analysis presented here, it is also possible to analyze the suggestion of Turcotte and Oxburgh that the observed exponential depth dependence of radioactive heat sources in the earth's crust arose from their equilibrium distribution under some appropriately fluid conditions. If their idea were correct, my Eq. 7, or approximately Eq. 9, would describe the result. This result follows directly from equilibrium thermodynamics; there is no need to employ the Boltzmann factor from statistical mechanics. Furthermore, the quantity in parentheses in their equation 3, the result of their hypothesis, has the wrong sign and the wrong density in the denominator; according to my Eq. 9, their result should read

$$\frac{X_i(y)}{X_i(0)} = \frac{H}{H_s} = \exp\left[\frac{(M_i - v_i\rho)gy}{RT}\right] = \exp\left(\frac{\Delta\rho M_i gy}{\rho_i RT}\right) \quad (12)$$

where H is the radioactive heat released in unit volume per unit time at the depth y; H_s is the value of H at the surface, where $y = 0$; $\Delta\rho = \rho_i - \rho$; ρ_i is the density of the molecular assembly under consideration; and ρ is the density of the solution. As Turcotte and Oxburgh observe, this distribution should hold for all chemical species frozen into an equilibrium distribution at a constant temperature and then not subsequently disturbed, provided they obey Henry's law and the solution is incompressible.

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1. W. O. Fenn, *Science* **176**, 1011 (1972). On the basis of my reading of Fenn's report, I believe the following changes should be made: line 4 after equation 1, change "g" to "mole"; equation 2, change "*Mx*" to "*Mgx*"; line 10 after equation 3, insert "any" after "at."
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Ragweed Hay Fever: Genetic Control and Linkage to HL-A Haplotypes

Abstract. *Clinical ragweed pollenosis (hay fever) and IgE antibody production specific for antigen E (the major purified protein antigen from ragweed pollen extract) correlated closely with HL-A haplotypes in successive generations of seven families. HL-A associated IgE antibody responsiveness was antigen specific and extended also to IgE antibody production. These data indicate an immune response (Ir) gene specific for antigen E necessary but not sufficient for the development of hay fever. This appears to be the first documentation of an Ir gene in man.*

Classical studies in clinical allergy (*1*) have shown a preponderence of atopic allergic diseases in successive generations of certain families. These statistical data have been interpreted as indicating a genetic predisposition to the later development of these diseases (*1*), although the nature of genetic factors involved has not been identified. The present study is on ragweed pollenosis (hay fever), an example of an allergic disease mediated by IgE antibodies (reagins) (*2*) to airborne allergens (pollen particles). Genetic studies on reagin (IgE) production in inbred strains of mice have demonstrated two kinds of genetic controls of reagin production (*3*). One genetic factor permits the production of high serum levels of reagin to many antigens (*3*). The other control system is by genes at a locus (or loci) closely linked to the H-2 major histocompatibility system, termed Ir (or immune response) genes (*4*). The Ir genes control immune responsiveness to synthetic polypeptide antigens and to minute doses of protein antigens (*4, 5*). This system shows antigen specificity. In most mouse strains, reagin production is a prominent part of the immune response (*6*). Ragweed hay fever in man shows many features similar to these immune response systems in the mouse (*6*). A small percentage of people exposed to minute doses of ragweed pollen particles develop an immune response in which IgE antibody is prominent. Antigenic specificity of ragweed hay fever is largely toward antigen E, a purified protein derived from ragweed pollen extract (*7*). Accordingly, we suggested that one kind of genetic control of ragweed hay fever might be by Ir genes specific for antigen E (*8*). We now report studies supporting this idea.

Seven families in which ragweed hay fever occurred in more than one member were studied for clinical ragweed hay fever, IgE response to antigen E, and HL-A haplotype (*9*). Figure 1 depicts one of the seven families. In this family, four of six (67 percent) family members having the HL-A1, HL-A8(1,8) haplotype had intense immediate wheal-and-flare skin reactivity to antigen E in dilute solutions, indicating IgE antibody (*9*), and severe ragweed hay fever. Neither of the two subjects having the other HL-A haplotype of the propositus (HL-A10,12) had immediate skin reactivity to antigen E or clinical ragweed hay fever. Of the blood relatives of the propositus who lacked the 1,8 haplotype, none of the seven had ragweed hay fever. Six of the seven did not have skin reactivity to antigen E. One of the seven (subject 11, a grandson of the propositus) had relatively weak skin reactivity to antigen E. In this exceptional case, another haplotype correlating with IgE antibody responsiveness to antigen E was inherited from the maternal side of the family. Table 1 and Fig. 1 show that the maternal grandfather, a maternal uncle, the mother, and subject 11 had the HL-A9,x haplotype, and that subject 11, his maternal grandfather, and his uncle had weak skin reactivity to antigen E without clinical hay fever.

