ON THE INHERITANCE OF NATURAL ANTISHEEP AGGLUTININS IN MICE OF INBRED STRAINS¹

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PREVIOUS studies of DAVIDSOHN and STERN (1949, 1950) and of STERN and DAVIDSOHN (1954) have demonstrated significant differences in distribution of titers of natural agglutinins for sheep red cells within and between inbred strains of mice. Results obtained on substantial numbers of mice of 13 strains are summarized in table 1. These mice, which consisted of approximately equal numbers of males and females ranging in age from three to six months, showed greater interstrain than intrastrain differences. This suggested the possible influence of genetic factors. The degree of genetic control of differences in distribution of agglutinin titers is the subject of this investigation.

The two strains selected for the present study, C57BL and C3H, were chosen because of the difference between them in antibody distribution and in several other biologic properties, such as incidence of spontaneous mammary tumors.

MATERIALS AND METHODS

The C57BL and C3H strains investigated are derived from mice obtained in 1946 from DR. A. TANNENBAUM, Cancer Research Department of Michael Reese Research Institute, Chicago, who in turn had bred these strains since 1940 from stock originally obtained from Jackson Memorial Laboratory, Bar Harbor, Maine. The animals of our colony were maintained by brother-sister mating, kept in stainless steel cages with food hoppers, and fed ad libitum Rockland mouse pellets and water. Biologic characteristics, such as fertility, incidence of spontaneous tumors, and longevity of our colonies, compare well with those recorded in the literature for these strains reared elsewhere. Transplantable mammary tumors EO 771 growing in Bar Harbor C57BL were found 100 percent transplantable to our C57BL strain, killing all inoculated animals; the same was true for lymphosarcoma C3HED (Bar Harbor) inoculated into our C3H mice.

Animals of both sexes were tested. Their ages ranged from three to six months. This age limit is important since in previous studies we found that the ability of mice to produce natural antisheep agglutinins is not developed fully until they are from 12 to 15 weeks old (DAVIDSOHN and STERN 1950). This phenomenon of "serologic maturation" applies to natural antibodies in general (HIRSZFELD 1928).

Blood was obtained either by decapitation or by bleeding from the tail. The latter technic permitted repeat testing of the same animals. The technic of testing for

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TABLE 1
Natural agglutinins for sheep red cells in mice¹

Strain	Total no.	Percentage distribution of titers											
		0	1	2	4	8	16	32	64	128	256	512	1024
C57BL	336	9.5	5.7	10.4	12.8	17.0	12.8	13.7	9.7	4.5	2.4	0.9	0.6
C3H	201	73.6	10.4	7.0	4.5	4.0	0.5	—	—	—	—	—	—
C57L	100	71.0	13.0	6.0	8.0	2.0	—	—	—	—	—	—	—
C57BR/cd	126	59.5	15.9	14.3	5.6	3.9	0.8	—	—	—	—	—	—
STOLI	88	26.1	23.9	10.2	23.9	6.8	8.0	1.1	—	—	—	—	—
NH	103	76.7	6.7	8.7	4.9	1.0	0	2.0	—	—	—	—	—
BALB/c	103	57.3	16.5	16.5	5.8	3.9	—	—	—	—	—	—	—
I	91	80.2	12.1	6.6	1.1	—	—	—	—	—	—	—	—
A	101	75.2	11.9	7.9	4.0	0	0	1.0	—	—	—	—	—
MA	128	55.5	9.4	16.4	14.8	3.1	0.8	—	—	—	—	—	—
DBA	261	43.3	18.8	19.2	9.5	4.2	1.9	2.3	0.8	—	—	—	—
CBA	120	87.5	7.5	4.2	0.8	—	—	—	—	—	—	—	—
AKR	116	66.4	11.2	9.5	8.6	4.3	—	—	—	—	—	—	—

¹ Modified from STERN and DAVIDSOHN (1954).

antisheep agglutinins has been reported in previous papers (DAVIDSOHN and STERN 1949, 1950).

