DISQUISITIONS ON ORIGINAL ANTIGENIC SIN

I. Evidence in Man*

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Given a dose of antigen, an organism may or may not respond by producing specific antibody. The contending theories disagree on almost every detail of the mechanism set in motion; they are one in assuming that whatever is or fails to be produced, is *specific*. Against the background of this tacit assumption any qualitative failure of the antibody response stands out as a major paradox.

Thus it has been known for over 10 yr now that humans vaccinated against influenza produce antibodies against the immunizing antigen, but produce antibodies of higher titer against the antigen that was their first childhood experience of influenza, even if that strain happened to be absent from the vaccine—hence the name Original Antigenic Sin (1). The phenomenon rests on solid experimental foundations (2–11), and has been reproduced in laboratory animals (10, 12, 13). The evidence is incompatible with either of the reigning immunological theories in their simplest form. On an instructive model the antibody molecule would be moulded on the antigen and hence, by definition, must be more complementary to it than to any other antigen. On a selective model the antigen acts as mitogenic stimulus for cells predestined to form that particular antibody; once again, the response should be most complementary to the antigen which triggered the proliferation of a particular clone of cells.

Our studies were prompted by this paradox, and undertaken in the hope that a satisfactory solution might also teach us something about the production of antibodies in general. The work falls into three parts: (*a*) reexamination of the sera on which the Doctrine of Original Antigenic Sin was founded; (*b*) demonstration of a basic difference between these and standard primary or secondary sera, together with a hypothesis to account for the observations; and (*c*) experimental tests of the new hypothesis.

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Materials and Methods

Diluents.—Normal saline (0.15 M NaCl and 0.02 M phosphate buffer at pH 7.2) was used in all tests, except when concentrating virus by adsorption-elution or when destroying nonspecific inhibitors by the neuraminidase of *Vibrio cholerae*. The diluent in these procedures was *Ca-Mg-saline* (14).

Viruses.—All strains of influenza virus were drawn from the collection of the Virus Laboratory, School of Public Health, Ann Arbor. Fresh stocks were grown from the original freeze-dried preparations of the same viruses used in the vaccine trials (9, 11). Dilute seed (about 10^2 ID_{50}) was inoculated into the allantois of 11-day-old chick embryos. After 2 days' incubation at 35°C the eggs were chilled, the allantoic fluids harvested aseptically, pooled, and titrated for hemagglutinin. For each 1000 hemagglutinating units of virus 1% v/v of washed and packed human erythrocytes were added, the suspension well mixed and incubated for 30 min at 4°C. The supernate was then pipetted off and the heavily agglutinated cells washed with two changes of approximately 20 times their volume of chilled normal saline. After the second wash the packed cells were taken up in Ca-Mg-saline ($\frac{1}{10}$ volume of the original allantoic fluid), and incubated for 30 min at 35°C under gentle stirring. At the end of this period the cells were spun out, 1% v/v of a 6% solution of NaN_3 added to the supernate and the preparation stored at 4°C. Such eluates contained 90 to 95% of the virus originally present in the allantoic fluid pool, and kept without change of titer for at least a year.

Sera.—Samples collected during field trials (9, 11) and stored without preservative at 4°C were inactivated by treatment with neuraminidase and subsequent heating at 62.5°C for 20 min (15). The nonspecific inhibitory titers of human sera were reduced by this treatment to levels of <5, without affecting their specific antiviral activity. Such inactivated sera were stored frozen at −15°C.

Test for Viral Hemagglutinin.—Serial 2-fold dilutions of the trial samples were set out in plastic trays (WHO model), in 0.25 ml of normal saline, with the aid of Takátsy-loops. One standard drop (0.025 ml) of 5% fowl red cells was added from a calibrated dropping pipette, the trays shaken and the pattern of settled cells evaluated after 35 min standing at room temperature. An *agglutinating dose* is that amount of virus which causes partial (+) agglutination of the cells under these conditions; it corresponds to about 6×10^6 electron microscopically visible particles. Nine intermediate degrees of agglutination were routinely read and the end point determined by interpolation where necessary. In this form the hemagglutinin test is reproducible to about ±10% (or ± 0.05 $\log_{10}$ units). As a rule, all titrations were done in duplicate.

