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Abstract

With the purpose of revealing the biological effects of the X-ray irradiation the authors extracted phospholipids from the liver of irradiated animals and proved that this substance has the action to inhibit the growth of the bone marrow cells, the motility of pseudo-eosinophilis and the erythropoiesis in tissue culture, suggesting that the injury will mainly be induced by the toxic substances produced by irradiation.

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MORPHOLOGIC STUDIES OF BONE MARROW CELLS EXPOSED TO THE PHOSPHOLIPID FRACTION FROM THE LIVER OF IRRADIATED ANIMAL, AN EXPERIMENT *IN VITRO*.

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The damage of blood cells by irradiation have been found soon after the very discovery of roentgen rays in 1895. Concerning the causative factor of this damage, many studies have been done revealing that the damage is attributable to the disturbances in the hematopoietic organs[1,9-10,11,12,13]. But the question whether the disturbances in bone marrow are of those induced directly by the action of X-rays or of those induced indirectly by some substance produced by X-ray irradiation is still problematical. Recently, we have discerned that some hemolytic substances are produced by irradiating the animals with X-rays[2] and attempted to observe the effects of those hemolytic substances extracted from the organs of irradiated animals on the rabbit bone marrow cells by tissue culture. The strong hemolytic power was found in the fraction of phospholipids from the organs of the irradiated animals. In this paper we describe the method of extracting the substance from the liver of irradiated animal and the morphologic change of the bone marrow cells exposed to this substance in culture.

MATERIALS AND METHODS

Preparation of phospholipid substrate : The 10 normal adult male rabbits were irradiated on the whole body with the dose of 3,000 r X-rays

and 24 hours later the animals were sacrifised by bleeding from the carotid artery. And the livers were taken out immediately and crashed by homogenizer, Sakuma Type, S. M. C. No. 500 and dried 30 minutes at about 80°C in a dryer. The dried substance was pulverized with mortar and pestle and the phospholipid was obtained by the SENO's method. The phospholipid obtained from 100 g. of pulverized liver was dissolved in 400 cc aseptic physiologic saline solution and used as substrate to be described later. For the control experiment the phospholipid fraction obtained from the non-irradiated animals by the same method was used.

Cells : For the observation the bone marrow cells of normal rabbits were used. The bone marrow tissues removed aseptically from both sides of the femur of a normal young rabbit were cultured in fluid medium or under cover-slide. For the culture media the serum of the same animal was used adding a trace of vitamin B_{12} as a growth promoting factor. To observe the effect of the substance in the phospholipid fraction obtained from the liver of irradiated animals, the solution mentioned above was used without dilution or diluting with the physiologic saline solution to 10-, 100-and 1,000-fold, in the control experiment using the solution of the phospholipids from the non-irradiated animals.

Method for culture : For the cover-slide culture the method devised by HIRAKI[2] is employed. Each drop of the original solution and of diluted one is added to each sample, respectively. Twenty samples of the cultured tissue are used for one observation, 10 samples in each for 2 series; one series added with the lipid solutions from irradiated animals, and another one with the lipid solution from nonirradiated animals. The samples in each series are divided further into 5 group, 2 in each; one group added with original solution and 3 groups with diluted ones and the last one with physiologic saline solution. The relative growth rate, cell density and the wandering velocity of the mature peudo-eosinophil are measured in each sample by the method reported by OFUJI[3,4]. For the tissue culure in fluid media, the method devised by IWASAKI-KUMEDA was used. In this case the original phospholipid solution is added to the medium in one third of volume. Twenty of the cultured samples are prepared as in the case of tissue culture under the cover sildes as described above. Twenty-four hours after the culture the percentage of erythrocyte increase are measured by the method of OFUJI[3,4]. And the increase in the hemoglobin contents of media are estimated on the hemolyzed sample by the method of OFUJI[3,4].

Method of observation (3, 4) : To know the grade of growing of cells in slide culture the "Relative growth rate" is obtained as the ratio of the area of the newly formed growth zone to that of the original tissue frag-

ment at the period of 3, 6, 12 and 24 hours after the onset of the culture. After 24 hours of culture the cell number on the growth zone has been counted in three optical fields, 100×5, each one of which is selected from three zones of the newly formed growing area, inner, middle and outer zones, respectively. The total number of the counted cells presents the cell density rate.

