showed a molecular weight of 128, which agreed with this assignment. In addition, a prominent peak at 100 *m/e*, corresponding to loss of ethylene from the molecular ion, indicated a MacLafferty rearrangement of a propyl ketone. Another prominent peak was at 110 *m/e*, corresponding to loss of water. The other prominent peaks were the ones expected by α cleavage at the carbonyl groups of 4-oxoheptanal: 85, 71, 57, 43, and 29. Thus, the sex pheromone is one of the *Z, E* isomers of 7-methyl-3-propyl-2,6-decadien-1-ol.

A mixture of *Z, E* isomers of the pheromone was synthesized by the sequence in Fig. 1. When the synthetic sex pheromone was subjected to gas chromatography on Carbowax 20 M, the first and second isomers, in order of elution, were entirely resolved while the third and fourth were only partially resolved. The second isomer had the same retention time as the pheromone on columns of Apiezon L, SE-30, OV-210, and cyclohexanedimethanol succinate as well as on Carbowax 20 M. The infrared and mass spectra of the second isomer were also identical to those of the pheromone. Since *E* isomers elute after *Z* isomers (*10*), the first isomer should be *Z, Z* and the fourth should be *E, E*. Also, the nuclear magnetic resonance chemical shift of the methyl group has been established as 1.65 for the *E* configuration and 1.70 for the *Z* configuration (*10*). Therefore, the structure of the sex pheromone is (2*Z*, 6*E*)-7-methyl-3-propyl-2,6-decadien-1-ol.

Attractancy tests in a field cage (7.9 m wide by 3.6 m high by 22 m long) indicated that the synthetic and natural pheromones were equally attractive. Each attracted about 15 percent of the males attracted by ten females per trap. Quantities ranging from 1 μg to 200 μg per trap were tested and there was no increase in attractancy above 10 μg per trap. Therefore some other material is also necessary for full attractancy.

The possibility that the sex pheromone was an artifact was considered. To test this possibility, condensed vapors from air passed over virgin females were analyzed by gas chromatography. Although enough pheromone to give a peak was not obtained, the bioassay indicated an active fraction with the same retention time as the pheromone on Carbowax 20 M. The active fraction was collected and reinjected into an Apiezon L column. Again the bioassay indicated an active fraction with the same retention time as the pheromone. Therefore, (2*Z*, 6*E*)-7-methyl-3-propyl-2,6-decadien-1-ol is a true sex pheromone of the codling moth.

E,E-8,10-Dodecadien-1-ol has been reported to be a sex attractant of the codling moth (*11*). Also, although structural studies of the sex pheromone were not carried out, it was proposed as the sex pheromone, based primarily on electroantennograms of model compounds (*11*). In work to be reported elsewhere, we found that the codling moth possesses multiple sex pheromones. As of this writing, however, we have not found the presence of *E,E*-8,10-dodecadien-1-ol in codling moths.

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5. The infrared spectrum was obtained from 80 μg of pheromone in a 1.5-mm KBr disc with a Perkin-Elmer model 337 equipped with a microbeam condenser.
6. The nuclear magnetic resonance spectrum was obtained from 900 μg of pheromone in a 60-μl microcell with a Varian model HA-100 equipped with a computer of average transients (40 runs).
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Genetics of the Antibody Response to Dextran in Mice

Abstract. *The immune response to dextran having the α-1,3 linkage may be under the control of antibody structural genes. Mice that respond well to this antigen produce antibody restricted with respect to light chain class (λ) and to an antigenic determinant resulting from a particular heavy and light chain interaction. The response to dextran is controlled by a locus linked to the heavy chain locus.*