Test for Antibody.—Antibody directed against the V antigen was assayed as antihemagglutinin. Samples of the inactivated sera were serially diluted in 0.25 ml normal saline and one standard drop (0.025 ml) of saline containing exactly 4 agglutinating doses of the test virus was added to each cup. The trays were shaken, and incubated at room temperature for 60 min. Then 0.025 ml of a 5% fowl red cell suspension was added to each cup, mixed, and the pattern of settled cells read after 35 min at room temperature. This test is as reproducible as are hemagglutinin titrations, but the absolute titers vary with different fowl cells. For that reason only selected birds with similarly reacting cells were used as donors, and all samples to be compared were always titrated (in duplicate) on the same day, using erythrocytes of a single bird.

Equilibrium Measurements.—The equipment (16) and the principles and practice of evaluation (17) have been fully described, and were used here without modification. An equilibrium test is basically a series of antibody titrations performed on the ultrafiltrates of an antiserum mixed with different doses of virus. As a rule each test was preceded by a range finding experiment. In this way the ten graded doses of antigen chosen for the main test were known to fall in the range of maximum information; i.e., where between 90 and 99.9% of antibody was

complexed. Two controls, with saline replacing the antigen, were included in each test. The time allowed for equilibration was 30 min (or about 3 times more than necessary), and the filters used for separating free antibody from the virus-antibody complex were Millipore VM membranes of an average pore diameter of 50 ± 3 mμ. Since large numbers of filtrations were performed each day, the data were processed on an IBM 7090 computer, the program performing the maximum likelihood fitting of the data to each of the four possible transforms of the mass law equation. Sets of data significantly deviating from linearity or not complying with the model on account of the intercepts or slope of the regression line are automatically rejected by this program. Such, however, were not found in this study. The final results are the weighted mean estimates of A, the number of antibody molecules/ml of serum, and of K, the equilibrium constant expressed in cgs-units.

RESULTS

Equilibrium Parameters of the Test Sera.—The vaccine trials were run in the years 1954 (9) and 1955 (11), covering a wide range of age groups, using both monovalent and polyvalent vaccines. For the sake of simplicity, the youngest age group (4 to 8 yr) was chosen for study. These children had their first exposure to influenza after the emergence of the A prime subtype (1946 to 1947), and could not have had experience of influenza A viruses other than those resembling the type strain FM1. Two subgroups, receiving either monovalent FM1 or SW vaccine were examined in detail. The first of these should represent a secondary response, the vaccine being homologous to the epidemic strains of the past 8 yr. The second, receiving a cross-reacting virus absent from human populations for at least 25 yr, should be regarded, formally at least, as responding primarily.

All prevaccination sera were first titrated against FM1 and SW virus. Forty-five of the 94 sera had measurable levels of antihemagglutinin against FM1 virus and none against SW. Those having undetectable levels of anti-FM1 were not tested further as there was no way of deciding whether these children had escaped natural infection with the prevalent epidemic strains and would thus respond primarily even to the FM1 vaccine, or whether they had been infected previously but either failed to respond or produced antibodies whose level was not maintained up to the time of the trials. All pairs of sera positive for anti-FM1 in the prevaccination bleed and available in quantities above 2.5 ml were tested by equilibrium filtration. The results obtained with sera from the 1954 trial (aqueous vaccines) are shown in Table I.

Looking at the prevaccination status first, we find that the antibody against FM1 made up rather less than 1% of the average gamma globulin in human plasma (about 10^{16} molecules/ml). The averages of the two groups of children do not differ significantly and the ranges largely overlap. The equilibrium constants are of the order of 10^{11} for both groups, indicating antibody of very high quality since, at least in rabbits and fowls, one finds equilibrium constants of the order of 10^{13} in primary and of 10^{11} in secondary sera (18, 19). Corresponding figures for human responses are not available, and thus this conclusion rests on

analogy. The same sera have no measurable antibody against SW virus, and hence the blank spaces in Table I: reliable equilibrium measurements cannot be made on sera of antihemagglutinin titers below 1:32.

