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# Studies on Recombination within the Mouse H-2 Complex

## II.

## Serological Analyses of Four Recombinants, H-$2^{al}$, H-$2^{ol}$, H-$2^{tl}$, and H-$2^{th}$

C. S. David and D. C. Shreffler

Department of Human Genetics
University of Michigan
Ann Arbor, Michigan, U.S.A.

Detailed serological analyses of four intra-*H–2* recombinants for 20 H–2 alloantigenic specificities were conducted. The H–2 alloantigenic profiles of these recombinants and resulting assignments to the *H–2* genetic fine structure map are presented.

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A preceding paper (Shreffler & David 1972) described the detection of three intra-*H–2* recombinants during mapping studies of the Ss serum protein variant system in the mouse. These recombinants were of particular interest and importance because they localized the *Ss* locus within the complex chromosomal region controlling H–2 antigens. They are also significant because of the information they can provide regarding detailed localization of determinants for the various H–2 alloantigenic specificities within the H–2 complex and were therefore analyzed serologically in rather great detail. This paper presents the results of the serological analyses on those three recombinants, designated H–$2^{ol}$, H–$2^{al}$, and H–$2^{tl}$, and on a fourth recombinant, H–$2^{th}$, detected by Stimpfling (1965), which was also of particular interest because of its great similarity to H–$2^{tl}$.

### *Materials and Methods*

The three recombinants detected in this laboratory are described in the preceding paper (Shreffler & David 1972). Recombinants *H–*$2^{ol}$ and *H–*$2^{al}$ were derived from crossing-over between *H–*$2^{d}$ and *H–*$2^{k}$. Recombinant *H–*$2^{tl}$ was derived from a cross-over between *H–*$2^{al}$ and *H–*$2^{s}$. The *H–*$2^{th}$ cross-over derived from the heterozygous parent, *H–*$2^{a}$/*H–*$2^{s}$ (Stimpfling 1965). This cross-over is carried in Stimpfling's strain

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*Table I*

*H–2 recombinant alleles analyzed*

| Strain | Recombinant allele | Derived from recombination between |
|---|---|---|
| A.AL | $H\text{–}2^{al}$ | $H\text{–}2^{d}/H\text{–}2^{k}$ |
| C3H.OL | $H\text{–}2^{ol}$ | $H\text{–}2^{d}/H\text{–}2^{k}$ |
| A.TL | $H\text{–}2^{tl}$ | $H\text{–}2^{al}/H\text{–}2^{s}$ |
| A.TH | $H\text{–}2^{th}$ | $H\text{–}2^{a}/H\text{–}2^{s}$ |

B10.S (1R) (Stimpfling & Reichert 1970). It was included in this study because preliminary data indicated that it was very similar to $H\text{–}2^{tl}$. The four recombinant chromosomes are now carried in congenic inbred strains, C3H.OL, A.AL and A.TL, described previously (Shreffler & David 1972), and a new strain, A.TH, which was derived from a cross to B10.S (1R) and produced in exactly the same manner as A.TL. The recombinant alleles and congenic strains are listed in Table I. Some of the studies described herein were carried out with the completed inbred strains. However, many of the analyses were performed on stocks derived at earlier stages during the course of inbreeding, which were homozygous for their *H–2* alleles, but not completely inbred. For the sake of convenience and simplicity, the strain designations for the completed inbred lines will be used throughout.

The details of the procedures used in producing H–2 antisera have been described (Shreffler et al. 1966). In some cases antisera obtained from the Transplantation Immunology Branch, National Institutes of Health, were used. The criteria for selection of antisera for each H–2 specificity, the methods for evaluation of the specificity of each antiserum, and the controls employed in all analyses were as described previously (Shreffler et al. 1966). All antisera were analyzed on a panel of inbred strains representing all of the defined *H–2* types. This panel of *H–2* "type strains" and synonyms used in this paper are listed in Table II. The antisera utilized in testing for each specificity are given under Results, by specificity.

