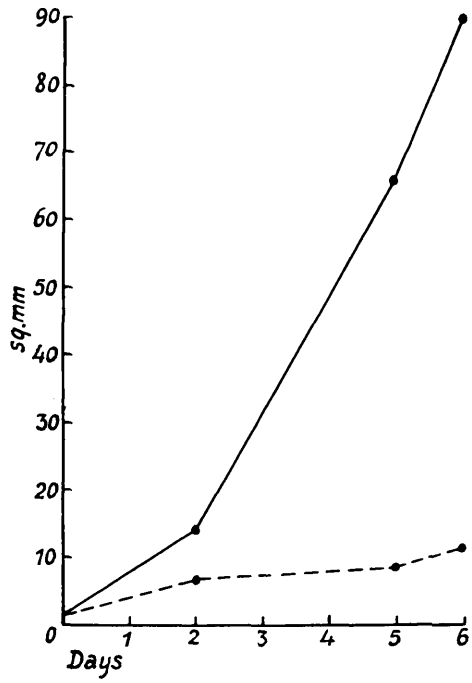

FIG. 1.
Experiment 11220/1 B—Growth curves of sister halves of fibroblast culture in protective medium and in medium containing solutions of alcohol precipitates treated with lipid solvents (acetone and petroleum ether).
———— Alcohol precipitates treated with lipid solvents.
..... Protective medium.

precipitated with 4 volumes of 96% alcohol. The precipitate obtained was treated successively for 3 hours in a Soxhlet apparatus with acetone and petroleum-ether. After drying *in vacuo* the extracted powder was suspended in Tyrode solution. The suspension was kept for 3 days in the refrigerator, centrifuged and the supernatant fluid was added to standardized cultures of chicken fibroblasts in Carrel flasks. The growth of the cell colonies in medium containing this solution as fluid phase was compared with the growth of controls (sister halves), growing in protective medium composed of plasma diluted with Tyrode in the proportions: 1:2. The growth of cultures was followed over a period of 6 days and recorded according to the method of Ebeling.

The curves of Fig. 1 show the growth of a culture stimulated with alcohol precipitate treated with lipid solvents (acetone and petroleum-ether), compared with that of a control in plasma-Tyrode medium. These curves illustrate the optimal activation obtained; the average stimulation amounted to 520% (13 experiments).

Summary. The experiments reported above indicate that in extracts of rat tumors the activity of the growth-stimulating principle is counteracted by an antagonistic factor. By precipitation with alcohol and subsequent treatment with lipid solvents (acetone and petroleum-ether) it is possible to remove the latter and to convert the originally inhibiting material into a definitely stimulating preparation.

15004 P

The Effect of X-Rays on the Mitotic Activity of Explanted Bone Marrow.*

M. RACHMILEWITZ, A. ROSIN,† G. GOLDHABER, AND L. DOLJANSKI. (Introduced by L. Halberstaedter.)

From the Department of Experimental Pathology, Department of Radiology, and Medical Department B, The Hebrew University, Jerusalem, Palestine.

The effect of X-rays on the mitotic activity of the bone-marrow has scarcely been studied. In his recent extensive review on the effect of X-rays on bone-marrow, Dunlap[1] mentions the findings of Mottram[2] who observed a

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† Working on a grant from Mrs. J. H. Stodel, Cape Town, South Africa.