Reciprocal crosses of C57BL and C3H mice produced F_1 offspring. Brother-sister mating of F_1 mice resulted in F_2 offspring, and breeding of F_1 females to the male parents, and of F_1 males to the female parents, furnished backcrosses B_1 and B_2 .

RESULTS

The distributions of agglutinin titers obtained for parent strains, crosses and backcrosses, are shown in table 2. Data for the parent strains refer to the total population tested instead of to the small number of actual parents of the F_1 mice. For the sake of easier handling of the data, titer was converted into "concentration", indicating amount of the serum in one ml of the highest dilution which yielded agglutination. For convenience, the negative scale, obtained by using the logarithm of the concentration, was converted into a positive scale by multiplying it with minus 10 (table 2). These data, plotted on a log scale (fig. 1), give some evidence that a threshold is involved which would render inappropriate analyses that assume a normal distribution. However, within the range of "positive" titers (one or higher), the data for C57BL showed a normal distribution, suggesting that within this range the two criteria of a satisfactory scale for genetic analysis are satisfied, namely, (1) additivity of genetic factors causing variation, and (2) independence of genetic and non-genetic variation.

Methods for the estimation of the mean and variance of data involving threshold effects have been developed by WRIGHT (1934). These methods involve the construction of an artificial second threshold at some arbitrary point along the scale of observed variation. The two thresholds divide the observed distribution into three percentage classes: (1) values below the lower threshold, representing in this study those animals in which no detectable agglutinin was found; (2) values be-

TABLE 2
Distribution of agglutinins in parents and crosses

Titer Concentration† Log ₁₀ concentration -10 log ₁₀ concentration	<1 >1	1 0 0	2 .5 3.01	4 .25 6.02	8 .125 9.03	16 .0625 12.04	32 .01325 15.0	64 .01562 18.1	128 .00712 21.1	256 .00306 24.1	512 .0019 27.1	1024 .00097 30.1
	Percentile Distribution											
C57BL No. 338	33	19	35	43	57	44	46	33	15	8	3	2
% 100	9.8	5.6	10.3	12.7	16.9	13.0	13.6	9.8	4.4	2.4	0.9	0.6
C3H No. 205	152	21	14	9	8	1						
% 100	74.1	9.1	6.8	4.4	3.9	0.05						
F ₁ No. 122	53	11	13	19	13	10	2	1				
% 100	43.4	9.0	10.6	15.6	10.6	8.2	1.6	0.8				
F ₂ No. 254	122	22	33	28	22	14	7	4	1	1		
% 100	48.0	8.7	13.0	11.0	8.7	5.5	2.7	1.6	0.4	0.4		
B ₁ * No. 93	27	6	11	7	11	14	11	6				
% 100	29.0	6.5	11.8	7.5	11.8	15.1	11.8	6.5				
B ₂ ** No. 141	85	16	17	6	10	5	2					
% 100	60.3	11.3	12.1	4.3	7.1	3.5	1.4					

† Represents amount of serum in 1 ml of test dilution.

* Backcross of F₁ to C57BL parents.

** Backcross of F₁ to C3H parents.

tween the two thresholds; and (3) values above the higher threshold. The three percentages delimited by these thresholds determine a distribution of unit area without limiting assumptions other than that the data are normally distributed.

Tentatively, let us accept the assumption that on a log scale there is a normal distribution in the parental strains, crosses, and backcrosses, although this is partially obscured by a threshold which prevents observation of part of the variation. Under this assumption and by following the method of WRIGHT, we construct an arbitrary second threshold at a point just greater than nine on the modified log scale. The percentile distributions of parental strains, of crosses, and of backcrosses are shown in table 3, and plotted on a modified log scale in figure 2. If we let the interval zero to nine inclusive on the modified log scale equal one unit on a new arbitrary scale, then each of these percentile distributions defines a unique normal distribution of unit area given by the formulae:

$$pri^{-1}p_1 = \frac{t_1 - m}{\sigma} \quad \text{and} \quad pri^{-1}p_2 = \frac{t_2 - m}{\sigma}$$

where $pri^{-1}p_1$ is the inverse probability integral of p_1 , t_1 is the observed threshold, t_2 is the artificial threshold, p_1 is the percentage below t_1 , p_2 is the percentage below t_2 , and m and σ are the mean and standard deviation, respectively, of the fitted normal distribution as measured in the units of the arbitrary scale.