The data for the seven families are totaled in Table 2. Of 26 family members having the hay fever–associated haplotypes, 20 members (77 percent) had ragweed hay fever and intense skin reactivity to antigen E (Table 2). By contrast, none of the 11 family members who had the other haplotypes of the propositi (and lacked the hay fever–associated haplotype) had clinical ragweed hay fever. Of these 11, 10 did not have skin reactivity to antigen E, and 1 had relatively weak skin reactivity to antigen E. This difference in the frequency of IgE antibody responsiveness to antigen E (and clinical ragweed hay fever) between the family members having the hay fever–associated haplotype and the family members having the other haplotype of

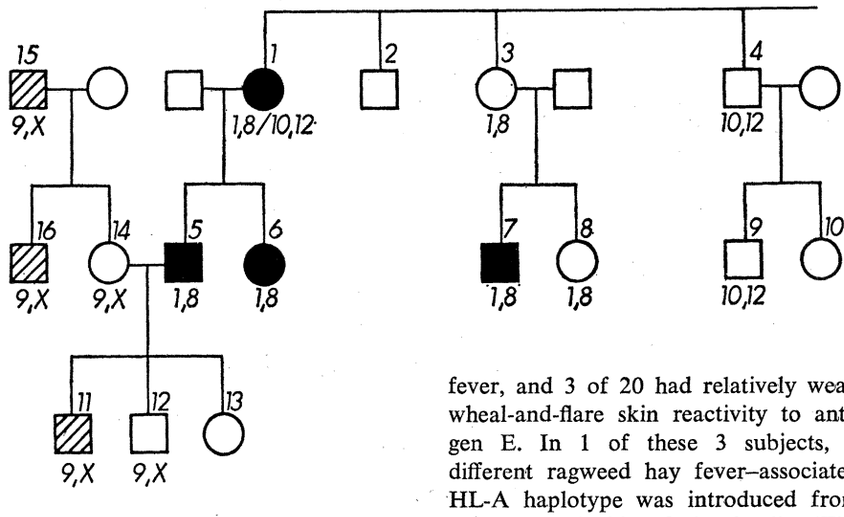

Fig. 1. Representation of family R. Solid circles or squares represent subjects with ragweed hay fever. Hatched squares represent subjects with relatively weak skin reactivity to antigen E but no hay fever. Open circles and squares represent subjects without skin reactivity to antigen E and without hay fever. Numbers below the symbols are haplotypes. Numbers above the symbols represent subject number for whom genetic and immunological data are in Table 1.

the propositus is statistically significant ($P < .01$). Table 2 shows also the frequency of IgE antibody responsiveness to antigen E and ragweed hay fever in the blood relatives of the propositus who had neither of the haplotypes of the propositus. None of the 20 subjects in this group had clinical hay fever, and 3 of 20 had relatively weak wheal-and-flare skin reactivity to antigen E. In 1 of these 3 subjects, a different ragweed hay fever–associated HL-A haplotype was introduced from the maternal side of the family (Fig. 1).

These results show a statistically highly significant association of clinical ragweed hay fever and associated intense IgE antibody immune response specific for antigen E with an HL-A haplotype in successive generations of the seven families studied. This close association between intense IgE antibody responsiveness to antigen E (and ragweed hay fever) and HL-A haplotype through successive generations of the seven families implies close genetic linkage betwen the HL-A system and a genetic locus controlling immune responsiveness to antigen E. If this immune responsiveness gene is similar to the Ir loci in the mouse (*4*, *5*), then it should also control the production of IgG antibody specific for antigen E, and immune responsiveness should be antigen specific. Preliminary studies on these two parameters have been done. First, serum IgG antibodies specific for antigen E was assayed (*10*) in subjects from the family depicted in Fig. 1. Three kinds of subjects were chosen. Subjects 1 and 6, having the hay fever–associated haplotype and intense IgE antibody responsiveness, were found to have small amounts of IgG antibody to antigen E in their serums.. Subjects 4 and 9, having the other haplotype of the propositus, had no detectable IgG antibody for antigen E in their serums. Subjects 3 and 8, having the hay fever–associated haplotype, but no hay fever or wheal-and-flare reactivity to antigen E, nevertheless had trace amounts of IgG antibody to antigen in their serums. Thus the gene controlling IgE immune responsiveness to antigen E also appears, by these preliminary data, to control IgG responsiveness to antigen E.

