intracellular phosphatase is not associated with bone formation and may largely disappear, to be succeeded by a second wave of formation of phosphatase, a great part of which is found outside the cells'. A point of discrimination is to be made about the nature of phosphatase reactions found in the cells before the disappearance and after the reappearance. Before the disappearance, the alkaline phosphatase localisation is seen to be mostly intracellular, whereas after the reappearance it is extracellular in nature. However, the deep phosphatase localisation of the mesenchymatous cells is throughout an important peculiarity of this study. The plastering cells are mesenchymatous cells and that is why they always show positive phosphatase reaction. The study confirms the view that alkaline phosphatase is associated in the development of the bone element of the vertebral column, and at the time of calcification, there is a phenomenon of reappearance of phosphatase in the cells. This study also supports the view previously expressed by LORCH [2] that, in the absence of extracellular phosphatase, there cannot be formation of bone.

This problem was suggested by Dr. SIVATOSH MOOKERJEE and I am indebted to him for his constant help and encouragement.

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Department of Zoology, Presidency College, Calcutta, June 19, 1959.

Zusammenfassung

Zu Beginn der Entwicklung der Wirbelsäure im Hühnerembryo findet sich die alkalische Phosphatase intrazellulär. Mit der späteren Verkalkung und Entwicklung des Knochenmarks ist die Reaktion extrazellulär nachweisbar.

Protective Action of Irradiated Bone Marrow Cells

The potential of the host's immunogenic tissue surviving following lethal irradiation determines whether a foreign graft will take. If it is feasible to completely suppress the immunogenic system of the host by x-ray, the immunologic potential of the graft determines how long the host will survive. Provided that the remaining host's immunogenic tissue after lethal irradiation is negligible, 'takes' and progressive growth in the new hosts of foreign bone marrow, if still functional, would ensue[1]. Even if the implants were hypo-functional, or afunctional, they would nevertheless modify the irradiation response.

Since it appears undesirable to inject cells fully capable of reacting immunologically against the host, in the present study, bone marrow was subjected to irradiation *in vitro* prior to transplantation. The degree of protection afforded by isologous (DBA_1), homologous (C57BL/6) and heterologous (WR) bone marrow exposed to fractionated doses was assessed from the number of mice (DBA_1) surviving lethally irradiated controls. The effects of all treatments are presented in tabular form (Table).

The observations made indicated that: (1) isologous bone marrow cells exposed *in vitro* up to 950 r exercised some protective effect in lethally irradiated mice, and (2) irradiation of homologous and heterologous bone marrow before injection, (a) did not render them incapable of reaction against the host; (b) were destroyed, or, (c) could not repair the host's radiation-injured cells.

Assuming that radiation is cumulative, the total amount of x-radiation to the animals' bone marrow is obtained simply by adding the doses of lethal body irradiation and the *in vitro* exposure of the isologous bone marrow injected[2].

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Experiment	No. of Mice	Treatment of host; X-irradiation*	Treatment of graft; X-irradiation	M S T of mice dying of exposure	No Survival over Total number
I Isologous bone marrow** (DBA/1)	5	509 r	No bone marrow	11	0/5
	5	509 r	None	Permanent	5/5
	5	950 r	475 r	28	1/5
	5	950 r	950 r	14	1/5
	5	950 r	1425 r	10	0/5
II Homologous bone marrow (C57B/6)	5	950 r	No bone marrow	11	0/5
	5	950 r	None	22	2/5
	5	950 r	475 r	15	0/5
	5	950 r	950 r	9	0/5
	5	950 r	1425 r	19	0/5
III Heterologous bone marrow	5	950 r	No bone marrow	11	0/5
	5	950 r	None	12	0/5
	5	950 r	475 r	9	0/5
	5	950 r	950 r	10	0/5
	5	950 r	1425 r	10	0/5

* $LD_{100/11}$ = 950 r; rate 190 r/min. 250 Kvp, 15 ma; 1/2 mm Cu + 1 mm Al filters ** 10^8–10^{12} cells/0·5 ml saline i.v. 24 h post-irradiation

The fact that *isologous* marrow exposed to 1425 r still contained viable cells is of considerable interest. It would indicate that lethality from total body irradiation is not the result of bone marrow depletion alone, but due to damage to various organs, the bone marrow being actually more resistant to radiation than the body as a whole.

