Reactivation of Erythropoiesis by Cobalt after Radiation Injury

By Jouko Saikkonen and Eino Marttinen

Of the various tissues of the body, the hematopoietic system is one of the most sensitive to ionizing radiation. Lymphocytes are the first cells to show a reduction following exposure, but erythropoietic cells are almost equally sensitive. Anemia does not usually appear until several weeks have elapsed, but it may run a chronic course and in some cases aplastic anemia may develop.

The response of the organism to total body irradiation can be modified by several means. Postirradiation factors which increase the regeneration and survival time are important from the practical point of view. Severely damaged hematopoietic tissue can be restored, in any case transitorily, by injection of bone marrow or splenic homogenates. Myelopoiesis is stimulated by sterile inflammation. Stohlman et al. have demonstrated that erythropoiesis can be stimulated by such methods as bleeding the animals shortly before or after exposure and by postirradiation administration of paraaminopropiophenone. Furthermore, plasma derived from donors with posthemorrhagic anemia is capable of stimulating erythropoiesis in the sublethally irradiated animal. The authors suggested that the acceleration of erythropoiesis in these cases is due to hemopoietin released by hypoxia.

Cobalt is a powerful accelerator of erythropoiesis and effective in situations in which other erythropoietic factors are valueless. After the discovery of the erythropoietic effect of cobalt, the mechanism of this phenomenon was poorly understood until Goldwasser et al. demonstrated that cobalt increases the production of erythropoietin. The increase in the erythropoietic activity of plasma after cobalt therapy has also been confirmed by Brown and Meineke.

If the production of erythrocytes after radiation injury can be stimulated by erythropoietin, we may suppose that cobalt can be used as an initiator of the reaction. This study was performed to reveal whether cobalt can prevent or ameliorate the anemia produced by total body x-irradiation.

Material and Methods

Two different series of experiments were performed. The first series consisted of 50 adult male albino rats of the Wistar strain. The animals were divided into five groups, each consisting of 10 rats. The animals in groups II to V were exposed, five at a time in cardboard boxes, to a single total-body x-irradiation. Radiation factors were: 190 kv., 15 ma., hvl of 1.6 mm. of copper, rate in air about 60.5 r/min., and target-skin distance 60 cm.
Groups II and III received 250 r and groups IV and V 350 r. Cobalt treatment was started after radiation in groups III and V. 0.8 mg. of cobalt chloride (CoCl₂ · 6H₂O) dissolved in 1 ml. of saline, which corresponds to 0.2 mg. of Co, was injected subcutaneously in each rat daily. In other words, when the average weight of the animals was 240 Gm., the daily dose was about 0.8 mg. per Kg. of body weight. The first injection was given 1 hour after irradiation and the treatment was continued over a period of 8 weeks. The same amount of cobalt was administered to the rats of group I, which were not irradiated.

Groups II and V were treated with saline injections for 8 weeks after exposure.

Blood samples were obtained from tail veins once a week for hemoglobin and red cell count determinations. The animals were weighed at the same time.

The purpose of the second series of experiments was to study the changes in the bone marrow. Forty adult female rats of the same strain were divided into four groups, denoted VI to IX, composed of 10 rats each. Groups VI and VII were exposed to a single total body irradiation of 350 r as before. Group VI was treated with daily saline injections, whereas rats in group VII received 0.8 mg. of cobalt chloride daily. The first injection was given 1 hour after exposure. Group VIII was treated with cobalt only and rats in Group IX served as controls, receiving a daily saline injection.

To make smears and histologic sections of the bone marrow, from one to two animals in each group were killed by decapitation 1, 2, 4, and 8 weeks after exposure. Bone marrow from the femur was examined. Smears were stained by the May-Grünwald-Giemsa method and material for sections was fixed in Zenker solution and stained with hematoxylin-eosin. Myeloid erythroid ratios (M:E) were calculated on 2000 marrow cells counted in each animal after the specimen had been labeled by code so that they could be counted "blind." At the same time, mitotic frequency was determined.

The management of different groups is summarized in table 1.

**RESULTS**

The results of the first series of experiment regarding the effect of cobalt on blood values are presented in figures 1 to 5. The mean values of each group are given. Hemoglobin is expressed in Gm. per 100 ml. of blood and erythrocytes in millions per 1 mm³.

