one may not use the characteristic to show that C is a consequence of A, B, D_i and so one obtains more or less by the same reasoning:

THEOREM. *N.a.s.c. in order that a connected, nonseparable graph G of Betti number R be imbeddable in an orientable surface of characteristic $2 - \epsilon$ (ϵ even) [in a nonorientable surface of characteristic $1 - \epsilon$] is that it possess $1 + R - \epsilon$ loops $\lambda_0, \lambda_1, \ldots, \lambda_{R-\epsilon}$ satisfying the only independent chain-relation $\Sigma\lambda_h = 0$ [$R - \epsilon$ independent loops $\lambda_1, \ldots, \lambda_{R-\epsilon}$] such that every arc of the graph belongs to exactly two loops of the set $\lambda_0, \ldots, \lambda_{R-\epsilon}$ [$\lambda_1, \ldots, \lambda_{R-\epsilon}$]. Moreover, at each vertex of the graph property C (umbrella uniqueness) holds relative to the respective set of loops.*

Examples: Imbedding in a projective plane: $\epsilon = 1$, with D_2, ($\chi = 1$); imbedding in a torus; $\epsilon = 2$ with $D_1(\chi = 0)$.

The author wishes to express his appreciation for valuable remarks received from Hassler Whitney and Saunders Mac Lane.

* Supported in part by AFSOR under contract 693-64.

[1] Kuratowski, K., "Sur le problème des courbes gauches en topologie," *Fund. Math.*, **15**, 271–283 (1930).

[2] Whitney, H., "Nonseparable and planar graphs," *Trans. Amer. Math. Soc.*, **34**, 331–362 (1932); Whitney, H., "Planar graphs," *Fund. Math.*, **24**, 23–34 (1933).

[3] Mac Lane, S., "A combinatorial condition for planar graphs," *Fund Math.*, **28**, 22–32 (1936); Mac Lane, S., "A structural characterization of planar combinatorial graphs," *Duke Math J.*, **3**, 460–472 (1937).

[4] The proof which is not elementary may be found in the author's book *Introduction to Topology*.

[5] For $|K|$ is connected as a surface and this readily implies the same for Γ.

[6] Note that the connectedness of G implies that of $|K_G|$.

---

*DIFFERENTIAL ACTIVITY OF ALLELIC γ-GLOBULIN GENES IN ANTIBODY-PRODUCING CELLS**

BY EBERHARDT WEILER

THE INSTITUTE FOR CANCER RESEARCH, PHILADELPHIA

Communicated by Thomas F. Anderson, August 30, 1965

For heterozygous individuals the question may be asked whether at a given locus both alleles are active in individual cells, or only one. A decision can be reached when methods are available to determine phenotypes at the cellular level. This has been done with glucose-6-phosphodehydrogenase, specified by an X-linked gene, and it has been found that in each cell of heterozygous females only one or the other allele is phenotypically expressed.[1, 2] Such "phenotypic mosaicism" has not been found with markers on autosomes.[2] The present work is concerned with an autosomal locus in mice,[3] which is active in the synthesis of γ-globulin.

In mice, gamma-globulin (of the 7Sγ2a class)[4] occurs in genetically segregating variant forms (allotypes). In a preceding paper, a new effect called facilitation of hemolysis was described, which makes it possible to determine the allotype of antibodies secreted by individual cells.[5] In experiments to be described in this paper, hemolysis facilitation was applied to study heterozygous cells, and it will be shown that in single cells only one of the two parental phenotypes can be recognized.

Nature of the Experiment.—Allotype antisera serve as specific reagents for the two γ-globulin variants of the two mouse strains used. In the preceding paper[5] it was shown that the addition of allotype antiserum to Jerne-type preparations (in which plaque formation demonstrates antibody production by single cells) led to the appearance of plaques that would otherwise not have been visible, and caused an enlargement of other plaques. This facilitation effect is allotype-specific, and depends on an interaction between the allotype antibodies and sheep red cell-sensitizing antibodies produced by single cells. Cells can thus be characterized with respect to the allotype of the antibody they produce.

