Passive Sensitization in vitro: Effect of Antibody Concentration on the Lag Period and Velocity

Abstract. *Strips of guinea pig ileum were sensitized in vitro in various concentrations of rabbit antibody prepared against ovalbumin. The dependence of the first-order velocity constants upon antibody concentration was hyperbolic. The limiting velocity constant was 0.0097 per minute, and the antibody concentration giving half the limiting velocity was 0.0035 milligrams per milliliter. The lag period varied inversely with the logarithm of the antibody concentration.*

The degree of immunological sensitization attained by incubating isolated tissues with antibody varies with the antibody concentration and the temperature (*1*). Although it is implicit in those studies that these two parameters affect the velocity of sensitization, most of the quantitative data have been expressed either as the time necessary for the production of a constant response at a given temperature or as the antibody concentration required to produce a given response at various temperatures in a constant time.

We now report that both the velocity of sensitization and the duration of the "lag" period are dependent on the antibody concentration. The evidence was obtained by determining the time courses of sensitization of bundles of ileum from the normal guinea pig, which had been incubated for various intervals of time with 0.004, 0.008, 0.016, 0.032, or 0.064 mg per milliliter of the γ-globulin fraction obtained from rabbit antibody against ovalbumin.

The ovalbumin used, for immunization as well as for the elicitation of tissue anaphylaxis, was made according to the method of Kekwick and Cannan (*2*) and recrystallized six times. The rabbit antibody to ovalbumin was made according to the method of Swineford and Samsell (*3*). Ultracentrifugal analysis showed a single peak having a sedimentation constant of 6.9*S* at 20°C. Immunochemical analysis by the quantitative precipitin technique showed that 40 percent of the total γ-globulin was specifically precipitable with ovalbumin at optimum proportions.

Guts obtained from three male (400 g) guinea pigs were thoroughly flushed with Tyrode's solution and conditioned for sensitization by storage in separate vessels for 2 hours at 4°C. Each gut was then cut into 10-cm segments, and bundles of tissue were assembled for incubation by tying together three strips selected at random from each of the three vessels. The bundles were first incubated for 45 minutes at 19.6°C in oxygenated Tyrode's solution and then transferred at random into one of five vessels containing 100 ml of oxygenated Tyrode's having a defined concentration of antibody. Tissue samples were withdrawn from each reaction vessel at intervals (Table 1), thoroughly washed by being moved successively through three 100-ml portions of fresh Tyrode's, and then challenged in 10 ml of a 0.1 percent ovalbumin–Tyrode's solution containing semicarbazide (2 μg/ml). The challenge reaction was permitted to develop for 10 minutes, and the tissue was withdrawn, blotted, and weighed in a covered vessel. The "release" fluid was centrifuged at 0°C, and portions of the supernatant were taken for the extraction of histamine with *n*-butanol (*4*).

Table 1. Time-course of sensitization for antibody concentrations (0.004 to 0.064 mg/ml) as indicated by the histamine released [10^{-9} mole per gram of tissue (wet weight)].

Incubation period (min)	Histamine released (10^{-9} mole/g)				
	0.004	0.008	0.016	0.032	0.064
45					2.16
60					2.98
75			1.15	3.00	4.54
90		1.06	3.16	3.90	5.20
105					6.97
120	1.15	2.39	4.24	5.73	7.18
150	2.60	4.09	5.63	5.82	7.80
180	4.15	5.68	7.40	7.45	8.82
210	5.20				9.55
240	6.80	7.24	8.62	9.40	9.50
270	8.04				
300		8.00	9.58		
360	9.25	9.25	10.90		

