population sample was divided into groups, those individuals heterozygous for that locus, and those homozygous. Phenotypic variance for the heterozygous and homozygous groups was estimated using all seven meristic variables with the average coefficient of variation or population parameter of Soulé[13] and the determinant of the variance–covariance matrix[14].

The null hypothesis tested here is that individuals heterozygous for an enzyme locus have the same level of morphological variation as individuals homozygous for that locus. There are at present no standard errors or tests of significance for either the average coefficient of variation or the determinant of the variance–covariance matrix, so that differences cannot be tested directly. Yet rejection or support of the null hypothesis may be judged by the sign test[15], evaluating the number of comparisons in which heterozygotes have lower phenotypic variance than homozygotes. When heterozygotes are compared with homozygotes using the mean coefficient of variation (Table 1), heterozygotes have lower phenotypic variation than homozygotes in 22 of 30 tests ($P<0.05$). The determinant of the variance–covariance matrix gives more striking results, heterozygotes being less variable than homozygotes in 26 of 30 tests ($P<0.001$).

Several of the loci surveyed in this analysis have been postulated to have an impact in the determination of fitness[12], but the evidence for lactate dehydrogenase (LDH) is most convincing. Gene frequencies differ between populations in artificially heated and control environments, and this observation is consistent with differences in gene frequencies between populations from natural environments that differ in temperature. Furthermore, kinetic differences between LDH genotypes are consistent with the observed geographical variation in allelic frequencies[16]. Yet in this study, as in most studies, it is not possible to discern whether the loci being analysed produce the differences observed, or whether they function in this analysis as markers of segments of chromosomes.

When soluble proteins were first used by population geneticists to assess levels of genetic variation, these markers were presented as a random sample of the genome[17]. Since then, several observations suggest, for a variety of reasons[18–22], that groups of enzymes differ in their levels of genetic variation. If groups of proteins differ in their levels of genetic variation, it is not likely that electrophoretically detectable genetic variation will give an accurate assessment of the total genetic variation of a population. Given this heterogeneity and doubt about just what is being measured, and further doubt concerning the adaptive value of protein variation[23], the results reported here are surprising. On the basis of genetic variation of a single locus, a population can be subdivided into two groups that differ in their levels of morphometric variation (Table 1). This dissection of a population is replicable in three localities, for five loci tested.

This first observation is consonant with observations compiled by Lerner[1], who extensively documented the relationship between higher heterozygosity and enhanced developmental homeostasis, but it raises several other questions. Is it the single protein locus that influences developmental homeostasis, is it loci linked to this locus, or is it heterozygosity correlated with this locus? Does normalising selection acting on morphological variation impinge on protein polymorphisms that influence developmental homeostasis? These data suggest that heterozygosity influences developmental homeostasis, but the source of this effect, and the impact of this effect on evolution is yet to be resolved.

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Immunological memory is regulated in the enhanced rat renal allograft recipient

SUCCESSFUL transplantation of renal allografts without compromising the immune system of the recipient is a goal of clinical transplantation. The enhancement of kidney allografts in the inbred rat provides an experimental system satisfying this goal. Pretreatment of recipient animals with antigen and antibody, alone or in combination, has produced the indefinite survival of renal allografts[1–4], but the mechanisms underlying this specific elimination of responsiveness to the donor graft have not been completely elucidated. Three alternative mechanisms have been proposed to explain the maintenance of the enhanced state: (1) deletion or alteration of donor antigens on the graft; (2) deletion of the relevant antigen-reactive cells; (3) regulation of the host response to the graft. We discuss these suggestions here, and report our own work on enhancement, giving evidence for the role of immunological memory regulation.

Deletion of graft antigens does not account for the maintenance of the enhanced kidney. Antigen in a form and amount sufficient to lead to rejection persists in the donor graft. Stuart *et al.* have shown that an enhanced Lewis × Brown Norway (LBN) F_1 hybrid kidney removed from an enhanced Lewis rat and retransplanted into a normal untreated Lewis recipient is rejected in only a slightly delayed fashion[5]. Furthermore, the accessibility of donor graft antigens in the enhanced animal has been demonstrated by *in vivo* antibody absorption studies[6].