The two-round facilitation procedure[5] makes it possible to apply both allotype antisera in sequence to single-cell assay preparations on microslides. The effect of each allotype antiserum on individual plaques is recorded in photographs taken after the first round (after the first allotype antiserum), and again after the second round (after the second allotype antiserum). Since heterozygous mice have γ-globulin of both parental allotypes in their serum,[3] two cases are of interest. Each cell might produce antibodies of only one of the two parental allotypes. Then the allotype antiserum applied first should facilitate one allotypic subpopulation of plaques, and the other allotype antiserum, applied second, should facilitate the other subpopulation. Alternately, if heterozygous cells each produce both allotypes (and are phenotypically all alike), then all plaques that respond to facilitation should become facilitated by either allotype antiserum in the first round; the allotype antiserum applied second should be without effect, or might possibly cause a slight enlargement of already facilitated plaques. Model experiments with mixtures of homozygous cells of both genotypes were performed to demonstrate that heterogeneous populations of antibody-producing cells can indeed be resolved into subpopulations by this method.

In additional experiments, fluorescent allotype antisera were applied to Jerne-type preparations after facilitation had occurred. The aim was to correlate the allotype of γ-globulin present in the cytoplasm of plaque-forming cells with the allotype of sheep-cell antibody secreted by such cells.

Materials and Methods.—The allotype antisera were the same as used in the preceding paper. The demonstration of facilitation in single cell assays has also been described, as well as the immunization of donors of spleen cells.[5] Heterozygous cells came from C3H ♂ × C57Bl ♀ F_1 females. The inbred strains were the same as used before.[5]

Abbreviations: As in the preceding paper,[5] the allotypes will be called *a*, for Ig-1a, and *b*, for Ig-1b. The corresponding genotypes will be referred to as *a/a* and *b/b*, for homozygous cells from C3H or C57Bl mice, respectively; *a/b* are heterozygous cells from F_1 hybrids. $N(a)$ is normal serum from *a/a* mice, $N(b)$ from *b/b* mice, and $N(a + b)$ is a mixture of both sera. *Aa* will stand for allotype antiserum against *a*, and *Ab* against *b*.

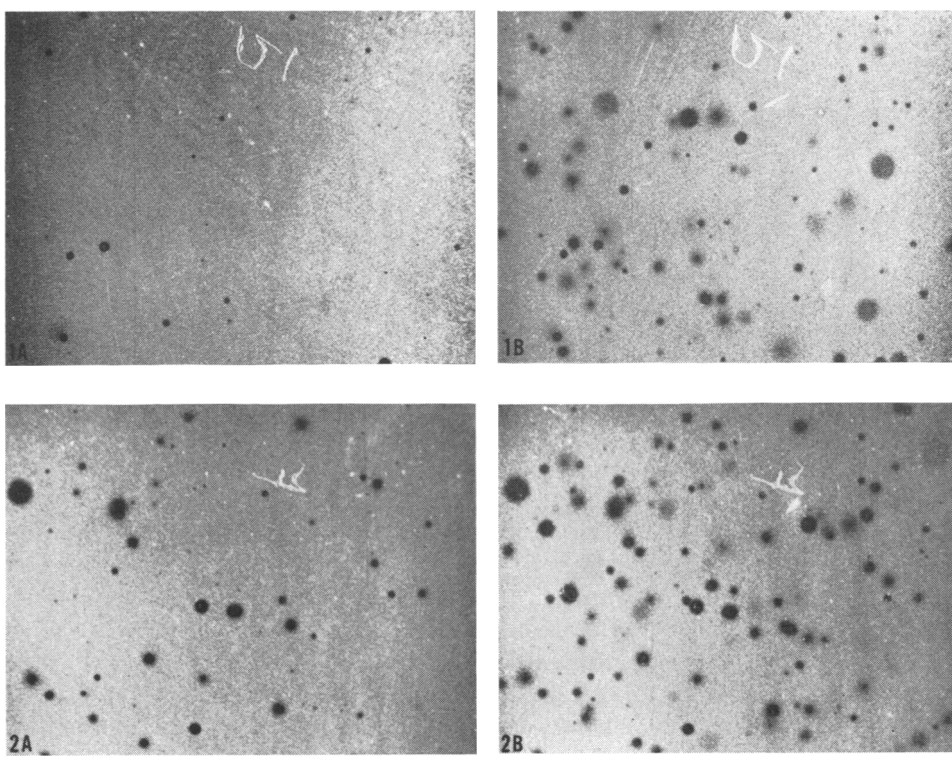
Fluorescent allotype antisera: γ-Globulin was purified from a pool of several individual sera by a modification of the method of Kekwik[6] and coupled to fluorescein isothiocyanate (BBL) according to the dialysis method of Clark and Shepard.[7] The fluorescein conjugates were dialyzed against phosphate-buffered saline for 4–5 days.

