feces was sharply reduced and that of potassium was markedly increased. These changes resulted in a negative potassium balance which was reflected by an unequivocal drop in serum potassium and by slight muscular weakness. Alterations in sodium balance were not significant although directed toward the negative state. Despite the latter tendency, serum sodium levels in FF dogs showed a significant increase; this, in combination with their apparent dehydration, indicated a depletion of fluid in excess of salt.

Discussion. It is generally accepted that adrenal cortical hormones diminish the renal excretion of sodium and enhance the renal excretion of potassium. FF is reported to be extremely active in this respect(5,6). FF exerted a similar influence on sodium and potassium voidance from the gastrointestinal tract as attested to by the fecal cation changes sustained by test dogs in our experiments. Apparently, the urinary excretion of sodium and potassium in these animals was altered to offset the shift of fecal cations. Since, however, a negative potassium balance and hypokalemia with muscular weakness were produced in FF dogs, it would appear that their renal mechanism was unable to limit adequately the excretion of potassium; this effect may have been due to the profound diuresis also noted in these animals. Conversely, since no significant change was produced in sodium balance and serum sodium became elevated in the presence of dehydration, it would also appear that their renal mechanism limited the excretion of sodium, despite the copious flow of urine. Thus, although an atypical urinary pattern of cations was observed in these experiments, it is considered that the effect of FF on cation transfer in the kidney was analogous to its effect on cation transfer in the gut; with both organs functioning to abase sodium and augment potassium excretion.

Summary. The daily intramuscular administration of large doses (1 mg/kg) of nine-alpha-fluorohydrocortisone (FF) to intact dogs resulted in a chronic state of polydipsia and polyuria with gross evidence of mild dehydration. With FF treatment the fecal excretion of sodium was reduced and that of potassium enhanced but the converse was true for urinary excretion. These changes did not significantly alter sodium balance although serum sodium was elevated, but resulted in a negative potassium balance which was indicated by a drop in serum potassium and muscular weakness.

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Thymus Specificity in Lethally Irradiated Mice Treated with Rat Bone Marrow. (23586)

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The transplantation and repopulation of erythrocytic, granulocytic, and platelet precursors in lethally irradiated mice injected with heterologous (rat) bone marrow has been demonstrated by immunohematologic, cytochemical, and chromosome marker tech-

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nics(1-5). Ford *et al.*(4) identified donor-type chromosomes in dividing cells of the thymus and lymph nodes of the protected mice and concluded that donor lymphatic tissue made up these organs; however, in the dividing cells, the actual cell type could not be identified. Merwin and Congdon(6) reported the presence of homologous donor cells in the bone marrow, spleen, blood, lymph nodes, and thymus of irradiated mice given bone marrow. Their work was based on the antigenicity of these cells in appropriate test animals. Direct evidence by another method for the repopulation of lymphatic tissues in these irradiated mice treated with bone marrow is needed.

The successful application of immunologic methods for determination of specificity of the red blood cells and platelets of lethally irradiated mice injected with rat bone marrow suggested the feasibility of similar *in vitro* methods for determining the specificity of thymus regeneration in mice given rat bone marrow.

Materials and methods. Antisera. For antirat thymus sera, thymuses from Sprague-Dawley rats were cleaned of connective tissues, weighed, "teased" apart, and made into a suspension with Tyrode's solution by flushing through 22- and 26-gauge needles. The cells were washed in 300-400 volumes of saline and adjusted to about 100 mg/ml after the last wash. Adult (101 x C_3H)F_1 mice were given 3 injections over a period of 30 days; the antigen-host relation was about 4 mg/g of body weight per injection. A modified Freund's adjuvant, consisting of antigen, Bayol F, falba, and killed tuberculosis organisms was used for the first subcutaneous injection. The second and third injections, given intraperitoneally at 10-day intervals, were cellular suspensions in Tyrode's solution. Sera were obtained 10 days after the third injection, pooled, inactivated at 56°C for ½ hour, and merthiolate added to give a final concentration of 0.1%. After standardization (see next section), aliquots of this pool were placed in small vials and frozen. Antimouse (101 x C_3H)F_1 thymus sera was obtained from Sprague-Dawley rats in a similar manner, except that the antigen-host relation was 1 mg/g of body weight per injection. Individual antisera for thymuses from 12-, 16-, and 20-week-old (101 x C_3H) F_1 mice were prepared to test the possible existence of antigenic differences for the various age groups. Cross-testing of the 3 antisera and thymuses revealed no difference in reactivity; and the antisera were pooled to form a standard reagent. A third standard antiserum consisted of a mixture of equal volumes of the antirat thymus sera and antimouse thymus sera.

