heavy growth of 3 strains of group A streptococci have been developed. The medium consists of either a) 21 amino acids, glutamine, 6 vitamins, salts, purines, pyrimidines and glucose or b) 13 amino acids and 4 vitamins. 2) Cysteine is important both as an essential amino acid and as a reducing agent. As an amino acid only a small amount (10 μg ml) is needed and this can be substituted by an equivalent amount of cystine. As a reducing agent it can be replaced by ascorbic acid and less effectively by thiomalic or thioglycollic acids. Concentration of cysteine was critical for initiation of growth from small inocula. With less than 5 x 10^6 ml cells at least 350 μg ml cysteine HCl are needed for obtaining visible growth. 3) Pyridoxal was necessary in a medium of 13 amino acids and 3 vitamins (nicotinic acid, pantothenate, riboflavin) whereas in a complete medium (22 amino acids) no need for pyridoxal was found. Pyridoxal could be replaced by L- or DL-alanine.

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Survival of Labeled Platelets in X-Irradiated Rabbits.* (23407)

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Although several excellent studies of radiation injury to animals have shown that the cause of the thrombocytopenia appears to be depression of platelet formation in the marrow(1,2,3), none of these studies showed the effect of radiation on a specific population of platelets. The development of a satisfactory method of labeling platelets made this type of study possible(4). This paper reports observations on the survival of Cr^{51} labeled platelets reinjected into rabbits irradiated with X-ray.

Methods and materials. 13 rabbits were exposed to 750 r of whole body X-ray, 24 hours after injection of autologous platelets labeled with sodium chromate^{51}. Twelve rabbits were subjected to the same procedure but with "sham" irradiation.

A Kellikoet X-ray machine was used set at 20 ma, 200 KV with HVL 1.1 mm of Cu using a 0.5 mm Cu filter. The unit was calibrated by the hospital physicist with a Victoreen 250 r chamber, and had an output of 12 r/min with back scatter. The tsd was 1 meter to the dorsal skin of the rabbit, and the animal was entirely within the 90% isodose line.

The platelets from 40 ml of rabbits heart blood were separated and labeled with 450-500 μc of sodium chromate^{51} (sp. activity 20-25 μc/μg) by the technic previously described(4). The labeled platelets

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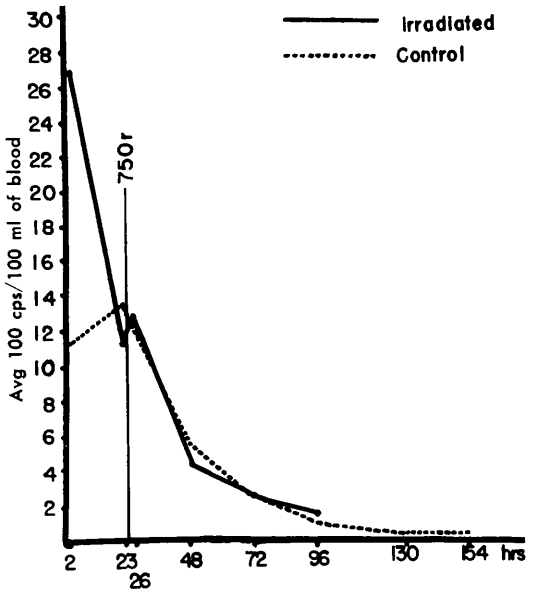

FIG. 1. Effect of 750 r X-ray on survival of Cr^{51} labeled platelets in rabbits. The higher initial value in the irradiated group is due to variations in the effectiveness of the initial tagging procedure (see ref. 4).

were washed once with 10 ml of the original plasma, reseparated, and suspended in the remaining plasma for reinjection into the rabbits' ear vein.

Ten ml blood samples were taken from the heart at 2, 23, 26 and 48 hrs after injection and daily thereafter as long as significant radioactivity persisted in the platelets.

Red blood cells were separated by slow centrifugation, and the platelets then separated by centrifugation at 2500 rpm for 30 min.

Since the amount of plasma radioactivity was negligible, the platelet button was measured directly after the removal of the platelet-poor plasma. All determinations were done in a well type scintillation counter and calculated as counts/second in 100 ml of blood.