Besides these, the wandering velocity of the cell has been calculated. The movement of neutrophil in the growing zone is traced at a period of 30 seconds for two minutes and the distance of wandering is calculated. More than five neutrophils are observed and a half of the mean value is recorded as the wandering velocity of the cell per minute.

In the fluid medium culture, the erythrocyte number and hemoglobin contents are calculated at a period of 0, 3, 6 and 9 hours after the culture. For the calculation of erythrocyte number the routine method of numbering of the peripheral blood cells is applied after shaking well. For the measurement of hemoglobin contents 20 cu. mm. of media with cells is taken by the melangeure for Sahli and hemoglobin coutents is measured by the ferrocyanide method by means of Beckman's spectrophotometer.

RESULTS

Tissue growth : In the case of the bone marrow tissue cultured under cover-slides, the addition of the phospholipids fraction from normal non-irradiated rabbits in media results in the decrease in the relative growth rate. The decreasing effect becomes marked with the increased concentration of the phospholipids. The same tendency is found in the two series of experiment as indicated Fig. 1 a, b. The cell density rate measured after 24 hours in culture shows almost the same level in the case added with phospholids fraction as in the case of control (Table 1).

In the cases of adding the extract from the liver of irradiated animals the relative growth rates decrease markedly. Addition of the original extracts results actually in no growth as indicated in Fig. 2 a, b. The cell density rate shows also a very low level in each case after 24 hours in culture as indicated in Table 2.

Similar observations have been carried out by using the phospholipid solution from the irradiated animals, which is left at room temperature for one week after the preparation. The results indicate that the depressing effect of this solution on the growth of cultured cells disappears giving almost the same results as in the case added with the extracts from the non-irradiated animals (Fig. 3 and Table 3).

Fig. 1. Addition of phospho-lipid extract of normal rabbit liver

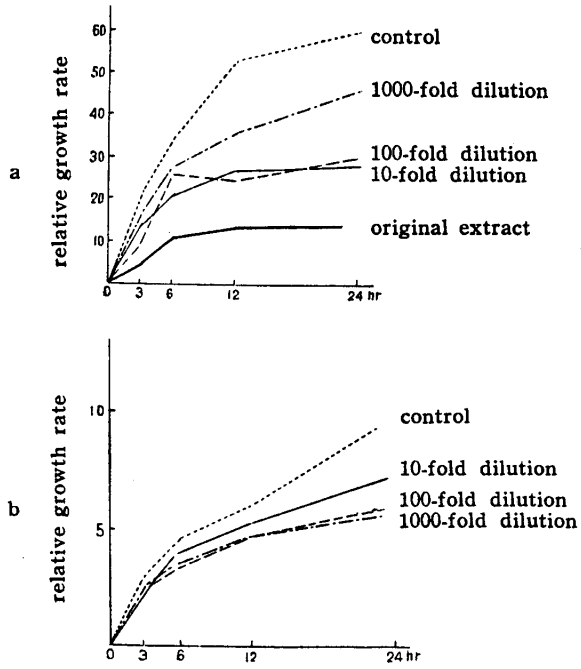


Table 1. Tissue growth with addition of phospho-lipid extract from normal rabbit liver

	Cell-density rate (24-hr value) a		Cell-density rate (24-hr value) b
Control	45	Control	62
Original extract	68	Diluted 10-fold	60
Diluted 10-fold	68	Diluted 100-fold	51
Diluted 100-fold	74	Diluted 1000-fold	71
Diluted 1000-fold	68		

Table 2. Addition of the liver extract from irradiated rabbit

	Cell-density rate (24-hr value) a		Cell-density rate (24-hr value) b
Control	116	Control	62
Original extract	28	Diluted 10-fold	30
Diluted 10-fold	46	Diluted 100-fold	33.
Diluted 100-fold	51	Diluted 1000-fold	72
Diluted 1000-fold	88		