In the postvaccination bleeds after monovalent FM1 vaccine the average antihemagglutinin titers have risen by over tenfold, and this is reflected also in the number of antibody molecules. These rises are significant: even the lowest of the postvaccination sera score higher than any in the prevaccination set, by either measurement, and we are bound to conclude that substantial amounts of new

TABLE I

Response to Aqueous Vaccines

Project: Lapeer, 1954; groups A and C (9)

Vaccine	Serum	No. of samples	Equilibrium parameters					
			vs. FM1 antigen			vs. SW antigen		
			K	A	aHA	K	A	aHA
FM1	Prevaccination	6	11.62 ± 0.21 (11.40–11.93)	13.48 ± 0.27 (12.87–13.62)	2.55 ± 0.21 (2.02–2.70)			<1.5
	Postvaccination	10	11.23 ± 0.18 (10.90–11.57)	14.65 ± 0.26 (14.33–15.03)	4.02 ± 0.22 (3.67–4.26)			<1.6 (8 × <1.5, 1.63, 2.41)
SW	Prevaccination	6	11.58 ± 0.24 (11.17–11.85)	13.85 ± 0.27 (13.01–14.11)	2.88 ± 0.36 (2.06–3.39)			<1.5
	Postvaccination	9	11.17 ± 0.13 (10.97–11.41)	14.92 ± 0.27 (14.41–15.48)	4.24 ± 0.29 (3.64–4.80)	12.34 ± 0.23 (11.97–12.83)	14.85 ± 0.31 (14.35–15.59)	3.51 ± 0.28 (2.96–3.99)

The mean equilibrium constants (K), concentrations of antibody molecules/ml (A), and antihemagglutinin titers/ml (aHA) are given in $\log_{10}$ units, followed by their standard deviation and, in parentheses, the range of observations.

antibody had been formed. The quality of this new antibody is only slightly better than it was before vaccination and this, once again, suggests that these children had already secondary anti-FM1 in their plasma when the project began.

The second group, given monovalent SW vaccine, reached almost equally high antihemagglutinin levels, even though initially none of the subjects had any measurable anti-SW, and it is unlikely that any of them would have had previous acquaintance with this virus. Yet, about 7% of their total gamma globulin turned into anti-SW antibody (range 2 to 11%, with one exceptionally high value, 38%). What is more, this antibody was of remarkably good quality, at least ten times better than what one should have expected. This combination of findings suggests that it was of secondary type, even though in response to a

nominally first experience. The left hand side of Table I reveals three remarkable things: there was as much anti-FM1 produced after monovalent SW vacnine as after homologous boosting; the quality of this antibody was just as good as after the homologous vaccine; and the number of antibody molecules was about the same when tested against one or the other antigen.

The second set of sera, collected in 1955, represents responses to monovalent vaccines given in a water-in-oil adjuvant. The postvaccination bleedings were obtained 6 wk after vaccination, at the height of the antibody response (11).

TABLE II

Response to Water-in-oil Vaccines

Project: Coldwater, 1955; groups I and III (11)

Vaccine	Serum	No. of samples	Equilibrium parameters					
			vs. FM1 antigen			vs. SW antigen		
			K	A	aHA	K	A	aHA
FM1	Prevaccination	6	11.37 ± 0.17 (11.14–11.67)	13.27 ± 0.14 (12.98–13.51)	2.92 ± 0.11 (2.80–3.21)			<1.5
	Postvaccination	7	11.15 ± 0.06 (11.08–11.33)	14.72 ± 0.27 (14.16–15.00)	3.76 ± 0.25 (3.34–4.22)			<1.5
SW	Prevaccination	5	11.28 ± 0.21 (11.01–11.64)	13.71 ± 0.22 (13.24–14.04)	3.19 ± 0.14 (2.87–3.40)			<1.5
	Postvaccination	9	11.34 ± 0.22 (11.01–11.67)	14.60 ± 0.23 (14.26–15.05)	3.88 ± 0.28 (3.26–4.43)	11.87 ± 0.23 (11.46–12.17)	14.65 ± 0.18 (14.36–15.17)	3.20 ± 0.12 (2.90–3.48)

The mean equilibrium constants (K), concentrations of antibody molecules/ml (A), and antihemagglutinin titers/ml (aHA) are given in $\log_{10}$ units, followed by their standard deviation and, in parentheses, the range of observations.