The antibody light chains, kappa and lambda, have been divided into subgroups on the basis of their variable region sequence similarity to various prototype sequences (*1*). A comparison of the sequences of mouse λ chains produced by plasmacytomas in BALB/c mice showed that they belong to one λ variable region (V_λ) subgroup. Since the variants within this subgroup were confined to hypervariable regions and could be accounted for by single base changes in DNA, we postulated that the V_λ subgroup is coded for by a single V_λ gene which evolves somatically by mutation and antigenic selection. The sequence coded for by germ-line gene, $V_{\lambda(0)}$, would be the one which occurred most frequently. This prototype sequence, $V_{\lambda(0)}$, was found to be a subunit of those myeloma proteins which bind to α-1,3-dextran (*2*). These proteins also show a similar antigenic determinant dependent upon a particular light chain and heavy chain combination. This determinant (that is, idiotype) is related to specific antigen binding since nigerose (a disaccharide with the α-1,3 linkage) will inhibit the reaction between idiotype and antiserum to the idiotype (*3*). Variable regions of these myeloma proteins could be coded for by two germ-line genes, one of which is $V_{\lambda(0)}$ and the other $V_{H(0)}$, one of the heavy chain variable (V_H) genes. If this were the case, BALB/c mice immunized with α-1,3-dextran should respond with antibody having the characteristics of the myeloma proteins which are antibodies to dextran. Furthermore, germ-line polymorphism in either gene $V_{\lambda(0)}$ or $V_{H(0)}$ might render mice unresponsive to α-1,3-dextran. Responsiveness would show dominant inheritance.

We have tested these possibilities by analyzing the response of inbred mice to dextran containing the α-1,3-glucosyl linkage. Mice were immunized subcutaneously with 100 μg of dextran B-1355S (57 percent α-1,6; 35 percent α-1,3; and 8 percent α-1,4 linkages) (*4*) in complete Freund's adjuvant. Antibody response to dextran was tested after 5 days by the Mishell-Dutton modification (*5*) of the Jerne plaque assay, with the use of sheep red blood cells (SRBC) sensitized with either dextran

B-1355S or dextran B-512S (95 percent α-1,6 and 5 percent α-1,4 linkages) (*4*) according to the method of Leon *et al.* (*6*).

The light chain class to which the antibodies to dextran belonged was determined by testing inhibition of plaques with either antiserum to lambda chains (R-132 from K. R. McIntire, National Institutes of Health), or antiserum to kappa chains (antiserum to P10, provided by Paul Knopf, Salk) added to the agarose solution. The idiotype of the antibody to dextran was analyzed as follows. A purified IgA myeloma protein, J558, with antibody activity to dextran, was used to make an antiserum to the idiotype by immunizing strain A/He mice by the method of Lieberman and Potter (*7*). The antiserum to J558 reacts uniquely with those myeloma proteins with antibody activity to α-1,3-dextran, whereas it does not react with normal BALB/c serum proteins (*3*). Myeloma proteins without antibody activity to dextran, yet containing the $V_{\lambda(0)}$ light chain, do not react; therefore this antiserum reacts with an idiotypic determinant which depends in part on the $V_{H(0)}$ domain. In order to decrease any nonspecific effects, all antiserums were inactivated by heating at 56°C for 30 minutes and absorbed at 0°C for 1 hour successively with packed cells (percent by volume) as follows: 10 percent P3 cells (BALB/c myeloma in tissue culture) (*8*), 10 percent S49 cells (a BALB/c lymphoma in tissue culture) (*8*), and 10 percent sheep red cells sensitized with dextran B-1355S.

The specificity of the dextran response was tested in BALB/c and C57BL/6 immunized with dextran B-1355S. Spleen cells from these mice were tested for their ability to form plaques against SRBC sensitized with either dextran B-1355S (containing α-1,3 linkages) or dextran B-512S (containing primarily α-1,6, but no α-1,3 linkages). Plaque formation can only be detected on SRBC sensitized with dextran B-1355S. No detectable plaques were found against B-512S–sensitized red cells although they were agglutinable by $\alpha\kappa$ (α heavy chain and κ light chain) myeloma proteins with specificity for dextran with the α-1,6 linkage, showing that determinants on B-512S are accessible. This suggests that the antibodies are primarily directed toward an antigenic determinant that depends on α-1,3 linkages.

