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# ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 114
10 April 1953

## FURTHER INVESTIGATIONS INTO THE MODIFICATION OF RADIATION SENSITIVITY AFFORDED BY COBALT*

*Subtask under Biological and Medical Aspects of Ionizing Radiation, AMRL Project No. 6-59-08-014, Subtask, Effects of Ionizing Radiation.

MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
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# FURTHER INVESTIGATIONS INTO THE MODIFICATION OF RADIATION SENSITIVITY AFFORDED BY COBALT*

by

W. H. Parr, Radiobiologist, T. O'Neill, Pfc, S. G. Bush, Pfc,
and A. T. Krebs, Chief Radiobiologist

from

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## ABSTRACT

# FURTHER INVESTIGATIONS INTO THE MODIFICATION OF RADIATION SENSITIVITY AFFORDED BY COBALT


### OBJECT

To study the influence of administering times and feeding conditions on cobalt afforded total body x-irradiation protection.

### RESULTS

The administration of a cobalt diet for 5 and 8 days before and for 15 days after x-irradiation increased the tolerance of female Swiss-Webster albino mice to total body x-irradiation (720 r). The administration of a cobalt diet for 36 days before and for 15 days after irradiation and the administration of a cobalt diet for 30 days beginning immediately after irradiation produced no pronounced beneficial effects.

### CONCLUSION

The results indicate that the initial events produced by the administration of cobalt rather than the events at later time intervals are decisive for the observed increase in radio-resistance. Which one of the initial events, stimulation of the hemopoietic system, interference with cellular respiration, inhibition of enzymatic activity, contributes, alone or in combination, to the protection cannot be said at present.

### RECOMMENDATIONS

Methods and times of administering cobalt, optimum quantity for protection, and the combination of cobalt with other protective agents should be investigated.




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Submitted by:

W. H. Parr, Radiobiologist
T. O'Neill, Pfc
S. G. Bush, Pfc
A. T. Krebs, Chief Radiobiologist

Approved: Ray G. Daggs
RAY G. DAGGS
Director of Research

Approved: Carl F. Tessmer
CARL F. TESSMER
Lt. Colonel, MC
Commanding



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# FURTHER INVESTIGATIONS INTO THE MODIFICATION OF RADIATION SENSITIVITY AFFORDED BY COBALT

## I. INTRODUCTION

The beneficial effect of cobalt against acute mortality of mice after total body x-irradiation was recently reported (1) and the possible mechanisms of action discussed (2). Cobalt may afford protection by producing a polycythemia, suggesting an active stimulus to the hemotopoietic system (3); by interfering with cellular respiration (4); or by blocking enzymatic activity (5). Each mechanism *per se* influences radiosensitivity.

Before a decision can be reached as to which mechanism is primarily involved, further investigations are necessary. If polycythemia is decisive for the change in radiation sensitivity, the oral administration of cobalt over long periods should increase its effectiveness since cobalt polycythemia generally approaches a maximum after weeks of oral administration (3, 6-11).

Evaluation of this possibility under controlled feeding conditions was the purpose of the experiments reported here.

## II. EXPERIMENTAL PROCEDURE

Four hundred female Swiss-Webster albino mice (25 $\pm$ 1 gm) were divided into four groups:

Group A - Purina Stock Chow Diet

Group B - Cobalt Diet

Group C - Purina Stock Chow Diet and Irradiation

Group D - Cobalt Diet and Irradiation

The normal diet consisted of Purina stock chow pellets. The cobalt diet was prepared daily by immersing Purina chow pellets for



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two minutes in an aqueous 2% solution of $CoCl_2 \cdot 6H_2O$* and then allowing them to dry. Under these conditions the pellets retain their original form and become enriched with cobalt. To give all animals easy access to food, the pellets were scattered in the cedar shavings covering the bottom of the cage. Thus, the cobalt fed animals could eat ad lib the cobalt treated surface or the less enriched core of the pellet. The animals were kept on this diet for 36 days or less before and for 15 days or more after irradiation.