*Table II*

*H–2 type strains*

| H-2 Chromosome | Type strain | Synonym |
|---|---|---|
| a | A/SnSf | A |
| b | C57BL/10JSf | B10 |
| d | DBA/2JSf | D2 |
| f | A/SnSf–*H–2*$^{f}$ | A.CA |
| g | HTG/AoSf | HTG |
| h | HTH/AoSf | HTH |
| i | HTI/Sf | HTI |
| j | (C3H/JSf × JK/St)$F_5$ | CJ |
| k | C3H/JSf | C3H |
| m | AKR/SnSf–*H–2*$^{m}$ | AKR.M |
| n | (C57BL/10JSf × F/St)$F_5$ | BF |
| oh | C3H/JSf–*H–2*$^{oh}$ | C3H.OH |
| p | P/JSf | P |
| q | DBA/1JSf | D1 |
| r | (C3H/JSf × RIII/WyJ)$F_6$ | CR |
| s | A/SnSf–*H–2*$^{s}$ | A.SW |
| u | PL/JSf | PL |
| v | SM/J | SM |

The PVP hemagglutination method, used for direct tests, and the absorptions *in vitro* and *in vivo* were performed as described in a previous paper (Shreffler et al. 1966). Unless otherwise stated, all direct tests and titrations were done by PVP hemagglutination. Antigenic specificities 32 and 33 were detected by a simple microcytotoxic test, modified from that described by Amos et al. (1969). Lymphoid cells were obtained by teasing the axial and mesenteric lymph nodes with forceps. The cells were washed and suspended in Hank's solution at a concentration of $2\times10^{6}$/ml. The test was carried out in microtiter plates (Falcon Plastics, Los Angeles, Calif.) with one lambda each of the cell suspension, an appropriate dilution of the antiserum, and undiluted rabbit complement

which had been absorbed with mouse tissues. A normal serum control was employed with each cell suspension. At the completion of the test 0.3 % trypan blue was added and the percentage of stained (dead) cells was determined.

## *Results*

Serological analyses were carried out for 20 of the defined *H–2* specificities. Appropriate antisera were not available for specificities 10,14,22,25,27,28,29, nor were they available at the time of these studies for the new specificities recently described by Snell et al. (1971) and Demant et al. (1971a, 1971b). Each of the specificities analyzed will be considered in numerical order below.

*H–2.1* – Antiserum (C3H.SW×B10.M) anti-C3H.NB was used to test for H–2.1. (This serum was obtained from the National Institutes of Health, Transplantation Immunology Branch.) This combination differs from that originally used to define H–2.1 [(C57×D2) anti-A (Amos 1962)], so the specificity may not be precisely the same. By direct hemagglutination test all four recombinants reacted strongly. On *in vivo* absorption, all four recombinants absorbed the reactivity of this serum against strain A erythrocytes, hence all four are classified as positive for H–2.1. Recently, however, Snell et al. (1971) have subdivided H–2.1-positive strains into 1-complete and 1-intermediate classes. Since our data do not permit such subclassification, further analyses for this distinction will be necessary. It should be noted, however, that Snell et al. classified $H\text{–}2^{tl}$ and $H\text{–}2^{oh}$ (which is very similar to $H\text{–}2^{ol}$) as 1-intermediate.

*H–2.2* – The antiserum employed was (B10.D2×A) anti-B10.A (2R), obtained from the National Institutes of Health, Transplantation Immunology Branch. Tests on our panel showed it specific for H–2.2. All four recombinants were consistently negative with this antiserum on direct hemagglutination test and failed to absorb (*in vivo*) the reactivity of this antiserum against B10 (H–2.2-positive) erythrocytes.

