A = 81 percent or (A + B − C)/A = 86 percent. The remaining part of the within-year variance, 19 or 14 percent, is due to differences between individual females. These differences between females may be hereditary, but may also be due to other factors, like differences in feeding efficiency.

If territory quality is an important factor in determining optimum clutch size, one should expect a generally smaller clutch size variation in colonial species, without exclusive foraging areas in the different breeding pairs, than in territorial species. Coefficients of variation of clutch size in species being both colonial and territorial, and of ecologically similar species, belonging to one of the two categories (Table 3), fit this prediction.

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Correction of Enzyme Deficiency in Mice by Allogeneic Bone Marrow Transplantation with Total Lymphoid Irradiation

Abstract. *Enzyme deficiency was corrected in mice after allogeneic bone marrow transplantation without occurrence of graft versus host disease. β-Glucuronidase-deficient C3H/HeJ mice were treated with total lymphoid irradiation. Normal bone marrow cells (30 × 10⁶) from BALB/c mice were infused 1 day after total lymphoid irradiation, with resulting stable BALB/c to C3H/HeJ chimeras (> 90 percent circulating donor-type cells) without graft versus host disease. β-Glucuronidase activity increased to normal levels in all chimeras as measured in the liver and in the plasma. Activity was maintained throughout an observation period of 7 months.*

Enzyme replacement therapy is the most desirable and the only rational goal for correction of the many varieties of enzyme deficiency disorders in man. There has been only limited success for enzyme replacement strategies that involve directly administered purified enzymes, enzyme-rich plasma, or placental fractions (*1–5*), enzyme-containing semipermeable microcapsules (*6*), erythrocyte and liposome-entrapped enzymes (*7–10*), and enzyme preparations attached to physiological carrier molecules (*11, 12*). The usefulness of some of these approaches has been limited by relatively short-circulating and intracellular half-life, susceptibility to various degradation processes—predominantly by the reactivity of the immune system against the foreign proteins—and the limited distribution into various cellular compartments. Enzyme-producing allogeneic cells or whole organs may provide a better potential intrinsic physiological source of deficient enzymes.

Whole organ (kidney, liver, and pancreas) and cellular (fibroblasts and bone marrow) transplantations have already been attempted experimentally for the treatment of enzymatic and metabolic disorders (*13–19*). Initial results were encouraging, but the long-term success of organ transplantation has been rather limited, mostly as a result of unsolved problems in overcoming rejection (*20–27*). We have explored the feasibility of enzyme reconstitution in deficient mice by evaluating the effectiveness of continuous enzyme supply by allogeneic bone marrow cells. The attempt to use allogeneic bone marrow transplantation for the reconstitution of enzyme-deficient recipients imposes obstacles, including the so-called double barrier of host versus graft (HVG) and graft versus host disease (GVHD). We now report a new approach to enzyme replacement therapy by allogeneic, strongly histoincompatible bone marrow grafts obtained from normal enzyme-producing donors after enzyme-deficient recipients were treated with total lymphoid irradiation to ensure engraftment and prevent GVHD. The preparation of recipients with a single high-dose whole-body irradiation or with a high dose of cyclophosphamide instead of total lymphoid irradiation resulted in a vigorous lethal GVHD after bone marrow transplantation.

Total lymphoid irradiation, a relatively safe form of radiotherapy originally used for the treatment of human malignant lymphomas (*28*), was developed in the last few years as a new regimen to condition recipients of subsequent bone marrow and organ allografts (*29–35*). Permanent and stable bone marrow allo-

Table 1. Hydrolase activities in the liver and plasma of untreated C3H/HeJ, normal BALB/c mice, and BALB/c → C3H/HeJ chimeras 50 days after total lymphoid irradiation and marrow transplantation. C3H/HeJ mice were treated with daily doses of 200 rads (for 17 days) to expose the major lymphoid organs, including the thymus and spleen [as described in (*29, 30*)]. One day after completion of irradiation, 30 × 10⁶ BALB/c bone marrow cells were infused. Individual samples of heparinized blood and liver biopsies were obtained from untreated C3H/HeJ (N = 20), BALB/c (N = 17), and BALB/c → C3H/HeJ chimeras (N = 6) 50 days after bone marrow transplantation. Hydrolase activities of β-glucuronidase and two unrelated enzymes (β-galactosidase and N-acetyl-β-glucosaminidase) were assayed simultaneously in the plasma and in the liver homogenate (*37*). Data are expressed as means ± standard deviations (S.D.).

