# REDUCTION OF ANTIBODY AFFINITY IN IMMUNIZED RABBITS BY ETHYLENIMINE AND ITS DERIVATIVES

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To investigate changes in the functional affinity of antibodies under the influence of immunodepressants, changes in the effective association constant, standard free energy, concentration of hapten-binding sites, heterogeneity index, and concentration equivalents of IgG, Fab, and Fc fragments were studied in 48 rabbits. Under the influence of ethylenimine and its derivatives the affinity indices were reduced and heterogenization of the antibodies took place. Changes in functional affinity of the antibodies were determined by administration of the immunodepressants at different periods of formation of the immune response.

KEY WORDS: antibodies; affinity; immunodepressants.

Changes in the structure of mitochondria with disturbance of the generation and transmission of energy under the influence of alkylating agents and, in particular, of ethylenimine (EI) [4], are the basic mechanism of alteration of protein synthesis [1]. The available data on the action of alkylating agents on antibody production [3, 4, 7, 10] are concerned with changes in secondary indices of the antigen–antibody reaction. Meanwhile, description of the immune response by means of primary indices is an essential condition not only for the study of the immune response [8, 10], but also for the evaluation of immunodepressants [11].

The object of the present investigation was to study the action of EI and its derivatives on antibody synthesis and to establish how changes in antibody production depend on administration of the various substances at different times of formation of the immune response.

## EXPERIMENTAL METHODS

The compound Σ-dinitrophenyl lysine, obtained by the method in [13], was purified to remove unconjugated lysine residues and 2,4-dinitrophenol (DNP) by chromatography on paper. The number of DNP groups per molecule of ovalbumin or bovine gamma globulin was determined spectrophotometrically [7, 15]. Proteins were injected intradermally in a dose of 1 mg at three points and intramuscularly in a dose of 1 mg (first course). A week later, at intervals of 3 days, 5 mg protein was injected three times intravenously [5]. Immunization with DNP proteins and Freund's complete adjuvant was carried out in the footpads of rabbits (2 mg). A further injection of 1 mg DNP protein was given 1 month later.

Experiments were carried out on 48 chinchilla rabbits divided into six groups: Group 1 was the control. The rabbits of group 2 received a single injection of 0.015 mg/kg body weight of EI or one of its derivatives – N-(N-diethylamino)-2,2-dimethylethylenimine (DEI) and N-(N-piperidino)-2,2-dimethylethylenimine (PEI) – was given two days before the first course of immunogenic injections. The compounds were obtained from the Department of Chemical Mutagenesis (Head, Professor I. A. Rapoport), Institute of Chemical Physics, Academy of Sciences of the USSR. The same compounds were injected into the animals of the remaining groups in the same doses, but simultaneously with the first course of antigenic injections (group 3), five days after the first course (group 4), simultaneously with the second course (group 5), and five days after the second course (group 6).

As indices of functional affinity of the antibodies, the mean effective association constant ($K_0$), the standard free energy ($\Delta F^0$), the concentration of hapten-binding sites of the antibodies, concentration equivalents of IgG, Fab, and Fc fragments, and the heterogeneity index ($a^c$) were determined 5, 10, and 20 days after injection of the preparations. Determinations and calculations of the first three indices were carried out in

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TABLE 1. Changes in Indices of Functional Affinity of Antibodies in Immunized Rabbits Due to EI and Its Derivatives

