the injection of EB + P. Similarly, insulin had little effect on gland growth in pregnant rats(1) but in ovariectomized rats, a significant 24% increase in DNA was observed in synergism with EB + P. In these studies L-T_4 had little effect in either type of animals. While GH stimulated a significant 13% increase, insulin had little effect in pregnant animals when administered separately. The combination of the two hormones had a significant mammary growth stimulating effect of 62% in pregnant(1) and 37% in ovariectomized rats. Similarly, while GH and L-T_4 had little value separately in pregnant animals, the combination stimulated an increase of 29% (3) when the latter were stimulated also with EB + P.

The combination of insulin and L-T_4 stimulated slight growth in both types of animals. Finally, the combination of GH, L-T_4 and insulin stimulated a significant 41% increase in DNA in pregnant animals(1) and a significant increase of 51% in ovariectomized rats in synergism with EB + P.

With the exception of GH alone and with L-T_4, the mean DNA of the pregnant rats considerably exceeded the mean DNA of the ovariectomized rats. This observation suggests that the pregnant state stimulates the increased secretion of endogenous hormones which are not stimulated by EB + P.

Summary. The synergistic effect of various hormones upon mammary gland growth has been studied in adult ovariectomized rats stimulated with estradiol benzoate (EB) and progesterone (P) for 19 days. The mean deoxyribonucleic acid (DNA) of 28 control rats was 5.57 mg/100 g bw. When treated with EB and P plus 3 units of insulin, a mean DNA of 6.89 mg/100 g bw, a significant increase of 24% ($P<0.05$) was observed. EB and P plus growth hormone (GH) plus protamine zinc insulin increased the mean DNA to 7.62 mg/100 g bw, a highly significant increase of 37% ($P<0.001$). When EB + P + GH + insulin + L-thyroxine (L-T_4) were added, a mean DNA of 8.40 mg/100 g bw was observed, a highly significant increase of 51% ($P<0.001$). Non-significant increases in DNA were observed with L-T_4 and with L-T_4 plus protamine zinc insulin.

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Erythropoietic Recovery of Chronically Radiated Rats. II. Response to Phenylhydrazine. (32145)

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Sustained depression of erythropoiesis and development of progressive anemia during continuous exposure to irradiation has been well documented by Lamerton(1). Resumption of erythropoiesis during continued irradiation was, however, observed by Brambel *et al* who exposed rats to 70 R per day(2). After 5 to 6 weeks of exposure, there was a rapid increase of normoblasts in the spleen, followed shortly thereafter by a sustained reticulocytosis and stabilization of the hemoglobin level in the peripheral blood. Brambel *et al* suggested that the observed resumption of erythropoiesis during continued irradiation might represent an adaptation phenomenon. The present paper will adduce evidence for the alternative hypothesis that erythropoiesis develops in the spleen in response to a severe degree of anemia which develops at about 5 weeks of chronic radiation. To test this hypothesis, severe anemia was produced early in the course of chronic radiation by the in-

jection of phenylhydrazine which is known to induce hemolysis of the majority of adult red blood cells.

Experimental. Anemia was induced in 2 groups of mature female Wistar rats, about 150 days old, by subcutaneous injection of phenylhydrazine (PH). In the first experiment, 20 rats were given 0.25 ml of a 1% solution of PH 3 times a week for a total of 10 doses. The first 2 doses were given before, the remainder after the rats were transferred to the continuous radiation environment and exposed to 70 R per day. 19 rats received PH but no radiation. Two animals were taken from both radiated and control groups 3 times a week during the first 3 weeks for examination. Subsequently, 2 animals each were examined on days 25, 29, and 43 of the experiment. The rats were anesthetized and blood taken by cardiac puncture for hematologic studies. The rats were then killed for histologic studies of bone marrow and spleen by section and imprint.

In the second experiment, 60 rats were given 4 doses of 0.25 ml of a 2% solution of PH on days 1, 3, 4 and 6 of the experiment. Forty rats were placed in the radiation environment 2 days after the last injection; 20 controls remained in the radiation-free environment. Four animals were removed from both the radiated and non-radiated group for complete hematologic and other studies on days 3, 5, 14, 44, and 58 of exposure, covering a range of accumulated doses up to 4,060 R.

Irradiation was accomplished with a 5.7 curies cobalt 60 source in the center of a 12′ × 12′ shielded room. Cages in vertical racks were placed circularly around the source and locked in place after dosimetry with a suitably calibrated Victoreen R meter. Variation in exposure between the 36 cages used did not exceed 10%. The source could be lowered into the ground during the 2 hours a day set aside for servicing. Radiation was continuous for the remaining 22 hours of each day.