This second set of children was essentially of the same age group as those receiving aqueous vaccines and, judging by their prevaccination status, of the same immunologic experience (Table II). At the inception of the trials their sera contained antibody of low titer but high quality against FM1, and none against SW. Their postvaccination responses are also much the same: a great deal of new antibody was produced, the increase in quality on homologous boosting even less striking than in the previous group. Once again the consequences of Original Antigenic Sin are evident: there is a marked rise of anti-FM1 after the SW vaccine, this antibody is of excellent quality and, as in the previous group, about the same number of molecules scoring as anti-FM1 and anti-SW. The conclusion that all sera contained secondary type antibody still holds, but its cogency is less in this group since the serum samples were taken 6 wk after immunization, and it is conceivable that there might have been a low primary

response which was then boosted by the antigen persisting in the water-in-oil depot.

Yet, the two sets of results (Tables I and II) are so similar that any interpretation based on disparate mechanisms would seem farfetched. The joint evidence indeed permits some quantitative conclusions. Thus, the effect of boosting with a cross-reacting antigen is manifested in (*a*) the appearance of secondary

TABLE III

Distribution of Antibody in Absorbed and Dissociated Samples of Postvaccination Sera

Sera C/6 and A/7; Lapeer, 1954 (9)

Vaccine	Absorbing antigen	Sample	Antihemagglutinin titer/ml*		Difference of titers*
			vs. FM1	vs. SW	
FM1	FM1	Supernate	3.96	2.05	1.91
		Dissociate	3.72	1.72	2.00
	SW	Supernate	3.99	<1.00	>2.99
		Dissociate	2.78	1.90	0.88
	(Control)		4.23	2.41	1.82
SW	FM1	Supernate	4.61	3.92	0.69
		Dissociate	4.38	3.57	0.81
		Supernate	4.12	3.41	0.71
		Dissociate	4.51	3.67	0.84
	SW	Supernate	4.60	3.62	0.98
		Dissociate	4.06	3.32	0.74
	(Control)		4.82	4.02	0.80

* Log_{10} units.

looking antibody against the vaccinating virus; (*b*) the simultaneous appearance of high quality antibody against the originally experienced virus; and (*c*) that these are carried by the same number of molecules. The simplest logical step from here is to suppose that they register in identical numbers because they are on one and the same molecule. The next set of experiments was designed to test this proposition.

Fractionation of Secondary Sera.—An antigen, when added to a mixture of antibodies, will combine with the homologous component, the fraction of molecules bound being different in kind from the fraction left free. By the same procedure subpopulations of cross-reacting antibodies can be removed from sera, this being the time-honoured method of preparing specific serological reagents.

In our experiments postvaccination sera were absorbed with different doses of virus, using both the vaccinating strain and the strain corresponding to the primary, natural exposure. The mixtures of virus and serum were held at room temperature for ½ hr and then spun at 35,000 *g* for 45 min. As shown by control tests, this treatment is sufficient to deposit over 99.9% of the virus and, a fortiori, virus-antibody complexes; it does not measurably reduce the antibody titer of a serum. Thus the *supernate* removed from the absorption tubes represents all of the unbound antibody. The deposit was then taken up in 0.1 M glycine buffer at pH 3.0 and dispersed by sonic treatment (Raytheon oscillator, 60 sec at maximal output, at 0°C). At this hydrogen ion concentration most of the formed antigen-antibody complexes dissociate, and thus after a second spinning at 35,000 *g* the supernate will contain the balance of antibodies present in the original serum. These *dissociates* were decanted from the virus pellets, adjusted to neutrality with 0.6 M Aronsson-Grönwall buffer (20), and made up to the original volume of serum. By determining equilibrium parameters on both samples against both viruses, we have a set of quantitative absorption data from which a complete balance sheet of antibodies can be drawn up. Table III shows the results obtained with a pair of sera from the group that had received aqueous vaccines.

Taking the response to the FM1 vaccine first, it is evident that an absorbing dose of FM1 virus which reduces the homologous titer to roughly half of its original value (54%), leaves about the same fraction of anti-SW behind (44%). The corresponding dissociate is also only slightly richer in antibody homologous to the absorbing virus, and this is shown by the closeness of ratios between homologous and heterologous titers; 66:1 in the original serum, 81:1 in the supernate, and 100:1 in the dissociate. When SW virus is used as absorbing agent, the shift is more striking. While the anti-SW component has completely disappeared from the supernate (>25-fold reduction), the anti-FM1 titer is not even halved. In the dissociate, on the other hand, the ratio of anti-FM1 to anti-SW titers is 7.5:1, as against 66:1 in the original serum, or >1000:1 in the supernate. This serum thus behaves as a mixture containing a majority of molecules reacting with the vaccinating virus only (i.e. nonabsorbable by SW), together with some cross-reacting molecules (absorbable by SW, but capable of neutralizing FM1). By and large this holds for all sera we have tested; in some instances there was also an indication that the cross-reacting antibodies belonged to the most avid sector of the antibody population: on more extensive homologous absorption the supernate was left relatively poorer in heterologous than homologous antibody.

