Summary. A method for the preparation of macroscopic leptospiral agglutinating antigens is described. Antigens of this type have been prepared from 9 leptospiral serotypes. Cross reaction patterns are essentially comparable to those observed in the agglutination-lysis technic. Antigens so prepared are sensitive, specific, and stable under protracted periods of storage, and produce easily discernible reactions in the presence of antibodies.

The authors acknowledge, with thanks, the advice of Dr. C. W. Hiatt and the technical assistance of Mr. LaRue B. Evans in the performance of microscopic agglutination-lysis examinations.

1. Starbuck, E. B., and Ward, T. G., *J. Inf. Dis.*, 1941, v70, 88.

2. Pot, A. W., *Lancet*, 1936, v1, 1290.

3. Gardner, A. D., and Wylie, J. A. H., *Lancet*, 1946, v1, 955.

4. Brown, H. C., *Brit. Med. J.*, 1939, v2, 1138.

5. Esseveld, H., Thesis, Amsterdam, 1937.

6. Van Thiel, P. H., *The Leptospiroses*, Universitaire Pers, Leiden, 1948, 31.

7. Wilson, G. S., and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity*, 3d ed., Williams and Wilkins Co., Baltimore, 1946, 211.

8. Northrop, J. H., and DeKruif, P. H., *J. Gen. Physiol.*, 1922, v4, 639.

9. *Ibid.*, 1922, v4, 655.

10. Stuart, R. D., *J. Path. and Bact.*, 1946, v58, 343.

11. Unpublished data.

12. Gochenour, W. S., Jr., Yager, R. H., Wetmore, P. W., and Hightower, J. A., *A. J. P. H.*, 1953, v43, 405.

13. Gochenour, W. S., Jr., and Yager, R. H., *Proc. A. V. M. A.*, 1952, 178.

Received May 22, 1953. P.S.E.B.M., 1953, v83.

Relative Biological Effectiveness of Radium and Other Alpha Emitters in CF No. 1 Female Mice. (20394)

MIRIAM P. FINKEL.

From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

Estimates of permissible levels of radioactive isotopes in man depend, in part, upon a comparison of the radiotoxic hazards of these elements and of radium. Radium has become the common denominator for experimental animals and man because of the information available from the study of individuals who were exposed accidentally or who were intentionally treated with it. The data at hand from a series of experiments in progress make possible comparisons a) of the effects of radium and some other alpha emitters on the survival of mice, and b) of their carcinogenic potentialities for the mouse skeleton.

Procedures. The dose ranges of the radioisotopes used are presented in Table I. Radium 226, polonium 210, and thorium 228 were injected as chlorides; plutonium 239 (plus 4) and the 2 uranium isotopes were given as nitrates. Except for uranium 232 and thorium 228, the doses were selected to range from rapidly lethal levels to levels below tolerance. The materials, in isotonic salt solution at pH 2-4, were injected into a lateral tail vein of CF No. 1 female mice that were 9-10 weeks old. Complete protocols and details concerning the handling of these duration-of-life experiments will be presented when the studies are finished. Briefly, all animals were autopsied, tissues were taken for histological examination, and roentgenograms were prepared. Whenever possible, animals were sacrificed when moribund.

TABLE I. Materials and Range of Doses.

Isotope	Range of doses (μc/kg)	No. of animals
Radium 226	.6 –4170	675
Plutonium 239	.04– 50.4	765
Polonium 210	.46– 100	645
Uranium 233	.1 – 100	675
" 232	.1 – 10	135
Thorium 228	6.6 – 360	75
Controls		450
Total		3420

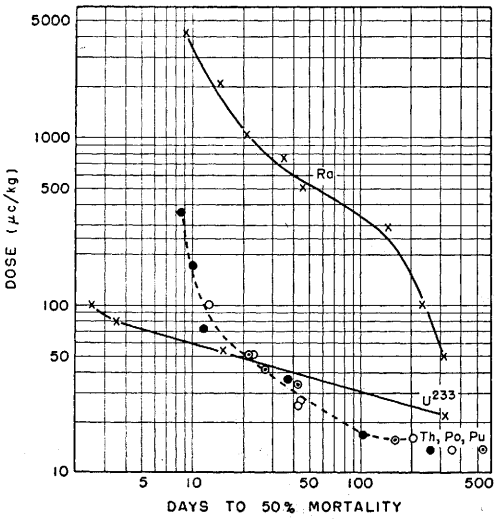

FIG. 1. Relationship between isotope-dose and time to 50% mortality.