The data for the non-PH injected controls were taken from a previous experiment. In the control curves illustrated in Fig. 1-6, each point represents the average of 4 animals. A second experiment using similar number of animals gave superimposable curves(3).

Results. Administration of 2 doses of PH produced a moderate anemia (hematocrit 34, hemoglobin 10 g) by day 3 of the experiment when rats were placed in the radiation environment. In the animals receiving both PH and radiation, the anemia progressed rapidly. The hematocrit fell to 14 and the hemoglobin to 3 g by day 18. At that time, phenylhydrazine administration was discontinued because of the severity of the anemia (Fig. 1). In contrast, the control animals that received phenylhydrazine but no radiation maintained their hemoglobin at about 8 g, in spite of continued PH administration. As is apparent from the reticulocyte data (Fig. 2), both irradiated and non-irradiated rats responded with reticulocytosis to the severe phenylhydrazine-induced anemia, but in the irradiated rat this response was insufficient to prevent a progressive anemia. When PH was discontinued, continued reticulocytosis restored the hemoglobin to normal in the non-irradiated controls, and led to a substantial improvement in the animals subjected to continuous radiation with a rise of the hemoglobin level from 3 to 10 g. Fig. 3 illustrates the striking increase in splenic erythropoiesis in both irradiated and non-irradiated animals injected with phenylhydrazine. There was no appreciable response in the bone marrow. No splenic erythropoiesis was noted in irradiated or non-irradiated control animals that did not receive phenylhydrazine until day 40. At that time, splenic erythropoiesis developed in the irradiated controls as previously described(2). Thus, splenic erythropoiesis can be induced quite early during chronic irradiation when a moderate anemia is induced by PH. In contrast, in animals that received radiation but no PH, there is a slowly progressing anemia which reaches a level of 10 g of hemoglobin after 40 days of irradiation. At that time the animal also responds with splenic erythropoiesis.

To buttress the contention that it is the degree of anemia which leads to the induction of splenic erythropoiesis in the chronically irradiated animal, and not some other late effect of PH, injections of PH were given prior to the initiation of chronic irradiation. The 3 injections lowered the hematocrit to 34 and

