M., Proc. Soc. Exptl. Biol. Med. **99**, 35 (1958).
13. Hazelwood, R. L. and Hazelwood B. S., Am. J. Physiol. **206**, 1137 (1964).
14. Nelson, N., J. Biol. Chem. **160**, 61 (1945).
15. Seifter, S., Dayton, S. Novic, B., and Muntwyler, E., Arch. Biochem. **25**, 191 (1950).
16. Bessman, S. and Anderson, M., Federation Proc. **16**, 154 (1957).
17. Lowry, O., Rosebrough, N., Farr, A., and Randall, R., J. Biol. Chem. **193**, 265 (1951).
18. Frame, E., Russell, J., and Wilhelmi, A., J. Biol. Chem. **149**, 255 (1943).
19. Beatty, C., Peterson, R., Bocek, R., and West, E., J. Biol. Chem. **239**, 2106 (1964).
20. Beatty, C. H. and Peterson, R. D., Am. J. Physiol. **183**, 325 (1955).
21. Scow, R. O. and Chernick, S. S., Recent Progr. Hormone Res. **16**, 497 (1960).
22. Winternitz, W. W. and Lattanzi, W. E., Endocrinology **58**, 232 (1956).
23. Friedmann, B., Goodman, E., Jr., and Weinhouse, S., J. Biol. Chem. **238**, 2899 (1963).
24. Scow, R. O., Wagner, E., and Cardoza, A., Endocrinology **61**, 380 (1957).
25. Lukens, F. D. W., Am. J. Physiol. **192**, 485 (1958).
26. Friedman, R. C. and Reichlin, S., Am. J. Physiol. **75**, 787 (1965).
27. Kessler, J. I., J. Clin. Invest. **42**, 362 (1963).
28. Lyon, J. B., Jr., Endocrinology **64**, 967 (1959).

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Autoregulation of Adipose Tissue Mass in the Mouse* (32714)

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Lipid deposition occurs at different rates in the inguinal and gonadal fat depots of NH and CBA mice during postnatal development, yet the lipid content of either fat depot represents a direct proportion of the total body lipid content during normal growth or the development of goldthioglucose-induced obesity (1,2). Surgical removal of the gonadal fat depots followed by a single injection of goldthioglucose results in a "compensatory hypertrophy" of the remaining fat depots during the development of obesity and an increased rate of converting dietary constituents into stored lipids (3). These data suggest that the anatomically dispersed fat depots are integrated into a functional total adipose tissue mass.

Syngeneic grafts of ovarian (4) and splenic (5) tissues atrophy in intact animals. However, ovarian and splenic grafts remain morphologically and functionally viable in ovariectomized or splenectomized hosts, respectively. Interpretations of these findings favor the existence of autoregulatory mechanisms determining tissue or organ mass (6). It was the purpose of the following studies to determine the fate of syngeneic adipose tissue grafts in intact mice and in mice in which a deficit in the total adipose tissue mass was created by surgical removal of specific fat depots.

Materials and Methods. Experiment A. A total of 84 (BALB/c Ki × CE/Ki) F_1 hybrid mice[1] of both sexes were used at approximately 90 days of age and were distributed into four comparable groups according to age, sex, and body weight. All animals were anesthetized with sodium pentobarbital and the peritoneal cavity was opened by a midline incision from the xiphoid process to the region of the external genitalia. Animals assigned to Group I had the paired epididymal or parametrial fat depots (GFO's) in males and females, respectively, lifted from the abdominal cavity without interfering with the blood supply and returned to the normal anatomical position. Group-II animals had one of the paired fat depots partially transected lateral to the main blood supply (tes-

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[1] Obtained from Kirschbaum Memorial Laboratory, Department of Anatomy, Baylor University College of Medicine.

ticular or uterine artery), but was left *in situ*. One of the paired fat depots was completely excised in animals of Group III and both fat depots were completely removed in Group-IV animals. Care was exercised in removing the fat depots in the latter two groups to avoid damaging the blood supply to the reproductive organs. The removed fat depots provided the source of adipose tissue for transplantation.

