been found that cortisone causes an increase in blood amino nitrogen levels(4) and an increase in the zymogen granulation of the pancreas(1,2,15).

From these data and those obtained previously(1,2) it may be stated: 1) Glucagon administration *in vivo* causes a decrease in protein synthesis of the pancreas, and in formation of zymogen granules, but does not alter the rate of exocrine secretion; this produces a histological and chemical depletion of the secretory digestive enzymes in the acinar cells. 2) This action of glucagon is secondary to a lowering of essential amino acid levels in the serum.

Summary. *In vivo* studies measuring DL-leucine-1-C^{14} incorporation into pancreatic protein helped confirm earlier histological and chemical observations that glucagon administration to rats and rabbits inhibited synthesis of pancreatic protein. *In vitro* studies revealed that glucagon had no direct effect on DL-leucine-1-C^{14} incorporation into protein of segments of rat pancreas. Incubation of pancreas in medium with lowered essential amino acid levels or in serum from glucagon-treated rats with lowered amino acid levels resulted in decreased incorporation of radioactive leucine into pancreatic protein. Neither lowering non-essential amino acids nor adding glucagon to incubation medium had any effect. It was suggested that glucagon administration *in vivo* causes a decrease in protein synthesis of the pancreas and that this action of glucagon is secondary to the lowering of essential amino acid levels in the serum.

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Graft-Host Response in Murine Radiation Chimeras.* (28730)

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A problem in bone marrow therapy of lethally irradiated mice is the "secondary disease" mortalities arising from a graft-host immune reaction(1,2). In an attempt to influence these delayed deaths, we administered hemopoietic tissues of strain (A) mice to newborn strain (B) animals. The bone marrow from (B) animals was later injected into lethally X-irradiated (A) animals. Seventeen out of 28 animals receiving bone marrow from donors neonatally treated with host tissue survived 90 days, whereas 9 out of 29 receiving bone marrow from normal

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TABLE I. Survival of Bone Marrow Treated Irradiated Mice.

(A) Host	No. of animals	(B) Donor	Pre-treatment of donor	90-day survival
C31	8	—	—	0/8
C31	15	LAF	None	7/15
C31	14	LAF	Neonatal C31 spleen implants + 1 or 2 later intraperitoneal injections of C31 lymphoid tissue	10/14
LAF	8	—	—	0/8
LAF	8	C31	None	1/8
LAF	8	C31	Neonatal LAF spleen implants + 1 or 2 later intraperitoneal injections of LAF lymphoid tissue	4/8
LAF	4	—	—	0/4
LAF	6	Sprague-Dawley rat	None	1/6
LAF	6	*Idem*	Neonatal LAF spleen implants + 1 or 2 later intraperitoneal injections of LAF lymphoid tissue	3/6

Host animals received between 2-10 $\times$ 10^6 viable bone marrow cells, intravenously, after 950 r whole-body x-irradiation.

(no pretreatment) donors survived (Table I). Skin transplantation studies revealed that all (B) animals rejected (A) skin grafts in the different strain combinations noted in Table I. We have assumed the skin graft rejection to indicate a general tissue histoincompatibility, and that the enhanced survival could, therefore, not have been due to tolerance of (B) for (A) tissue. To aid in interpretation of these results, further experiments were performed to assess the state of chimerism, body weight, hematocrit, and possible serum antibody titers arising from such pretreatment of donor animals. For these studies, a xenogeneic chimera (rat⟶mouse) was chosen, primarily for the ease with which hemagglutinating antisera could be produced with this combination. The results provide evidence in support of humoral antibody production in these chimeras against both host and graft tissues.

Materials and methods. Hybrid male mice of the LAF (C57L $\times$ A/He) and C31 (C3H $\times$ 101), obtained from Cumberland View Farms and Sprague-Dawley rats, were used. From one-third to one-half of an adult donor spleen was implanted intraperitoneally into newborn animals; this treatment was followed by one or 2 later intraperitoneal injections of lymphoid tissue homogenates, (lymph node, spleen and thymus) from donor animals. Donor skin grafts transplanted to these animals were rejected. Four to 8 weeks after the last injection, these treated animals were used as bone marrow donors (B) to lethally X-irradiated hosts (A).

