in utero* as a function of the number of x-ray films taken during pregnancy. Stewart's data have been regrouped into categories similar to those of MacMahon.

exposures of MacMahon's samples, there is no detectable deviation from linearity in the dose-response curve (Fig. 3). This is in agreement with other observations of dose-rate effects on genetic point-mutations, where changes in dose rates by three orders of magnitude result in effects differing only by factors of 2 to 4 (*11*, annex C, Table X).

A direct comparison with radiation from normal background sources and fallout at comparable total doses now appears possible. Thus, typical doses from normal background radiation (75 to 150 mr in 9 months) would suggest that about 5 to 10 percent of all childhood cancer and leukemia cases may be traced to the triggering action of natural ionizing radiation.

Total doses comparable to background radiation are encountered as the

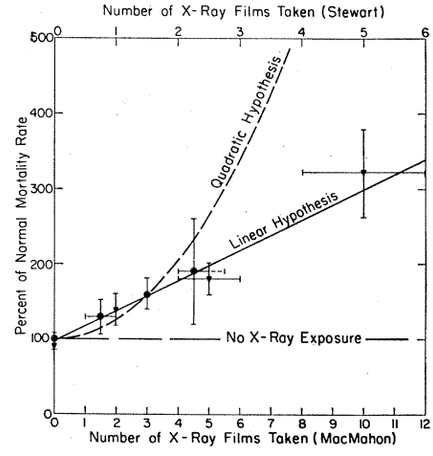

Fig. 3. Combined results of MacMahon and Stewart after application of linear change in abscissa scale by a factor of two for Stewart's data to correct for difference in average dose per x-ray picture (see top and bottom scales). Dashed curve represents quadratic relation of Fig. 1.

result of large-scale nuclear explosions in the atmosphere. Thus, it is now generally agreed (*12*) that the insertion of fission products into the low stratosphere at polar latitudes equivalent to 1 megaton explosive force results in an amount of additional radiation equivalent to 1 to 2 weeks of natural radiation (2 to 4 mr) to every individual in the northern hemisphere. Approximately 75 percent of this activity is contained in short-lived isotopes which deliver their dose in less than 6 to 12 months (*11*, annex F). Thus, a series of test explosions totaling 100 megatons of fission-product equivalent explosive force, comparable to the combined tests of the U.S.S.R.–U.S. in 1961–62, would be expected to produce a 9 months' dose to the bone marrow anywhere from 150 to 300 mr. This is of the order of magnitude of the dose to the embryo in a typical pelvic x-ray picture taken with the best modern techniques (*4*). If dose-rate effects do not reduce the risk by more than a factor of two to four, as the existing evidence indicates, then one would expect an increase in childhood cancer mortality between 2.5 and 10 percent for children born within about a year after the last atmospheric test series.

Doses considerably larger than these average worldwide values can result from heavy local fallout in certain areas because of unusual meteorological conditions even for relatively small nuclear explosions, such as occurred in the Troy-Albany (N.Y.) area, after a 40 to 50 k-ton low altitude explosion in Nevada in 1953 (*13*). Due to the short time between the formation of the fission products and the arrival of local fallout, the short-lived isotopes present special hazards to the embryo. Aside from the external γ-radiation originating mainly from short-lived Zr^{95} and Nb^{95}, estimated to have been about 100 mr for the first 13 weeks after arrival of the fallout in this particular case (*14*), a significant dose to the blood-forming bone marrow results from the short-lived isotopes Sr^{89}, Ba^{140}, and I^{131} ingested by the mother, in particular with fresh milk and vegetables.

The special hazard that these short-lived isotopes (half-lives 53 days, 12 days, and 8 days, respectively) present for the development of childhood cancer should be noted: (i) These isotopes give off their radiations in a time short compared with pregnancy and the critical phases of rapid cell division associated with organ and skeletal formation, unlike the much longer lived Sr^{90} and Cs^{137}, for which the appropriate integration time is the whole adult life-span. (ii) In particular I^{131} is known to have produced leukemia in adults (who are much less sensitive than children) when administered in large therapeutic doses (*15*). (iii) Starting close to the end of the first trimester the embryo has a tendency to accumulate radioactive iodine consumed by the mother in its own thyroid at a concentration 2 to 10 times that of the normal adult (*16*), and there is a similar tendency for greater concentration of Sr^{89} and Ba^{140} in the fetal bone structure (*17*). Although I^{131} concentrates primarily in the thyroid and gives a strong β-dose to this organ, it is also carried to the blood-forming centers. Thus, the centers nearest the thyroid are subjected to the γ-rays as well as to the β-decay of the radioiodine in the embryo's thyroid. The principal danger of I^{131} may therefore not be thyroid cancer, which is normally extremely rare in children and treatment for which leads to better than 90 percent recovery (*18*), but rather leukemia, which is the highest single cause of death in children 4 to 14 in the U.S. (other than accidents) and affects about one in every thousand (*18*).