By the *in vitro* exposure of *homologous and heterologous bone marrow* to various dose levels of x-rays, it was hoped to remove host specificity and to abolish graft-versus-host reaction through x-ray induced mutation, chromosomal aberrations, breaks, deletion, and/or ploidy[3]. Obviously, several gene requirements would have to be altered in order that the foreign bone marrow be compatible with the new host[4]. It would appear that either *in vitro* irradiation did not render homologous and heterologous bone marrow immunologically incompetent, or that a 100% lethal dose might not adequately suppress the host's immunogenic system. Also, it would be conceivable to suspect that desoxyribonuclease played an important part in destruction of DNA that follows irradiation[5]. The inability of irradiated homologous and heterologous bone marrow to persist and proliferate, even temporarily in the new host, similar to isologous bone marrow, or to stimulate regeneration of the host's bone marrow was presumably because of the degree of genetic dissimilarity.

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Zusammenfassung

Wurde *in vitro* röntgenbestrahltes Knochenmark (475 r, 950 r, 1425 r) in letal bestrahlte Mäuse (LD 100/11 = 950 r) injiziert, so ergab sich: Isologes Knochenmark, mit 950 r bestrahlt, besitzt noch beachtliche Schutzwirkung; bestrahltes homologes und heterologes Knochenmark war weniger oder ganz inaktiv.

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Immediate Effects of UV Irradiation on the Isolated Rat Blastocyst

It has recently[1,2] been reported that the inner cell mass of a rat blastocyst ($4^1/_2$ day after copulation) is destroyed after 1500 ergs/mm² of UV irradiation. By the tenth day of pregnancy, only cells descending from the trophoblast are found in a normally configurated egg-chamber.

The present study is an attempt to elucidate these results, analyzing the immediate cytological changes after irradiation. We tried to investigate the following possible cytological effects:

(1) since it is well known[3] that the UV irradiation can retard or inhibit cell division and cleavage, we counted mitoses and cells of the blastocysts.

(2) The inner cell mass of a normal mammalian blastocyst shows a pronounced affinity for pyronine[4]. Such cytoplasmic basophilia may disappear after UV irradiation, as has been shown in nucleate and anucleate fragments of ameba[5].

(3) The pycnotic cells are often found after irradiation[6] and the affinity of the nuclei for methyl green may disappear[7].

As before, we used the modified egg-tranfer method[8] together with irradiation. Owing to this technique, the transplanted eggs are always in the right uterine horn and those of the recipient in the left one.

In order to test these possibilities, we recovered the irradiated and transplanted blastocysts from the uterine horn of the recipient after 6 (Series 1) or 17 h following irradiation (Series 2). The eggs were fixed *in toto*[4] and stained by the usual mixture after Unna-Brachet.

Series 1. The eggs recovered 6 h after irradiation (i. e. still the fifth day of pregnancy) showed no changes at all, if 1500 ergs/mm² were used, but they had some rare pycnotic cells after 3000 ergs/mm². The average number of cells after both doses was 31·77 ± 1·19. Of 6 blastocysts (3000 ergs/mm²) in three there were 4 pycnotic cells. Controls had the same number of cells (33 ± 2·93), but no pycnotic cells. A total number of 17 blastocysts with 550 cells has been counted.

Series 2. After 16·17 or 19 h (i. e. already the sixth day of pregnancy) the average number of cells was as follows (dose 1500 ergs/mm²):

Experiment (right uterine horn)		Controls (left uterine horn)	
a transplantation and irradiation	*b* transplantation only	*b*	*a*
(8) 26 ± 1·57*	(7) 48·43 ± 1·98	(5) 51 ± 4·09	(7) 49 ± 0·03

* = standard error. ( ) = number of blastocyst.
Total number: 27 blastocysts with 1146 cells.

A considerable number of blastocysts were transplanted and irradiated, but only a small number was recovered and successfully fixed and stained.

Thus we may conclude that transplantation does not disturb the mitotic process, the number of cells being approximately the same as in the controls. Only the irradiated blastocysts have a smaller number of cells. The difference is highly significant ($P < 0{\cdot}01$). This number (26) is not very different from the number of cells from series 1, recovered the fifth day of pregnancy (the difference is not significant).

The relative number of cells of the inner cell mass is approximately the same in irradiated blastocysts as in the controls (about 32–37%).

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