The serum obtained in response to the SW vaccine behaved quite differently. The striking feature of these results is the constancy of the anti-FM1:anti-SW ratios. Whether we tested a large or a small fraction of the original antibody, and whether these fractions had been bound or left behind by one or the other

of the absorbing viruses, within statistical variation the relative titers remained the same. Their ratio was 6.3 in the original serum, and the range of scatter was bounded by the values of 4.9 and 9.5. When this finding is evaluated against absorption results on standard sera (an example of which is shown in the top half of Table III), there is no choice but to conclude that we are dealing with

TABLE IV

Equilibrium Parameters of Absorbed and Dissociated Samples of a Postvaccination Serum

Serum A/7; Lapeer, 1954 (9)

Vaccine	Absorbing antigen	Sample*	Test antigen	K		A	
					Difference		Difference
SW	FM1	Supernate	FM1	11.68	−0.48	15.49	+0.03
			SW	12.16		15.46	
		Dissociate	FM1	11.37	−0.53	14.88	−0.03
			SW	11.90		14.91	
		Supernate	FM1	11.55	−0.54	15.29	+0.32
			SW	12.09		14.97	
		Dissociate	FM1	11.28	−0.81	14.99	−0.03
			SW	12.09		15.02	
	SW	Supernate	FM1	11.59	−0.66	15.48	−0.05
			SW	12.25		15.53	
		Dissociate	FM1	11.39	−0.46	14.53	−0.33
			SW	11.85		14.86	
	(Control)		FM1	11.31	−0.66	15.53	−0.01
			SW	11.97		15.54	

The equilibrium constants (K), concentrations of antibody molecules/ml (A), and their differences are given in $\log_{10}$ units.

* The samples correspond to the entries in the lower half of Table III.

antibody molecules each of which is highly avid against two different antigens. This interpretation is the same as reached in the fundamental study of Jensen et al. (10), where the double specificity of antibodies characteristic of the Original Antigenic Sin was clearly recognized.

Yet, since this conclusion is unorthodox and would set apart responses to cross-stimulation from both primary and secondary sera, we also determined the equilibrium constants and the number of antibody molecules in each of the fractions (Table IV).

Of the two parameters estimated by equilibrium measurements, the number of antibody molecules can be determined with greater accuracy. This value, unlike antihemagglutinin titers, is independent of the quality of antibodies and thus a sounder criterion for comparing concentrations of antibody, even apart from its greater inherent precision. It is evident from Table IV that the pairs of values obtained for each of the fractions are identical: within the accuracy of the method the differences between the numbers of anti-FM1 and anti-SW molecules are not significant. This corroborates the conclusions drawn from antihemagglutinin tests (Table III).

The behavior of the equilibrium constants, on the other hand, reveals a new aspect of the effect of boosting with related antigens. It appears that antibody molecules made in response to heterologous stimulation are not only uniformly cross-reactive, but also form a homogeneous population. Instead of finding, as one would in a standard primary or secondary response, that the supernates after absorption contain antibody of poorer quality while the more avid fractions have been bound by the antigen and thus appeared in the dissociates, there is only the barest hint of such a trend in this serum. Thus the mean of the K values from the supernates is only 0.25 log units higher than the corresponding value for the original serum ($0.10 > P > 0.05$), while the dissociates are on the average 0.01 log units lower, which is statistically insignificant. A further point in favor of the homogeneity of this antibody population is the constancy of the ratio of the two equilibrium constants within each of the fractions. There is no indication of preferential binding of one or the other kind of antibody, a state of affairs difficult to visualize unless the two specificities are carried by the same molecule.