Results. Time to 50% mortality. The effectiveness of the isotope-doses as measured by the time required to kill 50% of the animals is presented in Fig. 1. A single curve expresses the thorium, polonium, and plutonium data since the values were close over the range where the doses overlapped. The differences in the slopes of the 3 curves during the first 15 days after injection can be explained on the basis of the μc:μg ratio of the various radio-isotopes, *i.e.*, whether death was due to irradiation, which kills relatively slowly, or to chemical toxicity, which can kill rapidly. Since one μc of thorium 228 is equivalent to only 0.0012 μg, death was due to irradiation alone. This is borne out by the slope of the curve, where it can be seen that 72 μc/kg was almost as effective as 360 μc/kg. On the other hand, 100 μc/kg of uranium 233 resulted in death almost immediately. In this case the amount of uranium injected (1 μc = 100 μg) was above the chemically lethal dose (3). The curve describing the radium data up to the fifteenth post-injection day is intermediate in slope, and it is likely that at these levels there was a chemical as well as a radiation effect. Because of these considerations, comparisons of μc dosages that resulted in 50% mortality in less than 20 days are not justified. In addition, the curves past 150 days have little significance since the control populations reached 50% mortality at 400-450 days. From 20 to 100 days the LD_{50} per given time for radium remained approximately 20 times greater than that for thorium, polonium, and plutonium and varied from 23 to 11 times greater than that for uranium 233. In other words, these alpha emitters killed CF No. 1 female mice approximately 20 times as efficiently as radium at levels high enough to halve the population in 20 to 100 days.

Life expectancy. Fig. 2 illustrates the expectation of life expressed as the percentage of the life expectancy of the control populations immediately after injection. Higher doses of radium than of any of the other materials were required to decrease by 50%

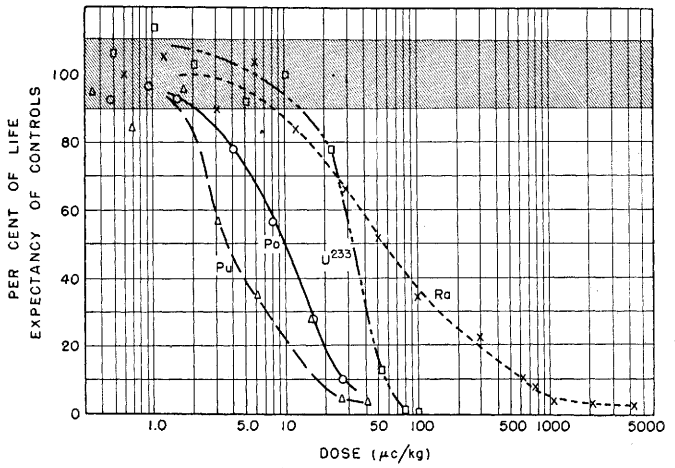

FIG. 2. Relationship between expectation of life and dose proportionate to the expectation of life of the control populations (100% $\pm$ 2 S.D. of mean).

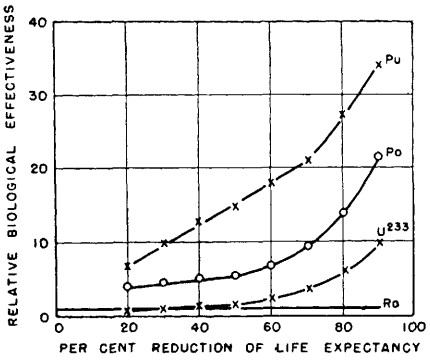

FIG. 3. Biological effectiveness relative to radium in reducing expectation of life.

or more the average number of days lived. These curves were used in the preparation of Fig. 3, which presents the effectiveness of plutonium, polonium, and uranium 233 relative to radium in reducing the expectation of life. The greater the effect, the greater the divergence between radium and the others. For example, the factors are 34 (Pu), 21 (Po), and 10 (U233) for 90% reduction and 8, 4, and 0.75, respectively, for 20% reduction. It is the minimal effect produced by very low doses that must be known in order to establish permissible levels in man. Unfortunately, these are the data that are most difficult to obtain. The values derived from the curves in Fig. 2 for 10% reduction are meaningless in view of the deviation among the control populations. However, if the curves in Fig. 3 are extended toward zero reduction, those for plutonium and radium meet at zero but that for polonium does not go below three. It appears, then, that as ineffectual levels are approached, polonium remains three times as effective as radium in reducing the expectation of life.

Malignant bone tumors. The carcinogenic property of bone-seeking radio-isotopes furnishes another criterion by which the hazards of different materials can be compared. Radium, plutonium, and uranium localize in bone while polonium and thorium do not. Although the incidence of malignant bone tumors after the injection of some levels of polonium was higher than that among the control animals, it was much lower than that following the injection of comparable doses of the bone-seekers. The data presented here are tentative in that every case of possible malignancy has not yet been verified histologically; only the positively diagnosed tumors have been included.* However, it is expected that the final tabulation of the number of animals with malignant bone tumors will not vary greatly from that now at hand.