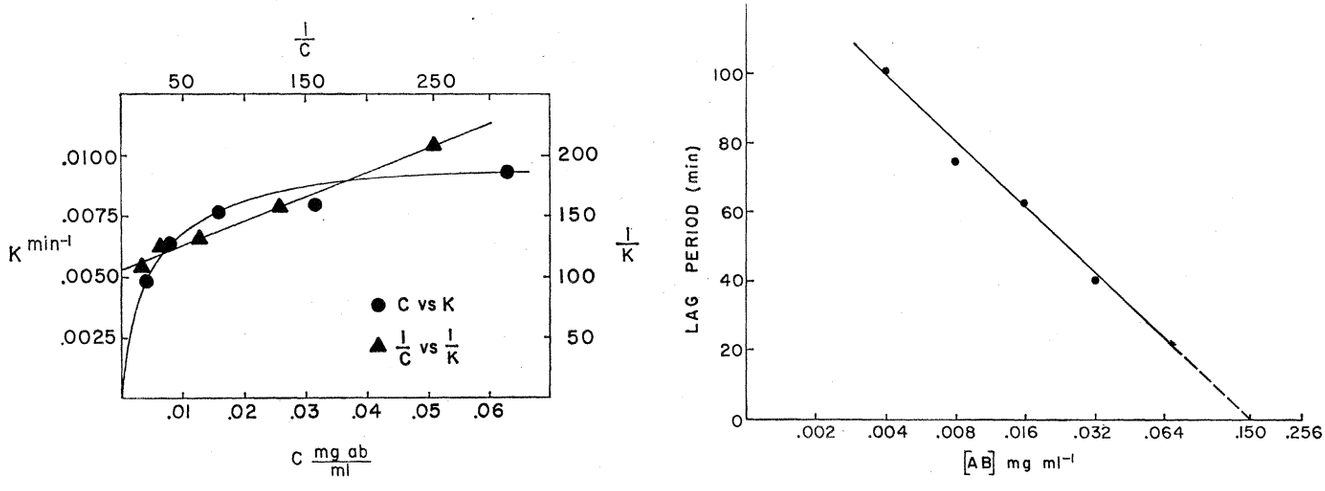

Fig. 1 (left). Direct and reciprocal plots of the variation of velocity constants with antibody concentration. Direct plot ● — ● on left and lower coordinates. Reciprocal plot ▲ — ▲ on right and upper coordinates. Fig. 2 (right). Variation of the calculated lag period. t^*, with antibody concentration $[AB]$. The line is projected to that antibody concentration at which $t^* = t_0$.

Table 2. Constants calculated from time courses. Values for H_{max} were obtained by successive approximation from the data.

Antibody (mg/ml)	H_{max} (10^{-9} mole)	t^*	k min^{-1}
0.004	13.5	102	0.00478
.008	11.0	75	.00637
.016	12.0	63	.00763
.032	11.5	40	.00798
.064	11.3	23	.00935

The butanolic phase was passed through columns packed with cotton acid-succinate (*5*), and the adsorbed histamine was liberated with 0.2*N* HCl, neutralized, and assayed on ileal strips obtained from normal guinea pigs according to the method used in this laboratory (*6*).

Curves of unit histamine release plotted against time are sigmoidal, the slope becoming steeper and the "lag" period shorter with increasing antibody concentration. The initial velocity constants for these curves were evaluated from the modified form of the first-order law as applied to growth data by Lotka (*7*) and Brody (*8*). In the present case the working expression can be written

$$1 - \frac{H}{H_{max}} = e^{-k(t-t^*)} \qquad (1)$$

in which H_{max} is the maximum unit histamine release, H is the release at time t, and t^* is the time at which the extrapolated curve crosses the time axis. The value for H_{max} was obtained graphically for each antibody concentration by successive approximations, differing in steps of 0.5 units, to reach that value which would linearize the curve of log $(1 - H/H_{max})$ plotted against $(t-t^*)$. The first-order constants were obtained by multiplying the slopes by -2.303. The values of H_{max}, t^*, and k for the respective systems are entered in Table 2.

The dependence of k upon antibody concentration (Fig. 1), is described by a curve which rises steeply from the origin and then becomes asymptotic to a line parallel to the x-axis. The curve evidently is an hyperbola since a plot of the reciprocals produces a straight line. Using the classical notation of Michaelis-Menten kinetics,

$$\frac{1}{V} = \left(\frac{1}{S} \cdot \frac{K_s}{V_{max}}\right) + \frac{1}{V_{max}} \qquad (2)$$

in which K_s/V_{max} is the slope of the reciprocal plot and $1/V_{max}$ its intercept, we can evaluate the limiting velocity, V_{max}, and the antibody concentration at which the velocity has one-half the maximum value, K_s. Putting k for V and the antibody concentration for S, and taking the intercept as 103 and the slope as 0.36, we obtain 0.0097 min^{-1} for the V_{max}, and 0.0035 mg/ml for K_s.

One of the features of passive sensitization is the so-called induction or "lag" period which elapses between the passive transfer of antibodies and the time at which a certain degree of reaction first becomes evident. Our experiments show this in the sigmoidal form of the function describing the time course of sensitization. The reduction of the "lag" period with increasing antibody concentration is quantitatively illustrated (Fig. 2) by the linear plot of t^* against antibody concentration on semilogarithmic coordinates. Assuming that the linear function continues to hold at antibody concentrations higher than those used in our study, we can see that the value of t^* approaches that of t_0 at about 0.15 mg of antibody per milliliter.