*H–2.3* – Since all four parental alleles determine H–2.3, the presence of this specificity was expected in all of the recombinants analyzed. The antiserum employed was (B10×A.CA) anti-A.SW, which contained anti-H–2.3 and anti-H–2.19. All four recombinant cell types reacted with this antiserum on direct test. However, as shown later, A.TL and A.TH are positive for H–2.19, so absorption analysis was necessary to test for H–2.3. This is shown in Table 3. After *in vivo* absorption, all four recombinants absorbed completely the reaction against cells for D2, D1, C3H and C3H.OH, indicating that all four have H–2.3. (A 24-hour *in vivo* absorption was

*Table III*

*Absorption in vivo of anti-H-2.3, (B10 × A.CA) anti-A.SW*

| Test cells | Reciprocal of titer after absorption in | | | | | Relevant H–2 specificities |
|---|---|---|---|---|---|---|
| | B10 | A.AL | C3H.OL | A.TL | A.TH | |
| B10 | 0 | 0 | 0 | 0 | 0 | – |
| A.SW | 320 | 160 | 40 | 0 | 0 | 3, 19 |
| D2 | 80 | 0 | 0 | 0 | 0 | 3 |
| D1 | 40 | 0 | 0 | 0 | tr | 3 |
| C3H | 160 | 0 | 0 | tr | tr | 3 |
| C3H.OH | 40 | 0 | 0 | 0 | tr | 3 |

necessary for clearance by C3H.OL.) Note also that A.TL and A.TH absorbed the reaction with A.SW cells as well, indicating that these two recombinants are H–2.19-positive.

Recent data of Demant et al. (1971b) have led these authors to subdivide specificity H–2.3 into a "family" of apparently related specificities, some of which were given new symbols. We also have data on the heterogeneity of H–2.3, especially as it relates to the C3H.OL recombinant strain. These data will be presented in a more comprehensive future paper. However, the present data clearly show that all four recombinants are positive for H–2.3 as it was originally defined (Hocker et al. 1954).

*H–2.4* – Antiserum (B10×AKR.M) anti-A was employed. This serum could also contain anti-H–2.10, but tests against the panel revealed no detectable anti-10. On direct hemagglutination test, this antiserum reacted very strongly with A.AL, A.TL and A.TH and failed to react with C3H.OL. The results of an *in vivo* absorption analysis indicated quite clearly that A.AL, A.TL and A.TH are H–2.4-positive, while C3H.OL is 4-negative.

*H–2.5* – By direct hemagglutination, all four recombinants were strongly positive with antiserum (B10.D2×HTG) anti-B10. This antiserum contains only anti-H–2.5 and anti-H–2.33. Since the four recombinants lack 33 (see below), this reaction must be due to anti-5. All four recombinants absorbed completely (*in vivo*) the reactivity of this serum with A cells (+5, —33).

*H–2.6* – At the time of these studies, a C3H anti-P serum was the only antiserum available which could be used for the detection of anti-6. (Because it is impossible to produce a monospecific anti-6 in any strain combination and because anti-6 titers are highly variable, relatively few antisera are useful for this specificity.) C3H anti-P could also contain anti-7 and anti-16. Absorption with the panel revealed anti-16, but no anti-7. On direct hemagglutination test, A.AL, A.TL, and A.TH were positive, and C3H.OL was negative. An *in vivo* absorption analysis (Table IV)

*Table IV*

*Absorption in vivo of anti-H-2.6,16 (C3H anti-P)*

| Test cells | Reciprocal of titer after absorption in | | | | | | | Relevant H–2 Specificities |
|---|---|---|---|---|---|---|---|---|
| | C3H | P | A | A.AL | C3H.OL | A.TL | A.TH | |
| C3H | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – |
| P | 40 | 0 | 80 | 320 | 160 | 40 | 40 | 6, 16 |
| A | 20 | 0 | 0 | 0 | 80 | tr | 0 | 6 |

showed that C3H.OL removed no activity for either P (+6, +16) or A (+6, —16), while A.AL, A.TL and A.TH absorbed for A, but not for P. This result indicates that A.AL, A.TL and A.TH are +H–2.6, —H–2.16, while C.3H.OL is apparently —H–2.6, —H–2.16. (Absence of H–2.16 in C3H.OL is based only upon failure of the antiserum to react in direct test, however. Since C3H.OL lacks H–2.6, the remaining activity for P, after absorption in C3H.OL, could be due to anti-6 plus anti-16, or to anti-6 only.)