In the xenogeneic chimera experiment, 7 groups, each of 6 male mice of the LAF strain, were used as irradiated hosts. The donor animals were either nontreated male Sprague-Dawley rats (Group 2 donors) or Sprague-Dawley rats which had received neonatal LAF spleen implantations followed by 2 later intraperitoneal injections of LAF lymphoid tissues (Group 1 donors). Host mice were injected intravenously with 1 $\times$ 10^5, 1 $\times$ 10^6, or 1 $\times$ 10^7 viable bone marrow cells from either Group 1 or Group 2 donors within 2 hours following 950 r total body X-ray. Survivors were bled at weekly intervals from the retro-orbital sinus and the sera were frozen until hemagglutination assays could be performed. The hemagglutination assays were performed according to the procedure of Stimpfling(3). The sera were diluted to 1/32 concentration with a 1.5 per cent polyvinyl pyrrolidone solution in phosphate-buffered saline.

To assess the degree of chimerism, antisera were produced by injecting Sprague-Dawley rats and LAF mice with their re-

TABLE II. Degree of Chimerism Following Treatment of Irradiated LAF Mice with Rat Bone Marrow.*

		Weeks after treatment										
		0	1	2	3	4	5	6	7	8	9	10
Group 1	1	1	1	2	2	3	4	5	4	4	3	3
	2	1	1	2	3	4	3	4	1	2	2	1
	3	1	1	2	3	3	4	5	5	5	5	5
	4	1	1	—	3	4	5	5	5	5	5	5
	5	1	1	2	4	4	4	5	5	dead		
	6	1	1	dead								
Group 2	1	1	1	2	3	4	3	2	5	5	4	5
	2	1	1	1	3	4	4	4	5	5	5	5
	3	1	1	3	3	4	4	4	dead			
	4	1	1	2	3	4	dead					
	5	1	1	2	dead							
	6	1	1	dead								

* Scoring of rbc chimerism: 1 = 100% LAF; 0% rat. 2 = >70% LAF; <30% rat. 3 = 70–30% LAF; 30–70% rat. 4 = <30% LAF; >70% rat. 5 = 0% LAF; 100% rat.

ciprocal lymphoid tissue. Injections were made weekly into footpads and intraperitoneally. Hemagglutinin titers in excess of 1/1000 were attained by the fourth week. Washed red blood cells of mouse:rat were mixed in proportions of 4:0, 3:1, 2:2, 1:3, and 0:4, and used as target cells for hemagglutination. Rat anti-LAF and LAF anti-rat sera in minimal concentrations necessary to attain a 4+ reading with their target erythrocytes were used. Stimpfling's hemagglutination assay(4) was modified by adding 0.4 ml of saline and mixing the cells before brief centrifuging. This resulted in a reproducible scoring of hemagglutination according to the following scheme:

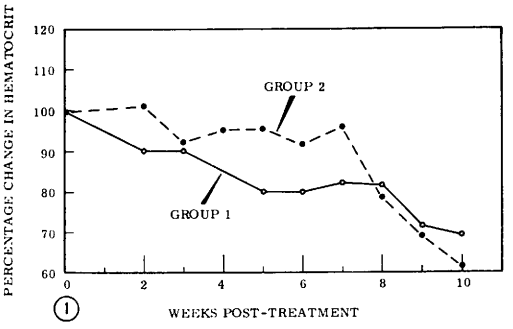
Ratio of rbc LAF/rat	4/0	3/1	2/2	1/3	0/4
Rat anti-LAF serum	4+	3+	2+	+	0
LAF anti-rat serum	0	+	2+	3+	4+

The experimental animals were scored for degree of erythrocyte chimerism, according to the following scheme:

(1)	100 % LAF	0 % Rat
(2)	>70 % LAF	<30 % Rat
(3)	70–30% LAF	30–70% Rat
(4)	<30 % LAF	>70 % Rat
(5)	0 % LAF	100 % Rat

A 100% score was given to chimeras showing 4+ to its antisera and negative to its reciprocal antisera.

Results. Mice receiving 1×10^5 or 1×10^6 bone marrow cells succumbed within 2 weeks post-irradiation, and hemagglutination titer was not detected. Results of studies on animals receiving 1×10^7 viable bone marrow cells are reported in terms of chimerism (Table II), serum hemagglutinin titers (Table III), hematocrit (Fig. 1), and weight of animals (Fig. 2). Table II shows that by the second week post-treatment, donor red blood cells are detectable in the host; by the fifth



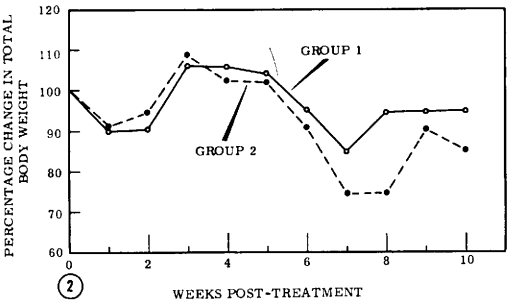


FIG. 1. Percent change in hematocrit values of survivors given 1×10^7 rat bone marrow cells after 950 r total body x-ray.