Within each experimental unit the expectancy of bone tumors remained relatively constant after the first malignancy appeared. A single figure for each dose-level group was obtained by averaging these daily tumor expectancies, and the data are plotted in Fig. 4. The expectancy value among the control animals was 3.3%. The unfinished, steeply declining line following the peaks of the curves indicates that at the next dose the tumor expectancy was zero or close to zero, either because the animals did not live long enough or because they were so debilitated that tumors did not appear. Such a peak was not obtained with the doses of uranium 232 used. On the assumptions that the slope of this curve is correct and that uranium 232 is no more toxic to the entire organism than ura-

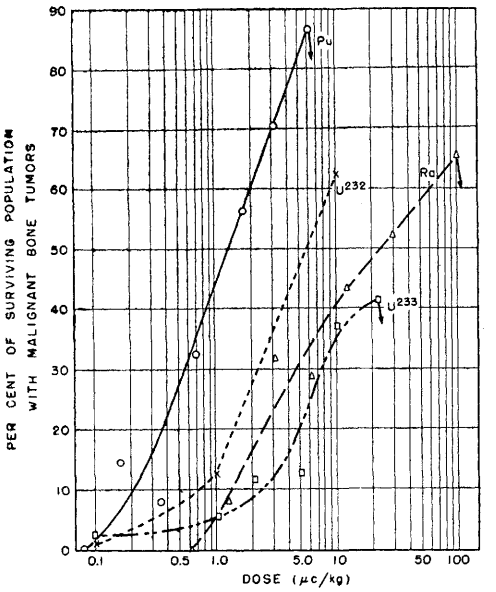

FIG. 4. Probability of developing a malignant bone tumor after the injection of various isotope-doses if surviving the latent period.

* The author is indebted to Dr. Donald Russ for examination of many of the histological preparations.

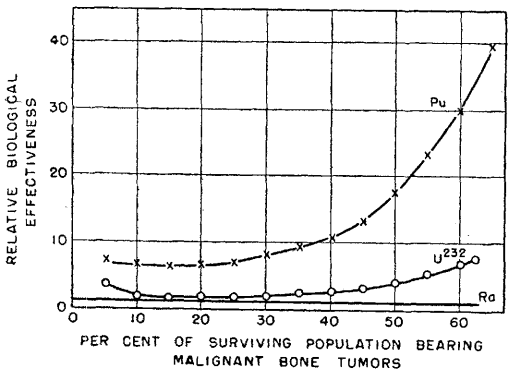

FIG. 5. Biological effectiveness relative to radium in producing malignant bone tumors.

nium 233, a maximum probability of tumor incidence of approximately 80% would be expected after the injection of 22 μc/kg of uranium 232. The difference in tumor producing effect between these two isotopes of uranium is probably due to the fact that uranium 232 (5.3 mev α) decays to the 1.9 year thorium 228 (5.4 mev α), while uranium 233 (4.8 mev α) decays to the 7000 year thorium 229 (4.85 mev α).

In Fig. 5 the radium values have been converted to unity and those for plutonium and uranium 232 plotted accordingly. As was noted previously, the effectiveness of the more potent isotopes relative to radium increases as the severity of the response increases. In this case, however, the curves describing the plutonium and uranium 232 data show no indication of decreasing to zero. At present the best estimate that can be made of their relative effectiveness in producing malignant bone tumors in CF No. 1 female mice at levels approaching those that are ineffectual is 7 (Pu):2 (U233):1(Ra).

Discussion. The relative biological effectiveness of radium and the other alpha-emitting isotopes studied varies with the criterion of effect and with the degree of the response being measured. Many factors enter into considerations of the reasons for these deviations, such as the biological half-time of the various radio-isotopes, their distribution, and the radio-sensitivity of the tissues in which they are most heavily concentrated. Boyd *et al.* noted similar variations in their studies which compared the effect of polonium and plutonium with that of radium on the median survival time in rats(1).

From the data on CF No. 1 female mice presented here, the best figures that can be derived for near-tolerance levels relative to radium are 7 for plutonium, 3 for polonium, 2 for uranium 232, and 1 for uranium 233. The data concerning time to 50% mortality and life expectancy are complete, but other considerations must enter into a final evaluation of the efficiency with which these radio-isotopes produce bone malignancies, *e.g.*, the length of the latent period and the number of tumors per animal. The available data suggest that the length of the latent period is independent of the dose level, but it may vary with the isotope. However, an eventual analysis in terms of the total number of primary malignancies per group may be of greater value than the present analysis, which is based on the total number of animals with tumors per group. Two or more bone tumors per animal were occasionally found among those that received some doses of radium or uranium, but multiple tumors were the rule after the most effective doses of plutonium.

These tentative factors will be subject to revision as other organs and tissues are studied. For example, in view of the relatively high, early concentration of polonium in the ovary(2) and of uranium in the kidney(3-5), these may prove to be the critical organs in establishing tolerance levels for these elements.