All animals in the four groups received a graft of adipose tissue weighing aproximately 150 mg wet weight. No recipient served as its own donor and the sex of donor and recipient were the same. Grafts were placed either into the peritoneal cavity as "free grafts" or were sutured to the liver capsule with a piece of 3–0 silk thread. The latter type of graft was used to avoid the increased possibility of the "free grafts" establishing a vascular attachment to the stumps of the removed fat depots in the operated as compared to the intact animals. Interrupted sutures of 3–0 silk were used to approximate the abdominal muscle layers and the skin separately. Animals were autopsied at 2, 4, 6, and 9 months after transplantation. On the basis of an arbitrary scale, the grafts were classified as being either "successful," "partially successful," or "unsuccessful." This scale ranged from 0–12 points and was based on gross morphological characteristics of the grafted tissue. If a transplant did not become vascularized in the case of "free grafts" is was given 0 points and considered "unsuccessful." Vascularized grafts were awarded 1 point and also graded on the degree of vascularization, necrosis, amount of normal appearing adipose tissue present, and presence or absence of a fibrotic capsule. Transplants with a total score of 0–3 points were considered as "unsuccessful"; those with 4–7 points as "partially successful" while those grafts scoring 8–12 points were classified as "successful." Representative grafts from all groups were processed for microscopic evaluation.

Experiment B. A total of 40 (BALB/c/Ki × CE/Ki) F_1 hybrid males and females 120 days of age were divided into four groups. Group I was made up of intact animals that received a graft of adipose tissue obtained from the inguinal fat organ (IFO). Group II animals had both IFO's removed, and received a graft of adipose tissue from a GFO. Group-III animals received an IFO adipose tissue graft and both GFO's were removed. Group-IV animals had both GFO's and IFO's removed, and received an IFO adipose tissue graft.

TABLE I. Relationship Between the Recovery of Adipose Tissue Grafts Classified as Successful in Different Experimental Groups Following Varying Time Periods After Transplantation.

Experimental groups[a]	No. of grafts classified as "successful" at varying time periods after transplantation (months)				Total No. of "successful" grafts
	2	4	6	9	
I	2/6[b]	0/5	0/4	1/5	3/20 (15)[c]
II	2/8	1/5	3/3	3/3	9/21 (43)
III	3/5	2/4	4/6	5/8	14/23 (61)
IV	3/6	3/4	4/4	6/6	16/20 (80)

[a] See text for description.
[b] "Successful" grafts/total number of grafts.
[c] Percentages are given in parentheses.

The removed fat depots were used for transplantation. No recipient served as its own donor and the sex of donor and recipient were the same. The wet weight of the grafts of IFO and GFO were approximately 100 mg. Autopsies were performed on all animals at 6 months after transplantation and the grafts were classified on the same basis as in the first experiment.

Results. The results of Experiment A are shown in Table I. No sex differences were noted, thus the data from males and females in each group were combined. A total of 3 out of 20 grafts (15%) in Group I were classified as "successful" as compared to 9/21 (43%) in Group II, 14/23 (61%) in Group III, and 16/20 (83%) in Group IV. However, the time at which the hosts were killed following transplantation was important. Two of the 3 grafts listed as "successful" in the intact animals (Group I) were recovered at 2 months post-transplantation and the third one was found at 9 months after transplantation. The latter mouse showed an exceptional increase in body weight and excessive lipid deposition as