The sensitizing power of a particular antibody preparation often has been given in terms of the minimum sensitizing dose. For example, Kabat and Landow (*9*) found that 30 μg of antibody nitrogen was sufficient to sensitize a guinea pig in 48 hours; Brocklehurst *et al.* (*10*) reported positive Schultz-Dale reactions in tissues containing 0.02 μg of antibody per gram; and, more recently, Humphrey *et al.* (*11*) showed that histamine release by isolated mast cells was still possible under conditions in which the maximum antibody load was less than 10^6 molecules of γ-globulin per cell. Owing to the kinetic basis of the sensitization reaction suggested by our present work, the minimum sensitizing dose can have only a practical meaning; presumably, a vanishingly small dose ought to be sufficient if the time allotted for incubation were long enough and if the rate of antibody disappearance were negligibly small.

GEORGE A. FEIGEN
CHRISTEN B. NIELSEN
Department of Physiology, Stanford University, Stanford, California 94305, and *Division of Neurology, Veterans Administration Hospital, Palo Alto, California 94304*

References and Notes

1. G. A. Feigen, C. B. Nielsen, G. Terres, *J. Immunol.* **89**, 717 (1962).
2. R. A. Kekwick and R. K. Cannan, *Biochem. J.* **30**, 227 (1936).
3. O. Swineford and D. Samsell, *J. Allergy* **33**, 423 (1962).
4. F. McIntire, L. W. Roth, J. L. Shaw, *J. Biochem.* **170**, 537 (1947).
5. Cotton acid–succinate [see (*4*)] was prepared by heating 10 g of U.S.P. cotton at 100°C for 46 hours in a solution consisting of 5 g of sodium acetate, 40 g of succinic anhydride, and 300 ml of glacial acetic acid.
6. G. A. Feigen, E. M. Vaughan Williams, J. K. Peterson, C. B. Nielsen, *Circulation Res.* **8**, 713 (1960).
7. A. J. Lotka, *Elements of Mathematical Biology* (Dover, New York, 1956).
8. S. Brody, *Bioenergetics and Growth* (Reinhold, New York, 1945).
9. E. A. Kabat and Harold Landow, *J. Immunol.* **44**, 69 (1942).
10. W. E. Brocklehurst, J. H. Humphrey, W. L. M. Perry, *Immunology* **4**, 67 (1941).
11. J. H. Humphrey, K. F. Austen, H. J. Rapp, *ibid.* 6, 226 (1963).

12. Supported by contract Nonr 225(46) between Stanford University and the ONR, and by PHS grant-in-aid HE-03693-06.

11 July 1966.

Reversal in Tactile and Visual Learning

Moffett and Ettlinger (*1*) conclude from study of four monkeys that "tactile and visual learning take place in independent functional systems." Their conclusion, however, should be held in doubt since their procedure permitted the possible presence of a confounding phenomenon. It has been shown that, after such extensive discrimination training as that used by them, discrimination-reversal learning is retarded in nonvisual discrimination-learning tasks (*2*).

In order that Moffett and Ettlinger may legitimately derive their conclusion, they must be able to draw a meaningful performance baseline for each task in reversal. That is, reversal of a tactile discrimination must be meaningfully compared with reversal of the same discrimination in light. If the former reversal task is handicapped by the effect mentioned above, comparison of reversal in light and darkness has little utility.

The validity of Moffett and Ettlinger's conclusion, therefore, awaits experimental proof that their procedure did not fall prey to the overtraining effect that has been described in the literature dealing with discrimination reversal.

GERALD B. BIEDERMAN
Department of Psychology, University of Toronto, Toronto 5, Ontario

References

1. A. Moffett and G. Ettlinger, *Science* **153**, 205 (1966).
2. W. F. Hill, N. E. Spear, K. N. Clayton, *J. Exp. Psychol.* **64**, 553 (1962); J. Komaki, *Ann. Anim. Psychol.* **12**, 1 (1962); K. N. Clayton, *Percept. Mot. Skills* **17**, 83 (1963); *J. Exp. Psychol.* **65**, 255 (1963); W. F. Hill and N. E. Spear, *J. Exp. Psychol.* **65**, 317 (1963).

11 July 1966