Since t_1 has a value of zero and t_2 a value of one on the arbitrary scale, the above expressions may be simplified to $\frac{t_1 - m}{\sigma} = \frac{-m}{\sigma}$, $\frac{t_2 - m}{\sigma} = \frac{1 - m}{\sigma}$ and $\frac{1}{\sigma} = pri^{-1}p_2 - pri^{-1}p_1$. Solving these for σ and m , we obtain $\sigma = \frac{1}{pri^{-1}p_2 - pri^{-1}p_1}$ and $m = -\sigma pri^{-1}p_1$.

FIGURE 1.—Observed titer distribution.

FIGURE 2.—Calculated titer distribution.

TABLE 3

Percentile distribution around two thresholds

	P_1^* $P_2 - P_1^*$ P_2^*		
	-10 log ₁₀ concentration		
	T_1	T_2	
	0	0-9	9
$P_1(\text{C57BL})$	9.76	45.56	44.67
$P_2(\text{C3H})$	74.15	25.37	0.49
$F_1(P_1 \times P_2)$	43.44	45.90	10.65
$F_2(F_1 \times F_1)$	48.03	41.34	10.63
$B_1(F_1 \times P_1)$	29.03	37.63	33.33
$B_2(F_1 \times P_2)$	60.28	34.75	4.96

* P_1 is the percentage of animals without detectable antibodies and may also be considered as the area (fig. 2) under a curve to the left of line T_1 which is the observed threshold. P_2 is the total percent or area to the left of the arbitrary threshold T_2 , and $P_2 - P_1$ is the area of percent between thresholds.

TABLE 4
*Means and variances of agglutinins calculated on an arbitrary scale**

Group	Mean	σ	σ^2	σ_G^2
C57BL	0.90657	0.69984	0.48978	
C3H	-0.42500	0.65587	0.43017	
F ₁	0.11716	0.70922	0.50299	
F ₂	0.04123	0.83808	0.70238	.22807
B ₁	0.56205	1.01729	1.03488	.56057
B ₂	-0.18772	0.72036	0.51892	.04461

* For explanation see table 3 and text.

The means and variances thus calculated from the data of table 3 are presented in table 4. The calculated distributions, as plotted on the modified log scale, are shown in figure 2. It may be noted that the calculated distributions show fairly good agreement with the observed distributions plotted in figure 1.

Using the values of the means and variances (σ^2) calculated above, we may now test the assumption that the log scale is one on which the two criteria of an adequate scale for genetic analysis are satisfied. We test the additivity of the genetic components of variation, in accordance with the method of MATHER (1949), by defining P₁, P₂, F₁, F₂, B₁ and B₂ as the means of parental strains, crosses, and backcrosses, respectively, and the variances of these variables as $\sigma_{P_1}^2$, $\sigma_{P_2}^2$, $\sigma_{F_1}^2$, $\sigma_{F_2}^2$, $\sigma_{B_1}^2$ and $\sigma_{B_2}^2$, in corresponding order. Accordingly, we express the three functions A, B, and C with their variances:

$$A = 2B_1 - F_1 - P_1 \quad \sigma_A^2 = 4\sigma_{B_1}^2 + \sigma_{F_1}^2 + \sigma_{P_1}^2$$

$$B = 2B_2 - F_1 - P_1 - P_2 \quad \sigma_B^2 = 4\sigma_{B_2}^2 + \sigma_{F_1}^2 + \sigma_{P_2}^2$$

$$C = 4F_2 - 2F_1 - P_1 - P_2 \quad \sigma_C^2 = 16\sigma_{F_2}^2 + 4\sigma_{F_1}^2 + \sigma_{P_1}^2 + \sigma_{P_2}^2$$