Alteration of donor graft antigens most certainly occurs in the long-term enhanced kidney through the loss of 'passenger leukocytes'. Passenger leukocytes provide a potent antigenic stimulus in the donor allograft and may be a sufficient antigenic stimulus alone to cause the rejection of a renal allograft[7]. Some kinds of leukocytes bear Ia antigens which may be necessary for, or at least augment the activation of amplifier T cells[8]. These amplifier T cells can heighten the response of killer T cells which react mainly with antigens defined by other regions of the major histocompatibility complex[8]. Batchelor *et al.* have proposed that kidney allografts in enhanced recipients are depleted of passenger leukocytes and thus are deficient in Ia antigens needed for the activation of amplifier T cells[9]. These authors suggest that enhanced kidneys are unable to stimulate an effective host response due to the deficit of Ia antigens and the suppressive effects of continued exposure to other MHC antigens. This explanation is supported by the observations that have been made in the thyroid allograft model in the mouse. The previous depletion of passenger cells by short-term *in vitro* culture results in successful long-term transplantation of thyroid allografts in mice, and injection of leukocytes bearing graft-type alloantigens results in rejection of the thyroid

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graft[10]. However, two findings indicate that this mechanism cannot account for renal allograft survival in enhanced recipient rats. As noted above, enhanced kidney allografts are rejected when retransplanted into untreated rats syngeneic with the original recipient[5]. Furthermore, fresh kidney allografts from the same strain as the original enhanced kidney and bearing a full complement of passenger leukocytes are accepted indefinitely by long-term enhanced rats without any additional treatment[5]. Thus, the maintenance of the enhanced state in the rat renal allograft model cannot be explained simply by deletion or alteration of donor graft antigens present either in the kidney or on passenger leukocytes.

Deletion of primary antigen-reactive cells in the host seems not to account for the maintenance of the enhanced kidney. Normal proliferative responses have been obtained with lymphoid cells from enhanced rats in local graft-versus-host assays as well as in mixed leukocyte cultures (MLC) (refs 11–14). Cytolytic responses towards the donor antigens in MLC have been observed with spleen cells from long-term enhanced rats, and the magnitude of the response is comparable to that obtained with spleen cells from normal rats[14]. However, the reactivity of these cell populations has been studied either in normal rats or in tissue culture. A limited number of experiments (3) has been performed to determine whether these cells can develop such reactivity in the enhanced animal, and it has not been definitively shown that such reactivities reflect the functional cell population(s) important in the rejection of an allograft. Nevertheless, the normal reactivity *in vitro* of lymphoid cells from enhanced rats towards the graft antigens indicates that antigen-reactive cells are not deleted in the recipient bearing an enhanced kidney allograft.

As neither deletion or alteration of graft antigens nor deletion of reactive cells account for the enhanced state, regulation of the host response to the donor graft antigens is the only tenable hypothesis. To characterise the regulatory mechanisms involved in allograft enhancement, we examined the reactivity of enhanced rats to a fresh challenge of donor antigen.

Long-term enhanced renal allograft survivors were prepared by pretreating Lewis rats with 5×10^7 LBN spleen cells and, 24 h later, with 1 ml of Lewis anti-BN serum. After 10 d, these rats were bilaterally nephrectomised and an LBN kidney was transplanted. Most rats receiving this treatment survive indefinitely without signs of rejection[12]. All long-term enhanced rats used in this study had survived 35 d or longer without elevation of their blood urea nitrogen (BUN).

We evaluated the reactivity of these animals to donor antigens using a local popliteal node host-versus-graft assay[15]. Normal Lewis or long-term enhanced Lewis rats received either 10^7 Lewis, LBN or Lewis×ACI (LACI) F_1 spleen cells in the hind footpads. Local popliteal nodes were removed after 5 d and weighed to assess node enlargement. As Table 1 indicates, the response of long-term enhanced rats to donor as well as third party alloantigens present on spleen cells was

Table 1 Local host-versus-graft response of normal Lewis and long-term renal allograft recipients*

Animal injected	Popliteal lymph node weight (mg) Spleen cells injected Lewis	LBN	LACI
Normal Lewis			
Group 1	6.4±0.4	35.2±5.1	
Group 2		33.4±0.6	42.3±0.9
Lewis with long-term enhanced LBN kidney			
Group 1	7.2±1.4	29.6±2.0	
Group 2		25.9±1.9	26.4±3.0

* Normal Lewis or Lewis rats bearing enhanced LBN renal allografts received in the footpad 10^7 spleen cells from the indicated donor. After 5 d the ipsilateral popliteal nodes were weighed. Values are means ± s.e.m.