FIG. 2. Percentage change in total body weight of survivors given 1×10^7 rat bone marrow cells after 950 r total body x-ray.

TABLE III

		Weeks post-treatment																			
		1		2		3		4		5		6		7		8		9		10	
		Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF	Rat	LAF
Group 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+	1+	3+	2+
	2	0	0	0	0	0	0	2+	2+	3+	3+	3+	3+	3+	2+	3+	3+	4+	3+	4+	3+
	3	0	0	0	0	0	0	0	0	2+	0	2+	0	0	0	2+	0	3+	0	3+	2+
	4	0	0	0	0	0	0	0	0	1+	0	1+	1+	0	0	2+	2+	2+	2+	3+	3+
	5	0	0	0	0	0	0	0	0	0	0	2+	2+	2+	2+						
	6	0	0																		
Ratio:																					
No. surviving		6/6		5/6		5/6		5/6		5/6		5/6		5/6		4/6		4/6		4/6	
No. reacting		0/6	0/6	0/5	0/5	0/5	0/5	1/5	1/5	3/5	1/5	4/5	3/5	2/5	2/5	3/4	2/4	4/4	3/4	4/4	4/4
Group 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+	3+	2+
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+	2+	3+	2+
	3	0	0	0	0	2+	0	1+	0	1+	0	0	0								
	4	0	0	0	0	0	0	0	0												
	5	0	0	0	0																
	6	0	0																		
Ratio:																					
No. surviving		6/6		5/6		4/6		4/6		3/6		3/6		2/6		2/6		2/6		2/6	
No. reacting		0/6	0/6	0/5	0/5	1/4	0/4	1/4	0/4	1/3	0/3	0/3	0/3	0/2	0/2	0/2	0/2	1/2	2/2	2/2	2/2

Serum hemagglutinins in heterologous chimeras reacting against donor (Rat) and host (LAF) red blood cells. All serum was diluted to 1/32 with 1.5% PVP in buffered saline and degree of hemagglutination scored according to Stimpfling(3).

week post-treatment, the circulating erythrocytes are predominantly rat type in both Groups 1 and 2. In Group 1, 2 out of 4 surviving animals showed a complete or partial reversion to LAF-type erythrocytes; survivors of Group 2 ultimately show a rat erythrocyte chimera by the tenth week.

Presence of serum hemagglutinins in these xenogeneic chimeras is shown in Table III. The earliest anti-graft reaction was seen at 3 weeks post-treatment in a Group 2 animal that later succumbed. A more typical result was obtained in Group 1 at 4 weeks post-treatment in which both anti-graft and anti-host reactions were detectable. As can be seen in the Table, these titers were sustained throughout the experimental period. Generally, animals in Group 1 showed detectable humoral hemagglutinins 2-4 weeks before animals in Group 2. In determining the titer of the hemagglutinins at 10 weeks(4), it was found that anti-LAF hemagglutinin titers were present in higher concentrations than anti-graft titers.

In Fig. 1 and 2 are plotted, at weekly intervals, the hematocrit values and weights of the animals that survived the experimental period. In Group 1, hematocrit values fell below 90% of controls at about the fourth week post-treatment, while Group 2 maintained its hematocrit values (ca 95%) until the eighth week post-treatment, following which values for both groups showed a further decline. After regaining their weight by the third week, the animals lost weight and remained subnormal throughout the remainder of the experimental period.

