larities with blood forming tissue (10). It remains to be determined whether humoral agents similar to erythropoietin (11) or granulopoietin (12) which are considered to play important integrative roles in hematopoiesis will be found to be performing similar functions for adipose tissue in lipid deposition. However, the role of hormones that are known to influence adipose tissue physiology such as insulin, epinephrine, and certain pituitary hormones must also be considered in any proposed mechanism (13).

Summary. Syngeneic adipose tissue grafts showed evidence of necrosis or lack of vascularization in intact, adult (BALB/c/Ki × Ce/Ki) F_1 hybrid mice between 2 and 9 months after transplantation. One or more fat depots were surgically removed to produce a deficit in the total adipose tissue mass, and this resulted in an increased number of vascularized and viable adipose tissue grafts in similar genetic hosts during a comparable period. The anatomically dispersed fat depots appear to be under some form of self-regulation and integrated into a total adipose tissue mass.

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Thymus–Marrow Immunocompetence III. The Requirement for Living Thymus Cells* (32715)

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The immunocompetence of thymus-marrow cell suspensions has been demonstrated (1,2). Suspensions containing both thymus and marrow cells produced more antibody to sheep erythrocytes (SRBC) when transferred to irradiated syngeneic hosts and stimulated with antigen than could be accounted for by the summation of the activities of thymus or marrow cell suspensions considered singly. Thymus–marrow interaction has also been demonstrated in other systems (3–6).

The nature of the thymus–marrow interaction is obscure. The purpose of these experiments was to investigate the system using the Jerne plaque assay method and to determine whether isologous living thymus cells could be replaced by irradiated cells, cell sonicates, or heterologous cells. The importance of the recipient thymus was also investigated.

Materials and Methods. LAF_1 mice were used and the description of the mice, method of cell suspension preparation and hemolysin assay have already been published (1,2). Immunization was with 0.2 ml of 10% washed SRBC. Rat thymus cell suspensions were made from freshly sacrificed young adult

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TABLE I. Immunocompetence of Thymus–Marrow Cell Combinations.

Group	No. of animals	Cells received on day 0[a]	Results day 8	
			PFC/spleen (±95% limits)	Log_2 hemolysins
A	5	Thymus iv + marrow iv + SRBC iv	892 (276–2233)	4
B	5	Thymus iv + marrow ip + SRBC iv	13 (3–68)	5
C	5	Thymus ip + marrow iv + SRBC iv	468 (86–2570)	4
D	4	Thymus iv + SRBC iv	11 (1–125)	0
E	4	Marrow iv + SRBC iv	110 (19–624)	2
F	4	SRBC iv	2 (1–5)	0

[a] All recipients were given 855r 250 kV_p X-rays on day 0 prior to injection of cells, and on day 4 were given SRBC ip. Doses of cells were: thymus (5.1×10^7) and marrow (3.6×10^7).

Sprague-Dawley Rats (Simonson Laboratories). Fetal liver cell suspensions were made by passing fetal LAF_1 livers through a graded series of needles as was done with marrow. "Minced" thymus preparations each consisted of a pair of thymic lobes cut into 8–12 pieces with scissors; these pieces were injected ip through a 20-gauge needle. Chilled thymus cells suspensions were disrupted by sonication for 5 min. Thymectomy was done under secobarbital anesthesia one week before irradiation and cell transfer. The sternum was split, the fascia incised, and the thymus aspirated. Sham thymectomies were done similarly except that aspiration was omitted.

Irradiation was carried out either with a 250 kV_p GE Maxitron or a 220 kV_p Westinghouse therapy unit. HVT for both units was 1.57 mm Cu. Radiation was delivered at 26 r/min.

In most of the experiments the recipient mice were irradiated on day 0 and cells from normal donors together with SRBC were injected iv several hours later. On day 4 recipients were given an additional ip injection of SRBC. At sacrifice on day 8 the mice were bled and the sera from each group were pooled for hemolysin titrations. The recipient spleens were removed and passed through a stainless steel screen to make a single cell suspension. Plates were made according to the method of Jerne *et al.* (7) as described in detail elsewhere (8). Plaques were counted with an image amplifier and the geometric mean number of plaque-forming cells (PFC) per spleen for each group was calculated together with the 95% confidence limits.

Histological examination of tissues was made by fixation in Zenker-formalin, imbedding in methyl methacrylate, sectioning and staining with hematoxylin–eosin–azure.