To study the effects of feeding conditions (12), another group of mice was kept in plastic cages. Their random choice of food was restricted by feeding them through the regular food baskets.

The irradiations were made with a Kelley-Koett deep therapy x-ray unit operated at 200 kv, 6 ma, inherent filtration equivalent 0.25 mm Cu., 1 mm Al. plus 0.5 mm Cu. added filter, target distance 28 cm., 48 r/m in air, total dose 720 r/in air. The animals were irradiated in a well ventilated lucite cage in groups of 10 (5 animals from Group C and 5 animals from Group D) and observed for 30 days after irradiation with the deaths being recorded every 24 hours.

## III. RESULTS

### A. Administering Period

Mice kept on cobalt food for a few days prior to x-irradiation show a greater resistance to radiation injury than mice kept on cobalt food over a longer period of time. The survival of animals receiving cobalt food for 5 to 8 days prior to and for 15 days after radiation (Fig. 1 and Fig. 2) was approximately 50 to 60% higher than the survival of the irradiated Purina chow fed animals in their respective groups.

The survival curves of the animals fed cobalt food for 36 days before and for 15 days after irradiation (Fig. 3) and the survival curves of the animals fed Purina chow and irradiated follow approximately the same pattern. Only in the latter part of the study did the cobalt fed irradiated groups show a modification in the curve. This also holds true for animals placed on cobalt food immediately after x-irradiation and restricted to the same diet until the termination of the study (Fig. 4).

*E&A Tested Purity Reagent, Cat. No. C-371, Cobalt (ous) Chloride, C. P. $CoCl_2 \cdot 6H_2O$ M. W. 237.95, Fisher Scientific Company.



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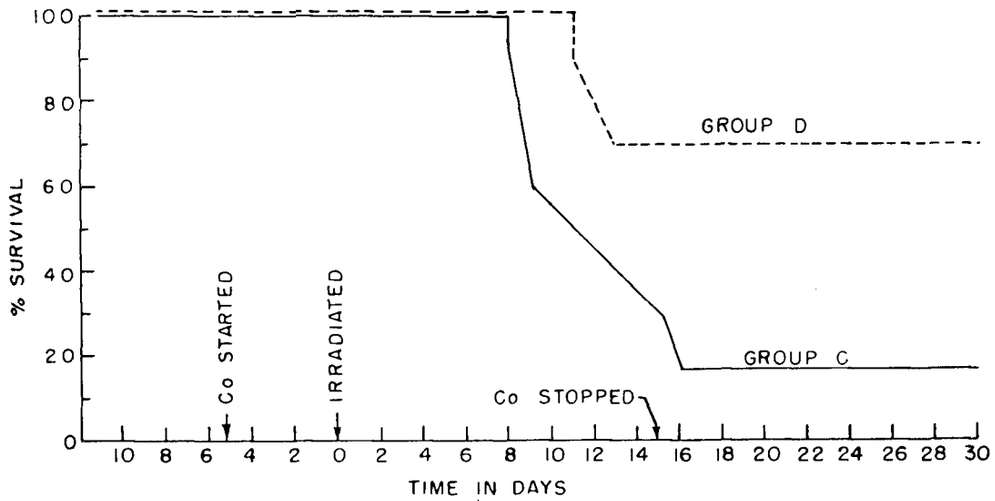


FIG. 1.-SURVIVAL OF MICE AFTER TOTAL BODY IRRADIATION (720r). Co FED TO GROUPS B AND D FOR 5 DAYS PRIOR TO AND FOR 15 DAYS AFTER IRRADIATION. GROUPS A & B (NON-IRRADIATED CONTROLS) HAD NO DEATHS DURING THE 35 DAY STUDY. GROUP C = STOCK FED IRRADIATED ANIMALS. GROUP D = Co FED IRRADIATED ANIMALS.

