the phospholipids is removed. Treatment of fresh serum under the same conditions results in an irreversible inactivation of the total complement due to protein denaturation.

While it is agreed that the phospholipids of the mid-piece may play a role in complementary activity,[6] it has not been possible to establish a lipid-fourth component relationship.

Summary. The fourth component of complement was not found to be in association with a lipid complex of serum.

11605

Effects of Irradiation on Leukemic Cells in Marrow Cultures.*†

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The experiments herein reported were undertaken to determine whether a small dose of irradiation would have a selectively greater effect than a large dose on a susceptible cell, such as the lymphocyte, in comparison with a less susceptible cell, such as the progranulocyte[1] (promyelocyte); whether an increase in dose of irradiation produced a corresponding increase in effect; and whether leukemic cells were more susceptible than nonleukemic cells of the same type.

The marrow culture technic[2] permits quantitative studies of the effect of irradiation on living normal or leukemic human cells. After an initial total and differential cell count, 50 cc of culture containing about 100,000,000 nucleated cells in a medium consisting of 65% balanced salt solution and 35% human cord serum is thoroughly mixed, and equal portions of about 8 cc are placed in each of several 30 cc vaccine vials. One of these is left as a control, and the others

6 Ecker, E. E., Jones, C. B., and Kuehn, A. O., in press.

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† Presented before Section N of the American Association for the Advancement of Science, Seattle, Washington, June 19, 1940.

1 The cell nomenclature used is the same as that which is found in Osgood, E. E., and Ashworth, Clarice M., *Atlas of Hematology*, pp. 6-8, J. W. Stacey, Inc., San Francisco, 1937; and Osgood, E. E., *A Textbook of Laboratory Diagnosis*, pp. 161-164, Ed. 3, The Blakiston Company, Philadelphia, 1940.

2 Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1937, **108**, 1793.

are given various doses of irradiation. All vials are handled identically except for the irradiation. The total and differential cell counts are repeated at intervals. Curves are plotted expressing the absolute number of each type of cell in the irradiated cultures in percentage of the number of the same type of cell in the control at the same time.

In a previous publication[3] it was shown that irradiation, in the doses used therapeutically, apparently does not kill living human

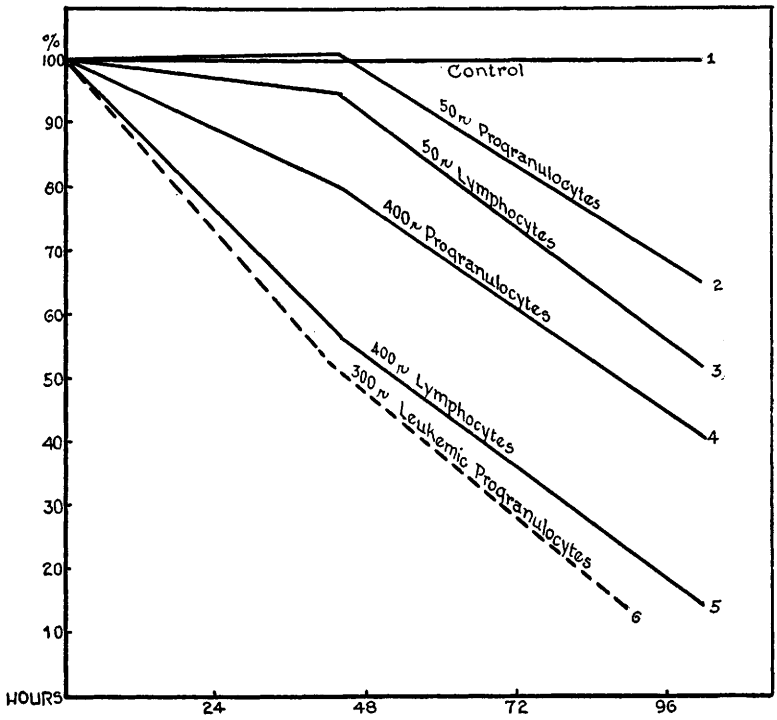

FIG. 1.

Effects of Irradiation on Progranulocytes and Lymphocytes. Curve 1 corresponds to the controls for all experiments, since the other curves are plotted in percentage of the absolute number of the same type of cells in the control at the same time. Curves 2-5 are the weighted averages for the cultures from eight experiments in which in addition to control vials an identical vial was given 50 r of irradiation and another vial 400 r of irradiation. The cell type and dosage of irradiation are indicated for each curve. Curve 6 is from a culture of the blood of a patient with subacute granulocytic (myelogenous) leukemia which contained 93% of progranulocytes A, in which one vial served as a control and an identical vial received 300 r of irradiation. In all experiments irradiation was given at 200 KV, 7 MA, 1 mm copper, and 1 mm aluminum, irradiation at 40 cm distance, 22 r per minute, and 0.063 Å minimum wave length, and a half value layer of 1.54 mm copper. The dosage recorded is that measured with a thimble ionization chamber inside a vial from which the bottom had been cut off.

[3] Osgood, E. E., and Bracher, G. J., *Ann. Int. Med.*, 1939, **13**, 563.

cells in marrow cultures, but does prevent the onset of mitotic or amitotic division. This results in a straight line decrease in progranulocytes and lymphocytes which continues for a period of several days. The more mature cells of the granulocyte series begin to decrease only after sufficient time has elapsed for maturation of progranulocytes to this stage. This suggests that irradiation does not directly affect the more mature cells which are not capable of division.