Results. Tables I and III show that the enhanced effect of thymus-plus-marrow combinations over the sum of thymus and marrow taken singly may be demonstrated using the Jerne plaque-assay method. The importance of the route of injection of cells is shown by comparing groups A, B, and C in Table I. If marrow was given iv thymus cells were effective if given either iv (A) or ip (C). If marrow was given ip, however, thymus cells iv were ineffective in augmenting the PFC in host spleens (B). The hemolysin titer of group B, on the other hand, was as great as A or C. We interpret this to indicate that antibody-producing cells were present in the body outside the spleen (see *Discussion*).

Table II shows that the sonicated cells of one thymus per day injected ip daily into each mouse for 8 days did not interact with iv marrow, while 3×10^7 iv thymus cells given once only on day 0 with iv marrow did result

TABLE II. Effects of Suspensions of Whole and Sonicated Mouse Thymus Cells.

Group	No. of animals[a]	Cells received on day 0	Daily	Results day 8 PFC/spleen (±95% limits)	Results day 8 Log_2 hemolysins
A	5	Mouse thymus (3×10^7) iv Marrow (9.1×10^6) + SRBC iv		383 (180–812)	3
B	6	Marrow (9.1×10^6) + SRBC iv	Sonicated thymus ip[b]	90 (33–249)	0
C	5	Marrow (9.1×10^6) + SRBC iv		76 (15–375)	0
D	4	SRBC iv	Sonicated thymus ip[b]	3 (1–17)	0

[a] All recipient mice received 855r on day 0.
[b] Each mouse in Groups B and D received the cells of one thymus, sonicated ip daily.

TABLE III. Effects of Rat Thymus Cell Suspensions.

Group	No. of animals[a]	Cells received on day 0	Daily	Results day 8 PFC/spleen (±95% limits)	Results day 8 Log_2 hemolysins
A	4	Mouse thymus (5×10^7) iv Marrow (10^7) + SRBC iv		404 (107–962)	4
B	5	Rat thymus (3×10^7) iv Marrow (10^7) + SRBC iv	Rat thymus ip[b]	42 (14–131)	0
C	3	Mouse thymus (5×10^7) + SRBC iv		12 (3–55)	0
D	5	Mouse (10^7) + SRBC iv		16 (6–38)	0
E	5	Rat thymus (3×10^7) + SRBC iv	Rat thymus ip[b]	8 (1–45)	0
F	5	SRBC iv		2 (1–3)	0

[a] All recipients given 855r irradiation and SRBC as in Table I.
[b] Each mouse in Groups B and E received 1/12 fresh rat thymus suspension ip daily, days 1–7.

in production of significant antibody.

Table III shows that daily injections of suspensions of rat thymus cells ip were unable to substitute for a single iv injection of mouse thymus cells.

Other workers have shown that the immunocompetence of thymus cells was resistant to 500r *in vitro* and may be associated with the radioresistant thymic epithelial and reticular cells rather than the radiosensitive thymic small lymphocyte (9). Our previous experiments, on the contrary, showed that the immunocompetence of the thymus component in this thymus–marrow system was abolished by 500r *in vivo* (2). One possible explanation for this discrepancy is that in using screened suspensions of thymus cells prepared after irradiation, a selected population of epithelial and reticular cells adhered to the stroma during preparation and was not passed through the screen nor injected into the recipient. To test this, minced whole thymus preparations, presumably containing all the cells of the thymus, were injected ip. While

these minced preparations showed immunocompetence when injected with iv marrow (Table IV, A), similar minced thymus preparations from irradiated donors (530r) showed very little antibody production (B) when injected with iv marrow.

Thymectomy 1 week prior to the injection of cells (Table V) had no significant effect on the antibody-forming potential of transfused thymus–marrow suspensions. Histological examination of tissue from the anterior mediastinum showed no thymic remnants in the thymectomized group.

Other experiments have shown that fetal liver may substitute for marrow since fetal liver-plus-thymus combinations were more immunogenic than the sum of both cell suspensions alone. Fetal liver suspensions were about half as effective as suspensions containing equal numbers of marrow cells.