Figure 1 shows the changes in group I, treated with cobalt only. Initially hemoglobin was 12.8 Gm. and the erythrocyte count 8.2 millions. A marked polycythemic reaction was observed after 4 weeks. The highest values, 14.5 Gm. and 9.2 millions, were reached after 8 weeks. The administration of cobalt was thereafter discontinued and 2 weeks later the hemoglobin and erythrocyte values were below the original levels. Three of the animals died during the fifth and sixth weeks. The weight of the rats decreased during cobalt treatment but when this was discontinued, the animals gained in weight. Some of the rats were infected at the sites of injections.

Figures 2 and 3 show changes in groups II and III irradiated with 250 r. In group II, x-irradiation caused a significant lowering of the hemoglobin and erythrocyte values. Hemoglobin dropped from 13.2 to 10.0 Gm. and erythrocytes from 9.2 to 6.4 millions in 4 weeks, whereafter the values began to rise slowly. Three animals died during the third and fourth weeks. In group III, exposed to a similar radiation dose but treated with cobalt afterwards, there was likewise an initial lowering of the hemoglobin value and erythrocyte count, but this was relatively small and transient and was followed by a marked polycythemic reaction. Before any treatment the mean hemoglobin was 13.7 Gm. and the erythrocyte count 9.3 millions. At the end of 8 weeks
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the hemoglobin was 15.7 Gm. and the mean erythrocyte count 9.6 millions. During the following 2 weeks, when cobalt administration was discontinued, hemoglobin dropped to 13.9 Gm. and erythrocytes to 8.6 millions. One rat in this group died during the fifth week. The animals lost weight in group III but not in group II.

Figures 4 and 5 show the changes occurring in groups IV and V, exposed to 350 r. Despite small fluctuations, a continuous fall in the blood values was observed in group IV. In 8 weeks hemoglobin dropped from 14.0 to 12.5 Gm. and erythrocytes from 9.4 to 8.4 millions. During the following 2 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>X-irradiation</th>
<th>Cobalt Injections</th>
<th>Saline Injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0.8 mg./Kg. daily</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>250 r</td>
<td>0</td>
<td>1 cc. daily</td>
</tr>
<tr>
<td>III</td>
<td>250 r</td>
<td>0.8 mg./Kg. daily</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>350 r</td>
<td>0</td>
<td>1 cc. daily</td>
</tr>
<tr>
<td>V</td>
<td>350 r</td>
<td>0.8 mg./Kg. daily</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>350 r</td>
<td>0</td>
<td>1 cc. daily</td>
</tr>
<tr>
<td>VII</td>
<td>350 r</td>
<td>0.8 mg./Kg. daily</td>
<td>0</td>
</tr>
<tr>
<td>VIII</td>
<td>0</td>
<td>0.8 mg./Kg. daily</td>
<td>0</td>
</tr>
<tr>
<td>IX</td>
<td>0</td>
<td>0</td>
<td>1 cc. daily</td>
</tr>
</tbody>
</table>

Each group contained 10 rats. The first injection was given 1 hour after exposure and treatment was continued over 8 weeks.

the hemoglobin was 15.7 Gm. and the mean erythrocyte count 9.6 millions. During the following 2 weeks, when cobalt administration was discontinued, hemoglobin dropped to 13.9 Gm. and erythrocytes to 8.6 millions. One rat in this group died during the fifth week. The animals lost weight in group III but not in group II.

Figures 4 and 5 show the changes occurring in groups IV and V, exposed to 350 r. Despite small fluctuations, a continuous fall in the blood values was observed in group IV. In 8 weeks hemoglobin dropped from 14.0 to 12.5 Gm. and erythrocytes from 9.4 to 8.4 millions. During the following 2 weeks

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Fig. 2.—The development of anemia in rats irradiated with 250 r. After the exposure, the animals were treated with daily saline injections. Three of the 10 rats died.

Fig. 3.—The effect of cobalt on rats irradiated with 250 r. The first injection was given 1 hour after the exposure and the treatment was continued daily over a period of 8 weeks. The dose of Co was 0.8 mg. per Kg. of body weight. Compare these curves with fig. 2.
Fig. 4.—The development of anemia in rats irradiated with 350 r. After the exposure the animals were treated with daily saline injections.