In Fig. 1, curves 2-5 were obtained by adding the absolute numbers of progranulocytes and lymphocytes in the controls and irradiated vials from 8 experiments, and then computing several points on each curve in percentage of the absolute number of cells in the control at the same time intervals. In each of the 8 experiments there was one control vial, one vial which was given 50 r of irradiation, and one vial which was given 400 r of irradiation, with the factors indicated in the legend. Therefore, any deviation from the 100% control line represents a difference due to the irradiation.

Curve 6 was obtained from a culture of the blood of a patient with subacute granulocytic leukemia which contained 93% of progranulocytes A^1 (promyelocytes II). All of these cells were peroxidase positive and had too coarse a chromatin structure to be blast cells, and yet contained either no granules or azurophil granules alone with no neutrophil, eosinophil or basophil granules. Because of the high percentage of progranulocytes the probable error of any point on the curve is relatively small.

Note from curves 2-5, Fig. 1, that lymphocytes from the same cultures were more affected by the same dose of irradiation than were progranulocytes; that all of the curves approach a straight line decrease beginning at the time of irradiation; and that the dose of 50 r of irradiation had a lesser effect than the dose of 400 r, but not in direct proportion to the dose; *e.g.:* from curve 2 at 102 hours there are 35% less progranulocytes in the cultures irradiated with 50 r than in the control, while from curve 4 there are 60% less progranulocytes in the cultures irradiated at 400 r than in the control, which is a ratio of 35 to 60 or 1 to 1.7. Similarly, from curves 3 and 5 the ratio of percentage decrease in lymphocytes is 48 to 85 or 1 to 1.8, but the ratio of 50 r to 400 r is 1 to 8, so that it is obvious that increasing the dose of irradiation does not produce a directly proportional increase in effect.

It seemed possible since lymphocytes are more affected than progranulocytes that a more selective action on the most susceptible cell would be obtained by decreasing irradiation. That this is not the case is shown by the fact that the ratio of decrease in progranulocytes

to decrease in lymphocytes with 50 r of irradiation is 35 to 48 or 1 to 1.4 which is, within the limits of error of the method, the same as the ratio of decrease in progranulocytes to decrease in lymphocytes with 400 r of irradiation which is 60 to 85 or 1 to 1.4.

From curve 6 in Fig. 1 it is apparent that the general type of curve obtained with leukemic progranulocytes is similar to that obtained with nonleukemic progranulocytes, which suggests that the mechanism of action is the same,[3] that is by arrest of mitotic division, permitting these cells to live out their normal life span and then to die. However, the slope of the curve is steeper than the slope of curve 4 which averages 8 experiments on nonleukemic progranulocytes, and is slightly steeper than the steepest of the curves from any of the 8 individual experiments. This suggests, but does not prove, that the effect of irradiation is quantitatively greater on leukemic cells than on nonleukemic cells. The most plausible explanation for this would be a naturally greater rate of cell division and a shorter life span for the leukemic cells.

It was interesting to note that neither in the irradiated nor in the control cultures of this leukemic blood, even though normal cord serum was used in the medium, was there any tendency for the progranulocytes to mature (Table 1), although maturation has

TABLE I.
Percentage of All Cells of the Granulocyte Series More Mature Than the Progranulocyte in the Experiment from Which Curve 6, Fig. 1, Was Obtained.

Time in hr after irradiation	0	18	66	90
Control	1.2	1.1	0.9	1.6
300 r	1.2	0.8	2.3	4.0

occurred in this type of medium with all of the more than 500 cultures we have made of nonleukemic cells or cells from chronic leukemias in which the mature stages are found in the blood. This suggests that the failure of maturation of the cells in acute and subacute leukemias is not due to any alteration in their environment but is due to a fundamental change in the cell itself, and supports the view that these are malignant cells.[4]

The failure of these cells to mature with irradiation is strong evidence against Isaacs' theory[5] that irradiation acts by hastening maturation to a stage at which cell division is no longer possible. However, our studies of the effects of irradiation on normal cells and

[4] Furth, J., *J. Exp. Med.*, 1935, **61**, 423; Osgood, E. E., and Ashworth, Clarice M., *Atlas of Hematology*, p. 36, J. W. Stacey, Inc., San Francisco, 1937.

[5] Isaacs, R., *Arch. Int. Med.*, 1932, **50**, 836.

cells from chronic leukemias explain Isaacs' observation that after irradiation the proportion of mature cells in the cell population is increased, for as our experiments with colchicine showed[3] X-rays prevent the onset of mitotic division. The immature cells capable of such division are therefore bound to decrease in numbers first, and only later the more mature cells developed from them.

Summary. In irradiated marrow cultures of nonleukemic cells the ratio of effect of 50 r of irradiation on progranulocytes to the effect on the more susceptible lymphocyte is the same as with a dose of 400 r of irradiation. An increase of 8 times in the amount of irradiation, that is from 50 r to 400 r, produced an increase of only about 1.7 times in the effect on either progranulocytes or lymphocytes. Leukemic progranulocytes showed a greater decrease from a dose of 300 r than nonleukemic progranulocytes from a dose of 400 r, but the character of the curves is similar which suggests that the mechanism of action of irradiation on leukemic cells is probably a prevention of the onset of cell division as was shown to be the case for nonleukemic cells. Leukemic progranulocytes which failed to mature in the patient also failed to mature in cultures in a medium containing normal human cord serum with or without irradiation. All of these observations are explained if the action of irradiation is to prevent the onset of mitotic or amitotic division and not by directly killing cells and if the leukemic process depends on a fundamental change in the cell affecting its rate of division and maturation and not on any alteration in the environment.

11606

Microscopic Lesions Without Functional Impairment of Striated Musculature of Suckling E-Low Rats.*

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In endeavoring to determine the minimal prophylactic dose of alpha tocopherol which would insure the normality of the cross-striated musculature in suckling young rats, alpha tocopherol dis-

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