MATHER has shown that if the genetic components of variation are additive, the values for each A, B, and C will equal zero. The values of A, B, and C, their variances, standard deviations, the ratios of the deviation of the variables from zero to their standard deviations, and the probability of these ratios as unit normal deviates, are shown in table 5. It may be seen that none of these deviations differs significantly from zero. Hence, there is no evidence to suggest non-additive interaction among the loci involved in the control of antibody production.

The second criterion for a satisfactory scale on which genetic analysis can be made is that of independence of the genetic and non-genetic components of variation. It may happen that the environmental variation is reduced or increased within a particular part of the range, or that an increase in genetic variation might cause an increase in sensitivity to non-genetic factors. Under such conditions a correlation between genetic and non-genetic variation might be observed.

The question of the independence of the genetic and non-genetic components may be tested by finding out whether the variances of the two parental strains and the F₁ population are equal. On a satisfactory scale there should not be any genetic variation within each of these three populations since each is genetically homogene-

TABLE 5
Calculated values of functions in additivity test

	A	B	C
Value	0.10037	0.06760	0.55087
Variance	5.13229	3.00883	14.23768
Standard deviation	2.26546	1.73459	3.77328
Value/standard deviation	0.0443	0.0389	0.1459
Probability	> .5	> .5	> .5

TABLE 6
Calculated values of test for independence of genetic and non-genetic components of variance using null hypothesis of independence ($\sigma_{P_1}^2 = \sigma_{P_2}^2 = \sigma_{F_1}^2$)

Variable	Value	Degrees of freedom	Probability
$F_{P_1 F_1}$	1.02699	$F_{1121} \ P_{1337}$	> .20
$F_{P_2 F_1}$	1.16930	$F_{1121} \ P_{2253}$	> .20
$F_{P_1 P_2}$	1.13858	$P_{1337} \ P_{2253}$	> .20

ous: members of the pure strains are homozygous, and F_1 individuals are uniformly heterozygous at each locus where the parents differ.

Applying FISHER's F test (1953), we find, as shown in table 6, that there is no significant difference between the three variances. Hence, we may conclude that the original assumption of independence of the genetic and non-genetic components of variation (cf. table 2 and fig. 2) is justified.

Since each of the parental and the F_1 populations is genetically homogeneous, variation within each of these three populations must be due to non-genetic factors. We may use an average of the three variances as an estimate of the degree of non-genetic variation found within F_1 and backcross populations. The average variance of the parental and F_1 populations, as calculated on the arbitrary scale, is $0.4743 \pm .0317$. Subtracting this value from each of the individual variances of the crosses and backcrosses, we obtain an estimate of the variance of each of these populations which may be attributed to heredity. These data are shown in the last vertical column in table 4 (σ_G^2).

By comparing the mean and estimated heritable variance of the backcross of the F_1 to C57BL with the mean and variance of the backcross of the F_1 to C3H, we may obtain an estimate of the dominance relations of the genetic factors involved. It must be kept in mind that the differences in the two parental strains are probably due to more than one genetic factor and that the dominance effects observed may be due to interaction of many different genes, each with its own dominance relations. Thus the dominance observed will probably not be complete, but be expressed as an intermediate quantitative effect.

The existence of dominance relations controlling agglutinin titer is clearly suggested by the large differences between the genetic variances of the two backcrosses (table 4). The backcross to C3H has a genetic variance only slightly greater than that of either parental strain, while the genetic variance of the backcross to C57BL is the largest observed in this series of experiments. This suggests that the C3H

parental strain carries genetic factors that are strongly dominant over those of C57BL and act to reduce the titer of antisheep agglutinins.

