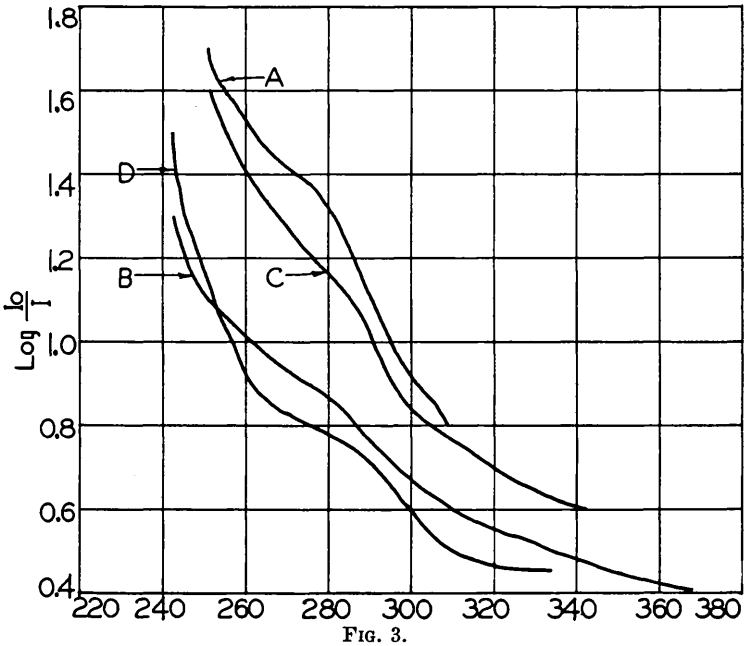

FIG. 3.

Curves A and B, two preparations of P5 (glycoprotein) examined at pH 7.85. Nitrogen content in A = 0.0022%; cell length 1 cm. Curves C and D, preparation of P4 (glycoprotein) examined at pH 7.85 and 12 respectively. Nitrogen content = 0.00136% in both; cell length 2 cm.

pH 12 there is a shift toward the longer wave lengths of the slight inflection at 285 mμ in the curve for glycoprotein.

We wish to record our appreciation to Dr. Hugh H. Darby for a discussion of the contents of this report.

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Studies on Synergism of Leukemogenic Agents in Mice.*

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Various agents have been shown to act synergistically with carcinogens when applied simultaneously to susceptible mice.[1,2] Furth and Boon[3] recently reported a synergistic leukemogenic effect of combined application of methylcholanthrene and irradiation in Rf/Ak hybrids. The incidence of leukemia was 6% in irradiated mice receiving a single dose of 175 r, 16% in others treated only with the carcinogen, and 64% in irradiated and carcinogen-treated mice. The onset of leukemia was also accelerated in animals receiving combined treatment. Preliminary work revealed a 59% incidence of leukemia in animals

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1 Berenblum, I., *Cancer Research*, 1941, **1**, 807.

2 Shear, M. J., *Am. J. Cancer*, 1938, **33**, 499.

3 Furth, J., and Boon, M. C., *Science*, 1943, **98**, 138.

treated with X-rays alone using a single dose of 300 r.

Strain Dba‡ and strain F mice were used in the present experiments to determine whether the findings of Furth and Boon are applicable to other mice known to develop leukemia. Strain Dba exhibits marked susceptibility to the induction of leukemia by chemical carcinogens,[4,5,6] and strain F mice have a high incidence of spontaneous leukemia,[7] the onset of which can be accelerated by treatment with methylcholanthrene or benzpyrene.[8,9] Except for the genetically different stocks of mice employed, the investigations being reported paralleled those of Furth and Boon. However, fractionated dosage technic of irradiation was used, and higher total doses were administered in an attempt to accentuate the effect of the physical agent. The irradiated animals received a daily dose of 80 r, using 140 K.V., 30 cm skin target distance, 2 mm Al filter, including the entire body in the field. Nine successive daily treatments were administered to the strain F mice for a total of 720 r, while the more hardy strain Dba animals received a total dose of 880 r. Three experimental groups were established. In one, irradiation alone was used; in a second, 0.5% methylcholanthrene was applied percutaneously twice weekly; and a third received both types of treatment, the first application of carcinogen being given on the first day of irradiation

Four deaths occurred in the group of strain Dba mice receiving combined treatment, and 14 of the surviving 24 animals, or 58%, developed leukemia at an average of 127 days after the inception of treatment. Thirty-eight, or 60%, of 63 non-irradiated, carcinogen-treated strain Dba mice developed leukemia at an average of 114 days after the first percutaneous application. Only one post-irradiation death occurred in the group subjected to X-rays alone; all of the remaining 22 animals are in excellent condition and none have developed leukemia 5 months after irradiation. However, since the leukemogenic effect of irradiation alone was reported by Furth and Boon to require a considerably longer period to become manifest, the results in this group must be regarded as preliminary and subject to revision.