*H–2.7* – Antiserum (A×B10.D2) anti-B10.M (kindly provided by Dr. George D. Snell) contained anti-H–2.7 and anti-H–2.9, based upon tests with the panel. This antiserum was negative by direct test

with all four recombinants. On *in vivo* absorption, none of the recombinants removed reactivity with cells of strain P (+7, —9) or A.SW (+7, —9). Therefore, all four recombinants must be H–2.7-negative.

*H–2.8* – Antiserum (B10×A.SW) anti-A.CA contains both anti-H–2.8 and anti-H–2.9. By direct hemagglutination, it was positive with A.AL and C3H.OH, but negative with A.TL and A.TH. The results of *in vivo* absorption with this serum

*Table V*

*Absorption in vivo of anti-H-2.8,9, (B10 × A.SW) anti-A.CA*

| Test cells | Reciprocal of titer after absorption in | | | | | | Relevant H–2 specificities |
|---|---|---|---|---|---|---|---|
| | B10 | D1 | A.AL | C3H.OH | A.TL | A.TH | |
| B10 | 0 | 0 | 0 | 0 | 0 | 0 | – |
| A.CA | 160 | 160 | 640 | 640 | 160 | 80 | 8, 9 |
| C3H | 40 | 20 | 0 | 0 | 160 | 20 | 8 |
| BF | 40 | 20 | 0 | 0 | 80 | 20 | 8 |

are shown in Table V. These indicate that A.AL and C3H.OH are +H–2.8, while A.TL and A.TH are —H–2.8.

*H–2.9* – Antiserum A.SW anti-A.CA, containing anti-8, 9 and 27, was absorbed *in vitro* with A tissues to remove anti-8 and anti-27. The absorbed serum failed to react with cells from any of the four recombinants, and the reactivity of this serum with A.CA (+9) cells was not absorbed by the recombinants. It is therefore concluded that all four are H–2.9-negative.

*H–2.11* – Tests against the panel of H–2 type strains indicated that antiserum (D2 ×BF) anti-CR was specific for H–2.11. Although anti-18 could have been present, it was not detected. On direct hemagglutination tests, this antiserum reacted strongly with erythrocytes of strain A.AL, but not of C3H.OL, A.TL or A.TH. *In vivo* absorption results confirmed the conclusion that A.AL is H–2.11-positive, while the other three recombinant types are H–2.11-negative.

*H–2.13* – Recombinants A.AL, A.TL and A.TH all reacted positively with antiserum (C3H×B10) anti-D1, while C3H.OL was negative. This antiserum contained anti-17 and anti-30 as well as anti-13. However, as shown below, all four recombinant strains are —17, —30, so the reaction must have been due to anti-13. This was confirmed in *in vivo* absorption analyses, in which A.AL, A.TL and A.TH absorbed reactivity for A and D2 cells (+13, —17, —30), while C3H.OL did not.

*H–2.16* – All four recombinants lack this specificity, as shown by the results presented for H–2.6.

*H–2.17* – An analysis with the H–2 panel showed that only anti-H–2.17 was present in serum (B10×AKR.M) anti-D1. This serum failed to agglutinate erythrocytes from any of the four recombinant types, and on *in vivo* absorption there was no reduction in activity for D1 cells by any of the recombinants, so all four are classified as H–2.17-negative.

*H–2.18* – Antiserum C3H anti-C3H.RIII is apparently a monospecific anti-18. The four recombinant strains were negative with the reagent, both by direct tests and by *in vivo* absorption, so are H–2.18-negative.