Standardization of antisera. Preliminary testing of both antirat thymus sera and antimouse thymus sera showed a strong cross reaction for the respective red blood cell (RBC) type. Although the RBC contamination of thymus suspensions to be tested was negligible (less than 3%), the removal of the RBC agglutinins would provide a more specific antisera. Two absorptions with equal volumes of packed RBC (4°C, 24 hours) removed completely the hemagglutinins and diminished the reactivity for the thymus cells only slightly. By the agglutination technic to be described, tests of the antisera on suspensions of rat and mouse thymus cells mixed in various proportions showed that each antiserum could detect the specific cell type if it was present in at least 16% concentration. The technic was thus limited by its inability to detect the presence of small amounts (less than 16%) of either cell type.

Agglutination technic. Thymus cell suspensions (approximately 3-4%) were prepared in Tyrode's solution as described and a drop pipetted on a microscope slide. To this was added an equal volume of serum, and the slide was rotated gently several times to ensure proper mixing. A cover slip was placed over the mixture and microscopic examination made for agglutination of cells. Readings were taken during a 15-minute period; and the final degree of agglutination scored as −, ±, 1+, 2+, 3+, and 4+ as judged by the relative number of free cells present. For each control and experimental thymus test, five slides were prepared with the following reagents: (a) normal rat serum, (b) normal mouse serum, (c) antirat thymus serum, (d) antimouse thymus serum, and (e)

TABLE I. *In Vitro* Agglutination Tests on the Thymuses of Lethally Irradiated Mice Treated with Rat Bone Marrow.

No. of thymuses tested	Days after treatment	Serum reagent: Antirat thymus	Antimouse thymus	Antirat, antimouse thymus	Normal rat	Normal mouse
		Degree of agglutination* (mean value)				
(2)†	7	—	3+	4+	—	—
9 (4)	12	±	3+	4+	—	—
3	14	—	4+	4+	—	—
7	15	±	3+	4+	±	±
2 (2)	18	1+	3+	4+	—	—
2	20	1+	2+	4+	—	±
2 (2)	21	2±	2+	3+	±	±
2	22	2+	2+	4+	—	—
2	23	3+	1+	3+	±	±
4	25	3+	—	3+	—	±
4	28	4+	±	4+	±	±
1 (6)	29	4+	—	4+	—	—
2 (6)	30	4+	±	4+	—	—
2 (2)(2)	40	4+	±	3+	±	1+
2	45	4+	±	4+	—	—
1	55	4+	±	4+	—	±
3	56	4+	—	4+	1+	1+
1	83	4+	—	3+	—	—

* Degree of agglutination graded from — to 4+.
† Parenthetical figures indicate No. of thymuses pooled and tested as a single suspension.

antirat-antimouse thymus serum. In general, the thymus of each individual animal was tested separately, although in a few instances, the small size of the organ necessitated the pooling of several thymuses for suitable cellular suspensions (Table I). *Chromosome analysis.* In a separate set of experiments, determination of the chromosome type was made on the reticular tissues of irradiated mice treated with rat bone marrow. *Irradiation and bone marrow treatment.* Twelve-week-old male and female (101 x $C_3H)F_1$ mice were placed in a circular, perforated lucite container attached to a revolving turntable and exposed to 950 r of total-body X radiation. Radiation conditions were: 250 kv; 15 ma; ~ 160 r/min. at 60 cm; inherent filtration, 1.0 mm of Al; added filtration, 1.0 mm of Al; and hvl, 0.5 mm of Cu. Rat bone marrow (RBM) obtained from one femur and one tibia of a Sprague-Dawley rat was made into a 1-ml suspension with Tyrode's solution and injected intravenously within 4 hours after X irradiation. Mice treated with isologous (101 x $C_3H)F_1$ bone marrow (IBM) received intravenously the contents of the shaft of one femur made up in 1 ml of Tyrode's solution. At various intervals, mice were sacrificed, body and thymus weights recorded, the reticular tissues fixed in Zenker formol solution, and hematoxylin-eosin stained sections prepared. The body- and thymus-weight data were expressed in mg of thymus weight per g of body weight (T/B). This procedure was used since it showed the regeneration of the thymus relative to the recovery of the irradiated, bone marrow-treated mice. When the size of the experimental thymus permitted, one lobe was fixed for histologic study and the other made into a suspension and tested with the various reagents described previously.