Discussion. These data clearly demonstrate that the phenomenon of thymus–marrow "synergism" may be seen using the Jerne plaque assay on recipient spleens. This technique is more readily quantitated than the previously used technique of Playfair *et al.* (9). A drawback of the Jerne technique, as used here and in other experimental systems, is that it measures antibody production only in the tissue sampled, e.g., spleen in these ex-

TABLE IV. Effects of Minced Normal or Irradiated Mouse Thymus Preparations.

Group	No. of animals	Cells received on day 0[a]	Results day 8	
			PFC/spleen (±95% limits)	Log_2 hemolysins
A	11	1 Normal thymus (minced) ip Marrow + SRBC iv	1006[b] (550–1756)	4.5[b]
B	8	1 Irradiated thymus (minced) ip Marrow + SRBC iv	209[b] (98–448)	1[b]
C	5	1 Normal thymus (minced) ip SRBC iv	15 (15–45)	0
D	5	Marrow + SRBC iv	69 (17–276)	0
E	3	SRBC iv	10 (2–47)	0

[a] All recipients were given 742–795r on day 0 prior to injections of cells, and on day 4 were given SRBC ip. Marrow dosage was 7.4–10.0 × 10^6. Group-B recipients received minced thymus from donors which had received 530r 2–3 hours before transfer of cells.

[b] Groups A and B are pooled from two experiments.

TABLE V. Effect of Thymectomy of Hosts.

No. of animals	Treatment	Cells received on day 0[a]	Results day 8	
			PFC/spleen (±95% limits)	Log_2 hemolysins
7	Thymectomy	Thymus + marrow + SRBC	1810 (920–3557)	4
6	Sham thymectomy	Thymus + marrow + SRBC	1566 (926–2650)	4
3	None	SRBC	11 (2–48)	0

[a] Hosts were thymectomized or sham thymectomized on day −7. On day 0 hosts received 742r and then were given 5 × 10^7 thymus cells plus 10^7 marrow cells plus SRBC iv.

periments. Serum-antibody titers probably reflect antibody production in the *whole* animal to a greater degree than do spleen PFC. On the other hand, it is known that the antibody response of mice to iv SRBC is concentrated in the spleen. These factors, together with the known migration of antibody-forming cells between tissues (8), complicate the interpretation of the numbers of spleen PFC. This is particularly obvious in Group B of Table I where the discrepancy between spleen PFC and serum hemolysins is large. We interpret this discrepancy to indicate that antibody is being formed by thymus- or marrow-cell descendants in tissues other than the spleen. Since this discrepancy was seen only with iv thymus and ip marrow, the route of injection of marrow becomes crucial. These data suggest that the marrow cells must be present within the spleen in order to get PFC in that organ while the thymus cells probably do not appear in the spleen in significant numbers. This conclusion is based on the findings that iv marrow is more effective in thymus–marrow interaction than ip marrow, and that dividing cells from iv marrow "home" to the spleen in 8 days (11). Marrow cells given ip "home" to the spleen in much less efficient manner. Dividing thymus cells given iv do not appear in the recipient spleen in significant numbers (11), and in our experiments, both ip thymus cells and ip minced thymus were effective in thymus–marrow synergism.

These data show that the thymus component of the "synergistic" combination must consist of living cells. Thymic cell suspensions from irradiated donors and thymic cell sonicates are ineffective. In view of the large amount of sonicated thymic tissue given (Groups B and D in Table II received approximately 10 times more thymus tissue than did Group A), it is unlikely that a "humoral factor" is involved. The absence of activity following large daily doses of living rat thymus cells indicates a need for genetically related thymus cells. Recent experiments in our laboratory have shown that allogeneic (parental) thymus cells are less effective than are syngeneic cells (12).

The failure of recipient thymectomy to alter the thymus–marrow synergism indicates that this synergism does not depend on the presence of recipient's thymus during the period following irradiation and cell transfer. The mechanism of the phenomenon of thymus–marrow interaction remains obscure.

Summary. The enhanced immunocompetence of transferred thymus–marrow cell combinations was demonstrated using the Jerne plaque-assay method. Suspensions containing both thymus and marrow cells produced more antibody to sheep erythrocytes when transferred to irradiated syngeneic hosts and stimulated with antigen than could be accounted for by the summation of thymus- or marrow-cell activities considered singly. Living homologous thymus cells were required, since the following materials were incapable of interacting with isologous marrow cells; sonicated or irradiated (530r) mouse thymus cells or living rat thymus cells. The thymus of the host was not necessary for demonstrating thymus–marrow immunocompetence.

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