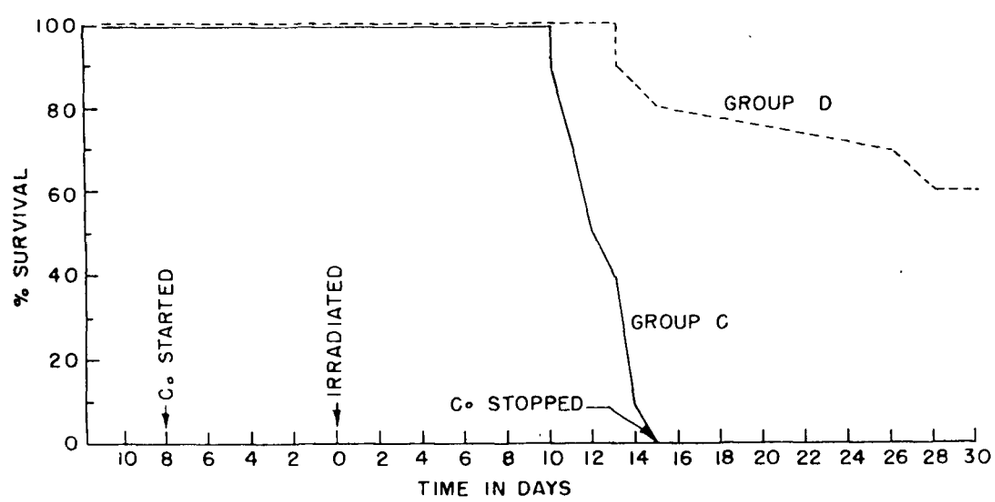


FIG. 2.-SURVIVAL OF MICE AFTER TOTAL BODY IRRADIATION (720r). Co FED TO GROUPS B AND D FOR 8 DAYS PRIOR TO AND FOR 15 DAYS AFTER IRRADIATION. GROUPS A & B (NON IRRADIATED CONTROLS) HAD NO DEATHS DURING THE 38 DAY STUDY. GROUP C = STOCK FED IRRADIATED ANIMALS. GROUP D = Co FED IRRADIATED ANIMALS.



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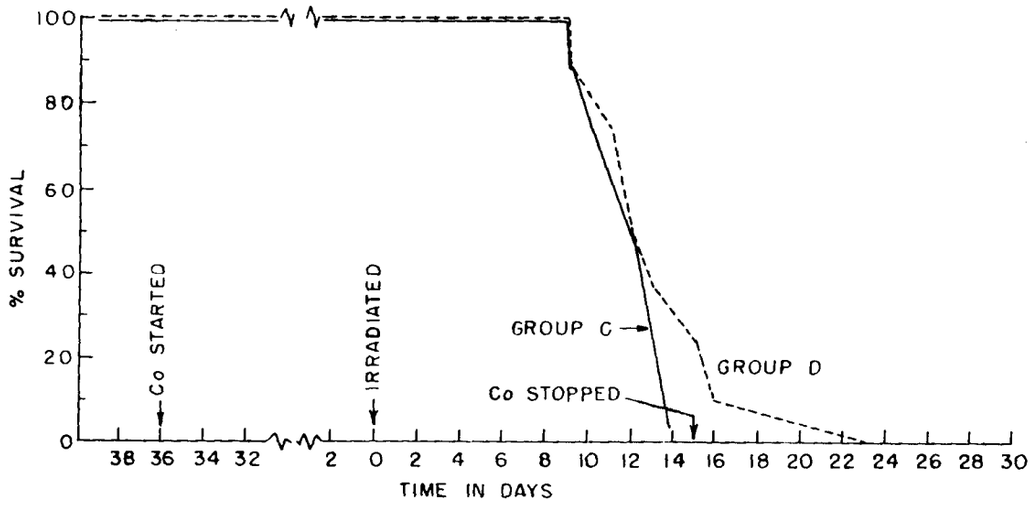


FIG. 3-SURVIVAL OF MICE AFTER TOTAL BODY IRRADIATION (720r). Co FED TO GROUPS B & D FOR 36 DAYS PRIOR TO AND FOR 15 DAYS AFTER IRRADIATION. GROUPS A AND B (NON-IRRADIATED CONTROLS) HAD NO DEATHS DURING THE 66 DAY STUDY. GROUP C = STOCK FED IRRADIATED ANIMALS. GROUP D = Co FED IRRADIATED ANIMALS.