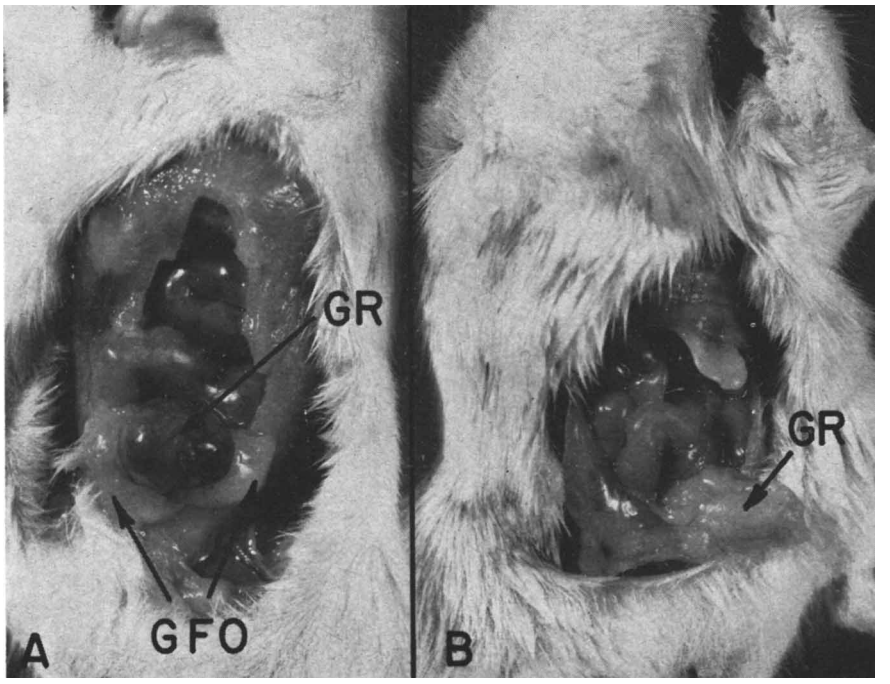

FIG. 1. A. An "unsuccessful" graft (GR) in a host with intact gonadal fat depots (GFO). Note extensive vascular pattern. B. A "successful" graft (GR) in a host with both gonadal fat depots surgically removed.

compared to the other mice killed at the 9-month period. The importance of the relationship between the time period after transplantation and the status of the graft was also seen in Group II animals (partially-cut GFO). Whereas only 3 out of 13 grafts were classified as "successful" at the 2- and 4-month periods, a total of 6/8 were found to be "successful" at the 6- and 9-month periods. Approximately 60% of the grafts were "successful" at all time periods in Group III (one GFO removed). All the grafts in Group IV (both GFO's removed) were classified as "successful" at 6 and 9 months after transplantation as compared to 50% at 2 months and 75% at 4 months. The majority of the "free grafts" classified as "successful" in all groups established vascular connections with liver, small intestine, and mesentric adipose tissue rather than with the stumps of removed gonadal fat depots. The grafts sutured to the liver accounted for the highest percentage of either "unsuccessful" or "partially successful" grafts as compared to the "free grafts" in all groups. Localized necrosis about the suture material was the primary basis for classifying grafts as "partially successful" especially in Groups III and IV.

The "unsuccessful" grafts were a yellowish-brown color with a grossly prominent blood supply and had a caseous type of consistency (Fig. 1). No normal appearing adipose tissue was grossly or microscopically visible. The "partially successful" grafts had localized regions of necrosis, but some normal appearing adipose tissue was always present in varying amounts. The "successful" grafts appeared as normal adipose tissue, and necrosis could not be detected either macro- or microscopically. No attempt was made to quantitate the amount of viable adipose tissue in any graft. However, "successful" grafts especially in Groups III and IV were uniformly larger than the original graft. Microscopic examination of the "unsuccessful" grafts revealed numerous macrophage-type cells filled with vacuoles plus fibroblast-like cells at the periphery presumably responsible for the formation of a fibrous capsule about the grafts. No lymphocytes or plasma cell accumulations were noted.

Unattached "unsuccessful" grafts were occasionally found to consist of a pearly sphere, freely movable within the peritoneal cavity and consisting of fibrous encapsulated lipid material.

The results of Experiment B were very similar to those groups in Experiment A autopsied at 6 months after transplantation. Only 1 out of 10 IFO grafts was classified as "successful" in intact animals (Group I), and this was found in an animal demonstrating excessive amounts of adipose tissue as compared to the others in the group. It appeared that IFO grafts were relatively more sensitive than GFO grafts to the amounts of adipose tissue removed. Five out of 10 IFO grafts were "successful" in animals with both GFO's removed (Group III), as compared to 9/10 in animals having had both IFO's and GFO's removed (Group IV). All GFO grafts were successful (4/4) at 6 months following transplantation after removal of both GFO's (Experiment A). On the other hand, 5/10 GFO grafts were "successful" in animals that had only the IFO's removed (Group III). In unpublished studies it has been found that the lipid content of IFO accounts for 7% of the total body lipid content of mice of this strain as compared to 17% in the GFO. Additional animals will have to be studied at different time intervals to establish any quantitative differences between IFO and GFO responsiveness to deficits in adipose tissue mass.