*H–2.19* – For this specificity, an antiserum (C57BL/6J×A/J) anti-A.SW was employed. This could contain anti-7 and

anti-19, but in this serum, comprised predominantly of early bleedings after immunization, no anti-7 was ever detectable, e.g. the serum was consistently negative against A.CA and P cells. On direct tests, A.TL and A.TH were positive, while A.AL and C3H.OL were negative. The results of an *in vivo* absorption analysis showed clearly that A.TL and A.TH are +19, A.AL and C3H.OL are —19.

*H–2.23* – This specificity was rather recently rediscovered (see Snell et al. 1971 for details). It was detected in these studies by antiserum (B10×LP.RIII) anti-B10.BR, obtained from the Transplantation Immunology Branch, National Institutes of Health. This serum agglutinated erythrocytes of A.AL strongly, of C3H.OL very weakly, and was negative with A.TL and A.TH. In the strain combination employed, both anti-23 and anti-32 could be present; however, anti-32 is usually very weak or negative by hemagglutination. Absorption results with this antiserum are shown in Table VI. They indicate that A.AL is +23, while C3H.OL, A.TL and A.TH are —23. The weak direct hemagglutination with C3H.OL was probably due to anti-32 (see below).

*Table VI*

*Absorption in vivo of anti-H-2.23 (B10×LP.RIII) anti-B10.BR*

| Test cells | Reciprocal of titer after absorption in | | | | | | | Relevant H–2 specificities |
|---|---|---|---|---|---|---|---|---|
| | B10 | C3H | C3H.OH | A.AL | C3H.OL | A.TL | A.TH | |
| B10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – |
| A | 80 | 0 | 40 | 0 | 20 | 5 | 20 | 23 |
| C3H | 40 | 0 | 80 | 0 | 80 | 5 | 20 | 23, 32 |
| C3H.OH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 |

*H–2.30* – Antiserum A anti-D1 contained both anti-H–2.17 and anti-H–2.30. Cells of the four recombinant types were negative with this antiserum. In *in vivo* absorption, the four recombinants also failed to remove activity against AKR.M test cells ( —17, +30), so it seems clear that all four are H–2.30-negative.

*H–2.31* – The A anti-D2 serum employed was a specific anti-31. It reacted with C3H.OL on direct test, but was negative with A.AL, A.TL and A.TH. *In vivo* absorption results confirmed that C3H.OL

*Table VII*

*Direct cytotoxicity test of anti-H-2.32, (A anti-C3H)*

| Test cells | Antiserum dilution | | | | Normal serum dilution | |
|---|---|---|---|---|---|---|
| | 1/2 | 1/4 | 1/8 | 1/16 | 1/4 | 1/8 |
| A | 10[a] | 20 | 20 | 10 | 10 | 10 |
| C3H | 80 | 80 | 70 | 50 | 20 | 20 |
| A.AL | 30 | 30 | 20 | 20 | 40 | 20 |
| C3H.OL | 50 | 80 | 80 | 80 | 10 | 10 |
| A.TL | 30 | 40 | 20 | 20 | 20 | 20 |
| A.TH | 30 | 20 | 20 | 20 | 30 | 20 |

[a] Percent dead cells.

is H-2.31-positive, while the other three recombinants are H-2.31-negative.

*H-2.32* – This specificity was tested in the microcytotoxic test with antiserum A anti-C3H, a specific anti-32. The results, shown in Table VII, indicate that C3H.OL has H-2.32, while A.AL, A.TL and A.TH lack this specificity. Unfortunately, the titer of this antiserum was too low to permit confirmation of the direct test result by *in vivo* absorption.

*H-2.33* – The antiserum employed was (B10.D2×A) anti-B10.A (5R), obtained from the Transplantation Immunology Branch, National Institutes of Health. This serum could also contain anti-H-22, but as it failed to react with HTG cells, it apparently does not. On direct cytotoxic tests, this serum gave about 20 % cytotoxicity with negative control cells and with cells of the recombinants, whereas it gave 90 % cytotoxicity with B10 cells. Furthermore, *in vivo* absorption in the four recombinants removed no reactivity with H-2.33-positive cells. It is therefore concluded that the four recombinant strains are H-2.33-negative.