Since the indication of a linear response relationship does not support the hypothesis that multiple hits are required (*11*), MacMahon's and Stewart's data tend to support the "single hit" or genetic point defect theory (*19*) of the radiation damage responsible for childhood cancer and leukemia. The important implications of this result, both for the etiology of cancer and the long-range after-effects of fallout from the detonation of nuclear weapons, would seem to make it desirable to extend studies of this whole problem to still larger population samples. It would seem especially important to initiate carefully controlled surveys of childhood leukemia and cancer deaths among children born in areas known to have received heavy fallout doses in the preceding 6 to 9 months (*20*). As a further test of the present interpretation of the different rates of incidence in Stewart's and MacMahon's investigations, a follow-up study, with a test-population of children born after the introduction of improved diagnostic x-ray methods, should be undertaken, accompanied by detailed checks of the average dose received under the prevailing examination conditions. If the present interpretation of the difference between Stewart's and MacMahon's results is correct, such a study should reveal an increased mortality approximately half that observed by MacMahon, which would correspond to an average increase of only 20 percent for all irradiated children.

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References and Notes

1. B. MacMahon, *J. Natl. Cancer Inst.* **28**, 1773 (1962).
2. A. Stewart, J. Webb, D. Hewitt, *Brit. Med. J.* **1**, 1495 (1958).
3. It should be noted that because of this statistical uncertainty, MacMahon did not draw any conclusions as to the nature of the dose-response relationship.
4. G. M. Ardran and H. E. Crooks, *Brit. J. Radiol.* **26**, 352 (1953).
5. E. D. Trout, J. P. Kelly, G. A. Cathay, *Am. J. Roentgenol.* **67**, 946 (1952). The widespread change to heavier filtration appears to have occurred very rapidly after publication of this article in June 1952. Dr. Elliott C. Lasser, Department of Radiology, University of Pittsburgh Medical School, brought this fact to my attention.
6. J. Schubert, in *Congressional Hearings, Joint Committee on Atomic Energy*, 5–8 May 1959, vol. 2, p. 1652.
7. V. W. Ritter, S. R. Warren, E. P. Pendergrass, *Radiology* **59**, 238 (1952). These investigators found variations in output of different machines of $\pm$ 20 percent, and they estimate the typical spread in dose from calculated values at $\pm$ 33 percent. Table V (*5*) for equal dose transmitted through the pelvis at 85 kv (peak) gives a dose 3 cm below the surface of 576 mr with 1 mm of Al and 302 mr for 3 mm of Al filtration. Somewhat larger reductions occur for 60 kv (peak) radiation, and somewhat smaller changes for 100 kv (peak). The percentage reduction is not very sensitive to depth below the surface.
8. J. Lejeune and R. Turpin, *Sang* **29**, 730 (1959).
9. R. Heyssel, A. B. Brill, L. A. Woodbury, E. T. Nishimura, T. Ghose, T. Hoskino, M. Yamasaki, *Blood* **15**, 313 (1960).
10. W. M. Court-Brown and R. Doll, *Med. Res. Council, Spec. Rep. Ser. No. 295* (1957); *Brit. Med J.* **2**, 181 (1958).
11. *Report of United Nations Scientific Committee on the Effects of Atomic Radiation* (1962), annex H, p. 415.
12. "Radiation Standards, Including Fallout," *Hearings before the Subcommittee on Research, Development and Radiation of the Joint Committee on Atomic Energy, 4–7 June 1962*, Summary-Analysis, p. 8.
13. R. Lapp, *Science* **137**, 756 (1962).
14. The 14th semiannual report of the Atomic Energy Commission (July 1953) specifies a gross activity in this area of 100 to 200 c/mi^2 and exposures of 0.1 r for the first 13 weeks after arrival of the fallout on 26 April 1953.
15. W. H. Beierwaltes, *Med. Clin. N. Am.* **45**, 1055 (1961).
16. W. H. Beierwaltes, H. R. Crane, A. Wegst, N. R. Spafford, E. A. Carr, Jr., *J. Am. Med. Assoc.* **173**, 1895 (1960).
17. J. L. Kulp, A. R. Schubert, E. J. Hodges, *Science* **129**, 1249 (1959).
18. "End Results and Mortality Trends in Cancer," *Natl. Cancer Inst. Monograph* **6**, 34, 293 (1961).
19. E. B. Lewis, *Science* **125**, 965 (1957); see also the original genetic mutation hypothesis due H. J. Muller, *Science* **66**, 84 (1927).
20. Other areas that have received heavy fallout doses are mentioned by Lapp (*13*) and Eisenbud (*1* of Lapp's article).

4 March 1963