Results. Immunologic tests. The results of the agglutination tests on the thymuses of 950 r-RBM mice are shown in Table I. On days 12 and 15, tests with antimouse thymus serum showed strong agglutination, but only slight reactivity was obtained with antirat thymus serum. From days 18 through 30, there was a gradual increase in the degree of agglutination when the thymuses were tested with antirat thymus serum and a corresponding decrease in reactivity with antimouse thymus serum. On the basis of these tests, the 50% level of rat and mouse thymus cells present in the test organ occurred on about the 21st day after treatment; by 30 days, all the thymus cells appeared to be of the rat

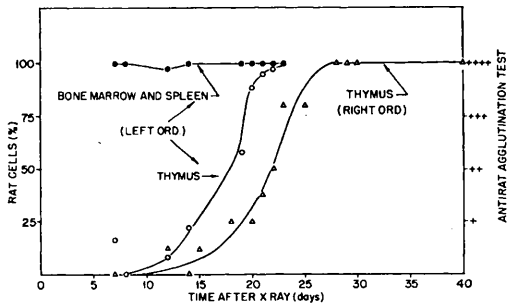

FIG. 1. Temporal relation of cytologic and immunologic identification of rat cells in bone marrow, spleen, and thymus of lethally irradiated mice treated with rat bone marrow (RBM). Cytologic, left ordinate (circles); immunologic, right ordinate (triangle).

type, as indicated by maximum agglutination. Beyond 30 days, occasional positive tests (±) were obtained with antimouse thymus serum, indicating either residual mouse thymus cells or a nonspecific type of agglutination. The positive reactions obtained with normal rat or mouse serum in several of the tests point to the latter. Although the exact cause of these reactions is not clear, their inconsistency would suggest nonspecific factors. The thymuses of 950 r-IBM mice were also tested at varying intervals after treatment. All tests showed strong agglutination with antimouse thymus serum and no reaction with antirat thymus serum (data not tabulated).

Cytological identification of tissue repopulation. Mice treated identically (950 r-RBM) to the immunologic experiment were used in a separate experiment for cytologic analysis of the proportion of mouse and rat cells in the bone marrow, spleen, lymph nodes, and thymus at increasing intervals after irradiation. By the technic of Ford and Hamerton(7), we found, as they have stated, that cells in division could be classified easily and unambiguously as either mouse or rat, on the basis of chromosomal morphology. The time course of appearance of dividing rat cells in the bone marrow, spleen, and thymus is shown in Fig. 1. Each data point for bone marrow and spleen is based on observation of 100 or more dividing cells; thymus data are from 100 cells from day 19 onward and 30 cells prior to that. On the same figure we have included, to show the temporal correlation between the effects, the appearance of rat cells in the thymus as detected immunologically (taken from Table I). It is obvious that replacement of mouse cells by rat cells occurs much sooner in the bone marrow and spleen than in the thymus. Both the bone marrow and spleen were pure rat, or almost so, by the 7th day after irradiation, whereas the thymus does not achieve this until about the 21st-22nd day. The thymus is 50% rat on about day 18, the bone marrow sometime before day 7.

Thymic weight recovery. The thymic weight pattern of lethally irradiated mice treated with RBM or IBM is shown in Fig. 2. As recorded by the average ratio of mg of thymus weight per g of body weight (T/B), a sharp reduction in size occurred within a few days after irradiation, followed in the 950 r-IBM mice by almost complete recovery within 15 days after treatment. In contrast, thymic recovery of the 950 r-RBM mice was only partial, reaching a peak value (~ 40% of normal) on the 15th-18th day, followed by a second period of relative weight loss from the 19th through the 25th days. Although the data of Fig. 2 suggest that the thymuses of these mice never completely recover, the thymus weight response varied extremely; and a few animals showed thymic recovery

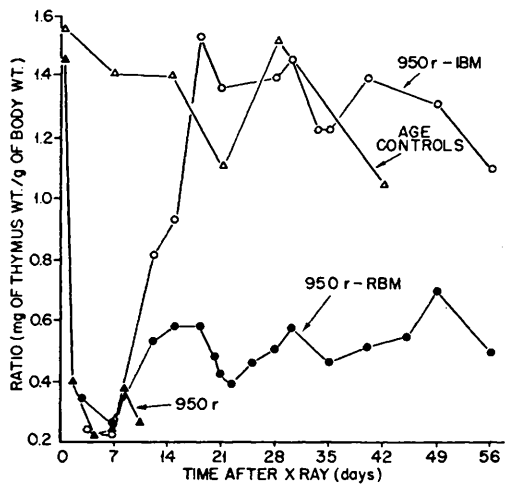

FIG. 2. Thymic recovery of lethally irradiated mice treated with rat bone marrow (RBM) or isologous bone marrow (IBM). Data represent average weights of 5-10 mice/point.

comparable to that of the IBM mice. Such variation in the T/B ratios did not occur among the 950 r-IBM mice. It was also noted that the T/B ratios of the 950 r-RBM mice that died during the experiment were well below the average value obtained with sacrificed animals.