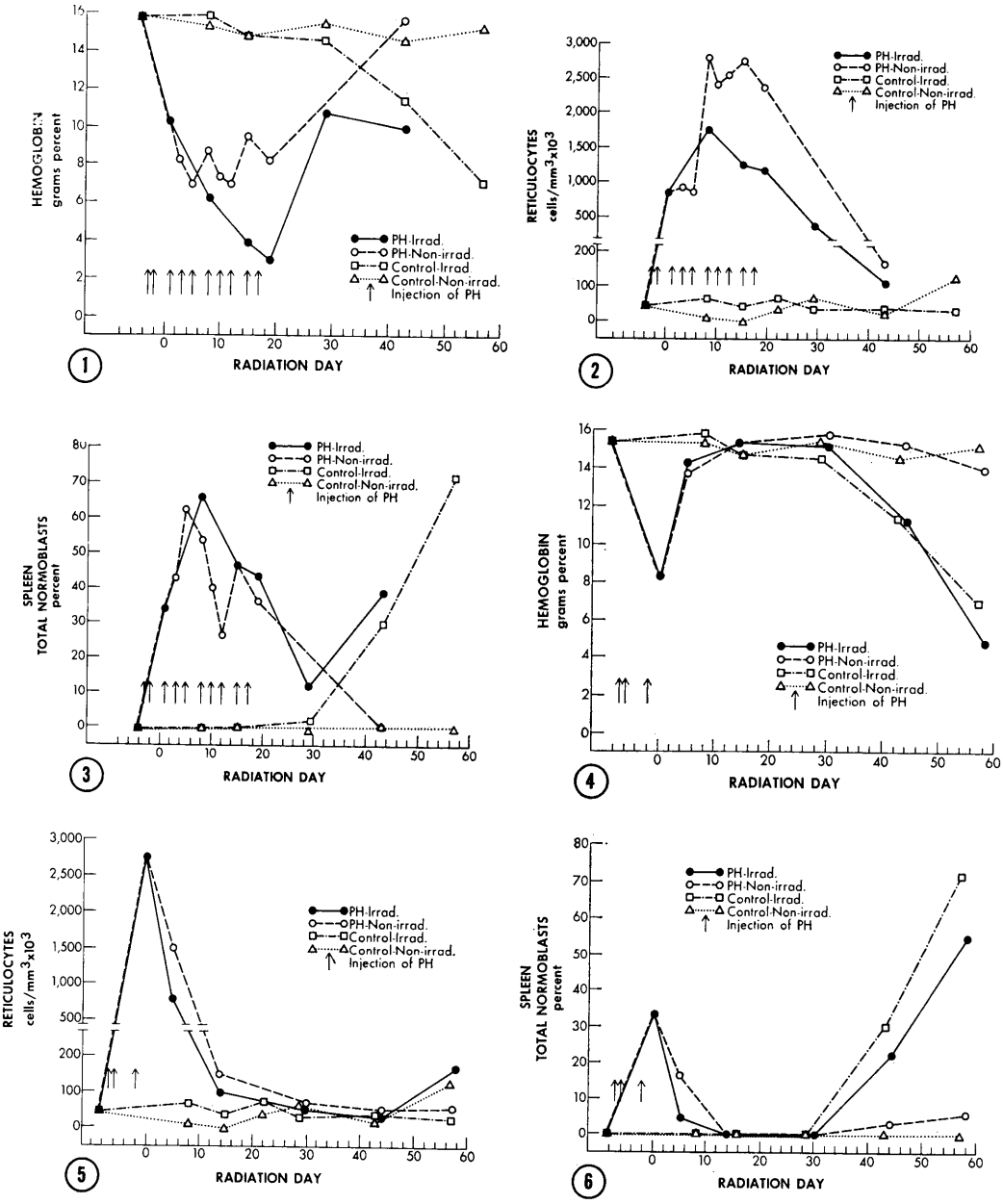

FIG. 1. Hemoglobin concentration during and after repeated phenylhydrazine (PH) administration in irradiated and non-irradiated animals. Arrows indicate days of injection of 1.0% PH solution. Changes in hematocrit paralleled those in hemoglobin. A substantial anemia was already present at beginning of irradiation in the PH treated animals.

FIG. 2. Reticulocyte response in PH injected irradiated and non-irradiated rats. Arrows indicate days of injection of 1.0% PH solution.

FIG. 3. Total normoblasts in the spleen from PH injected irradiated and non-irradiated rats. Arrows indicate days of injection of 1.0% PH solution.

FIG. 4. Hemoglobin concentration for PH treated irradiated and non-irradiated female rats compared to corresponding control animals. Arrows indicate days of injection of 2.0% PH solution. Changes in hematocrit values paralleled those of hemoglobin. PH had produced a substantial anemia in PH treated animals at start of irradiation.

FIG. 5. Reticulocytes in PH treated irradiated and non-irradiated female rats compared to

corresponding control animals. Arrows indicate days of injection of 2.0% PH solution.

FIG. 6. Normoblasts in the spleen for PH treated irradiated and non-irradiated rats compared to corresponding control animals. Arrows indicate days of injection of 1.0% PH solution.

the hemoglobin to 8 g (Fig. 4). The anemia again induced reticulocytosis (Fig. 5) due to splenic erythropoiesis (Fig. 6). The splenic erythropoiesis ceased when the peripheral hemoglobin and hematocrit values returned to normal. The return to normal occurred rapidly in both irradiated and non-irradiated rats. Reticulocytes dropped to control levels by the 14th day of irradiation (Fig. 6) as did splenic erythropoiesis. This was in contrast to the first experiment in which phenylhydrazine administration was continued and in which marked splenic erythropoiesis was present in the spleen up to day 22 of irradiation.

The red cells produced in response to phenylhydrazine were macrocytic in both the radiated and non-radiated animals. The MCV remained at levels of over 70 μ^3 (controls 56 μ^3) in the radiated and phenylhydrazine treated animals even after the reticulocyte level had dropped to 5% or less. This is in keeping with the previously reported macrocytic response to intense erythropoietic stimulation(4).

Discussion. It has been generally assumed that radiation suppression of erythropoiesis is only reversible after discontinuation of irradiation. The initial observation of Brambel *et al* that splenic erythropoiesis occurs in the rat after 5 to 6 weeks of continued radiation exposure opened the possibility of adaptation or repair during continuous radiation. The present observation suggests that chronic radiation though it suppresses erythropoiesis does not do so unconditionally, but permits quite significant levels of effective erythropoiesis in the spleen, when a sufficiently severe anemia provides a strong stimulus for erythropoiesis. This interpretation is supported by experiments now under way in which either bleeding or anoxia induced significant splenic erythropoiesis and reticulocytosis in rats continuously exposed to 70 R per day. The recovery of splenic erythropoiesis in the chronically irradiated animal after 5 or 6 weeks of exposure may now be interpreted as being due to the moderately severe anemia of approximately 10 g of Hb reached at about that time.