Fixation of specimens for fluorescent antibody work: The best fixation was achieved by freeze-substitution[8] followed by acetone: after being rinsed in veronal buffer, slides from single-cell assays were immersed in absolute alcohol at −79°C and left there overnight at −54°C. They were then transferred to: absolute acetone (2 min), 96% acetone (10 min), absolute acetone (2 min) (all at room temperature), and dried under a fan. They were incubated with fluorescent antiserum globulin, diluted 1/4, for 2 hr at room temperature. Fluorescent slides were examined and photographed in dark field, Leitz Ortholux, with the light of a mercury arc HBO 200. Filters were UG 1 (2 mm), and Euphos (2.5 mm) in the eyepiece.

The specificity of fluorescent staining was tested with a/a cells facilitated by Aa and with b/b cells facilitated by Ab. Sixty-eight per cent of a/a plaque-forming cells became stained by fluorescent Aa, and 79% of b/b plaque-forming cells by fluorescent Ab. As a criterion, a central cell was judged positively stained when it was significantly brighter than the majority of other nucleated spleen cells. (As one would expect, there were also stained cells not in the center of a plaque: cells producing γ-globulin that is not sheep cell-sensitizing antibody. In sample counts, their number was of the order of 1% of the input spleen cells.) Nonspecific staining (a/a cells with fluorescent Ab, or vice versa) was low; cells were either completely dark, or had only a slight greenish hue (Fig. 4*B*). (The fluorescent reagents were therefore not absorbed with tissue powder.[7]) Yet, when a/a cells were treated with fluorescent Ab, or b/b cells with Aa, 8 out of 184 cells, or 9 out of 149 cells, respectively, appeared weakly stained and could be classified as positive according to the criterion used. While specificity of staining has thus been demonstrated, these small numbers of "false positives" indicate a limit to the strictly quantitative interpretation of results.

(Fluorescent allotype antiserum, when present during the secretion phase, did not stain the intact spleen cells. Facilitating allotype antibody, had it entered the cells during the secretion phase, could conceivably have mediated a "sandwich"-type staining[9] by the counterspecific fluorescent allotype antibody for which, it will be recalled, it is an antigen. But apparently no antibody is taken up by the intact cells. "Sandwich"-type staining was, however, observed on red-cell ghosts in close vicinity to plaque-forming spleen cells [Fig. 4*B*].)

Results and Conclusions.—Sample preparations from a two-round facilitation experiment with a/b cells are shown in Figures 1 and 2. Relatively few plaques appeared in the first round in the presence of normal serum [$N(b)$] (Fig. 1*A*). Appli-



FIGS. 1 and 2.—First-round and second-round facilitation with heterozygous a/b cells in Jerne-type preparations. Fig. 1*A:* Normal serum in the first round; (*B*) Allotype antiserum Ab in the second round of the same preparation. Fig. 2*A:* Allotype antiserum Aa in the first round; (*B*) additional facilitation by Ab in the second round. Dark field illumination from one side. About $^1/_3$ of the preparations are shown. Magnification 3.6×.

cation of allotype antiserum *Ab* in the second round [sequence *N(b), Ab*] caused facilitation: appearance of new plaques, and enlargement of small ones (Fig. 1*B*). When *Aa* was given in the first round, more and larger plaques were produced than after normal serum (Fig. 2*A*). Yet, the subsequent application of *Ab* (sequence *Aa, Ab*) resulted in a new crop of facilitated plaques (Fig. 2*B*); large plaques already facilitated by *Aa* remained unchanged. Analogous results were obtained when the antiserum sequence was reversed (*Ab,Aa*).