Fig. 2. Addition of phospho-lipid extract of irradiated rabbit liver

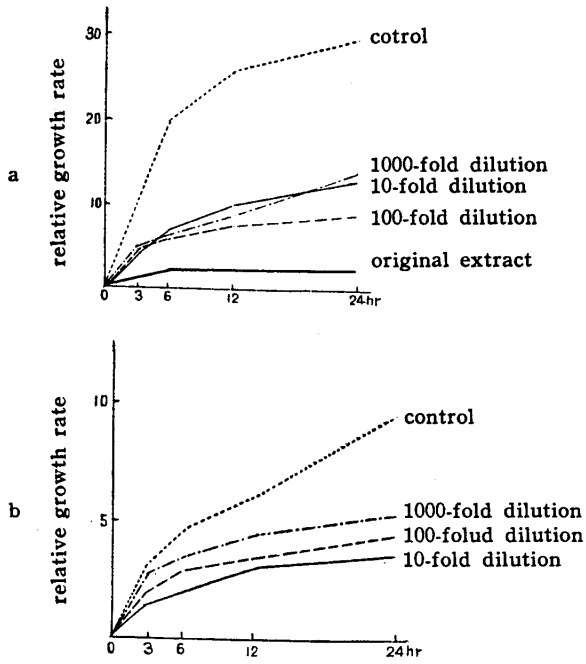


Fig. 3. Addition of phopho-lipid extract of irradiated rabbit liver left standing for one week at room temperature

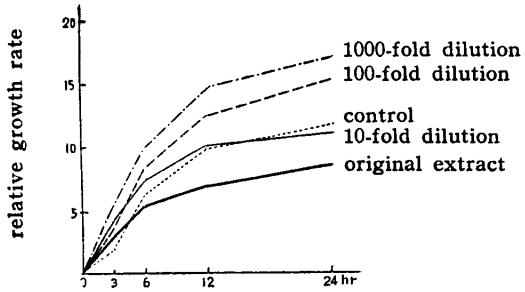
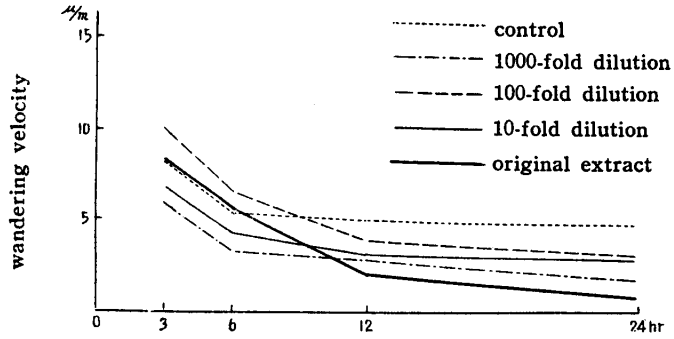


Table 3. Addition of the liver extract left standing for one week at room temperature

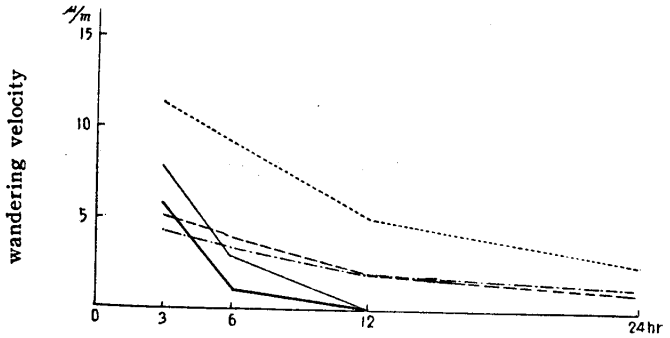
	Cell-density rate (24-hr value)
Control	66
Original extract	68
Diluted 10-fold	65
Diluted 100-fold	60
Diluted 1000-fold	53

Fig. 4. The wandering velocity of pseudo-eosinophils

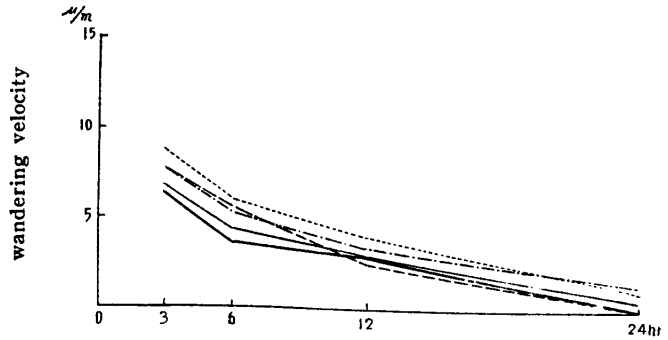
a) Addition of phospho-lipid extract of normal rabbit liver



b) Addition of phospho-lipid extract of irradiated rabbit liver



c) Addition of phospho-lipid extract of irradiated rabbit liver left standing for one week at room temperature