Results. Survival of labeled platelets in rabbits exposed to 750 roentgens following reinjection of the labeled platelets was normal. The platelet survival curve of the irradiated animals showed a transient increase in amount of platelet radioactivity 2 hours after exposure (Fig. 1). The 2 curves are otherwise identical except for a higher initial level in the irradiated group due to variations in efficiency of labeling(4). The radioactivity present at 96 hours was sufficient to indicate a survival period of 6 days or more, as was actually demonstrated in a few of the control animals.†

Discussion. Studies on irradiation injury by others have shown the syndrome to have several phases. With very large doses of X-ray death occurs in a few hours. With somewhat smaller doses gastrointestinal hemorrhages and necrosis develop, 3-5 days after exposure. Hematologic effects become marked in the second week with thrombocytopenia developing after 5 days and reaching minimal levels between 8 and 10 days following exposure. Megakaryocytes disappear from the marrow about the 3rd-5th day. During the period of marrow depression the animal may die from hemorrhage or infection.

Lawrence rendered cats thrombocytopenic by irradiation and then restored their platelet counts by cross circulation with normal animals(7). The platelet count returned to the pre-transfusion levels in 2 to 4 days. Although no one has reported survivals of platelets in humans suffering from acute radiation thrombocytopenia, 4-6 day survivals of transfused unlabeled platelets have been reported in thrombocytopenia resulting from marrow failure due to idiopathic or toxic causes(8). In studying platelet life span in animals irradiated sufficiently to make them thrombocytopenic, the incidence of early death from intestinal necrosis, and the presence of hemorrhagic diathesis are hindering factors. If sufficient platelets are given to maintain hemostasis(9) the animals are no longer thrombocytopenic.

† In our paper describing the method of labeling platelets with Cr^{51} we reported the measured survival of the tagged platelets in rabbits as 3-4 days (4). This short period was attributed to the volume of blood removed from the animals by frequent sampling. With improvements in technic, the use of sodium chromate^{51} of higher specific activity, and lowering the background radioactivity, the average survival of *in vitro* labeled platelets in rabbits is 6 days, a figure in agreement with the platelet life span reported by others using other methods of measurement(5,6).

In the pilot experiments for the present study, of 7 fasted rabbits given 600 r of X-ray and injected with labeled platelets after thrombocytopenia had developed, 2 showed a normal survival of labeled platelets, 3 animals died before platelet radioactivity had disappeared from the circulation, and 2 showed an early disappearance of platelet radioactivity coincidental with hemorrhage. When 3 larger rabbits, not fasted, were used, 2 doses of 750 r 2 weeks apart were required to produce persistent thrombocytopenia below 100,000/mm^3. None of these developed the intestinal necrosis syndrome, or generalized bleeding and injected labeled platelets survived normally for 5, 6, and 7 days. These studies, while incomplete, do not suggest any circulating anti-platelet factors resulting from radiation.

There is some evidence that irradiation in large doses has a damaging effect on circulating erythrocytes(2,10), in addition to the effect on erythropoiesis. The normal platelet survival reported here does not support such a contention with respect to platelets, at least with the doses of X-ray employed. No satisfactory explanation is available for the observed post-irradiation rise in circulating platelet radioactivity. It was present in 9 of 13 irradiated animals, and only 3 of 12 controls, but was not statistically significant. It was not accompanied by any change in the peripheral platelet count.

Conclusions. The life span of circulating platelets labeled *in vitro* with sodium chromate^{51} was normal in rabbits exposed to 750 r of X-ray subsequent to injection of the labeled platelets.

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A Modified Live Newcastle Disease Virus Vaccine.* (23408)

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Use of viable, virulent virus vaccines can increase incidence of respiratory disease in poultry, perpetuate disease in birds or produce a serious respiratory disease complex when superimposed in an already diseased flock. Failure to induce adequate immunity with inactivated Newcastle disease virus vaccine makes desirable an attenuated nonspreading virus strain; for vaccine production the strain should be propagable in nonchicken tissue to avoid contamination of poultry with egg-borne infections. This report describes modification of Newcastle disease (ND) virus and preliminary results of immunization of chickens.

Materials and methods. Modification of the virus. The California strain 11914 of Newcastle disease virus was modified by serial passage through tissue cultures composed of minced chicken embryo tissues suspended in Simm-Sanders medium containing bovine ultrafiltrate. Virus of passage 47 to 55 when

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