Table 1 gives the numbers of plaques present after the first round and remaining essentially unchanged, and of plaques new or enlarged after the second round. Plaques were classified as described[5] (see Table 1). Enlargement of plaque di-

TABLE 1

PLAQUES AFTER THE SECOND ROUND COMPARED TO PLAQUES AFTER THE FIRST ROUND

Serum*		No. of Plaques after Second Round†				Second-round facilitation‡
First round	Second round	Same	Larger	New, small	New, large	
			a/b Cells			
N(b)	*Ab*	32	37	71	87	124
Aa	*Ab*	91	29	64	54	83
Aa	*Aa*	130	4	18	3	7
N(a)	*Aa*	51	11	33	22	33
Ab	*Aa*	205	9	13	12	21
Ab	*Ab*	246	0.3	6	0.7	1
N(b)	*N(a)*	77	4	22	1	5
			a/a Cells + *b/b* Cells			
N(b)	*Ab*	45	12	37	39	51
Aa	*Ab*	152	13	21	27	39
Aa	*Aa*	164	2	7	1	4
N(a)	*Aa*	45	9	44	44	54
Ab	*Aa*	168	22	29	27	48
Ab	*Ab*	179	1	8	0.3	1
N(b)	*N(a)*	51	5	14	0.7	6

* For abbreviations see *Materials and Methods.*

† Individual plaques after the second round were classified in comparison to their first round image: "same," plaques unchanged, or increased in diameter by less than 2.5×; "larger," increase in diameter after second round 2.5× or more; "new, small," plaques were new after second round, with a diameter of less than 0.16 mm; "new, large," new after second round, diameter 0.16 mm or larger (compare Fig. 2). All numbers are averages of triplicate determinations. First-round facilitation can be derived from the total number of plaques present after the first round = sum of "same" and "larger" plaques, comparing, for example, *N(b), Ab* with *Aa, Ab.* Second-round facilitation is given as the sum of "larger" and "new, large" plaques.

‡ In some cases, a deviation of the facilitation value from the sum of "larger" and "new, large" is due to averaging and rounding off to whole numbers.

ameter by 2.5 × served as the criterion to distinguish "larger" from "same" plaques. Histograms of diameter changes demonstrate the differential response to second-round facilitation in sequences *Aa, Ab* and *Ab, Aa.* Among plaques classified as "same," 90 per cent had not changed in size, or had increased in diameter by less than 1.3 ×. Control *N(b), N(a)* shows only a small number of "false positives" in the second round, in the absence of allotype antiserum. Controls *Aa, Aa* and *Ab, Ab* show that first-round facilitation was essentially complete, and could not be increased substantially by the same allotype antiserum in the second round. Sequences *Aa, Ab* and *Ab, Aa* (as compared to sequences *N(b), Ab* and *N(a), Aa,* respectively) demonstrate two subpopulations among plaques caused by *a/b* cells; one can be facilitated in the first round, and the other in the second. This indicates two phenotypic subpopulations among the heterozygous cells, different with respects to the allotype of the antibody produced. In the same way the two genotypic subpopulations could be recognized in the experiment with a

1:1 mixture of a/a and b/b homozygous cells (Table 1). Three other experiments with a/b cells and one other experiment with $a/a + b/b$ cells gave the same result.

Two aspects of the hybrid cell experiment require special consideration. One is that Ab, whether applied in the first or in the second round, caused 3–4 times as many plaques to become facilitated as did Aa. One might suspect, as a reason, that Ab is intrinsically a more efficient facilitating agent than Aa. In the preceding work, however, both antisera were equally effective in single-cell assays, producing on the average a 3.8-fold increase in the number of first-round plaques; and in assays with antisera to red cells, Aa was a more effective agent than Ab. It appears, then, that more hybrid cells produced antibody of b allotype than of a allotype. The same was found to be true in three other experiments with hybrid cells, although the differences were not quite as large: the factors were 2×, 1.5×, and 2.2×, respectively.

