Factor reducing ^{59}Fe Incorporation into Mammalian Cells

AFTER local X-irradiation of the spleen of rabbits, a factor inducing a considerable drop of the reticulocyte number is released into the blood serum[1]. This factor is either gamma-globulin or perhaps a small molecular compound attached to it[2]. We have investigated whether serum or gamma-globulin from X-irradiated rabbits[1] influences iron incorporation into red blood cells *in vivo* and into bone marrow cells *in vitro*.

In preliminary experiments, rabbits were given, per kg body weight, either serum containing 100 mg protein or 3 mg gamma-globulin from X-irradiated rabbits. Controls were given the same amount of serum or gamma-globulin from unirradiated animals. Three h after these injections each animal received 4·5 μCi/kg ^{59}Fe citrate intravenously. The radioactivity in red blood cells withdrawn from the ear vein of the animals and washed twice with saline was determined 1, 2, 3, 4 and 5 days after ^{59}Fe injection and was found to be reduced following either the injection of serum or gamma-globulin from irradiated rabbits, as compared with the controls.

In other *in vivo* experiments either serum containing 60 mg protein or 6 mg isolated gamma-globulin from X-irradiated rabbits was given per 100 g body weight to five rats subcutaneously. In every experiment the same number of control animals received the same amount of serum or gamma-globulin from unirradiated rabbits. Three h after the injection of the serum and 2 h after the injection of gamma-globulin, each animal received 1 μCi ^{59}Fe citrate, intravenously. ^{59}Fe activity in red blood cells obtained by the decapitation of the rats was measured 24 h after treatment with serum or 4 h after treatment with gamma-globulin. ^{59}Fe incorporation was depressed after injection of serum or gamma-globulin from irradiated animals, as compared with the controls (Table 1).

Table 1. ^{59}Fe INCORPORATION INTO CIRCULATING RED BLOOD CELLS FOLLOWING INJECTION OF SERUM OR GAMMA-GLOBULIN FROM SPLEEN X-IRRADIATED RABBITS

Number of experiments	Number of Serum Normal	Number of Serum Irradiated	Number of Gamma-globulin Normal	Number of Gamma-globulin Irradiated	^{59}Fe activity in total red blood cells in per cent of injected activity Normal	^{59}Fe activity in total red blood cells in per cent of injected activity Irradiated	Decrease per cent
1	26	25			22·5	14·1	−38
2	26	25			38·1	19·0	−51
3	30	31			29·8	15·2	−49
4			30	31	2·5	1·3	−30
5			38	40	3·5	2·6	−25
6			43	41	1·8	1·5	−17
7			45	44	3·1	2·0	−35
8			45	44	3·6	2·4	−32

According to Koch *et al.*[3], the decreased iron incorporation found in circulating red blood cells following irradiation indicates a decrease of erythropoiesis. Donati *et al.*[4], however, have shown that reticulocytes may incorporate iron not only while they are still in the bone marrow but also in the circulating blood. To exclude the role of any higher regulatory mechanism, we experimented with bone marrow cells *in vitro*.

Our system consisted of: $1·5-2·0\times10^7$ nucleated bone marrow cells suspended in 1 ml. 0·15 M phosphate buffer, *p*H 7·4, to which we added 60 mg protein in 1 ml. serum or 6 mg isolated gamma-globulin in 1 ml. saline, and 0·5 μCi ^{59}Fe citrate dissolved in 1 ml. saline. After incubation for 2 h the acidhaematin was extracted[5] and its radioactivity determined. The spectra of these extracts were identical with those of crystalline haem[6]. The equilibrium of the ^{58}Fe and ^{59}Fe incorporations is reached after 40–60 min in the *in vitro* system. No decrease of iron incorporation was found with sera or gamma-globulin from animals the liver of which instead of the spleen was irradiated (Table 2).

Four gamma-globulin batches (numbers 25, 31, 41 and 61) used in these experiments were also checked for their influence on the reticulocyte count. We found that they depressed the reticulocyte count of healthy rabbits to 39–70 per cent[1].

Table 2. HAEM SYNTHESIS IN RAT BONE MARROW CELLS *in vitro* INFLUENCED BY SERUM OR GAMMA-GLOBULIN FROM SPLEEN X-IRRADIATED RABBITS

Number	Number of Serum Normal	Number of Serum Irradiated	Number of Gamma-globulin Normal	Number of Gamma-globulin Irradiated	^{59}Fe activity (c.p.m./10^7 bone marrow cells) Normal	^{59}Fe activity (c.p.m./10^7 bone marrow cells) Irradiated	Decrease per cent
1	26	25			5,818	4,750	−19
2	26	25			7,176	4,588	−36
3			30	31	6,894	5,710	−25
4			30	31	6,770	5,212	−23
5			38	40	3,615	3,501	−3
6			38	41	8,971	6,189	−31
7			43	41	3,648	3,101	−15
8			45	44	6,090	3,471	−43
9			49	61	5,740	2,975	−48

These experiments show that the factor found in the sera of spleen-irradiated animals depresses the reticulocyte count[1,2] and reduces ^{59}Fe incorporation both into circulating red blood cells and into the haem of bone marrow cells; the latter observation indicates a drop in haem synthesis in bone marrow cells.

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B. DALOS
ANNA DÖKLEN
MAGDOLNA HORVÁTH

"Fréderic Joliot-Curie",
National Research Institute for
Radiobiology and Radiohygiene,
Budapest, Hungary.

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Sensitivity of Neurones of the Insect Central Nervous System to Iontophoretically Applied Acetylcholine or GABA

NEURONES of the insect central nervous system have proved to be relatively insensitive to applied acetylcholine[1–5]. The threshold to acetylcholine applied to the nerve cord ranged from 10^{-2} M to 10^{-5} M. An explanation of this is that there is a high concentration of choline esterase around the nerve cell which effectively reduces the concentration of acetylcholine at the neurone surface to considerably below that applied to the whole central nerve cord[6].

Until recently it has been difficult to penetrate the neurones in the insect central nervous system with microelectrodes and obtain stable membrane potentials and action potentials. There is now, however, considerable evidence that microelectrode recordings can be obtained from insect central neurones[7–9] and it is possible to obtain action potentials from such cells for periods of several hours[10].

Fig. 1*A* shows the activity of a nerve cell in the sixth abdominal ganglion of the cockroach *Periplaneta americana*. The resting potential was 55 mV and the action potential 104 mV. The duration of the action potential was 3–5 ms. In many preparations it was possible to see post synaptic activity (EPSP and IPSP). When the microelectrode was filled with Procion yellow, similar recordings could be obtained. The cell could then be filled with the dye and later histological investigation showed the position of the electrode. Fig. 1*B* shows a