Histologic findings. During these experiments, one or both lobes of the thymus from several sacrificed mice treated with either RBM or IBM were taken for histologic examination. For RBM mice, thymus sections were available at intervals from days 7 through 56 after irradiation. For mice treated with IBM, thymus sections were available at intervals from days 7 through 21 after irradiation.

A. *Treatment with isologous bone marrow.* On day 7 the thymus of the one animal examined showed marked atrophy (4.8 mg) with loss of cortical thymocytes. Some small, very dark-staining thymocytes were present in the medulla. In one portion of the cortex, large pale-staining thymocytes were present, suggesting early regeneration. The thymus of a mouse sacrificed on day 12 weighed 23.7 mg, and the one lobe taken for sectioning had a normal histologic architecture. On day 18, the thymus from the sacrificed mouse weighed 31 mg, and the lobe taken for sectioning was also normal histologically. The thymus on day 21 weighed 42.4 mg, and the lobe examined histologically was normal.

B. *Treatment with heterologous bone marrow.* The animal sacrificed on day 7 had a 5.6-mg thymus. One lobe was sectioned; it resembled the 7-day thymus of the animal treated with IBM. Regeneration of the cortex in some areas was more advanced, however; and several cortical cells were in mitosis. Two mice sacrificed on day 12 had thymuses weighing 17.6 and 18 mg. One lobe of each was sectioned; in both, the architecture was normal. On day 15, one lobe from each of four sacrificed animals was sectioned. These thymuses were all large, but only one was weighed (20.3 mg). Mild, irregular atrophy of the cortex was seen in one thymus. The others were normal histologically. Two animals sacrificed on day 18 had thymuses weighing 10.9 and 16.4 mg. Both showed mild to moderate irregular atrophy of the cortex in the lobe examined. On day 22, two animals sacrificed had thymuses weighing 3.1 and 7.2 mg. The smaller one showed extreme atrophy with perivascular necrosis of cells in a focal area. The larger thymus showed marked atrophy of the cortex. Many thymocytes were present, however; some of these were pycnotic, and others appeared to be normal. On days 25, 28, 40, and 56, the single animals sacrificed showed thymus weights of 9, 15, 11.9, and 17.9 mg, respectively. The 25-day thymus showed early regenerative activity in the cortex, and the medulla was packed with large thymocytes. Scattered eosinophilic leukocytes were present throughout the organ. Nearly complete regeneration of the cortex was observed in the 28-day thymus. A few eosinophils were present. Two foci of granulopoiesis in the medulla were also noted. In addition, several multinucleate giant cells were scattered through the medulla. The thymus of the 40-day mouse was normal in architecture but had slight thinning of the cortex. A few eosinophilic leukocytes were seen. In the 56-day animal, the thymus looked normal. Some eosinophilic leukocytes were present.

Discussion. The specific *in vitro* agglutination tests and the cytologic analyses indicate a repopulation of the thymus by rat-type cells in lethally irradiated mice treated with rat bone marrow. The agglutination tests showed that repopulation by rat cells was 50% complete on about the 21st day after treatment; and by 30 days, all the cells in the thymus appeared to be of the rat type. Cytological identification of dividing rat and mouse cells in the thymus showed that they were 50% rat by day 18 and almost 100% by day 21. This time difference in detection of donor cells by cytologic versus immunologic technics may be explained by the fact that cytological identification involves only the dividing cells, whereas the immunologic test is applicable to both dividing and nondividing cells. Thus, detection of remaining nondividing host cells along with the repopulating rat cells in the thymus could ac-

count for this time lag in the agglutination test.

The presence of donor-type cells in the thymuses of mice injected with homologous bone marrow (HBM) 12-14 days after treatment, as determined by their antigenicity in appropriate test animals, was reported by Merwin and Congdon(6). We verified those results in similar tests involving the experimental thymus tissue obtained from 950 r-RBM mice on the 15th and 45th days after treatment. Thus, the injections of these thymus cells into normal mice induced formation of agglutinins to rat thymus tissue, as indicated by the positive *in vitro* agglutination tests. further substantiating the heterologous origin of these cells.