Confirmatory evidence for the dominant role of the C3H genotype is found in the position of the means of the F_1 and F_2 populations. In the absence of dominance the means of these populations should both fall on the mean of the parental means as calculated on a satisfactory scale. In the presence of dominance the F_1 mean should be closer to the dominant parent, as should be also the F_2 , though to a lesser degree. The mean of the parental means as calculated on the arbitrary scale is 0.24076. The observed means of F_1 and F_2 (table 4) are both on the C3H side of this value, confirming the dominance of this parental genotype as found in the analysis of variance. It is an anomaly that the F_2 mean is closer to that of C3H than is that of the F_1 , but this may be partially due to restricted sample size.

A method for the estimation of the number of genetic units, which may be blocks of genes acting as one physiological unit, involved in the difference of the two parental strains, has been developed by WRIGHT (1934). His method requires only data for the parental, F_1 and F_2 populations as distinguished from the usually more satisfactory method of MATHER which requires F_3 and double backcross data. WRIGHT's method is critically dependent on several assumptions, the failure of any one of which will cause a great reduction in the estimate of the number of genes involved in the trait studied. These assumptions are: independence, additivity and equality of the genes involved; and that one parental strain contains all the alleles tending to increase the trait while the other contains all the alleles tending to reduce it.

It is evident from table 1 that C3H is not at an extreme of the observable scale of titer variation and hence is not as different in genetic makeup from C57BL, as are some of the other strains tested (e.g., CBA and I). It must, therefore, be recognized that the estimate obtained by WRIGHT's method will be lower than the actual number of genetic units in which the parental strains differ.

Let D be the difference between the parental means measured on a satisfactory scale, and $\sigma_{F_2}^2 G$ (cf. table 4) the genetic component of the variance of the F_2 population, and N the number of genetic factors causing difference in the two parental strains. Then, provided that criteria mentioned above are satisfied:

$$N = \frac{D^2}{8\sigma_{F_2}^2 G} \quad (\text{WRIGHT})$$

Solving for N gives a value of 0.9486. Hence, we may conclude that there is a difference between the parental strains which involves at least one genetic factor, possibly many more.

The population data presented are compatible with the hypothesis that the strains differ in a single incompletely dominant factor. Pedigree analysis did not further elucidate this question because of a high "within litter" correlation of backcross mice.

DISCUSSION

There is general agreement in the literature that "constitutional" variation limits the ability of individuals to produce antibodies. WIENER (1951) proposed that

environmental factors, such as subclinical infections, exposure to saprophytes, or food constituents, are responsible for development of "natural" antibodies. Nevertheless, he recognized that these factors act as triggers which stimulate a genetically determined immune response. There is difference of opinion regarding the specificity of gene action: one school proposes direct gene-antibody interaction (FURUHATA 1927), while others suggest various degrees of indirect relationships (SCHIFF and ADELSBERGER 1924; WIENER 1951).

CARLINFANTI (1948) examined titers of isoagglutinins anti-A and anti-B in human parents and their children and found that there was a sufficient parent-child correlation to warrant the assumption that genetic factors control production of these antibodies. His population data showed that the isoagglutinin titer is distributed normally on a logarithmic scale, as would be expected on the basis of a multigene hypothesis with genes acting in a geometric way, as observed in this study.

Several investigations have been concerned with genetic control of immune antibodies. FJORD-SCHEIBEL (1944) observed considerable variation in the ability to produce diphtheria antitoxin in a non-inbred guinea pig colony. She undertook to breed selectively a high-titer and a low-titer strain. The F_1 individuals selected for their high titer contained over 90 percent producing antibody. This percentage remained stable in their offspring for six generations when the experiment terminated. Selection for low titers progressed more slowly. The point at which 90 percent of animals did not produce antibody was not reached until the fifth generation, and was preceded by a gradual decline in frequency of antibody producers. The author considered these results as evidence for a dominant gene controlling production of antitoxin. CARLINFANTI proposed a more likely hypothesis, i.e., that the gene controlling production of antitoxin is recessive, in which case the "high-titer" animals would be homozygous recessives and their offspring would therefore be "high-titer" animals, whereas the "low-titer" individuals with the dominant gene for inhibition of antitoxin production might be heterozygous. This picture of gene action is similar to that reported in the present study of natural antisheep agglutinins in the mouse.