Discussion. Serological assays have been useful in following the development of radiation chimerism (see Table II of ref. 1, p. 313). In radiation chimeras, the possibility of both antibody production by the host against graft hemopoietic tissue and by graft against host tissues has been suggested by Barnes and Loutit(5). The present report offers evidence indicating both donor- and host-type serum hemagglutinins. Similar findings in pigeons have been reported(6). Although nonspecific hemagglutination cannot be ruled out with certainty, we have assumed that these titers reflect the presence of both host- and donor-antibody producing cells against their reciprocal tissues. The sera of these chimeras have been shown to agglutinate other mouse red blood cells, as AKR/J, C57BL/6J, A/HeJ, C3H × 101/Cum, BALB/cJ, at the same serum dilution as LAF cells. Indeed, at 1/32 dilution, *in vitro*, hemagglutination was observed against its own red blood cells(4). Arguing against nonspecific hemagglutination is the knowledge that anti-LAF titers are appreciably higher than anti-rat titers, and anti-rat titers are generally detectable in the serum earlier than anti-LAF titers. In other experiments with animals treated similarly to those of Group 2, as early as 2 weeks post-treatment, 3 out of 6 showed hemagglutinins reacting against rat cells, whereas 1 out of 6 animals showed hemagglutinins against LAF cells.

It is noteworthy that a drop in hematocrit and a decrease in weight generally agreed with the emergence of humoral hemagglutinins in these chimeras. Serological assays have hitherto been utilized as markers for demonstration of the incompatibility of donor-host strains. The agreement of the combined data noted above suggests the possibility of considering such serological reactions as causal factors in development of "secondary disease." The direct demonstration of host anti-graft and graft anti-host antibodies in the sera of these radiation chimeras within 2 weeks post-treatment affords increased understanding of the host-graft interactions. The present studies on radiation chimeras are consistent with the idea that graft-host response is bi-directional and we have ascribed host- and donor-type hemagglutinins present in the sera of the chimeras to the presence of donor and host-antibody producing cells.

Summary. After lethal total body X-irradiation, host mice received bone marrow from normal donors as well as from donors pretreated with host tissue; the results indicated a higher percentage of survivors in the latter case. To aid in interpretation of those results, a xenogeneic chimera (rat⟶mouse) was assessed for the state of erythrocyte chimerism body weight, hematocrit values and serum antibody titers. The results are in

agreement with the idea that the surviving chimeras were immunologically reactive, since both host- and graft-type hemagglutinins were found in their sera 3-4 weeks following treatment. Generally, subnormal hematocrit and body weight values coincided with the emergence of antibody titers.

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Tumor Induction in Hypophyseal Grafts in Radiothyroidectomized Mice; Hypothalamico-hypophyseal Relationships.* (28731)

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Secretory tumors of the anterior pituitary gland can be induced in laboratory rodents by a variety of methods(1,2). Thyrotropin-secreting tumors (TtT) develop in mice given thyroid-destroying doses of I^{131}; tumors (MtT) of the mammotropin-secreting cells develop in both rats and mice subjected to chronic estrogen treatment and in grafts of normal pituitary tissue in estrogenized rats; pituitary tumors of a variety of secretory cell types develop in mice after exposure to ionizing radiation; and transplantation of mouse pituitary tissue to extracranial sites has recently been shown to be tumorigenic in intact females and estrogen-treated males. The initial events and factors involved in the neoplastic transformation have not, however, been fully clarified. For example, the importance of the radiation exposure of the anterior pituitary gland during radiothyroidectomy to the development of TtT has been disputed(3,4).

The experiments described here were initiated in an attempt to clarify: a) the role of pituitary irradiation in TtT induction by I^{131}, and b) the importance of an immediate circulatory connection between the pituitary and hypothalamus in tumorigenesis.

Materials and methods. Female LAF_1 and male C57Bl/6 mice were received† at 6 to 8 weeks of age. Those to be radiothyroidectomized were fed a low iodine diet‡ and distilled water from the day of receipt. Radiothyroidectomy was performed 10 to 21 days later by an intraperitoneal injection of 60-65 μc I^{131}. Grafts were made 14 to 33 days after I^{131} treatment. Pituitary glands from mice of the same age and sex as the recipients were transplanted beneath the left kidney capsules with a 14-gauge trochar dampened with isotonic nutrient medium. Thirty to thirty-one days prior to grafting some of the male mice were castrated.

The animals were inspected frequently and those *in extremis* or with external evidence of tumors were sacrificed. Animals found soon after death were also autopsied. Surviving female mice (Exp. 1) were sacrificed 600 days after grafting; remaining males of

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† LAF_1 mice were from Jackson Memorial Laboratories, Bar Harbor, Maine, or from Cumberland View Farms, Clinton, Tenn. C57Bl mice were from Jackson Laboratories.

‡ Nutritional Biochemicals, Cleveland, Ohio.