Another important point concerns the question whether or not there are also cells producing both allotypes, as antibodies, in about equal amounts. One aspect of the data seems to indicate that this possibility is real. In the Aa, Ab sequence, the number of plaques per slide facilitated in the second round was less, by 41 plaques, than in the $N(b)$, Ab sequence. (Aa, when present in the first round, reduced the number of plaques facilitated by Ab in the second round.) This difference might represent "double producers," which can be facilitated by either allotype antiserum. In the analogous comparison Ab, Aa with $N(a)$, Aa, the difference was 12 plaques per slide. The discrepancy between 41 and 12 plaques casts doubt on the interpretation, because the number of double producers should be the same. Second-round facilitation in Aa, Ab was 67 per cent of that in $N(b)$, Ab; it was 64 per cent in Ab, Aa as compared to $N(a)$ Aa. The analogous value, taken as an average from all four experiments with a/b cells, is 66.6% ± 3.2% (± standard error of the mean), significantly different from 100 per cent. However, a similar reduction of second-round facilitation by first-round allotype antiserum occurred also with homozygous cells.[5] The percentage values obtained in two experiments with mixtures of homozygous cells, and in three experiments with pure homozygous cells[5] are, on the average, 69.4% ± 8.8%, also significantly different from the theoretical value of 100 per cent ($p < 0.02$), but not significantly different from the average value for heterozygous cells ($p < 0.8$). Populations of heterozygous cells were thus phenotypically indistinguishable from populations of homozygous cells. The resolution of the method, however, was not sufficient to substantiate or exclude the possibility that a minority of heterozygous cells produce antibodies of both allotypes in about equal amounts.

In additional experiments, allotype antiserum was first added to Jerne-type preparations during the secretory phase (first round), to facilitate, selectively, plaques produced by red cell antibody of the corresponding allotype; after fixation, the slides were incubated with fluorescent allotype antiserum of the same or of the alternate specificity. The numbers of stained cells among all cells in the centers of plaques ("plaque-forming cells") were recorded. With heterozygous cells, two possible results could be expected. There could be a correlation between facilitation and cytoplasmic staining if one of the two Ig-1 alleles is active and the other quiescent in directing the synthesis of red-cell antibody and of all 7Sγ2a globulin as well. Alternately, staining by the fluorescent reagents could be independent of the

specificity of the allotype antiserum used for facilitation; in that case both alleles would be active in γ-globulin synthesis, but only one of them in the synthesis of red-cell antibody.

One experiment with a/b cells, and one model experiment with a mixture of a/a and b/b cells are presented in Table 2; results of the two experiments were similar.

TABLE 2

STAINING OF CELLS BY FLUORESCENT ANTIBODY AFTER FACILITATION

Facilitating serum	Fluorescent serum	Cells/slide* Neg.	Pos.	% Pos.	No. slides
		$a/a + b/b$ Cells			
$N(a + b)$	Aa	25	16	39	2
	Ab	39	12	24	1
Aa	Aa	37	67	64	2
	Ab	94	19	17	1
Ab	Aa	100	23	19	2
	Ab	48	89	65	1
		a/b Cells			
$N(a + b)$	Aa	19	8	30	2
	Ab	8	16	67	1
Aa	Aa	45	83	65	2
	Ab	108	35	24	1
Ab	Aa	57	20	26	2
	Ab	26	75	74	1

After treatment with "facilitating serum" (or normal serum), replicate Jerne-type preparations were fixed and treated with either of the two fluorescent allotype antisera. (For abbreviations, see *Materials and Methods*.) The numbers of stained ("pos.") and unstained ("neg.") plaque-forming cells per slide are given, as well as positive cells in per cent of all cells scored. The number of slides scanned is given in the last column. (Fluorescent Ab was in short supply, therefore only one slide was used for each determination.) Both experiments were repeated, giving essentially the same result.

* Numbers are averages, when two slides were scanned.

When Aa had been the facilitating agent, 65 per cent of the a/b cells and 64 per cent of the $a/a + b/b$ cells were stained by fluorescent Aa, but only 24 per cent and 17 per cent, respectively, were stained by the counterspecific fluorescent Ab. In the reverse situation, after facilitation by Ab, 74 per cent of the a/b cells and 65 per cent of the $a/a + a/b$ mixed cells were stained by fluorescent Ab, while the counterspecific Aa stained only 26 per cent and 19 per cent, respectively. The experiments were repeated, and gave the same result. It appears that in both experiments the facilitating allotype antiserum selected plaques out of a heterogeneous population, and the allotype of cytoplasmic γ-globulin in central cells corresponded to the allotype of the secreted red-cell antibody. This was expected with mixtures of a/a and b/b cells. With heterozygous cells, the result indicates that in each cell only one of the Ig-1 alleles is active and the other (relatively) quiescent. (Cf. Figs. 3 and 4.)