DAVIDSOHN and STERN (1949, 1950, 1954) found striking interstrain differences in titers of immune antibodies for sheep and chicken erythrocytes in mice of 10 inbred strains. With one exception (strain A), mice of strains with high incidence of spontaneous tumors showed lower levels of immune antibodies than did mice of strains with low incidence of spontaneous tumors. Genetic factors may be assumed to play a role in these interstrain variations, as well as in the observation by FINK and QUINN (1953) on strain differences of mice in their ability to produce antibodies for egg albumin and pneumococcus polysaccharides.

In studying genetic control of immune response in rabbits, SANG and SOBEY (1954) reported that the secondary antibody response to tobacco mosaic virus (TMV) depends on inheritable factors. Results of immunization with diphtheria toxoid and beef red cells ran parallel to the immune response to TMV, but this was not the case for other antigens such as bovine albumin and human red cells. Nevertheless, the authors concluded that an inheritable general predisposition for good or poor antibody response existed in rabbits.

Thus, various investigations using different approaches have brought forth evidence for genetic factors controlling at least in part "natural" antibodies in man

(anti-A and anti-B) and in the mouse (agglutinins for sheep red cells), and immune antibodies in guinea pigs, mice and rabbits. A study of immune antibodies for sheep and chicken red cells in hybrids and backcrosses of inbred mice, similar to the present investigation, is underway in our laboratory.

Analysis of the data of this study had to take into consideration the question as to whether presence of the "milk agent" (mammary tumor inciter, MTI) had an effect on distribution of sheep agglutinin. MTI, present in our C3H mice, was presumably transmitted to F₁ offspring with maternal C3H parent, but not in the reciprocal cross, and similarly it was presumably present in backcrosses to the maternal C3H parent. Separate tabulation of data obtained for the reciprocal hybrids and backcrosses failed to reveal any significant differences, and hence the data were pooled for presentation and analysis. Likewise, no difference was observed in antibody dependent on the sex of the hybrids. The failure of the MTI to influence sheep agglutinins is in good accord with results previously reported by DAVIDSOHN, STERN and BITTNER (1950). Furthermore, the similar antibody distribution in reciprocal crosses appears to eliminate the possibility that cytoplasmic inheritance affects formation of antibodies, since this would be expected to lend greater influence to the maternal parent furnishing the large cytoplasm of the ovum.

Finally, mention may be made of strain-dependent differences in mice found in plasma proteins (JOHNSON, ALBERT, PINKUS and WAGSHAL 1954) and in the excretion of amino acids (HARRIS and SEARLE 1953). Total plasma protein, and especially the globulin fractions, were found to drop with advancing age in cancer-susceptible C3H mice, even prior to development of tumor, but not in C57BL mice characterized by low spontaneous tumor incidence. Urinary taurine excretion was ten times as high in C57BL mice as in mice of strains A or CBA. This cumulative evidence points to fundamental differences in protein anabolism and catabolism in mice of different strains, which possibly may also be reflected in production of antibodies.

It is tempting to suspect a relationship among these biologic properties, and the resistance or susceptibility of mice to cancer, since genetic factors are operative in both types of phenomena. Analyses of genetic factors in incidence of mouse tumors by HESTON (1944) brought out data similar to the classic observations of WRIGHT (1934) on polydactyly in guinea pigs, interpreted as involving polygenic inheritance with a physiologic threshold, similar to the mechanism suggested in the present study for inheritance of natural agglutinins for sheep red cells.

At present the evidence is certainly insufficient for permitting any generalization about the genetic relationship between antibody production and tumor formation. If such a relationship exists, it probably operates on the level of interaction of gene products rather than of the genes themselves, a possibility that may deserve further study.