	C3H/HeJ		BALB/c		BALB/c → C3H/HeJ chimeras	
	Liver*	Plasma†	Liver	Plasma	Liver	Plasma
β-Glucuronidase	23.4 ± 7.0	9.5 ± 2.0	135.0 ± 30.2	20.4 ± 4.6	207.8 ± 82.2	16.5 ± 5.0
β-Galactosidase	146.9 ± 50.0	34.7 ± 13.2	137.0 ± 27.0	34.4 ± 27.0	155.3 ± 62.3	22.0 ± 4.3
N-Acetyl-β-glucosaminidase	1257.2 ± 476.0	1151.3 ± 204.6	1082.1 ± 224.6	854.6 ± 177.6	1320.6 ± 411.1	975.0 ± 261.9

*Specific activity is expressed as nanomoles per hour per milligram of protein. †Specific activity is expressed as nanomoles per hour per milliliter of plasma.

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grafts were successfully established after total lymphoid irradiation in mice [C57BL/Ka (H-2^b) → BALB/c (H-2^d)] (*29–31*), in rats [ACI (AgB^4) → Lewis (AgB^1)] (*32*), and in dogs (mongrel → unrelated, sex mismatched recipient mongrel) (*33–34*). Despite strong histocompatibility barriers between the donor and recipient, no GVHD was apparent in these recipients. None of the existing alternative conditioning regimens provides a safe approach for either prevention or effective treatment of GVHD (*36*). The bone marrow chimeras induced by total lymphoid irradiation developed permanent and specific transplantation tolerance to tissues, from the bone marrow donor, as shown by permanent (more than 1-year survival) acceptance of skin (*29–32*), heart (*32*), and kidney allografts (*35*) without maintenance immunosuppressive treatment.

Inbred C3H/HeJ (H-$2^{k/k}$) mice, deficient in β-glucuronidase, were used as a model of enzyme-deficient recipients. Normal enzyme-producing BALB/c (H-$2^{d/d}$) mice were used as donors (Table 1). Four-month-old C3H/HeJ mice were anesthetized and positioned in a lead apparatus that exposed the major lymphoid organs, including the thymus and spleen, to x-ray irradiation (*29, 30*). Irradiation dosage was 200 rads per day, six times per week, to a total of 3400 rads. One day after total lymphoid irradiation, 30×10^6 nucleated BALB/c bone marrow cells were administered intravenously.

Fresh plasma and liver tissue samples were obtained from experimental mice and from intact age- and sex-matched BALB/c and C3H/HeJ controls at various time intervals after bone marrow was inoculated. β-Glucuronidase activity and activities of two additional hydrolases (β-galactosidase and *N*-acetyl-β-glucosaminidase) were measured in parallel in each sample (*37*). Chimerism was detected by measuring the percentage of donor-type (BALB/c) cells in the peripheral blood of bone marrow recipients; for this purpose a complement-dependent microcytotoxicity assay with specific alloantiserum was used (*30, 31*). All six recipients (BALB/c → C3H/HeJ) were fully reconstituted with BALB/c cells 30 days after bone marrow transplantation (Table 2). Chimerism persisted throughout a period of 7 months, and no GVHD was noted. Enzyme activity increased significantly ($P = .00005$) to normal BALB/c levels in all chimeras tested (Table 1). The mean specific enzyme activity increased approximately ninefold in the liver and twofold in the plasma. Increased enzyme activity persisted throughout the 7-month period. The normal enzyme activity of the two lysosomal hydrolases measured in parallel was unaltered (Table 1). The increase of enzyme activity was attributable to the allogeneic marrow engraftment, since no increased activity was noted in C3H/HeJ mice undergoing similar radiotherapy, with or without syngeneic marrow reconstitution (data not shown).

Table 2. Evidence for chimerism in BALB/c → C3H/HeJ mice at various intervals after total lymphoid irradiation (TLI) and transplantation of 30×10^6 allogeneic BALB/c bone marrow cells. Percent chimerism was determined with Ficoll-Hypaque purified peripheral blood leukocytes. BALB/c cells were lysed with C3H/HeJ-anti-BALB/c serum, and C3H/HeJ cells were lysed with BALB/c-anti-C3H/HeJ serum, using a dye exclusion, complement-dependent, microcytotoxicity assay (*30*). Tests were done in duplicates, with peripheral blood samples obtained from the same six animals that were tested for enzyme replacement.