Fig. 5.—The effect of cobalt on rats irradiated with 350 r. The first injection was given 1 hour after the exposure and the treatment was continued daily over a period of 8 weeks. The dose of Co was 0.8 mg. per Kg. of body weight. Compare these curves with fig. 4.
Table 2.—Comparison between Values in Different Groups before and after Irradiation

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After 1 Week</th>
<th>After 2 Weeks</th>
<th>After 4 Weeks</th>
<th>After 8 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb</td>
<td>E</td>
<td>Hb</td>
<td>E</td>
<td>Hb</td>
</tr>
<tr>
<td>Group:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II (250 r)</td>
<td>13.2</td>
<td>9.2</td>
<td>12.1</td>
<td>8.3</td>
<td>12.0</td>
</tr>
<tr>
<td>III (250 r + Co)</td>
<td>13.7</td>
<td>9.3</td>
<td>13.0</td>
<td>8.8</td>
<td>13.8</td>
</tr>
<tr>
<td>Difference:</td>
<td>+4%</td>
<td>+1%</td>
<td>+7%</td>
<td>+6%</td>
<td>+15%</td>
</tr>
<tr>
<td>Adjusted difference:</td>
<td>+3%</td>
<td>+5%</td>
<td>+11%</td>
<td>+9%</td>
<td>+32%</td>
</tr>
<tr>
<td>Group:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV (350 r)</td>
<td>14.0</td>
<td>9.4</td>
<td>13.1</td>
<td>7.8</td>
<td>12.8</td>
</tr>
<tr>
<td>V (350 r + Co)</td>
<td>13.3</td>
<td>8.6</td>
<td>12.2</td>
<td>8.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Difference:</td>
<td>−5%</td>
<td>−8%</td>
<td>−7%</td>
<td>+3%</td>
<td>−2%</td>
</tr>
<tr>
<td>Adjusted difference:</td>
<td>−2%</td>
<td>+11%</td>
<td>+3%</td>
<td>+11%</td>
<td>+5%</td>
</tr>
</tbody>
</table>

The differences are calculated as percentages which show how much higher the values are in groups treated with cobalt as compared with untreated animals. The small initial differences have been taken into account and corresponding corrections made.
**Reactivation of Erythropoiesis by Cobalt**

Table 3.—Quantitative Data Regarding the Myeloid:Erythroid Ratio (M:E) and Mitotic Frequency (MF per 1000 cells) in Different Groups

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>3.0 1.5</td>
<td>2.0 2.5</td>
<td>1.1 3.0</td>
<td>1.5 3.0</td>
<td>350 r</td>
</tr>
<tr>
<td>VII</td>
<td>1.9 4.5</td>
<td>0.8 3.0</td>
<td>1.7 6.3</td>
<td>0.9 5.5</td>
<td>350 r + Co</td>
</tr>
<tr>
<td>VIII</td>
<td>1.8 7.5</td>
<td>0.5 5.0</td>
<td>0.9 3.8</td>
<td>1.2 3.0</td>
<td>Co</td>
</tr>
<tr>
<td>IX</td>
<td>2.5 3.0</td>
<td>1.8 2.0</td>
<td>2.1 3.5</td>
<td>2.1 3.0</td>
<td>control</td>
</tr>
<tr>
<td></td>
<td>1.5 2.0</td>
<td>2.3 4.0</td>
<td>2.0 2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For further explanation, see text.

The anemia became still more pronounced: hemoglobin 11.5 and erythrocytes 7.2 millions. No loss in weight of the animals was observed. The animals in group V, treated with cobalt after exposure, showed the same transient fall in hemoglobin and erythrocyte count as those in group III. Here also, the fall was slight and followed by a polycythemic reaction. The initial values of hemoglobin and red cell count were 13.3 Gm. and 8.6 millions. After 8 weeks of cobalt treatment the corresponding values were 14.2 Gm. and 9.5 millions. When cobalt injections were discontinued, the polycythemia subsided as in groups I and III. The weight of the rats in group V was reduced and one animal died in the third week and two in the sixth week.

The values in groups II to V initially and after 1, 2, 4, and 8 weeks are summarized in table 2, where the differences between cobalt-treated and untreated groups are expressed as percentages.

The bone marrow in group VI, which belonged to the other series of experiments, contained very few erythroblasts 1 week after exposure. The youngest basophilic cells especially were lacking. In group VII, afterwards treated with cobalt, the findings were very similar. In group VIII no effect of cobalt was seen either and the bone marrow looked like that of the control.