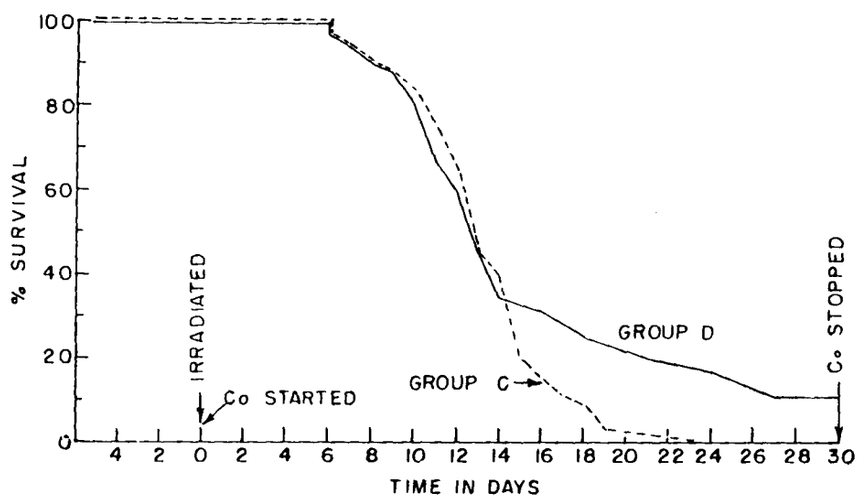


FIG. 4-SURVIVAL OF MICE AFTER TOTAL BODY IRRADIATION (720r). Co FED TO GROUPS B AND D FOR 30 DAYS AFTER IRRADIATION. GROUPS A AND B (NON IRRADIATED CONTROLS) HAD NO DEATHS DURING THE 30 DAY STUDY. GROUP C = STOCK FED IRRADIATED ANIMALS. GROUP D = Co FED IRRADIATED ANIMALS.



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B. Feeding Conditions

Animals having easy access to the treated food (pellets offered on the bottom of the cage) showed a significant increase in resistance against total body x-irradiation damage (Fig. 1). Animals forced to consume cobalt treated food through the regular food baskets died earlier than their respective controls (Fig. 5).