Homogeneity of the Antibody Population.—In view of the broad distribution of binding strengths in all antisera hitherto examined, a further set of results bearing on this point will be presented. The actual tests were done at the very beginning of this work, essentially to show how much the equilibrium parameters varied by varying the concentration of serum. Such a control was needed since the homologous and heterologous antihemagglutinin titers of any serum are not the same, and thus the two sets of equilibrium measurements were always performed at different concentrations of serum. We expected considerable heterogeneity as antihaptenic sera were shown by several workers (21–25) to have a standard deviation in equilibrium constants of about 16-fold, and we have looked into this problem on rabbit sera against three strains of influenza virus and obtained values between 6- and 10-fold (19).

The method of testing was the conventional way of demonstrating heterogeneity: the equilibrium constant against the homologous antigen was determined at several concentrations of the serum. A trend in the observed values (lowering of K with increasing dilution of serum) is the mark of heterogeneity.

The measurements in Table V show that a typical cross-stimulated serum

scores as completely homogeneous: there is no more than ± 4% variation, whether the equilibrium constants or the number of antibody molecules at each level of the 16-fold range are considered. We have done similar tests on a set of sera, with two measurements at serum concentrations 20- to 100-fold apart; the results were the same. This finding, entirely unexpected, is in line with the evidence of Table IV and sets such sera apart from all immune responses, primary or secondary.

To complete the picture, it should be added that this behavior holds strictly only for the 14-day bleeds after vaccination. In some of the sera taken from the same group of children 42 days after vaccination there were signs of

TABLE V

Dependence of Equilibrium Parameters on Concentration

Serum A/7; Lapeer, 1954 (9)

Dilution of serum	K	A	aHA
1/100	11.10	15.50	4.82
1/300	11.04	15.58	4.79
1/500	11.08	15.54	4.83
1/750	11.01	15.46	4.82
1/1200	11.01	15.58	4.82
1/1600	11.05	15.51	4.88

The equilibrium constants (K), concentrations of antibody molecules/ml (A), and antihemagglutinin titers/ml (aHA) are given in $\log_{10}$ units.

inhomogeneity: apart from the bulk of antibody molecules behaving like those shown in Table V, there was also a small fraction with properties of anti-SW antibodies. In the other test group which had received the SW-vaccine in a water-in-oil adjuvant and where samples of serum were collected only in the 6th wk after vaccination, all sera we examined contained a minority of antibodies which reacted only with the vaccinating antigen, but not with the "original" antigen, FM1 virus. For reasons which will be developed in the Discussion, and on experimental evidence to be presented in the companion paper, we believe this to be a superimposed primary response and not a necessary consequence of stimulation with a cross-reacting antigen.

DISCUSSION

As far as interpretation goes, we have to make a new start since any simple instructive or selective hypothesis was untenable before we set out on the experiments. They have become even less tenable as both postulate, for different reasons, antibody populations that are heterogeneous, and neither can account for antibody of directed double specificity. Although all our observations were

made at the immunological level and specify events in immunologic terms, a hypothesis accounting for them would remain precarious if it did not also tally with what we know about the production of proteins.

The cell, as we see it today, is a repository of information only part of which is expressed at any one time. The induction of protein synthesis starts in the nucleus when the repressive control is lifted from a segment of DNA. This segment is then copied into a messenger RNA which, in its turn, is translated into the gene product by the appropriate cytoplasmic machinery. In microorganisms at least, the derepressed segment may allow production of several proteins, usually involved at consecutive stages of a particular cellular function. If removal of a repressor is mediated through an extrinsic inducer molecule, it is common to find among the series of induced proteins also a permease. Permeases are specific for the inducer and capable of trapping and concentrating it inside the cell. Thus, while primary induction requires a relatively high concentration of inducer, once a permease has been produced that cell will be more prone to make the same series of proteins subsequently since it can be derepressed at lower external concentrations of the inducer.

Translating all this into immunologic terms, we have a large number of cells that come into contact with the primary antigen. Some may take it up, some may not, but when the antigen gets into the cells there is a second hurdle to overcome: it must combine with and remove a repressor from the DNA. If both operations are successful, the cell will start producing antibody, and we postulate that it will also produce some sort of trapping mechanism, which in this case may be the antibody molecule itself. The population of antibody molecules produced primarily will be heterogeneous as there are many ways in which a gamma globulin can be more or less complementary to an antigenic determinant, and all of these possibilities have a chance of being realized. When the same antigen is given as secondary booster, considerably smaller doses will suffice to produce anamnestic response since the antigen will be trapped by those cells (and their offspring) which have been successfully induced by the primary exposure. And even among these the most avid trappers will get the lion's share of antigen, with the consequence that the secondary response should be due to the most avid sector of the antibody-producing population. Both these consequences of the hypothesis are well established experimental facts, as is a third, namely that a smaller booster dose gives a more specific response than a larger one.