After 2 weeks the effect of cobalt became apparent. In group VI signs of spontaneous recovery were seen. There were numerous young erythroblasts and many mitoses. In group VII, however, the erythropoiesis was more active, as indicated by the presence of many large erythroblast groups. In group VIII the erythropoiesis was as active as in group VII.

After 4 weeks the differences were clearer. Groups VII and VIII, treated with cobalt, showed a marked erythropoietic activity in contrast to the untreated animals in group VI, where the formation of red cells was still partly inhibited. Furthermore, the erythropoiesis was more active in group VII than in the control group IX. In group VIII the bone marrow was already polycythemic.

Eight weeks after the exposure the situation was very like that after 4 weeks. The erythropoiesis in group VI was still below the control level but the bone marrow in group VII was beginning to become polycythemic.
Quantitative data regarding the M:E ratio and mitotic frequency are summarized in table 3. In group VII, treated with cobalt after exposure, the M:E ratio in almost every case was smaller than in group VI. The mitotic frequency was higher in group VII than in group VI. These figures indicate that cobalt stimulates the production of erythroblasts.

**Discussion**

Because the radiosensitivity of our rats was not exactly known beforehand and the animals were expected to develop anemia and survive for at least 8 to 10 weeks, two different doses, 250 r and 350 r, were used. The results were satisfactory because a moderate anemia developed in both cases and the mortality rate was quite low.

Constant and Phillips tested the effect of cobalt, copper and manganese on the erythrocyte fragility and x-irradiation mortality. They concluded that long-term feeding of polycythemic levels of cobalt increased the mortality rate of animals given whole-body irradiation. Later, Gessert and Phillips reported that after exposure to 750 r of total body x-irradiation the average mortality in rats with cobalt-induced elevation of hemoglobin levels is increased rather than decreased as compared with that of similarly irradiated controls. However, no statement was made about the erythropoiesis of the rats after exposure. The intake of cobalt by the rats in their experiments is not exactly known because cobalt chloride was incorporated in the food. Since, however, the rate of growth was slightly inhibited at the higher concentrations of dietary cobalt, these dosages must be regarded as toxic and it is very natural that the animals die more readily when they are under a double stress.

According to Parr et al., however, the resistance of mice to irradiation is significantly increased when the animals are given cobalt-supplemented diet for 8 days prior and 15 days following irradiation.

In our experiments, three animals died in group II, irradiated with 250 r, whereas only one died in group III, afterwards treated with cobalt. All rats exposed to 350 r survived except three in group V, treated with cobalt. In addition, three rats in group I died under cobalt treatment due to abscesses at the sites of the subcutaneous injections. In all groups the weight of the animals decreased during cobalt administration and when this was discontinued the animals gained in weight again. Our material is too small for accurate calculation of mortality percentages. It seems, however, that cobalt administered in a dose of 0.8 mg. per Kg. of body weight daily over a period of 8 weeks has toxic effects.

There are data which indicate that erythropoiesis can be stimulated by cobalt with smaller side-effects. It was later demonstrated that when cobalt chloride was used, only 0.1 to 0.2 mg. of cobalt per Kg. of body weight was needed to obtain a maximal erythropoietic effect in a normal rat. These dosages caused no apparent toxic symptoms in 5 weeks. Furthermore, by using some other chemical combination of cobalt, such as cobalt glutaminate, the toxicity can be still further reduced. According to Undritz, a polycythemic reaction produced by cobalt is itself a toxic symptom which is not necessary in therapeutic administration.
REACTIVATION OF ERYTHROPOIESIS BY COBALT

Despite the toxic side-effects observed in our experiments, the results deserve further investigation. In figures 2 to 5 and in table 2 we can see that erythropoiesis is markedly stimulated by cobalt after a dose of 250 r. After 350 r the effect is less rapid, but nonetheless significant. The reduction of the effect of cobalt after higher doses of x-irradiation suggests, however, that when the injury to the bone marrow cells becomes severe enough, no further stimulation can be achieved with the metal.

Bone marrow findings confirmed that a more active formation of red cells actually took place and the possibility of hemoconcentration due to dehydration and plasma loss could be eliminated.

When normal animals are treated with cobalt, it usually takes 2 to 3 weeks before the polycythemic reaction can be observed. Irradiated animals showed this latent period too. The blood values were at first decreased, although the reduction was of smaller magnitude than in untreated rats. The results indicate that before reactivation can be achieved, cobalt must be administered in very low doses for several weeks. The results obtained in animal experiments inspired us to test whether cobalt can be used to counteract the bone marrow depressing action of x-rays in human therapy.2 Cancer patients treated with high doses of x-rays were given cobalt, copper and iron perorally in the form of CCF 37 and Ferronicum tablets.* The daily dose of cobalt was 20 mg. and that of copper 2 mg. The effect of cobalt, copper and iron administered simultaneously was superior to the effect produced by iron alone.