Time after TLI	Mean percent cytotoxicity (± S.D.)	
	H-2^d type cells	H-2^k type cells
Experimental BALB/c → C3H/HeJ mice		
1 month	90.1 ± 6.2 ($N = 12$)	
7 months	100 ± 0 ($N = 2$)	4.5 ± 1.6 ($N = 2$)
Untreated BALB/c (H-$2^{d/d}$) mice	90.7 ± 11.0 ($N = 4$)	4.5 ± 0.7 ($N = 2$)
Untreated C3H/HeJ (H-$2^{k/k}$) mice	1.3 ± 1.1 ($N = 3$)	87.1 ± 1.1 ($N = 3$)

Our data indicate that total lymphoid irradiation can be used for the establishment of stable GVHD-free bone marrow chimeras, with histoincompatible marrow cells used as a continuous source of β-glucuronidase in enzyme-deficient recipients providing enzyme activity extracellularly in the cell-free plasma and in the liver tissue. The increased enzyme content in chimeric liver homogenate may be due to the presence of donor-type reticuloendothelial cells or else, although less likely, to the induction or transfer of lysosomal enzyme activity to the host's hepatocytes. The cellular mechanisms involved in increasing enzyme activity, the exact type and location of the enzyme-producing cells, and the specific tissue and subcellular distribution of the enzyme remain to be elucidated.

Induction of transplantation tolerance with total lymphoid irradiation is being extensively investigated in large outbred animals and man, as it seems to enable successful kidney transplantation across strong transplantation barriers (*35*). Our model indicates that a similar approach could be used potentially for the correction of certain metabolic deficiency disorders, with normal incompatible bone marrow cells or organ allografts (or both) used as a source of the missing factors. The mechanisms of the immunoregulatory effects of total lymphoid irradiation remain to be elaborated; however, the role of specific, as well as nonspecific, active suppression and clonal deletion of alloreactive cells by suppressor T cells is supported by a number of in vivo and in vitro studies (*38, 39*).

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Hormone Binding Alters the Conformation of the Insulin Receptor

Abstract. *Fat cells or fat cell membranes were briefly subjected to mild proteolysis under conditions where insulin receptors were either free or bound to* ^{125}*I-labeled insulin. When receptors were then affinity-labeled to visualize the effects of this treatment, it was observed that receptors that had been occupied by ligand during proteolysis exhibited greater rates of degradation than unoccupied receptors. These results demonstrate that insulin-receptor interaction induces a change in receptor structure that may be related to signal transmission.*

The binding of insulin to specific receptors on the membranes of target cells such as adipocytes initiates a broad range of well-known metabolic effects (*1–5*). However, the molecular events that occur after binding and before the onset of the biological response remain undescribed. Presumably, insulin binding triggers a structural change in the receptor that results in the generation of the transmembrane signal. We have recently developed affinity cross-linking techniques that allow the identification of insulin receptors in cells and membranes (*6, 7*). These procedures have provided information on receptor subunit composition, indicating that the subunit exists in native membranes as a disulfide-linked complex containing a total of four subunits (*7, 8*). Purification of the [^{125}I]insulin-receptor complex by ligand-directed affinity chromatography has also been achieved (*9*). By combining the affinity cross-linking approach with biological assays after limited proteolytic digestion of adipocytes, we have also been able to correlate aspects of insulin receptor structure with biological functions (*10*). In the present experiments, we sought to extend the combined approach of limited tryptic digestion with affinity cross-linking in an attempt to detect differences in proteolytic sensitivity between occupied and unoccupied insulin receptors.