The possibility that among a/b cells there were a minority of "double producers" should be considered again. If all cells belonged to one or the other of the two phenotypic subpopulations, then the number of cells stained by Ab should be the same, whether normal serum or facilitating Aa was present during the secretion phase, and vice versa. However, 35 cells per slide were stained by Ab after facilitation by Aa, as compared to 16 cells after $N(a + b)$ (Table 2). Twenty cells per slide were stained by Aa following facilitation by Ab, and only 8 following treatment with $N(a + b)$. It is possible that this discrepancy is caused by some double producers. However, since there was also a small discrepancy, in the same direction, when mixed homozygous cells were used (Table 2), the discrepancy might be due to some "false positives" (see *Materials and Methods*), which would be more

numerous in facilitated than in normal serum preparations, because the total number of plaques is larger.

Discussion.—The conclusion from the foregoing is that populations of heterozygous antibody-producing cells are phenotypic mosaics with respect to allotype. There is a correlation between the allotype of antibodies secreted by single cells (as revealed by facilitation) and the allotype of cytoplasmic γ-globulin (as revealed by fluorescent allotype antibody). Therefore, it appears that of the two allelic genes specifying γ-globulin allotype, one is (relatively) active and the other (relatively) quiescent in the majority of plaque-forming cells. The Ig-I mouse allotypes are expressed on the H- (A-) chains of 7Sγ2a globulins.[10] If, in mice, allotypic dif-

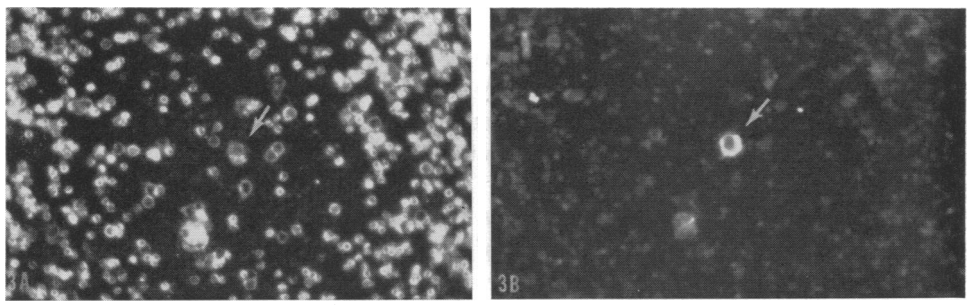


FIG. 3.—(*A*) A plaque in dark field, visible illumination. The central cell (arrow) is of *a/b* genotype, facilitation was by *Ab*. (*B*) The same plaque in UV illumination. The central cell is stained by fluorescent *Ab*, indicating γ-globulin of *b* allotype in its cytoplasm. Magnification, 240×.

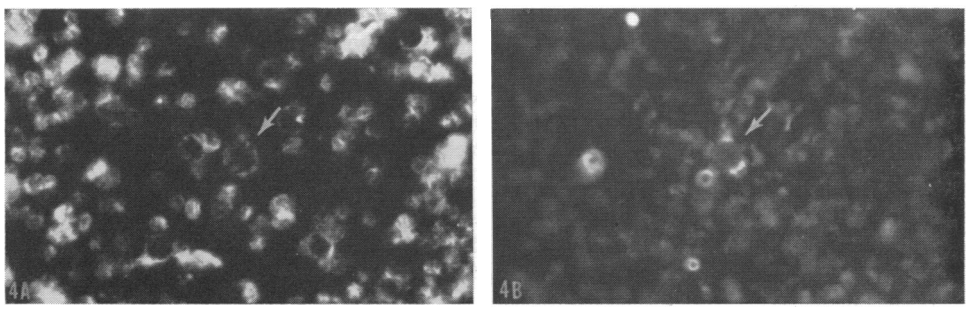


FIG. 4.—As in Fig. 3, but the preparation was facilitated by *Aa*. The central cell was not stained by fluorescent *Ab*. (Some red-cell stromata in the immediate vicinity were stained, indicating a "sandwich" effect; see *Materials and Methods*.) Magnification, 520×.

ferences among γ-globulins are due to differences in amino acid composition or sequence (as has been found in other species),[11, 12] the finding of differential gene activity would refer to the structural genes specifying the amino acid sequence on H-chains.

Phenotypic mosaicism exists in female mammals with respect to genes located on the X chromosome.[1, 2, 14] It can be understood as a dosage regulation effect, since males have only one X chromosome but females have two. On the other hand, such a phenomenon has not been demonstrated previously for autosomal genes. In fact, there are examples where both parental alleles are known to be expressed in each cell, such as transplantation and blood group antigens, or polypeptide chains of hemoglobin.[2] The differential activity of genes specifying γ-globulin, as reported in

the present work, so far constitutes an exception among autosomal loci. This finding may be related to the fact that in plasma cell tumors only one of two parental allotypes is expressed (literature in Mårtensson[13]).