The light chain class and idiotype of the antibody to α-1,3-dextran were determined by testing the inhibition of plaque formation with antiserums. The majority of cells induced by dextran B-1355S in BALB/c mice produce antibody in the lambda class (since plaques are inhibited with antiserum to lambda but not with antiserum to kappa) which shares the idiotypic determinant of the myeloma protein J558, which has antibody activity to dextran (Table 1). Because the lambda class comprises only 3 to 5 percent of mouse light chains, the response to α-1,3-dextran is highly restricted with respect to the light chain. That the unique idiotypic determinant common to myelomas with antibody activity to dextran is found for normal antibody to dextran suggests that the V regions of antibodies to dextran are homogeneous.

Table 1. Characteristics of the antibody response to dextran: inhibition of plaques.

Strain	Percent inhibition by antiserum to		
	Lambda chain	Kappa chain	Idiotype
BALB/c	86–92	0–10	86–100
F_1(BALB/c × C57BL/6)	90–97	4	90
BAB/14*	95–97	10–12	90
C58	95	20	91
129	97	16	
C57BL/6	15–20	100	0–20
AKR		95	
NZB		90	

* See (*12*).

These characteristics, however, are only found for certain strains of mice, whereas other strains produce antibody to α-1,3-dextran mainly in the κ light chain class and without the test idiotype (Table 1). This difference is related to responsiveness to dextran B-1355S. Those strains which produce an antibody restricted to the λ class that also has the test idiotypic determinant respond well, whereas those strains that express antibodies in the κ class without the test idiotype respond poorly (Table 2). The ability to respond well is dominant, as shown by the response of the F_1 hybrid of a high responder (BALB/c) and low responder strain (C57BL/6).

The linkage relationships of the dextran response have been investigated by testing seven recombinant inbred (RI) strains derived from the F_2 generation of C57BL/6 × BALB/c crosses (*9*). Mice were assayed for their response to dextran 5 days after immunization by a hemagglutination assay and by the plaque assay. Of the seven strains only two (C × BG and C × BJ) respond in a manner characteristic of BALB/c in terms of the level and idiotype. The BALB/c H-2 allele, d, is found only in strains C × BD and C × BH (*9*). Lack of concordance of the response to dextran with the H-2 locus

Table 2. Response to α-1,3-glucosyl linkages in dextran. The range for BALB/c (20 mice) was 1000 to 2800 plaque-forming cells per 10^6 cells; for C57BL/6 (15 mice) 50 to 160 plaque-forming cells per 10^6 cells.

Strain	Plaques per 10^6 cells at 5 days (mean)	H-2*	Allotype†	Light chain	Idiotype
BALB/c	1710	d	BALB/c	λ	J558
129	1635	b	BALB/c	λ	J558‡
C58	685	k	BALB/c	λ	J558
BAB/14	2080	d	C57BL/6	λ	J558
F_1 (BALB/c × C57BL/6)	1140	d/b	BALB/c C57BL/6	λ	J558
C57BL/6	110	b	C57BL/6	κ	Not J558
SJL	40	s	C57BL/6	κ	
CBA	41	k	BALB/c	κ	
AKR	40	k	AL	κ	
A/He	10	a	AL	κ	
NZB	33	d	AL	κ	

* The H-2 typings have been taken from (*13*). † Designated by prototype (*14*). ‡ This idiotype determination is described in (*3*).

shows that the locus determining response to dextran is not linked to the H-2 locus (linkage group IX), confirming this conclusion as based on the strain survey (Table 2).

The strain survey of the dextran response suggested that this locus was unlinked to the heavy chain allotype locus (C_H) as based on the response characteristics of CBA and BAB/14 (Table 2). The CBA strain is a low responder, yet has the BALB/c allotype, and BAB/14 is a high responder with C57BL/6 allotype. This conclusion, however, is not supported by the linkage analysis in the RI strains. Complete concordance is found for the strain distribution patterns of responsiveness and heavy chain allotype (*10*), demonstrating that the response locus is linked to the genes controlling the heavy chain constant regions. The response characteristics of CBA and BAB/14 mice are explainable if a recombinational event occurred between the locus for response to dextran and the genes coding for the heavy chain constant regions.