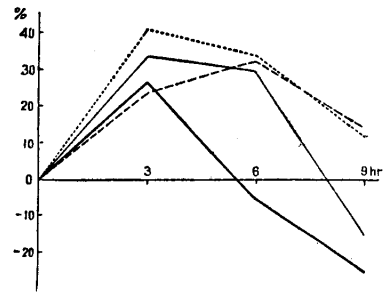


The wandering velocity of pseudo-eosinophilis : In the case of addition of phospholipids extracted from normal rabbit liver no significant differences have been found during the first 6 hours from that of control experiment added physiologic saline solution as can be seen in Fig. 4. However, after 12 hours the wandering velocity of the cells show some delay comparing to that of control with the lowest level in the case added with the original solution (Fig. 4 a). In the case added with the extracts from the irradiated animals a marked drop in the wandering velocity occurred already after six hours culture and no wandering has been recognized after 12 hours when the extracts are added in a high concentration, though some activity has been kept in the case added with the extracts in a low concentration (Fig. 4 b). The addition of the extracts from the irradiated animals which have been left at room temperature for one week proves to lose almost completely their supressing effect on the wandering activity of pseudo-eosinophils giving the same results as in the case added with the extracts from the nonirradiated animals (Fig. 4 c).

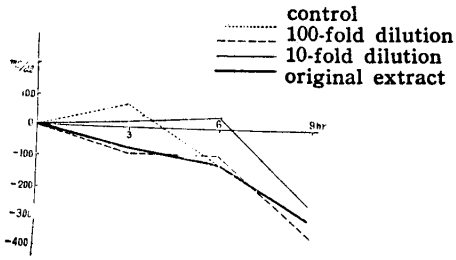
Bone marrow tissue cultured in fluid media : In the cases, in which the extracts from the nonirradiated animals are added, increasing rate in erythrocyte shows a little low level as compared with that of control experiment in one series (Fig. 5 a) but in another experiment the level

Fig. 5. Addition of phospho-lipid extract of normal rabbit liver

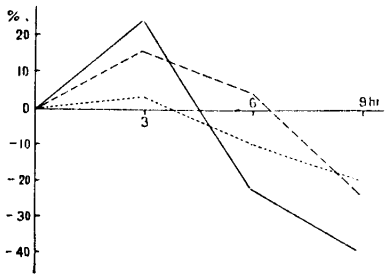
a) Increasing rate of erythrocyte count



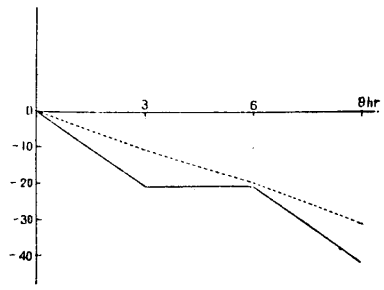
c) Increase of Hb content



b) Increasing rate of erythrocyte count



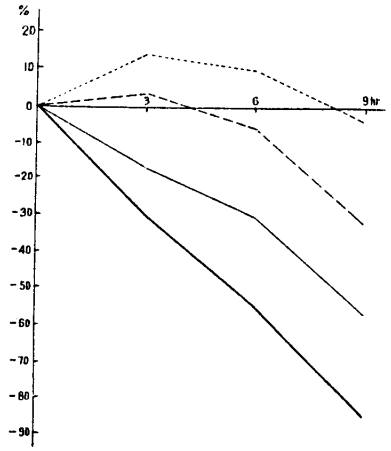
d) Increase of Hb content



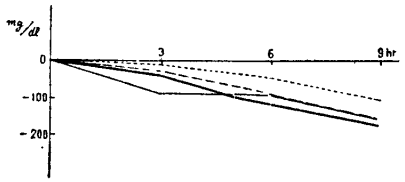
measured at 3 hours shows somewhat higher value than that of the control (Fig. 5 b), showing actually no difference between these added with the extract and those added with physiologic saline solution. In the hemoglobin level there is no difference between those added with the extract and those of control (Fig. 5 c, d). In the cases added the extracts from the irradiated animals, a marked drop in the increasing rate of the erythrocyte count has been observed (Fig. 6 a, b), but the hemoglobin contents show actually no difference from the control (Fig. 6 c, d). The observation on