Discussion. Transplantation of adipose tissue in experimental animals has been primarily restricted to autogeneic or allogeneic systems (7). The results of this study indicate that the removal of one or both gonadal or inguinal fat depots enhances the successful transplantation of syngeneic grafts of adipose tissue obtained from either fat depot. This suggests that creating a deficit in the total adipose tissue mass of the host results in an altered physiologic state favoring the incorporation of adipose tissue grafts into the total adipose tissue system. Conversely, the failure of the majority of adipose tissue grafts to become incorporated into the total adipose tissue mass in intact animals suggests the existence of some type of "suppressing" mechanism regulating the total complement of fat depots during a given physiologic state.

The length of time the graft was permitted to remain in the host influenced the results. A greater number of "successful" grafts were generally found in all groups except the intact animals from 4 to 9 months after transplantation. The amount of body adipose tissue of the hosts also increased during this time period. This suggests that the expansion of the total adipose tissue mass with increasing age favors incorporation of excess adipose tissue into the total mass. It has been shown that relatively small grafts of syngeneic adipose tissue (approximately 20 mg) can be transplanted successfully into the ears of young adult, intact hosts, but the number of successful grafts decreased with increasing age and adiposity of the host at the time of transplantation (1). Also it has been reported that the final weight of autogeneic grafts of adipose tissue placed subcutaneously into immature rats can be influenced by the amount of tissue originally transplanted (8).

The surprisingly high number of successful grafts at 6 and 9 months after transplantation in hosts with partially cut gonadal fat depots in Experiment A deserves some comment. It is possible that any type of trauma per se rather than removal of adipose tissue from the body's complement initiates the development of a physiological environment that enhances a successful transplantation of the graft. However, it is also possible that disruption of the blood supply to a large portion of the fat depot, at least temporarily, causes in effect a removal of a given functional quantity of the fat depot from the systemic metabolism of the host, thus creating a temporary deficit in the total adipose tissue mass. This latter explanation is supported by the occurrence of fatty necrosis and fibrosis in the traumatized fat depots at varying time periods after partial transection.

The mechanism by which this proposed regulation of adipose tissue mass takes place is unknown. The idea that anatomically dispersed tissues of a similar type can be integrated into a functional whole is not new since myeloid tissue which is widely distributed throughout the body functions as an integrated unit as proposed in the erythron concept (9). The embryological development of adipose tissue suggests numerous simi-

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.

1. Liebelt, R. A., Am. J. Anat. **105**, 197 (1959).

2. Liebelt, R. A., Ann. N. Y. Acad. Sci. **110**, 723 (1963).

3. Liebelt, R. A., Ichinoe, S., and Nicholson, N., Ann. N. Y. Acad. Sci. **131**, 559 (1965).

4. Krohn, P., "Transplantation of Tissue," Peer, L., ed., Vol. 2, p. 401. Williams and Wilkens, Baltimore, Maryland, 1959.

5. Metcalf, D., Transplantation **2**, 387 (1964).

6. Liebelt, R. A., Liebelt, A. G., and Gulledge, D., "Proliferation and Spread of Neoplastic Cells," Univ. of Texas Press, Austin, Texas, in press.

7. Peer, L., "Transplantation of Tissues," Peer, L., ed. Vol. 2, p. 165. Williams and Wilkens, Baltimore, Maryland, 1959.

8. Hausberger, F., Anat. Record **127**, 305 (1957).

9. Wintrobe, M., "Clinical Hematology," 5th ed., p. 181. Lea and Febiger, Philadelphia, Pennsylvania, 1961.

10. Wassermann, F., "Handbook of Physiolgy," Sec. 5, p. 87. Am. Physiol. Soc., Washington, D. C., 1965.

11. Gordon, A. S., Physiol. Rev. **39**, 1 (1959).

12. Bierman, H. R., Ann. N. Y. Acad. Sci. **113**, 753 (1964).

13. Hausberger, F., "Handbook of Physiology," Sec. 5, p. 519. Am. Physiol. Soc., Washington, D. C., 1965.

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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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