We favor the following interpretation of our results: A restricted response to dextran is due to selection of products of the germ-line $V_{\lambda(0)}$ gene or germ-line $V_{H(0)}$ gene or both. As the locus determining responsiveness is C_H-linked, if $V_{\lambda(0)}$ is necessary for response, the $V_{\lambda(0)}$ gene in mice is linked to the heavy chain locus. A recombinational event to explain the results in BAB/14 and CBA would then have occurred either between the locus for V_λ and the C_H locus or between the V_H and C_H loci. If response is determined by a $V_{\lambda(0)}$ gene, alleles of this gene (in unresponsive strains) code for the λ subunit of germ-line antibodies with different specificities.

It has been proposed that antibody variability is due to somatic mutation and selection; by this model a variety of germ-line antibodies exist prior to diversification equal to the number of germ-line V_L genes multiplied by the number of germ-line V_H genes (*11*). For example, if there are five different variable heavy chain genes and one lambda variable gene then five antibody specificities can be generated. The homogeneous response to dextran in certain strains of mice, such as BALB/c, suggests that such a germ-line antibody, $V_{\lambda(0)}V_{H(0)}$, is selected since it is unlikely that an identical antibody would be repeatedly generated by random somatic mutation. The low response in the kappa class in some strains, such as C57BL/6, suggests that they do not have a $V_{\lambda(0)}V_{H(0)}$ antibody with dextran specificity. The antibodies to dextran of these strains could be of the $V_{\kappa(0)}V_{H(0)}$ type (germ line) or these antibodies may have been selected for as a result of somatic diversification of germ-line genes. The light chain class of such somatically derived antibodies is more likely to be kappa since the ratio of germ-line V_κ genes to V_λ genes is at least 20 : 1 in the mouse (*2*).

Since high response is limited to a particular light chain class and idiotype, differences between strains must involve the bone marrow–derived cell. This is consistent with our initial results where immunizing C57BL/6 and BALB/c mice with sheep erythrocytes coupled to dextran B-1355S does not abolish the response difference. Both strains, because they respond well to SRBC, must have thymus-derived cells capable of recognizing the carrier determinants on SRBC. Difference in responsiveness to dextran in this case is due to differences expressed in the bone marrow–derived cell.

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Benzodiazepines: Anxiety-Reducing Activity by Reduction of Serotonin Turnover in the Brain

Abstract. *The anxiety-reducing effects of minor tranquilizers in the rat conflict test were mimicked by serotonin antagonists and by* p-*chlorophenylalanine, an inhibitor of serotonin synthesis; the depressant effects of the minor tranquilizers were mimicked by norepinephrine antagonists. Intraventricular injections of serotonin led to a suppression of behavior, and also antagonized the anxiety-reducing action of benzodiazeprines. Intraventricular injections of norepinephrine led to a release of punished behavior from suppression, and also antagonized the depressant action of benzodiazepines. The anxiety-reducing activity, and the decrease in serotonin turnover induced by benzodiazepines, were maintained over repeated doses, whereas depressant activity, and the decrease induced in norepinephrine turnover, both rapidly underwent tolerance. Tranquilizers may exert their anxiety-reducing effects by a reduction of serotonin activity in a behaviorally suppressive punishment system, and they may exert their depressant effects by a reduction of norepinephrine activity in a behaviorally facilitatory reward system.*

We attempt here to relate the behavioral actions of the minor tranquilizers (benzodiazepines, barbiturates, and meprobamate) to their recently discovered effects on monoamine turnover in the brain. Studies from several laboratories indicate that benzodiazepines and barbiturates decrease the turnover of norepinephrine, serotonin, and other biogenic amines in the brain (*1–3*). Because norepinephrine and serotonin play an important role in the control of behavior (*4, 5*), the decreased turnover of these substances may be at least partly responsible for some of the behavioral effects of tranquilizers. To test this idea, we varied the activity of norepinephrine and serotonin in the brain in several ways, and compared the behavioral effects of these changes with those of the benzodiazepines. On the assumption that