How would all this work when a cross-reacting heterologous antigen is introduced secondarily? The first consequence we can foresee is that since it is cross-reacting it will be cross-trapped. Thus, given a smallish dose, it will not reach those cells where it would normally induce a primary response, but end up in some others which had some past experience of the antigen given first. Once in these cells, there is the second hurdle to overcome, and here we can

distinguish three possibilities. If every cell that has trapped the second antigen can also be derepressed by it, the response should be essentially the same as it would be to homologous boosting. We have no instance of this in our material, but this is precisely what Dubert has found some years ago (26), and what has since been puzzling many immunologists. He used closely related modified albumins as primary and secondary antigens; his rabbits produced antibody to the first antigen only, the extreme case of the Original Antigenic Sin. Then we can envisage another situation where only a small fraction of the cells that successfully trap the second antigen will also be derepressed by it. (This is a more likely alternative since trapping is a simple binary reaction, while derepression is ternary, the inducer competing with the operator for the repressor). Such a state of affairs would make for a more homogeneous response since it originates in a narrow sector only of the primary population. This would fit the case of the FM1-SW pair. And there is also a third possibility, when the antigen is trapped, but incapable of inducing antibody production in the cell that has trapped it. In this form the Original Antigenic Sin would be manifested by rendering the vaccine less effective than it would be in a virgin population of cells, i.e., in a nonvaccinated host. We have a case approaching this situation in the pair FM1-PR8 (13, 27), and this area is obviously open to direct testing by using a wide range of related antigens and appropriate methods of evaluation.

The presence of a specific trapping mechanism distinguishes the sensitized from the nonsensitized organism, and on it rests the qualitative difference (28) between the primary and secondary response. Simple instructive models do not allow for such differences, and their inductive-instructive modification by Monod (29) and Pappenheimer (30) would account for quantitative differences only. So would all selective models, whether they envisage selection and proliferation at the cellular or subcellular level. The development and nature of the trapping mechanism itself is left deliberately unspecified: the experimental material, restricted to anamnestic responses, can do no more than reveal or rule out its presence. Indeed, a sequence involving two kinds of cells for the primary response is entirely compatible with the model, and the differential radiosensitivity of the primary response as well as Fishman's recent results (31, 32) would render this alternative the rather more likely. Whether the trapping occurs through a permease unrelated to antibody (29, 30), through sessile antibody (33), through excreted and homing antibody (34) or through the ingenious mechanism proposed by Eisen and Karush (35), remains to be decided by future work.

SUMMARY

When primary immunity is boosted not by the homologous but by a cross-reacting vaccine, the newly formed antibodies react better with the primary

antigen than with the antigen actually eliciting the response. This phenomenon bears the name of Original Antigenic Sin (1).

It is shown that the number of antibody molecules produced against the original and the vaccinating antigen is the same; that each of these molecules is capable of reacting with both antigens; that the activity of an antiserum can be completely absorbed with either antigen; that both residual and adsorbed-dissociated fractions of antibody exhibit the same relative affinities towards the two antigens as did the native serum; that, unlike standard primary and secondary responses, the population of antibody molecules characterizing the Original Antigenic Sin is homogeneous; that each molecule has a lower equilibrium constant (i.e. higher avidity) against the original antigen than against the antigen stimulating the present response; and that all equilibrium constants are typical of secondary antibody.

It is concluded that the Original Antigenic Sin is a partial anamnestic response, a related antigen stimulating that sector only of the originally primed cells which is destined to produce cross-reacting antibody.

A hypothesis is developed according to which the basic difference between primary and secondary reactivity rests on the presence of a trapping mechanism that allows anamnestic production of antibody against lower doses of the homologous antigen. Such a mechanism is capable of cross-trapping related antigens, thus preventing a standard primary response and allowing manifestations of Original Antigenic Sin.

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