Fig. 6. Addition of phospho-lipid extract of irradiated rabbit liver

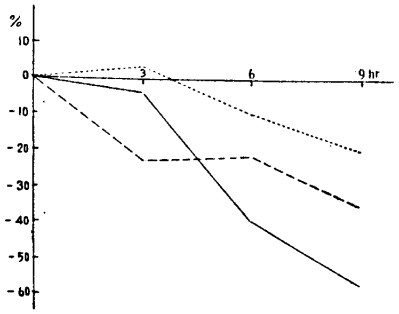
a) Increasing rate of erythrocyte count



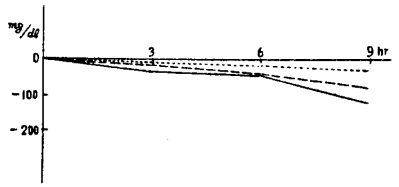
c) Increase of Hb content



b) Increasing rate of erythrocyte count



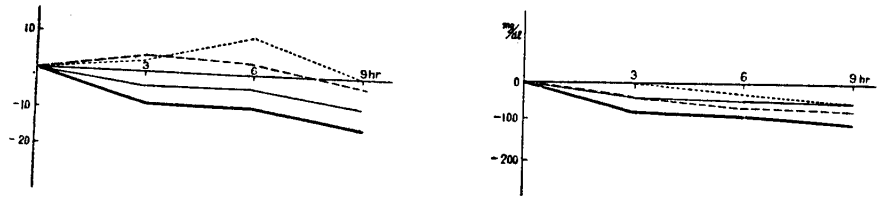
b) Increase of Hb content



the cultures added with the extract from the irradiated animals, but left standing for one week at room temperature, show a marked decrease in the activity revealing a very slight decreasing effect on the increasing rate of erythrocyte (Fig. 7 a).

Fig. 7 Addition of phospho-lipid extract of irradiating rabbit liver left standing for one week at room temperature

a) Increasing rate of erythrocyte count b) Increase of Hb content



COMMENT

Since the experiment of BARRON[5], WEISS[6,7] and LEA[8] the effect of the ionising radiation on the living cells has been largely attributed to the oxidative damage of some substance, especially the demolition or the inactivation of SH-enzymes. This theory is generally accepted at present, but this theory does not explain how the damage induced by X-rays irradiation is protracted for a long period showing almost no tendency for recovery or the severe symptom of the irradiation appears rather in the later stage, because the change occurring in SH group, if it would be the first and the most significant change after the irradiation, it is expected to be recovered in the course of time with the most severe symptoms just after the irradiation. Therefore, the delayed effects of the X-ray irradiation should be attributed to another unknown element. In our experiment it has been clearly shown that the X-ray irradiation results in produceing some toxic substance to the bone marrow cells, one of which is extracted with the phospholipid fraction. The true nature of this substance is unknown at present, but the effect of this substance on the living cells is very similar to that of X-ray irradiation inhibiting the growth and the motility of the white blood cells and the erythropoiesis on the cultured tissue. The results strongly suggest that the biological effect of irradiation is of rather indirect ones caused by some substance produced by irradiation, but not by the inactivation of some enzymes. Our experience, that the irradiation of some limited part of the body causes the disturbances of the hematopoietic organs, also supports this view.

SUMMARY

With the purpose of revealing the biological effects of the X-ray irradiation the authors extracted phospholipids from the liver of irradiated

animals and proved that this substance has the action to inhibit the growth of the bone marrow cells, the motility of pseudo-eosinophilis and the erythropoiesis in tissue culture, suggesting that the injury will mainly be induced by the toxic substances produced by irradiation.

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