Colberg and Dray,[15] studying all γ-globulin-containing cells in the lymph nodes of heterozygous rabbits, found both parental allotypes (which are expressed on L-chains[16]) present in individual cells. However, the data obtained by Ingraham *et al.*[17] with antibody-producing rabbit spleen cells can be interpreted in the same way as the present results.

It has been proposed[18, 19] that random variability of γ-globulin genes is responsible for the great variety in antibody specificities; and that antigen acts as a selective, not as an instructive agent. If that were so, it would follow from the great number of different antibody specificities that the random occurrence of a chromosomal template prescribing a given specificity is a rare event. One would expect that among cells exposed to a given antigen only a few can react, and that in the majority of those that can react (or in their descendants), only one of two allelic genes happens to be the template corresponding to the test antigen. The present work is in agreement with this expectation.

Summary.—In individual heterozygous mouse cells, the γ-globulin allotype of secreted antibody to sheep red cells and of cytoplasmic γ-globulin was determined. It was found that of the two parental alleles determining allotype, one or the other is relatively active in individual cells, while its allele is relatively quiescent.

Note added in proof: As the galleys of this paper were being read, a paper appeared by B. Pernis, G. Chiappino, A. S. Kelus, and P. G. H. Gell [*J. Exptl. Med.*, **122**, 853 (1965)], who studied the cellular localization of rabbit γ-globulin allotypes by means of immunofluorescence. These authors also found phenotypic mosaicism among γ-globulin-containing cells.

The author is greatly indebted to Drs. Jack Schultz, Thomas F. Anderson, and Ivan Jeanne Weiler for their interest in this work and for valuable criticisms of the manuscript. He also thanks Mrs. Elsa Melletz and Mrs. Evelyn Breuninger-Peck for their excellent assistance.

* This investigation was supported by Public Health Service research grants CA 04946 and CA 06927.

1 Davidson, R. G., H. M. Nitowsky, and B. Childs, these PROCEEDINGS, **50**, 481 (1963).

2 Beutler, E., in *Human Genetics*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 29 (1964), p. 261.

3 Herzenberg, L. A., N. L. Warner, and L. A. Herzenberg, *J. Exptl. Med.*, **121**, 415 (1965).

4 Fahey, J. L., J. Wunderlich, and R. Mishell, *J. Exptl. Med.*, **120**, 243 (1964).

5 Weiler, E., E. W. Melletz, and E. Breuninger-Peck, these PROCEEDINGS, **54**, 1310 (1965).

6 Day, E. D., J. Planinsek, L. Korngold, and D. Pressman, *J. Natl. Cancer Inst.*, **17**, 517 (1956).

7 Clark, H. F., and C. C. Shepard, *Virology*, **20**, 642 (1963).

8 Weiler, E., *Brit. J. Cancer*, **10**, 560 (1956).

9 Weller, T. H., and A. H. Coons, *Proc. Soc. Exptl. Biol. Med.*, **86**, 789 (1954).

10 Mishell, R. I., and J. L. Fahey, *Science*, **143**, 1440 (1964).

11 Meltzer, M., E. C. Franklin, H. Fudenberg, and B. Frangione, these PROCEEDINGS, **51**, 1007 (1964).

12 Reisfeld, R. A., S. Dray, and A. Nisonoff, *Immunochemistry.*, **2**, 155 (1965).

13 Mårtensson, L., *J. Exptl. Med.*, **120**, 1169 (1964).

14 Lyon, M. F., *Nature*, **190**, 372 (1961).

15 Colberg, J. E., and S. Dray, *Immunology*, **7**, 273 (1964).

16 Kelus, A. S., *Biochem. J.*, **88**, 4P (1963).

17 Ingraham, J. S., A. A. Biegel, and C. W. Todd, *Federation Proc.*, **24**, 180 (1965).

18 Burnet, M., in *The Clonal Selection Theory of Acquired Immunity* (Nashville: Vanderbilt University Press, 1959).

19 Lederberg, J., *Science*, **129**, 1649 (1959).