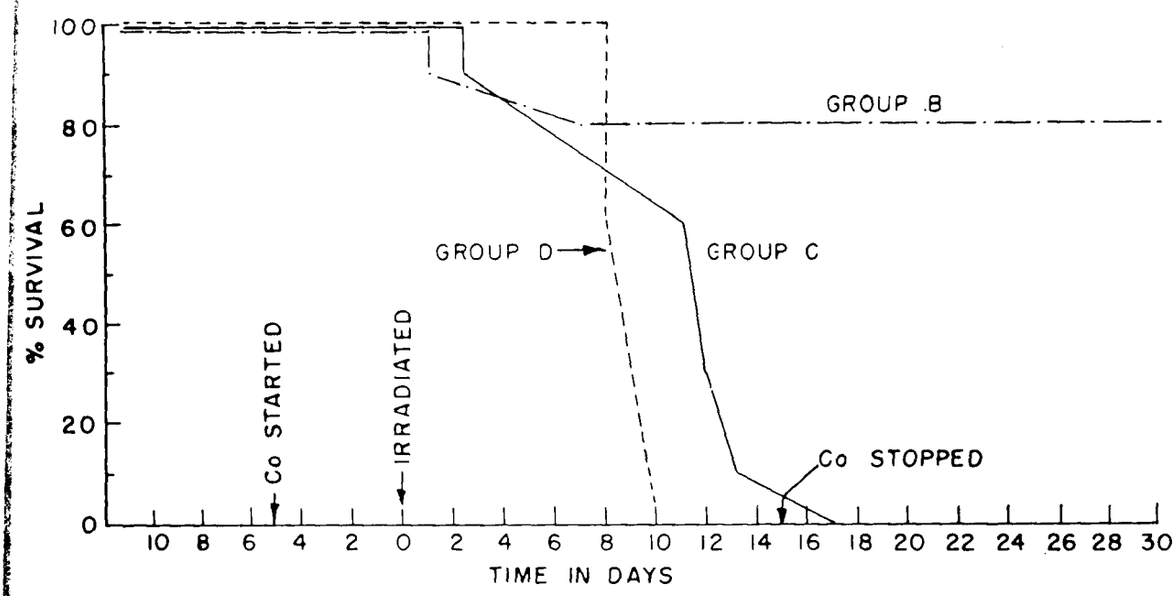


FIG. 5. EFFECT OF FEEDING METHOD ON SURVIVAL. CONDITIONS WERE THE SAME AS FOR FIG. I EXCEPT THAT THE ANIMALS OF ALL GROUPS WERE FED THROUGH REGULAR FOOD BASKETS. (GROUP A HAD NO DEATHS).



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## IV. DISCUSSION

### A. Administering Period

The reported data indicate that the beneficial effect of cobalt on radiation sensitivity does not occur when cobalt is given over long time periods. The highest protection is afforded with short time feeding experiments (5 to 8 days before and 15 days after irradiation) in accordance with Seyss (13) and Weissbecker (14) who maintain that a cobalt induced "pseudo globulie" builds up in the first 2-4 days after administration. This could mean that the greatest protection is not reached at the height of the true cobalt polycythemia (after application of cobalt over several weeks) but at a time when the blood forming apparatus is being stimulated. In support of this hypothesis are the findings by Bethard, Skirmond, and Jacobson (15). They found no significant difference in the sensitivity of blood forming tissues to $P^{32}$ between normal rats and rats with an established cobalt induced polycythemia. However, when the application of $P^{32}$ and the administration of cobalt were begun simultaneously, the rats showed some protection against the destructive effects of $P^{32}$. This protection, according to Bethard, Skirmond, and Jacobson, may result from the initial hyperactivity of the bone marrow following cobalt administration.

### B. Feeding Conditions

The feeding conditions are decisive for influencing radiosensitivity. Figures 1 and 5 show clearly that animals with easy access to food showed an increase in radio-resistance while animals fed through food baskets could not develop a resistance against the ionizing radiation. An explanation may be found for the food basket studies in that 20% of the cobalt fed animals (Group B) died indicating a probable toxicity from the ingested cobalt. In this feeding method the animals are forced to eat whatever part and surface of the pellet is presented to them through the openings in the food basket; whereas in the other method, the food is available ad lib giving the animals a choice of the pellet surfaces or the less cobalt enriched cores of partly eaten pellets.

The amount of cobalt necessary for the beneficial effect is probably relatively small. One of the latest publications on the clinical usage of cobalt by Gardner (16) reports that a dose of 50 to 150 mg given daily in enteric coated tablets (and not in liquid form) with meals is without noticeable ill effects. From this, assuming the average weight of the patients to be 60 kg, it can be tentatively estimated that



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a mouse may tolerate about 0.05 mg cobalt per day without noticeable ill effects. This is far under the previously estimated 3 to 4 mg (2) of cobalt probably consumed by the animals in these studies. The exact toxic level for mice remains to be determined.

## V. CONCLUSIONS

The results indicate that the initial events produced by the administration of cobalt rather than the events at later time intervals are decisive for the observed increase in radio-resistance. Which one of the initial events, stimulation of the hemopoietic system, interference with cellular respiration, inhibition of enzymatic activity, contributes, alone or in combination, to the protection cannot be said at present.

## VI. RECOMMENDATIONS

The following factors should be studied:

1. Administering time and quantity of cobalt necessary for maximum x-irradiation protection.

2. Augmentation of the action of cobalt by other protective agents.

3. Action of cobalt on sulfhydryl containing enzymes and substrates.

4. Influence of cobalt on cellular respiration.

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