## *Discussion*

In Table VIII, a summary is presented of the H-2 alloantigenic specificities found in the four recombinants and the distribution of these specificities in the parental H-2 types from which they were derived. Table VIII also includes Ss types reported in the preceding paper (Shreffler & David 1972), and Slp types from Passmore & Shreffler (1970). Several points are worth noting in these results.

Three different pairs of H-2-identical, but Ss-Slp distinctive recombinants have now been detected. These are $H\text{-}2^{a}$ and $H\text{-}2^{al}$, $H\text{-}2^{th}$ and $H\text{-}2^{tl}$, and $H\text{-}2^{ol}$ and $H\text{-}2^{oh}$ (see Shreffler et al. 1966 for H-2 type of the latter). The existence of such

*Table VIII*

*Alloantigenic profiles of the four parental and four recombinant H-2 chromosomes*

| Chromosome | H-2 specificity | | | | | | | | | | | | | | | | | | | Ss | Slp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 13 | 16 | 17 | 18 | 19 | 23 | 30 | 31 | 32 | 33 | |
| d | – | – | 3 | 4 | – | 6 | – | 8 | – | – | 13 | – | – | – | – | – | – | 31 | – | – | h | a |
| k | 1 | – | 3 | – | 5 | – | – | 8 | – | 11 | – | – | – | – | – | 23 | – | – | 32 | – | l | o |
| al | 1 | – | 3 | 4 | 5 | 6 | – | 8 | – | 11 | 13 | – | – | – | – | 23 | – | – | – | – | l | o |
| ol | 1 | – | 3 | – | 5 | – | – | 8 | – | – | – | – | – | – | – | – | – | 31 | 32 | – | l | o |
| al | 1 | – | 3 | 4 | 5 | 6 | – | 8 | – | 11 | 13 | – | – | – | – | 23 | – | – | – | – | l | o |
| s | 1 | – | 3 | – | 5 | 6 | 7 | – | – | – | – | – | – | – | 19 | – | – | – | – | – | h | a |
| tl | 1 | – | 3 | 4 | 5 | 6 | – | – | – | – | 13 | – | – | – | 19 | – | – | – | – | – | l | o |
| a | 1 | – | 3 | 4 | 5 | 6 | – | 8 | – | 11 | 13 | – | – | – | – | 23 | – | – | – | – | h | a |
| s | 1 | – | 3 | – | 5 | 6 | 7 | – | – | – | – | – | – | – | 19 | – | – | – | – | – | h | a |
| th | 1 | – | 3 | 4 | 5 | 6 | – | – | – | – | 13 | – | – | – | 19 | – | – | – | – | – | h | a |

pairs strongly supports the supposition that *Ss–Slp* is located in the middle of the *H–2* complex. The apparent identity of $H\text{–}2^{a}$ and $H\text{–}2^{al}$ also lends credence to the long-held view that $H\text{–}2^{a}$ arose as a cross-over between $H\text{–}2^{d}$ and $H\text{–}2^{k}$.

It is worthwhile noting that the serological properties of the members of these pairs seem unaffected by the Ss and Slp differences, either quantitatively or qualitatively. Thus, all specificities of $H\text{–}2^{al}$ appear to be as strongly expressed and qualitatively identical to those of $H\text{–}2^{a}$, etc. Likewise, as discussed below the serological peculiarities of $H\text{–}2^{oh}$ are also observed with $H\text{–}2^{ol}$.