As an initial study of antigenic specificity of system, the 37 family members with either haplotype of the propositi were skin tested for wheal-and-flare reactivity with dilute [10 protein nitrogen units (PNU)/ml] solutions of timothy grass pollen extract and cat dander (hair) extract. Intense skin reactivity (3+ to 4+) to dilute solutions of timothy pollen extract was found in 7 of 26 (27 percent) of the family members with the hay fever–associated haplotype and in 2 of the 11 (18 percent) of the family members with the other haplotype of the propositus. Intense skin reactivity (3+ to 4+) to dilute solutions of cat dander extract

Table 1. Genetic and immunological data for subjects in family R (Fig. 1); PNU, protein nitrogen unit.

Subject	Age (years)	HL-A haplotype	Ragweed hay fever	Immediate wheal-and-flare skin reactivity			
				Ragweed extract (PNU/ml)		Antigen E (μg/ml)	
				100	1	10^{-3}	10^{-5}
1	60	1,8/10,12	Severe	—	4+	3+	1+
2	48	10,x/9,5	None	0	0	0	0
3	49	1,8/10,12	None	0	0	0	0
4	58	10,x/10,12	None	0	0	0	0
5	37	1,8/10,x	Severe	—	3+	2+	1+
6	35	1,8/10,x	Severe	—	4+	4+	1+
7	24	1,8/5,x	Severe	—	4+	3+	1+
8	19	1,8/11R	None	0	0	0	0
9	25	2,7/10,12	None	0	0	0	0
10	26	2,7/10,x	None	0	0	0	0
11	12	9,x/10,x	None	—	2+	2+	0
12	16	9,x/10,4	None	0	0	0	0
13	14	10,x/1,Mapi	None	0	0	0	0
14	36	9,x/1,Mapi	None	0	0	0	0
15	59	9,x/x,x	None	4+	2+	1+	0
16	24	9,x/1,Mapi	None	3+	1+	1+	0

Table 2. Association of IgE immune response to antigen E—as detected by direct wheal-and-flare skin reactivity to dilute solutions of allergens (*9*)—and ragweed hay fever with HL-A haplotype in successive generations. Data for seven families are totaled. Group A consists of those family members, including the propositus and blood relatives of the propositus, having ragweed hay fever and intense wheal-and-flare reactivity to antigen E; Group B consists of those family members, including the propositus and blood relatives of the propositus, having relatively weak wheal-and-flare reactivity to antigen E, but without ragweed hay fever. For definition of "intense" and "relatively weak" wheal-and-flare skin reactivity to antigen E, see (*9*) and the legends to Table 1 and Fig. 1.

Family members	Group A		Group B	
	Ratio	Percent	Ratio	Percent
Hay fever–associated haplotype	20/26	77		
Other haplotype of the propositus*	0/11		1/11	9
No hay fever–associated haplotype†	0/20		3/20	15

* Excluding family members with both haplotypes of the propositus. † This group of 20 includes the 11 family members with the other haplotype of the propositus.

was found in 2 of the 26 (8 percent) of the family members with the ragweed hay fever–associated haplotype and in 2 of the 11 (18 percent) family members with the other haplotype of the propositus. Thus the HL-A linked genetic control of immune responsiveness to antigen E appears to show antigenic specificity, that is, IgE antibody responsiveness did not extend to two unrelated antigens.

The data presented above thus indicate the presence in human beings of a genetic locus closely linked to the HL-A system which controls IgE antibody responsiveness to antigen E and permits the development of ragweed hay fever. Preliminary data further indicate that this genetic factor controls immune responsiveness in at least two antibody classes, IgE and IgG, and shows antigenic specificity. This genetic factor thus is similar in immunological properties to H-2 linked Ir genes in mice (*4*, *5*). We tentatively refer to it as a human Ir gene and call this gene the "Ir–antigen E" gene. This appears to be the first documentation of an Ir gene in man.

Several points merit brief discussion. First, in the seven families studied, the Ir–antigen E gene was linked to seven different HL-A haplotypes [W-28,x; HL-A10,Da (*6*); HL-A3,7; HL-A3,5; HL-A1,8; HL-A2,x; and HL-A2,W-14]. Whether one or more of these haplotypes will be frequently or always linked to an Ir–antigen E gene in this or in other populations remains to be determined. In this regard, it is not known whether only one or several different Ir–antigen E genes exist. Further, we presume that Ir genes specific for other antigens exist in man, and that these may be necessary for the development of immunological diseases specific for other antigens. Second, a rough estimate of the frequency of Ir–antigen E genes in our local population can be given. Approximately 10 percent of our local adult population shows intense wheal-and-flare reactivity to antigen E and has clinical ragweed hay fever (*11*). If we assume that the expressivity of the Ir–antigen E gene (in an intense IgE antibody response, see below) is 0.2 to 0.4 (*12*), then 22 to 45 percent of the population may possess an Ir–antigen E gene (*13*).