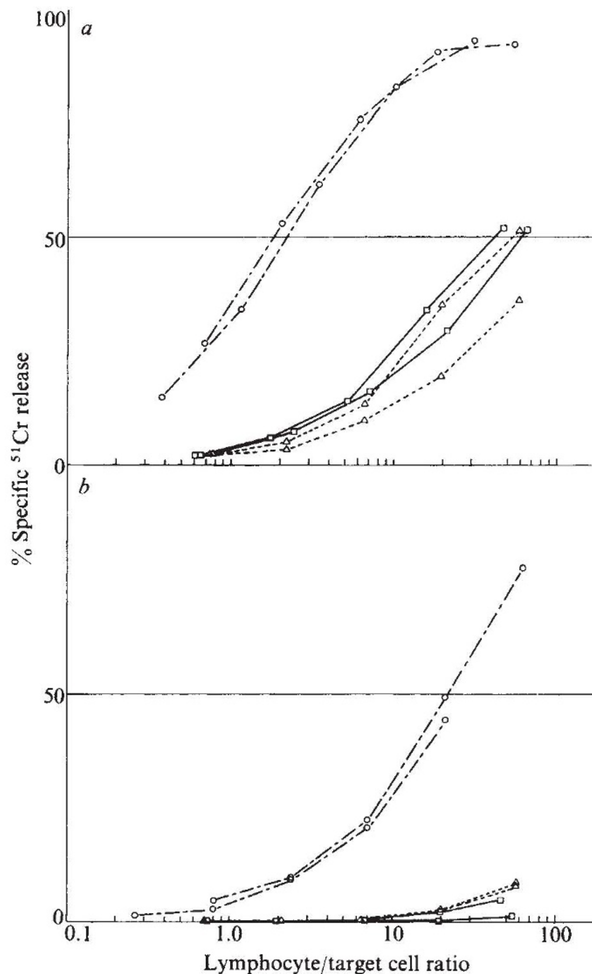

Fig. 1 Generation of cytolytic activity in cultures prepared with spleen cells from normal Lewis rats (□), or Lewis rats (○) and long-term enhanced renal allograft recipients (△) challenged with LBN spleen cells subcutaneously 3 weeks previously. MLC were prepared with carbonyl iron-treated spleen cells from two animals of the indicated group and either X-irradiated BN cells (*a*) or subcellular BN antigen (*b*). Cytolytic activity generated was assessed after 7 d in a 6-h ^{51}Cr release assay using cloned BN lymphoma targets. Subcellular antigen preparations were used to distinguish further the secondary CTL precursors from the primary CTL precursors[18].

nearly equivalent to that of normal rats, as shown by the increase in popliteal node weights. Thus, the enhanced renal allograft recipient can recognise and respond to donor alloantigens on leukocytes. In other experiments, long-term rats were injected with donor leukocytes subcutaneously, and BUN was measured frequently over a period of 30 d. No increase in BUN was observed. Thus, despite the ability of rats bearing enhanced renal allografts to respond to donor antigens on leukocytes, the activation of antigen-reactive cells in these rats did not lead to the rejection of the kidney allograft.

We examined whether memory cells for cell-mediated immune responses were generated in enhanced renal allograft recipients which had been challenged with antigen. MLC were prepared with spleen cells from normal Lewis rats, or normal Lewis rats and long-term enhanced Lewis rats which had been injected subcutaneously three weeks previously with 10^7 LBN spleen cells. Secondary cytolytic responses were obtained with spleen cells from normal Lewis rats which had been challenged with LBN spleen cells. However, the level of cytolytic activity generated in cultures prepared with cells from long-term enhanced rats which had been challenged with LBN antigen were comparable with those generated in cultures of normal spleen cells (Fig. 1). Secondary cytolytic responses were obtained with cells from long-term enhanced rats when the

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response to third-party antigen (LACI) was examined (data not shown). Thus, an inability to develop or maintain memory cells for cell-mediated responses such as generation of cytolytic T lymphocytes (CTL) is an apparent defect in the long-term enhanced rat. These results are particularly interesting in relation to the results obtained previously with skin grafts of long-term enhanced rats. Although LBN skin grafts were rejected in only a slightly prolonged fashion[5], rejection of a second LBN skin graft was further prolonged in enhanced rats in contrast to the accelerated rejection of second set skin grafts in normal Lewis rats (F.P.S., unpublished data).