1 Dunlap, C. E., *Arch. Path.*, 1942, **34**, 562.

2 Mottram, J. C., *Arch. Radiol. and Electroth.*, 1920, **25**, 197 (quoted by Dunlap[1]).

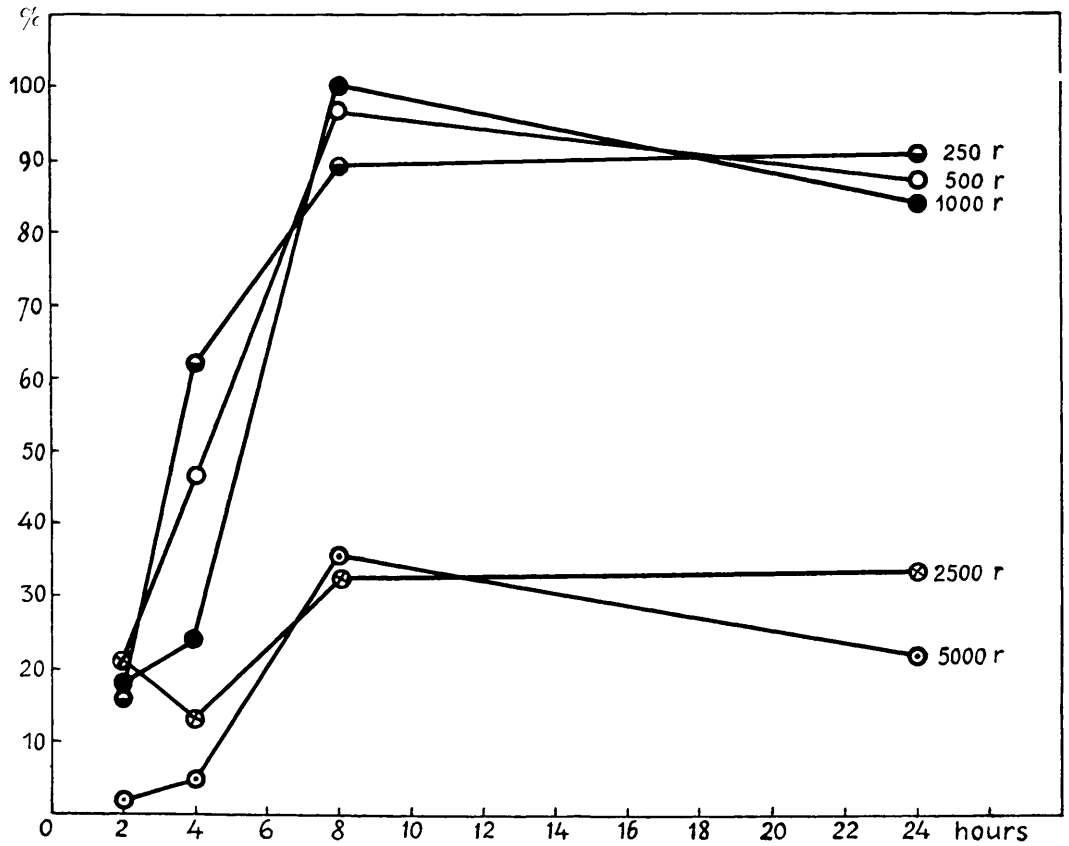

FIG. 1.

Effect of X-rays on Mitoses in Bone-marrow Cultures.

Abscissæ—Time after irradiation in hours.

Ordinate—Number of mitoses in irradiated cultures in percents of number of mitoses in non-irradiated controls.

depression of the mitotic activity in the marrow 2 days after irradiation. Hsü and Ma[3] report that after irradiation of rat bone-marrow with doses of 2500 and 5000 r units mitoses are rare or entirely absent, but they are present in bone-marrow treated with 1000 r units. Osgood and Bracher[4] found a decrease in mitoses after irradiation of suspensions of human bone-marrow cells *in vitro*, but no figures are given to substantiate this observation.

Methods. The present report deals with the mitotic behavior of bone-marrow cultures x-rayed *in vitro*. Experiments were performed on rabbit's bone-marrow cultured in plasma according to the method described elsewhere.[5] Before planting, the bone-marrow was irradiated with the following x-ray doses: 250, 500, 1000, 2500, 5000 r units. The irradiated cultures as well as the non-irradiated controls were incubated for 2, 4, 8, and 24 hours. After incubation the cultures were fixed in Zenker's solution, embedded in paraffin-celloidin, cut in 4 μ sections, and stained with hematoxylin-eosin and Giemsa's stain. The mitotic activity of the bone-marrow cultures was estimated by counting the number of mitoses in irradiated and control cultures respectively, the number of mitoses present in 10 high-power fields being determined in each culture.

Results. All doses applied provoked pro-

[3] Hsü, C. L., and Ma, W. C., *Am. J. Cancer*, 1940, **39**, 319.

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

[5] Rachmilewitz, M., and Rosin, A., *Am. J. Med. Sci.*, 1943, **206**, 17.

found decrease in the number of mitoses in marrow cells 2 hours after irradiation. The number of mitoses in the cultures irradiated with the doses of 250-2500 r units was reduced to approximately 20% of that in non-irradiated controls. In cultures irradiated with 5000 r units the mitoses disappeared almost completely at this time.

There was a definite recovery of the mitotic activity 4 hours after irradiation in cultures treated with 250 and 500 r units. The process of the recovery in cultures irradiated with these doses continued and the mitoses reached 8 hours after irradiation values equal to those of the controls. Cultures irradiated with 1000 r units showed no significant recovery after 4 hours, but only after 8 hours; in this case also the recovery was complete. After 24 hours the culture irradiated with 1000 r units and less showed an abundance of mitoses and normal cellularity.

A distinctly different behavior was observed in cultures irradiated with 2500 and 5000 r units. In cultures treated with these doses the recovery took place 8 hours after irradiation; it was incomplete, the number of mitoses rising only to 30% of the values in controls. No further increase of the mitotic count could be observed 24 hours after irradiation. In contrast to the cultures treated with doses of 1000 r units and less, the explanted bone-marrow fragments irradiated with 2500 and 5000 r units, presented after 24 hours a picture of complete or almost complete aplasia.

The mitotic behavior of irradiated bone-marrow cultures in relation to the control cultures is represented in Fig. 1.

Summary. The effect of direct irradiation with X-rays on mitoses in cultures of rabbit's bone-marrow was investigated. The X-rays in all doses applied (250, 500, 1000, 2500, 5000 r units) produced at first a profound decrease in the number of mitoses. In cultures irradiated with doses of 1000 r units and less the decrease in mitoses was followed by complete recovery of the mitotic activity. The recovery of mitoses in cultures irradiated with 2500 r units and more was only incomplete and the irradiation resulted in aplasia of the bone marrow.

15005

Effect of Drop Size and Wall Solution Concentration on Determinations Made with the Hill-Baldes Method.

ROBERTA FOLLANSBEE. (Introduced by M. B. Visscher.)

From the Department of Physiology, Division of General Physiology, University of Minnesota, Minneapolis.

The Hill-Baldes vapor tension method of determining osmotic pressure has been used extensively for determining the osmotic activity of biological fluids. This method involves a comparison of the rates of evaporation from or condensation on drops of the sample and of a reference solution when placed on opposite junctions of a thermocouple enclosed in a chamber whose walls are lined with moistened filter paper. The deflection of the galvanometer to which the thermocouple is connected is a measure of the difference in vapor pressure and therefore of osmotic pressure of the two solutions, and is calibrated by using 2 known or reference solutions.[1]

During a recent investigation of the osmotic activity of gastrointestinal fluids following the ingestion of distilled water,[2] osmotic activity readings were made at intervals over a period of from one to 3 days following drop deposition on the thermocouple loops, whereas ordinarily readings are completed within one

[1] Roepke, R. R., and Baldes, E. J., *J. Cell. and Comp. Physiol.*, 1942, **20**, 71.

[2] Follansbee, R., *Am. J. Physiol.*, in press.