tween normal sera and sera from patients with cancer were, of course, attributable to the fact that sera from the cancer cases were assayed shortly after venipuncture and compared with control serum that had been stored in the refrigerator.

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Manuscript received July 23, 1952.

A Study of the X-Irradiation Protection Afforded by Cobalt

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It is generally believed that hemopoiesis is the key to recovery after irradiation injury. Even if this fact does not assure survival of irradiated animals (*1*), it seemed of interest to study the influence of polycythemia-producing substances on irradiation effects. Such a substance is cobalt, which is known to produce polycythemia. The mechanism of this cobalt polycythemia is unknown. It is assumed that cobalt interferes with cellular respiration (*2*), a process of decisive importance again for the magnitude of the irradiation damage. With these facts in mind, investigations on the influence of cobalt in total body irradiation effects were undertaken.

Approximately 400 female Swiss Albino mice (25 ± 1 g) were used. They were divided into four groups for each experiment: group 1 on Purina stock chow diet, group 2 on cobalt diet, group 3 on Purina stock chow diet plus irradiation, group 4 on cobalt diet plus irradiation. The cobalt diet was prepared by immersing the Purina chow pellets in a 2% cobalt chloride solution ($CoCl_2 + 6\ H_2O$) for a 2-min period and then allowing them to dry. The mice were kept on this diet 5 and 8 days before irradiation and for about 15 days after irradiation. Food and water were given *ad lib.*

The mice were irradiated in groups of 10, each group consisting of 5 on Purina chow and 5 on the cobalt diet. The irradiations were done with a Kelley-Koett deep therapy x-ray unit, operated at 200 kv, 6 ma, inherent filtration equiv 0.25 mm Cu, 1 mm Al plus 0.5 mm Cu added, 30 cm target distance, 48 r/min in air; a total of 720 r/air. The mice were observed for 30 days after irradiation, and the mortality rate was recorded every 24 hr.

The results obtained with animals receiving cobalt food for 5 and 8 days prior to irradiation are presented in Figs. 1 and 2. The animals kept on cobalt diet before irradiation show in both cases a significant increase in their resistance against irradiation in comparison to the irradiated groups on Purina chow only.

The general appearance of the cobalt-fed irradiated

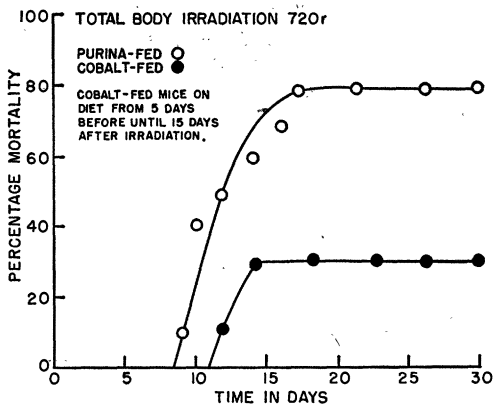

FIG. 1. Mortality rates.

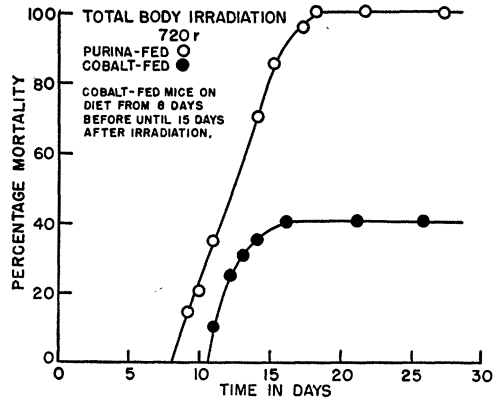

FIG. 2. Mortality rates.

animals was quite similar to that of the nonirradiated animals. They exhibited smooth fur and normal vitality, whereas the irradiated animals on normal food and the few animals that died in the cobalt-fed irradiated group showed all the signs of heavy irradiation damage. To obtain the beneficial effect the weight of the animals, the preparation of the cobalt food, and ease of accessibility to food and water are of decisive importance.

The protective effect exhibited by cobalt lends itself to discussion and comparison with present irradiation protection investigations.

An oxygen deficiency increases the resistance against irradiation damage (*3, 4*). Compounds such as cysteine and glutathione counteract irradiation effects on sulfhydryl enzymes (*1, 5*). The proper support of the hematopoietic system stimulates and speeds recovery (*6, 7*). In cobalt administration experiments, similar processes are going on. According to Barron and Orten (*8, 9*), cobalt interferes with cellular respiration and produces anoxia. According to Burk *et al.* (*10*), cobalt may block sulfhydryl groups and perhaps other groups necessary for tissue metabolism. According to Orten (*9*), cobalt produces a strong polycythemia, indicating an active stimulus to the hematopoietic system.

Which of these mechanisms is decisive in the ob-

served cobalt protection effect remains to be investigated. It may be that not one mechanism alone but all three together, working in a functional correlation, produce the effect. The parallelism is so close, and the possibilities of obtaining more information on certain phases of the protection mechanics are so obvious, that further investigations are indicated.

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Manuscript received July 25, 1952.

Collection and Storage of Chicken Plasma for Tissue Culture

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Since Carrel and Burrows introduced the use of chicken plasma in tissue cultures, many attempts have been made to replace it by something more readily available, such as agar (*1*), cellophane (*2*), or commercial thrombin and fibrinogen (*3*). These agents have proved fairly successful in the hands of their sponsors, but generally speaking the chicken plasma clot is still the most satisfactory substrate for tissue culture.

It has been said that experienced operators seem to draw blood without difficulty by intuition rather than by method. We hope that the method here described will be of help to the inexperienced and simplify the procedure for the experienced. Steps eliminated in the collection of plasma are based on our observations and are supported by information obtained from a survey of nine tissue culture laboratories.

Bleeding methods. Carrel and Burrows (*4*) in 1911 recommended collecting blood from the carotid artery by "the method similar to one used by Delezenne and by Gengou" (no reference given). The bird is etherized, and the carotid artery is exposed. The vessel is rubbed with dry gauze and covered with olive oil. A glass cannula sterilized in olive oil is used to run the blood into chilled paraffin-coated tubes, and after centrifugation plasma is removed with paraffin-coated pipettes. This is a delicate and time-consuming operation and is used in only three of the nine laboratories that we surveyed.

In 1928 M. R. Lewis (*5*) introduced the cardiac puncture method, using a lateral approach. This simplified the procedure but introduced the need for an anticoagulant. No toxic effects are observed when heparin is used, and six of the nine laboratories surveyed use this method.

The survey confirmed our observations on the following points:

1) Fasting is not essential to collection of clear plasma and does not affect clotting quality of blood. The fat content of chicken feed is not high enough to result in chylous plasma. Overnight fasting, however, is advantageous in the interclavicular approach to the heart. A full crop might get in the way. Fasting also eliminates unassimilated digestion products from the circulation. (One laboratory bleeds without fasting.)

FIG. 1. Bird in position for bleeding.

2) Paraffin coating of tubes and pipettes is unnecessary; the important thing is to keep them well chilled. Three laboratories use plain tubes; one collects in plain tubes but stores in paraffin-coated tubes; five use paraffin-coated tubes.

Modified MacArthur (6) technique of collecting blood. Briefly, this is a cardiac puncture method of reaching the heart through the interclavicular space. The Pavlech modification uses the same approach, but the blood is collected in tubes instead of in a syringe.

Birds selected for bleeding should be 10–12 months old, as management of the bird is easier and the