We believe these data suggest that one of the effects of irradiation is to alter the responsiveness of the erythroblasts in such a way that they no longer respond to the minor stimuli which ordinarily restore normal blood cell levels rapidly even after slight blood loss. The erythroid compartment can, however, as the present experiment shows, still respond to moderate or severe degrees of anemia with erythroid proliferation and reticulocytosis even while irradiation is continued. A reduced but not abolished responsiveness to erythropoietic stimuli is also in keeping with earlier observations in which accelerated recovery from whole body irradiation followed induction of anemia by bleeding or phenylhydrazine either before(5) or after(6) radiation exposure. No explanation is at hand why this response should be readily evident in the spleen but not in the marrow of the rat.

Summary. Administration of phenylhydrazine during chronic irradiation of rats with 70 R per day stimulates red cell production markedly and results in final hemoglobin levels comparable to those of untreated radiated controls, even though the PH administration itself produces a significant initial anemia. There is a marked increase in splenic normoblasts in the PH treated animals in spite of continuous irradiation. The data are interpreted as evidence for the ability of irradiated erythroid cells to respond to strong stimuli, although they are unresponsive to the lesser degrees of stimulation which lead to automatic correction of mild degrees of anemia in normal animals.

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Erythropoietin-Like Effect of a Polycythemic Virus.* (32146)

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In a hypertransfused-polycythemic (HP) state erythropoietin production and morphological evidences of erythropoiesis disappear(1,2). The only substance reported to date that can initiate erythropoiesis in a HP state is erythropoietin, whether administered exogenously or produced through the effects of other substances(1-4). Consequently, the HP state has been used as a reliable assay system for detection of erythropoietin activity in plasma, urine, and other body fluids. In the course of working with a murine virus causing polycythemia(5), we noted the ability of this virus to initiate erythropoiesis in HP mice. The purpose of this communication is to report our findings on the ability of this erythropoietic virus to initiate erythropoiesis in a HP state.

Material and methods. Animals. Ha/ICR Swiss mice, 5-6 weeks old, were used throughout the investigation. They were fed pellets of Derwood-Morris Diet (TEKLAD) and water was available to them at all times.

Hypertransfused-polycythemic (HP) state. The HP state was induced in the mice by giving intraperitoneal injections of 0.5 ml of washed, packed, homologous red blood cells on 3 consecutive days and repeated on day 5(1). This resulted in hematocrits in the vicinity of 70-75%. The virus was given to each group when the HP state was achieved. The HP state in infected mice was prolonged by giving red cell transfusions twice a week to maintain the hematocrit above 70%.

Virus. The polycythemic virus, which could be a variant of Friend virus(6) or a passenger virus(5), present in spleen filtrate prepared originally from Friend virus infected Taconic Swiss, has been passed in our laboratory since 1961 and it is in its 96th passage generation. The erythropoietic pattern induced by Friend virus as originally reported by Friend(6) and that induced by the polycythemic virus is considerably different. Friend virus does not produce polycythemia, increase in granulocytes or an increase in platelets.

The mice in the experiments described herein were inoculated intraperitoneally with 0.2 ml of filtrate made from spleens of animals infected with the polycythemic virus. The titer of the 10% splenic filtrate was $10^{4.5}$ ID_{50}/ml.

Erythropoietic parameters. Fe_{59} uptakes(7) and reticulocyte counts were used primarily to determine erythropoiesis. All mice were injected intravenously with 0.5 μc of Fe_{59} at various intervals following virus infection and Fe_{59} uptake in the femur, blood, and spleen was determined 24 hours later.

Erythropoietin assay. HP mice were used for assaying the presence of erythropoietin in plasma and urine of mice infected with virus(8). Plasma and urine were inactivated for virus activity by subjecting the plasma and urine to 56°C for 30 minutes.

Results. Characteristics of hypertransfused-polycythemic (HP) state (Table I): Attention should be directed to the characteristics of erythropoiesis one encounters in the HP mouse in order to understand the effect the polycythemic virus has in this state.

In the HP mouse all evidence of active erythropoiesis in the bone marrow, blood,

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