SUMMARY

(1) Breeding experiments have demonstrated a genetic difference in the ability of mice of strains C3H and C57BL to produce natural agglutinins for sheep erythrocytes.

(2) The differences in agglutinins have been analyzed according to methods of WRIGHT and MATHER, and it has been shown that they are controlled by one or more genetic factors.

(3) The genetic factors associated with low or absent antibody levels are incompletely dominant over those which allow the production of antibodies.

(4) In addition to genetic factors, a strong independent environmental effect was found to affect the antibody distribution.

(5) The findings of this study are discussed in relationship to observations by other authors on genetic control of antibody production and in connection with other biologic differences found in inbred mice, with particular reference to incidence of spontaneous tumors.

LITERATURE CITED

- CARLINFANTI, E., 1948 The predisposition for immunity. *J. Immunol.* **59**: 1-7.
- DAVIDSOHN, I., and K. STERN, 1949 Hemagglutinins in the serum of mice of low and high mammary tumor strains. *Proc. Soc. Exp. Biol. Med.* **70**: 142-146.
- 1949 Natural and immune antibodies in mice of low and high tumor strains. *Cancer Research* **9**: 426-435.
- 1950 Further studies on natural antish sheep agglutinins in mice of inbred strains. *Cancer Research* **10**: 571-576.
- 1954 Heterohemoantibodies in inbred strains of mice. II. Immune agglutinins and hemolysins for sheep and chicken red cells. *J. Immunol.* **72**: 216-223.
- DAVIDSOHN, I., K. STERN, and J. J. BITTNER, 1950 Milk agent and natural antish sheep agglutinins in mice of inbred strains. *Proc. Soc. Exp. Biol. Med.* **74**: 356-357.
- FINK, M. A., and V. A. QUINN, 1953 Antibody production in inbred strains of mice. *J. Immunol.* **70**: 61-67.
- FISHER, R. A., and F. YATES, 1953 *Statistical Tables for Biological, Agricultural, and Medical Research*, 4th edition. New York: Hafner Pub. Co.
- FJORD-SCHEIBEL, I., 1943 Hereditary differences in the capacity of guinea pigs for the production of diphtheria antitoxin. *Acta Pathol. Microbiol. Scand.* **20**: 464-484.
- FURUHATA, T., 1927 The heredity of blood groups. *Japan Med. World* **7**: 197-208.
- HARRIS, H., and A. G. SEARLE, 1953 Urinary amino acids in mice of different genotypes. *Ann. Eug.* **17**: 165-167.
- HESTON, W. E., 1944 The role of heredity in tumor development. *J. Natl. Cancer Soc.* **5**: 161-171.
- HIRSZFELD, L., 1928 *Konstitutionsserologie und Blutgruppenforschung*. Julius Springer: Berlin.
- JOHNSON, R. M., S. ALBERT, H. PINKUS, and R. R. WAGSHAL, 1954 Serum electrophoretic patterns in tumor-susceptible and tumor-nonsusceptible mice. *Proc. Am. Assoc. Cancer Res.* **1**: 23.
- MATHER, K., 1949 *Biometrical Genetics*. London: Methuen and Co. Ltd.
- SANG, J. H., and W. R. SOBEY, 1954 The genetic control of response to antigenic stimuli. *J. Immunol.* **72**: 52-65.
- SCHIFF, F., and L. ADELSBERGER, 1924 Über Blutgruppenspezifische Antikörper und Antigene. *Ztschr. Immunitätsforsch.* **90**: 335-367.
- STERN, K., and I. DAVIDSOHN, 1954 Heterohemoantibodies in inbred strains of mice. I. Natural agglutinins for sheep and chicken red cells. *J. Immunol.* **72**: 209-215.
- WIENER, A. S., 1951 Origin of naturally occurring hemagglutinins and hemolysins; a review. *J. Immunol.* **66**: 287-295.
- WRIGHT, S., 1934 The results of crosses between inbred strains of guinea pigs, differing in number of digits. *Genetics* **19**: 537-551.