Although donor antigens on leukocytes are recognised and antigen reactive cells are activated in the enhanced rat, the subsequent development or maintenance of memory CTL precursors seems not to occur. These results demonstrate that the host immune response to donor antigens is regulated in long-term enhanced rat renal allograft recipients. This defect in development of immunologic memory may be important for the maintenance of the enhanced state. Memory CTL may represent a highly differentiated population of cells important in the rejection of renal allografts because of their heightened cytolytic activity. Failure to develop memory may be sufficient to account for the survival of renal allografts. Indeed, rejection of most tissue allografts is accompanied by the appearance of memory cells.

The mechanisms accounting for the failure to develop immunological memory are not known. Although anti-idiotypic antibody has been detected in the serum of antigen and antibody pretreated Lewis rats at the optimal time for transplantation,[12] a direct role for such antibody in enhancement or in the suppression of memory cells has not been shown. Occasionally, suppressive activity was observed when spleen cells from long-term enhanced rats were adoptively transferred to new renal allograft recipients[16]. Batchelor *et al.* were unable to detect suppressor cell activity in rats bearing enhanced renal allografts[9]. However, their interpretations seem somewhat limited, as skin graft survival was used to measure suppressor cell activity from renal allograft recipients, and it is well known that skin grafts are not readily accepted by long-term enhanced renal allograft recipients[5]. The possible role of suppressor cells in prevention of immunological memory is now under investigation. An alternative mechanism for the failure to develop immunological memory could involve the opsonisation of antigen-reactive cells in long-term enhanced rats, as proposed by Hutchinson and Zola[17]. As normal primary responses are obtained with cells from long-term enhanced rats, such a mechanism would have to eliminate selectively only memory cells that might be generated. Whatever the mechanism, regulation of the host immune response seems to be an important and perhaps major process involved in rat renal allograft enhancement.

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Immune response (*Ir*) genes expressed at macrophage–B lymphocyte interactions

THERE are two major types of genetic control of antibody responses. One is linked to immunoglobulin (Ig) allotype, and the relevant genes are Ig heavy-chain variable-region genes, which control receptor structures on B cells[1]. The other major region controlling immune responses lies in the major histocompatibility complex (MHC), a genetic region discovered due to its predominant influence on graft reactions, but also containing immune response (*Ir*) genes[2]. The mode of action of the MHC-linked *Ir* genes is not known. Many hypotheses have been proposed both for their cellular site of action, that is, on T cells, B cells or macrophages, and for their mechanism of action[2–4]. Recently, we discussed the possibility that as macrophages are required for T and B lymphocyte activities, the available data were compatible with *Ir* genes expressed at the

Table 1 Induction of response to (T,G)-A--L by F_1 or R macrophages from F_1 (R×NR) B cells *in vitro*

Antigen	Stimulus HF	PE cell	Anti-(T,G)-A--L response IgM AFC per $15\times10^6\pm$s.e.
–	–	–	57±7
+	–	–	47±13
+	+	–	30±15
–	–	F_1	37±3
+	–	F_1	40±10
+	+	F_1	217±34*
–	–	B10	13±13
+	–	B10	47±12
+	+	B10	193±18*
–	–	B10.A	30±15
+	–	B10.A	50±6
+	+	B10.A	57±18

Response of $(B10\times B10.A)F_1B$ cells from mouse spleen to (T,G)-A--L. T cells were removed by lysis with sheep anti-mouse T-cell serum[11] and complement, and then macrophages were removed by using carbonyl iron[11]. 40% of cells were lysed with anti-T cell serum, and a 50% cell loss with the carbonyl iron. Helper factor (HF) to (T,G)-A--L obtained from CBA helper cells made as described earlier[23] were used at a concentration of 1/1,000, batch 237, the optimum found previously[9]. Response was measured in Marbrook flasks, with 15×10^6 cells cultured in 1 ml HEPES-buffered medium containing 5% foetal calf serum, and 1 $\mu g\ ml^{-1}$ of antigen, at day three. Sheep red cells were coated with (T,G)-A--L using $CrCl_3$, as described by Taussig[24] in a Cunningham type plaque assay[25]. The efficiency of the anti-T serum treatment was monitored by inhibition of the anti-SRBC response *in vitro*, without inhibiting the response to DNP acrylamide bends[25]. Macrophages used were from normal peritoneal washings; 3×10^5 cells were added for the restoration. Three experiments of this type have been carried out with analogous results, using $(B10\times B10.Br)F_1$ mice also.

* $P<0.01$, numbers statistically significantly different from the appropriate background by *t*-test. Background is control+appropriate PE cells+antigen alone.

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