The separations of certain specificities in these recombinants permit the genetic determinants for these specificities to be assigned to defined regions of the *H–2* complex. These assignments were discussed in previous publications (Shreffler 1965, Shreffler 1967, Shreffler 1970), and more recently have been somewhat reinterpreted (Shreffler et al. 1970, Klein & Shreffler 1971). Only a simple summary of the recombinants described in this paper will be presented here. It is quite clear that the *H–2* complex is devisible by recombination into at least three regions, *H–2K, SS–Slp* and *H–2D*. On the basis of the four recombinants analyzed, the determinants of

*Table IX*

*Assignment of H–2 specificities and Ss-Slp types to regions of the H–2 complex*

| H-2 Chromosome | Region | | | Unassigned |
|---|---|---|---|---|
| | H-2K | Ss-Slp | H-2D | |
| d | 8, 31 | $Ss^{h}Slp^{a}$ | 4, 6, 13 | 3 |
| k | 8, 11, 23 | $Ss^{l}Slp^{o}$ | 32 | 1, 3, 5 |
| al | 8, 11, 23 | $Ss^{l}Slp^{o}$ | 4, 6, 13 | 1, 3, 5 |
| ol | 8, 31 | $Ss^{l}Slp^{o}$ | 32 | 1, 3, 5 |
| a | 8, 11, 23 | $Ss^{h}Slp^{a}$ | 4, 6, 13 | 1, 3, 5 |
| s | 19 | $Ss^{h}Slp^{a}$ | 7 | 1, 3, 5, 6 |
| th | 19 | $Ss^{h}Slp^{a}$ [a] | 4, 6, 13 | 1, 3, 5 |
| tl | 19 | $Ss^{l}Slp^{o}$ | 4, 6, 13 | 1, 3, 5 |

[a] Position with respect to Ss-Slp cannot be determined because both $H\text{-}2^{a}$ and $H\text{-}2^{s}$ are $Ss^{h}Slp^{a}$.

certain *H–2* specificities and Ss-Slp types can be assigned to these regions, as shown in Table IX. The postulated cross-over position in each recombinant is indicated by a vertical bar. The separation of the *H–2K* and *H–2D* specificities is for the most part self-evident. H–2.8 is assigned to *H–2K* of $H\text{–}2^{k}$, $H\text{–}2^{al}$ and $H\text{–}2^{a}$ because of its loss from $H\text{–}2^{th}$ and $H\text{–}2^{tl}$ along with H–2.11 and 23. It is assigned to *H–2K* of $H\text{–}2^{d}$ and $H\text{–}2^{ol}$ because of its association with H–2.31 in $H\text{–}2^{g}$ (cf. Shreffler 1970). The problem of assignment of H–2.1, 3 and 5 to *H–2K, H–2D* or to separate regions has been discussed in detail elsewhere (Shreffler 1970, Shreffler et al. 1970, Klein & Shreffler 1971). In Table IX, these specificities are simply listed as unassigned. H–2.6 of $H\text{–}2^{s}$ cannot be definitively assigned because both $H\text{–}2^{a}$ and $H\text{–}2^{al}$ also have H–2.6 (located in *H–2D* of these chromosomes) and therefore the $H\text{–}2^{th}$ and $H\text{–}2^{tl}$ recombinants are not informative on the location of H–2.6 in $H\text{–}2^{s}$.

Snell et al. (1971) and Demant et al. (1971b) have presented evidence for heterogeneity among specificities H–2.1, 3 and 5 of different H–2 types. Included in

their investigation was $H\text{-}2^{oh}$, which seems to be serologically identical to $H\text{-}2^{ol}$, and which demonstrated an "intermediate" form of these three specificities. Earlier (Shreffler et al. 1966), the atypical behavior of H-2.3 of $H\text{-}2^{oh}$ had been noted. While the present studies did not undertake to define these subtleties in detail, our expericence with $H\text{-}2^{ol}$ suggests that it is identical to $H\text{-}2^{oh}$ in the weak and atypical properties of its H-2.1, 3 and 5 specificities. Further analyses of these differences, as well as for specificities H-2.27, 28 and 29 and the newly-defined specificities of Demant et al. (1971a, 1971b), must still be completed. These will be reported at a later time.

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Address:

*C. S. David*
Department of Human Genetics
University of Michigan
Ann Arbor, Michigan
U.S.A.