If the binding of insulin to its receptor results in a conformational change or if insulin binding stabilizes a preexisting conformational state of the receptor, we reasoned that differences in proteolytic susceptibility of the receptor in the occupied and unoccupied states may be detectable. Adipocytes were prepared by subjecting tissue obtained from the epididymal fat pads of male Sprague-Dawley rats (150 to 200 g) to collagenase digestion (*11*). The cells were then briefly treated with trypsin either before or during the last 2 minutes of a 30-minute incubation period with [^{125}I]insulin. The adipocytes were cooled to 15°C, and the cross-linking agent disuccinimidyl suberate was added in order to affinity-label the receptor. The cells were washed and disrupted, and a crude membrane fraction was prepared by centrifugation at 30,000*g* for 30 minutes. Figure 1 shows data for such fat cell membranes derived from adipocytes that were briefly exposed to trypsin in the absence or presence of receptor-bound [^{125}I]insulin and then subjected to electrophoresis (*12*) and autoradiography. At both times of trypsin treatment, the affinity-labeled receptor subunit, M_r 125,000, was less abundant when the insulin was receptor-bound than when the receptor was free during proteolysis, indicating a more complete tryptic digestion in the presence of bound insulin.

As previously observed, several receptor fragments that remain bound to the cell membrane are generated under these experimental conditions. The major receptor fragments are more abundant when proteolysis is carried out in

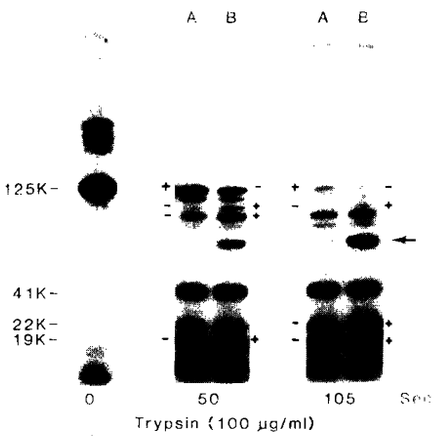

Fig. 1. Tryptic digestion of the adipocyte insulin receptor in the presence or absence of receptor-bound hormone. Fat cells in Krebs-Ringer phosphate buffer, *p*H 7.4, containing bovine serum albumin (20 mg/ml) were treated with trypsin (100 μg/ml) for the times indicated either before (A) or during (B) the last 2 minutes of a 30-minute incubation period at 24°C with 5×10^{-9}*M* [^{125}I]insulin. The cells were then cooled to 15°C and the receptor and fragments thereof were affinity-labeled by cross-linking bound [^{125}I]insulin with 0.5 m*M* disuccinimidyl suberate (*6*). A crude membrane fraction was obtained by centrifugation of disrupted cells. The membranes were solubilized in sodium dodecyl sulfate and subjected to electrophoresis (*12*) on a 5 to 15 percent acrylamide gradient gel and autoradiography as described (*6*). The molecular weights ($\times 10^{-3}$) of the receptor subunit M_r 125,000 and its major low-molecular-weight fragments are indicated on the left of the figure.

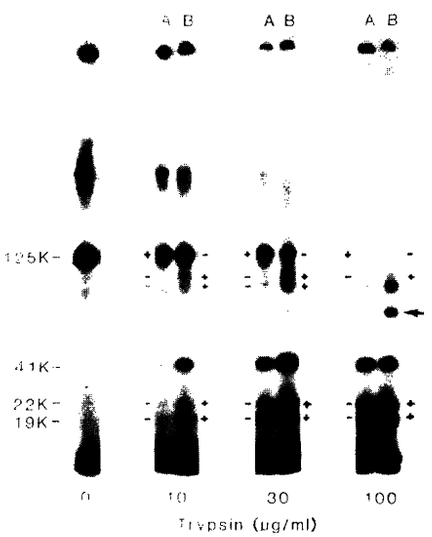

Fig. 2. Tryptic digestion of the insulin receptor in plasma membranes isolated from fat cells. A purified plasma membrane preparation was obtained from adipocytes by the procedure of Kono *et al.* (*13*). These membranes were suspended in Krebs-Ringer phosphate buffer, *p*H 7.4, containing bovine serum albumin (10 mg/ml), and were digested with the trypsin concentrations indicated either before (A) or during (B) the last 2 minutes of a 30-minute incubation period at 24°C with 5×10^{-9}*M* [^{125}I]insulin. Affinity labeling was accomplished by using 0.5 m*M* disuccinimidyl suberate as described in the text and elsewhere (*6, 7*). Depicted is an autoradiograph of 5 to 15 percent acrylamide gradient gel prepared after solubilization of the labeled membranes in sodium dodecyl sulfate. The molecular weight of the intact receptor subunit M_r 125,000 and the major tryptic fragments are given on the left of the figure.