Summary. 1. Plutonium 239, polonium 210, uranium 233, and thorium 228 were 20 times as effective as radium 226 in killing 50% of a population of CF No. 1 female mice in 20 to 100 days after intravenous administration. At levels approaching those that produced no effect, polonium was 3 times as effective, plutonium was equally effective, and uranium 233 was slightly less effective than radium in decreasing the expectation of life. The ratios relative to radium of the proportion of mice that developed malignant bone tumors after the latent period were 7:1 for plutonium and 2:1 for uranium 232. Uranium 233 and polonium were less effective than radium in this instance. 2. On the basis of these criteria, the best present estimate of the

relative biological effectiveness at near-tolerance levels in CF No. 1 female mice is 7 (plutonium 239):3, (polonium 210):2, (uranium 232):1 uranium 233):1 (radium 226).

Grateful acknowledgment is made to Mrs. H. Scribner for her assistance in all phases of the work and to Mrs. D. Bansch, Miss J. Lestina, and Mrs. P. Sieck for their technical aid.

1. Boyd, G. A., Silberstein, H. E., Fink, R. M., Frenkel, A., Minto, W. L., Metcalf, R. G., Casarett, G., Suter, G. M., Williams, A., and Tiedeman, D. V., Chap. 7 and 8, in *Biological Studies with Polonium, Radium, and Plutonium,* Ed., R. M. Fink, National Nuclear Energy Series, VI-3, McGraw-Hill, New York, 1950.

2. Finkel, M. P., Norris, W. P., Kisieleski, W. E., and Hirsch, G. M., *Am. J. Roentgenol. Radium Therapy Nuclear Med.,* in press.

3. Hodge, H. C., *Introduction to Pharmacology and Toxicology of Uranium Compounds,* Ed., C. Voegtlin and H. C. Hodge, Vol. I, National Nuclear Energy Series VI-1, McGraw-Hill, New York, 1949.

4. Kisieleski, W. E., Faraghan, W. G., Norris, W. P., and Arnold, J. S., *J. Pharmacol. Exp. Therap.,* 1952, v104, 459.

5. Tannenbaum, A., *Toxicology of Uranium,* National Nuclear Energy Series, IV-23, McGraw-Hill, New York, 1951.

Received June 3, 1953. P.S.E.B.M., 1953, v83.

Absorption of Cholesterol-4-C^{14} in Rats Fed Mixed Soybean Sterols and β-Sitosterol.* (20395)

H. H. HERNANDEZ, D. W. PETERSON, I. L. CHAIKOFF, AND W. G. DAUBEN.
From the Department of Physiology, School of Medicine, Department of Poultry Husbandry, College of Agriculture, and Department of Chemistry, University of California.

The hypercholesteremia and atherosclerosis observed in the cholesterol-fed chicken can be prevented by the addition of soybean sterols to the diet. To explain this phenomenon the suggestion has been made that the plant sterols interfere with the absorption of cholesterol(1,2). In the experiments presented here we have tested this hypothesis by feeding rats C^{14}-labeled cholesterol together with either β-sitosterol or soybean sterols and determining the recovery of the C^{14} in thoracic duct lymph.

Experimental. Rats of the Long-Evans strain weighing 250-300 g were used. They were maintained on a stock diet with the following composition: ground whole wheat, 64.3%; casein, 14.3%; skim milk powder, 6.8%; NaCl, 0.7%; $CaCO_3$, 1.4%; Wesson Oil, 11.5%; fish oil, 1%; and a trace of KI. Forty-eight hours before the experiment all food was removed from the cages, but for the 24 hours just preceding the operation the animals again had access to the stock diet. This feeding procedure ensured the presence of food in the intestinal tract, and consequently a copious lymph flow, at the time the cannulae were inserted into the thoracic ducts. The procedure used for collection of thoracic duct lymph has been described elsewhere(3). Rats with poor lymph flow were discarded at the time of operation. Immediately after the cannulation a test meal containing labeled cholesterol (Table I) was administered by stomach tube. During the rest of the experiment the rats had access to the stock diet and 0.9% saline for drinking purposes. The synthesis of cholesterol-4-C^{14} used in this study was described earlier(4). *Total C^{14} content of lymph* samples was determined as described elsewhere(5). Free and total cholesterol was determined by the procedure of Sperry and Webb(6). The C^{14} content of the cholesterol digitonide was determined by dissolving it in methanol and a suitable aliquot mounted on an aluminum plate for counting.

Results and discussion. The effects of mixed soy sterols and of β-sitosterol upon the recoveries of C^{14} in thoracic duct lymph were studied. The design of the 2 experiments is shown in Table I. It is clearly shown there that the simultaneous administration of C^{14}-cholesterol with either mixed soy sterols on

* Aided by grants from the U. S. Public Health Service and the Life Insurance Medical Research Fund.