The thymus weight and histology of the lethally irradiated mice treated with IBM showed a complete recovery within 21 days after exposure. In the lethally irradiated mice treated with RBM, thymus weight recovery and histologic appearance approximately paralleled that of the IBM mice for nearly 14 days after exposure. Subsequently, the thymus weight declined to a minimum by day 22. During this decline in weight, however, the immunologic test and the chromosone analysis demonstrated that the number of rat-type thymocytes was increasing relatively in the thymus, concomitant with an absolute decrease in host-type cells. A second partial recovery in thymus weight and histologic appearance took place 22-30 days after exposure. This partial recovery was, however, caused by regeneration of repopulated donor-type thymocytes.

In view of the extreme variation observed in the thymic recovery of heterologously treated mice as contrasted to the isologously treated mice, much more data are required to establish this secondary regression and recovery of the thymus as a real phenomenon. It is to be noted, however, that the time sequence in degeneration is quite similar to that observed with the secondary body weight losses occurring in 950 r-RBM mice, a phenomenon that has been attributed to the effects of a delayed *in vivo* antigen (transplanted foreign hematopoietic tissue)-antibody (recovering host's immune mechanism) reaction in the irradiated host(1,8,9). Thus the incomplete recovery of the thymus could be related indirectly to this stress mechanism within the animal. The more complete recovery of thymus tissue observed in a few animals is correlated with the failure of these mice to undergo the typical secondary loss of body weight; this emphasizes the relation of this organ's condition to the health of the irradiated host. Hirsch *et al.*(10) reported the failure of homologous and heterologous bone marrow to promote thymic regeneration after multiple sublethal total-body X irradiation, whereas regeneration was complete with isologous marrow. They suggest that different mechanisms operate in promoting thymic regeneration and survival for the different types of bone marrow treatment. Congdon and Urso(11) noted the incomplete recovery often observed with the thymus in foreign bone marrow-treated animals. They suggest that this is a result of the delayed foreign bone marrow reaction occurring in these mice.

The *in vitro* immunologic tests suggest that the temporary regeneration of thymus tissue occurring from the 7th through the 14th day after treatment is caused by a regeneration of host-type thymocytes; the chromosome analyses seem to substantiate this. However, although the latter studies indicate a greater percentage of mouse cells during this period, the low frequency of mitotic figures observed in the tissues at this time tends to negate the interpretation that this marked regeneration is caused entirely by a proliferation of host cells within the organ. The actual mechanism and factors contributing to this temporary recovery phase are still uncertain; and further experimentation is necessary to resolve this problem.

Kaplan *et al.*(12) reported that injection of parent mouse bone marrow (C_{57}BL) into X-irradiated F_1 hybrids (C_{57}BL X BALB) promoted regeneration of the irradiated thymus. Subsequent transplantation of this regenerated thymus into normal F_1 hybrids and parents resulted in a "take" and growth in the former but not in the latter hosts, suggesting that the regenerated thymus was com-

posed of host rather than donor cells. In similar experiments by Wolff and Upton (personal communication) with C_3H and (101 X C_3H)F_1 mice, the regenerated thymus grew when transplanted in the parent host, although the percentage of such takes was low.

In the experiments reported here, the designation of these foreign rat cells as "thymus" tissue is based primarily on their location and histologic appearance. Accepting this, one may surmise the presence of a multipotent cell in the bone marrow capable of transforming into the varying cell types and elements, as evidenced by the presence of foreign red blood cells, granulocytes, and platelets in lethally irradiated-rat-marrow-treated mice. It is also conceivable that lymphoblasts in the donor bone marrow seed the thymus gland in the irradiated host. The degree of functional ability of these foreign thymus cells as contrasted to the other transplanted foreign hematopoietic tissues is, however, open to question in view of the incomplete recovery and secondary degeneration processes often observed in this organ.

Summary. Immunologic and cytologic tests indicate that the thymuses of lethally irradiated mice protected with rat bone marrow are repopulated by rat-type cells. The agglutination tests showed this repopulation of the thymus by donor cells to be 100% complete 30 days after treatment, and the cytologic analysis of dividing cells showed 100% rat-type cells 21 days after treatment. Although the histologic architecture of these thymuses was often normal, recovery of the thymuses in terms of weight was incomplete.

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Phosphorylase and Glycogen Levels in Skeletal Muscle of Mice with Hereditary Myopathy* (23587)

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The activity of the enzyme phosphorylase, determined in biopsy samples of skeletal muscles of man with progressive muscular dystrophy, is markedly lower than normal(1). The discovery of a strain of mice with a hereditary myopathy (dystrophia muscularis), and the observation that the myopathy is in some ways similar to progressive muscular dystrophy in man(2) suggested an investigation on muscle phosphorylase in these animals. This report concerns the determination of both active and total phosphorylase and of glyogen concentration in certain skeletal muscles of these mice.

Methods. Phosphorylase activity was determined by the method of Sutherland as previously described(3) but with minor

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