The presence of an Ir–antigen E gene is viewed as necessary but not sufficient for the development of an intense IgE immune response to antigen E and clinical hay fever. The trait controlled by the Ir gene is inherited as a Mendelian dominant. Its expression, to permit the individual to mount an IgE immune response to repeated minute doses of antigen E, requires additional factors, both genetic and environmental. Among required environmental factors would be adequate exposure to airborne allergens, and its diffusion through mucous membranes to lymphoid cells. An additional genetic factor might be one similar to a genetic control described in mice, which permits a strong IgE antibody response to a variety of antigens (*3*). That such a genetic factor might also be operative in man is suggested by the present observations that of the 26 subjects possessing an Ir–antigen E gene, significantly more of the 20 subjects with ragweed hay fever had also an intense IgE antibody response to timothy pollen (7 of 20) than of the 6 subjects who did not have ragweed hay fever (0 of 6). Also, Hamburger *et al.* (*14*) have suggested a genetic control of basal IgE levels in man on the basis of statistical analysis of serum IgE levels in normal adults. In addition, factors unrelated to function of lymphatic tissues have been suggested as influencing immune responsiveness to airborne allergens, that is, permeability of mucous membranes (*15*). Other nonimmune factors are probably also operative in determining, in patients with intense IgE antibody responses to ragweed antigens, the nature of the clinical result (for example, rhinitis as compared to rhinitis plus asthma) and its severity (*16*).

Finally, we wish to point out a potential clinical usefulness for these findings. Haplotyping of families having high incidences of antigen-specific allergic diseases might serve to identify those young family members at risk for these diseases in which preventive measures might be instituted.

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7. T. P. King, P. S. Norman, L. M. Lichtenstein, *Ann. Allergy* **25**, 541 (1967).
8. B. B. Levine, John Sheldon Memorial Lecture at the annual meeting of the American Academy of Allergy, Chicago, February 1971.
9. Clinical hay fever was based on history of seasonal rhinitis (some with asthma or conjunctivitis, or both) 10 August to 30 September. Skin testing was with antigen E (provided by Dr. T. P. King) and ragweed pollen extract. Patients with clinical hay fever gave positive wheal-and-flare reactions at concentrations down to 10^{-4} to 10^{-5} μg/ml. Negative skin reactors were those failing to react to antigen E at 0.1 μg/ml. Hay fever patients reacted to ragweed to 0.1 to 0.01 PNU/ml. Positive skin reaction is generally accepted as an assay for IgE antibodies fixed to skin receptors. Lymphocyte typing was done on lymphocytes separated from peripheral blood by means of a Ficoll–Isopaque centrifugation procedure [M. Fotino, E. J. Merson, F. H. Allen, Jr., *Vox Sang.* **21**, 469 (1971)]. A two-stage microtoxicity assay procedure was used [M. Fotino, E. J. Merson, P. Benoit, A. W. Bowe, F. H. Allen, Jr., in *Histocompatibility Testing*, E. S. Curtoni, P. L. Mattiuz, R. M. Tosi, Eds. (Munksgaard, Copenhagen, 1967), p. 429]. Sixty typing serums were used. These were obtained from the New York Blood Center and the Transplantation Immunology Branch Serum Bank of NIAID. Twenty-two antigens were typed for.
10. These assays were performed by Dr. Kimishage Ishizaka. A double antibody radioimmunoprecipitation method was used with an antigen D preparation as antigen. Antigen D is a protein fraction purified from ragweed pollen extract and consists of antigen K and antigen E in equal amounts. Antigens E and K are partially cross-reactive IgG antibody concentrations by this technique are currently being expressed in relatively qualitative terms.
11. R. H. Stember and B. B. Levine, unpublished.
12. In the families studied, 20/26 (77 percent) of the subjects possessing the Ir–antigen E gene had ragweed hay fever. The seven families were not randomly selected however, but were basically selected for "expressivity." That is, an equal number of families were interviewed but not selected for study because clinical hay fever, while present in the propositus, did not appear in other members of the family. Thus, the 0.77 genetic expressivity observed in this study may be about twice or three times as high as in the general population.
13. About 40 percent of patients with ragweed hay fever also have intense wheal-and-flare skin reactivity and clinical hay fever to timothy grass pollen allergens. By contrast, only 4 percent of subjects without ragweed hay fever have grass pollen hay fever and intense skin reactivity (R. H. Stember and B. B. Levine, unpublished). These data are consistent with a high (about 40 percent) prevalence of individuals possessing Ir–antigen E genes in our population, along with a relatively low expressivity as an intense IgE antibody response (0.2 to 0.3) of the gene.
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17. Supported by contract DADA 17-67-7119 from the U.S. Army Medical Research Development Command, by grant HE 09011 from National Heart and Lung Institute, and a grant from the Irwin Strasburger Memorial Medical Foundation.

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