Although a smaller dose of X-rays was administered to the strain F mice, it proved lethal to almost one-half of the mice which were also treated with methylcholanthrene. Of 21 mice surviving 100 days or more after combined treatment, only 2 have developed leukemia, whereas 12 of 39 non-irradiated mice treated only with the carcinogen have died of leukemia (190 days have elapsed since beginning of experiment), with 20 animals still alive. Inasmuch as the effect of combined X-ray and methylcholanthrene treatment proved lethal to such a large proportion of mice, these results may be open to question. The experiments on strain F mice are therefore being repeated using a single small dose of X-rays. Only 4 of 38 strain F mice receiving irradiation alone died of the treatment, and none of the survivors have developed leukemia (5 months after the first treatment).

Contrary to the experience of Furth and Boon, no synergistic effect of irradiation, when used in combination with a chemical carcinogen, was noted on the induction of leukemia. The only non-genetic difference between the otherwise similar experiments lies in the higher dosage and somewhat different method of irradiation. The importance of the dosage factor is now being tested. The difference in genetic constitution might well account for the antithetical results of the present experiments as compared with those of Furth and Boon. The leukemogenic effect of carcinogens varies widely for different strains.[4,8] Among those susceptible, synergistic factors such as X-irradiation seem to

‡ Subline 12.

4 Morton, J. J., and Mider, G. B., *Science*, 1938, **87**, 327.

5 Engelbreth-Holm, J., and Lefevre, H., *Cancer Research*, 1941, **1**, 102.

6 Law, L. W., and Lewisohn, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 143.

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8 Kirschbaum, A., Strong, L. C., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 287.

9 Kirschbaum, A., and Strong, L. C., *Cancer Research*, 1942, **2**, 841.

be of variable significance. The problem of leukemogenesis is further complicated by results such as those obtained with strain C3H mice which were susceptible to the induction of leukemia with estrogens but not with methylcholanthrene.[8,9]

Summary. Irradiation, when used in conjunction with methylcholanthrene, failed to increase the incidence or to accelerate the onset of carcinogen-induced leukemia in strain Dba and strain F mice.

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Simplified Procedures for Ascertaining Concentration of and Bacterial Susceptibility to Penicillin.

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The simplified procedures for determining the concentration of and bacterial resistance to penicillin presented herein, utilize only such equipment and supplies as are already available in all bacteriological laboratories and the results obtained are well within the limits of accuracy reported for the "serial dilution" and "cup assay" technics.

The method is based on the following principles and observations:

1. If a stipulated volume, or concentration, of penicillin solution is placed on the surface of an agar plate previously seeded with a susceptible organism, a circular growth-inhibition zone (area) is produced which is *directly* proportional to the concentration, or volume, respectively, of penicillin applied.

2. If a definite volume (0.01 or 0.02 cc) of a stipulated concentration of penicillin is employed, the growth-inhibition zone (area) will be inversely proportional to the resistance of the test organism.

3. A 4 mm (22-23 gauge) platinum loop was found to deliver a sufficiently constant quantity, from a solution containing 1 to 10 Oxford units of penicillin per cc, to warrant its employment for determination of bacterial resistance to and concentration of penicillin within the limits of accuracy required for routine clinical purposes. The inhibition zones produced by application of such a loop were generally perfectly circular, if care were taken to keep the petri dish flat during incubation.

4. Penicillin is sufficiently stable, in standard culture media stored in a refrigerator, to permit the use of such media for quickly ascertaining the relative susceptibility of bacteria (such as are encountered in lesions and exudates) to this bacteriostatic agent.

The technic is briefly as follows: To 15 cc melted nutrient agar cooled to about 45°C (2-3% blood is added when the test organism is a hemolytic streptococcus) is added 0.05 cc of a 20-24 hr 37°C broth culture of the test organism. The contents are then poured into a Petri dish, allowed to harden and placed in the refrigerator until ready for use (1-4 hours).

To determine the resistance of an organism, 4 mm loops of one or more concentrations of penicillin, in M/50 phosphate buffer at pH 7.2, are placed on the surface of the seeded agar plate (within a few minutes after it is removed from the refrigerator) which is then immediately incubated at 37°C, care being taken to see that the plates are horizontal in order to insure that the inhibition zones will be circular. The diameters of the zones of growth-inhibition are measured the following day (18-22 hr incubation) to the nearest 0.5 mm.

The authors naturally entertained some misgivings as to the constancy and duplicability of the inhibition zones which would be obtained with this loop technic. Repeated observations have demonstrated, however,