Hammett3° and Barron31 have suggested that one of the effects of ionizing radiation in vivo is the oxidation and inactivation of compounds containing the –SH group. This stimulated the use of –SH compounds against injurious radiation and many such substances have been shown to give protection when administered before exposure. On the other hand, it has been suggested by Orten and Bucciero22 that cobalt may produce polycythemia by binding –SH or perhaps other groups involved in cellular respiration, thus leading to a simulated cellular anoxia, and in turn to compensatory polycythemia. It seems peculiar to think that erythropoiesis could be stimulated after radiation injury by inactivating still more –SH groups with cobalt. Of course, it is very possible that other important groups are involved.

Our findings are in good agreement in one respect with earlier investigations made by Stohlman et al.15,16 and in another with the works of Goldwasser et al.21,22 and Brown and Meineke.23 We believe, therefore, that cobalt liberates or activates erythropoietin, which in turn accelerates the formation of red cells. But in which cells or tissues the liberation of erythropoietin by cobalt occurs is not known.

We must remember, however, that cobalt is in many respects a toxic metal28,34 and its value in human therapy must be carefully studied before it can be used generally.

SUMMARY

The ability of cobalt chloride to prevent the anemia following total body

*CCF 37 tablets (Sandoz A. G., Basel, Switzerland) contain 5 mg. of cobalt, 0.5 mg. of copper and 22 mg. of iron. Ferronicum tablets (Sandoz A. G.) contain 22 mg. of iron.
irradiation injury was tested. Laboratory albino rats were exposed to a single x-ray dose of 250 or 350 r. After the exposure, one-half of the rats were treated with daily subcutaneous injections of cobalt while the others were given saline injections at the same time. The first injection was administered 1 hour after the exposure and the daily dose consisted of 0.8 mg. of cobalt per Kg. of body weight. The treatment was continued over a period of 8 weeks. Hemoglobin and the erythrocyte count were measured once a week.

Rats exposed to 250 r and thereafter treated with cobalt showed reactivation of erythropoiesis after 1 to 2 weeks and the animals then rapidly developed polycythemia, in contrast to the similarly irradiated rats afterwards treated with saline, which after 8 weeks still had blood values below the original level. In rats irradiated with 350 r, the same phenomenon was seen, although the effect of cobalt became apparent slowly. The findings observed in the peripheral blood were confirmed by checking the bone marrow of rats given 350 r.

Besides the erythropoietic effect, toxic symptoms were observed. The toxicity and mode of action of cobalt are discussed.

**SUMMARIO IN INTERLINGUA**

Esseva testate le capacitate de chloruro de cobalt de prevenir le anemia que seque le vulneration causate per irradiation del corpore total. Rattos albin de laboratorio esseva exponite a un dose unic de radios X de 250 a 350 r. Post le exposition, un medietate del rattos esseva tractate con diurne injectiones subcutanee de cobalt, durante que le alteres recipeva al mesme tempores injectiones de solution salin. Le prime injection esseva administrate 1 hora post le exposition, e le dose diurne consisteva de 0,8 mg de cobalt per kg de peso corporee. Le tractamento esseva continuare durante 8 septimanas. Hemoglobin a le numeration de erythrocytos esseva determinate un vice per septimana.

Rattos exponite a 250 r e tractate subsequentemente con cobalt manifestava un reactivation del erythropoiese post 1 a 2 septimanas. Postea le animales disveloppava rapidemente polycythemia. Per contrasto con isto, le similemente irradiate animals que esseva subsequentemente tractate con injectiones de un solution salin monstrava valores de infra le nivellos original ancora 8 septimanas post le exposition. In rattos irradiate con 350 r, le mesme pheno-meno esseva constatare, ben que le effecto del cobalt sub iste conditiones esseva apparentemente relentate. Le datos obtenite in studios del sanguine peripheric esseva corroborate per le examine de medulla ossee ab rattos tractate con 350 r. A parte le effecto erythropoietic, symptomas de toxicitate esseva observate. Le toxicitate e le modo de action de cobalt esseva discutite.

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