| Experimental conditions | $\frac{K_0}{\cdot 10^4} \pm$ $\pm < 0,23$, ml/mmole | $\Delta F^0$ $\pm < 0,31$, kcal/mole | Concentration of hapten-binding sites of antibodies $\cdot 10^{-6} \pm < 0,17$, mmole/ml | Concentration equivalent of Fab fragment $\frac{\cdot 10^2}{\cdot 10^3} \pm 0,19$, mg/ml | $a^c$ $\pm < 0,09$ |
|---|---|---|---|---|---|
| Normal globulins | $3,4 \cdot 10^4$ | 5,1 | $7,9 \cdot 10^{-6}$ | $4,26 \cdot 10^2$ | 0,87 |
| Immunoglobulins | $8,7 \cdot 10^4$ | 5,4 | $8,1 \cdot 10^{-6}$ | $4,6 \cdot 10^2$ | 0,85 |
| Two days before first course: | | | | | |
| EI | 8,0 | 4,8 | 7,6 | 3,72 | 0,66 |
| | 6,7 | 9,6 | 6,7 | 2,72 | 0,76 |
| DEI | 7,8* | 4,7* | 7,6 | 4,2 | 0,68* |
| | 6,8 | 8,8 | 6,6 | 3,26 | 0,73 |
| PEI | 8,4 | 5,3 | 7,9 | 4,26 | 0,61 |
| | 6,4 | 9,4 | 6,5 | 3,12 | 0,78 |
| Simultaneously with first course | | | | | |
| EI | 4,6 | 4,9 | 7,7 | 3,52 | 0,69 |
| | 4,1 | 6,3 | 5,4 | 2,46 | 0,49 |
| DEI | 5,5 | 5,2 | 7,9 | 3,4 | 0,68 |
| | 3,7 | 6,4 | 4,3 | 1,72 | 0,39 |
| PEI | 6,1 | 4,8 | 7,5 | 4,0 | 0,67 |
| | 4,5 | 7,6 | 6,6 | 2,26 | 0,44 |
| Simultaneously with second course: | | | | | |
| EI | 6,0 | 4,8 | 7,1 | 3,26 | 0,66 |
| | 3,7 | 6,9 | 5,2* | 2,32 | 0,47 |
| DEI | 4,0 | 4,7 | 6,7 | 3,46 | 0,74 |
| | 5,2 | 6,4 | 6,1 | 1,86 | 0,54 |
| PEI | 6,0* | 4,6* | 6,5 | 3,6 | 0,72* |
| | 4,6 | 7,5 | 4,4 | 2,26 | 0,79 |
| Five days after second course: | | | | | |
| EI | 6,9 | 5,0 | 6,8 | 4,2 | 0,72 |
| | 6,5 | 9,6 | 6,2 | 3,06 | 0,76 |
| DEI | 7,6 | 5,1 | 6,9 | 4,12 | 0,74 |
| | 6,2 | 9,5 | 6,2 | 3,0 | 0,75 |
| PEI | 7,6 | 5,2 | 7,4 | 4,32 | 0,77 |
| | 6,6 | 9,9 | 6,1 | 2,86 | 0,71 |

*$P > 0.05$.

Legend. Top figure is index determined 5 days after injection of substances, bottom figure that determined 20 days after injection.

accordance with known recommendations [12, 16] by the method of equilibrium dialysis. All experiments were carried out in 0.05M phosphate buffer, pH 7.4, for this concentration of phosphate is sufficient to ensure a negligible Donnan correction [7, 9]. Calibration of the samples for spectrophotometry was carried out with respect to protein nitrogen, tested by the micro-Kjeldahl method [3]. The heterogeneity index $a^c$, described by Sips [15], reflects dispersion of the various equilibrium constants.

## EXPERIMENTAL RESULTS

The experimental data and their statistical analysis by the Student–Fisher method are given in Table 1.

As Table 1 shows, changes in an index of specific antibody activity so important as functional affinity bear a complex relationship to the times of administration of EI and its derivatives relative to the day of immunization, to the time elapsing after their administration, and to the concrete immunodepressant properties of the substances tested.

The different forms of the reduction in the mean effective association constant, the standard free energy, concentration of hapten-binding sites, and heterogeneity index under the influence of EI and its derivatives, when given at different times of immunization, indicate differences in the sensitivity of the antibody-forming cells at different periods of antibody formation to the action of the substances used. The greatest decrease in the indices of functional affinity of the antibodies was observed as a result of simultaneous administration of the test compounds and the immunogens. For instance, when the test substances were injected 5 days after the first course and simultaneously with the second course of immunization the reduction in the indices of functional affinity of the antibodies were relatively more intensive. Injection of the test compounds 2 days before and 5 days after immunization led to the smallest changes in the indices of affinity.

Analysis of the changes in functional affinity of antibodies found 5, 10, and 20 days after injection of the mutagens indicates that their effect is stronger at relatively late periods of antibody formation. For instance, during the action of EI and its derivatives on the tenth and, in particular, on the 20th day after their injection lower indices of functional affinity of the antibodies were obtained. In particular, the greatest decrease in the mean effective association constant and standard free energy was observed on the 20th day after injection of the compounds, and the greatest changes in the heterogeneity index toward heterogenization of the antibodies were observed in this case also. Furthermore, disparity between the changes in functional affinity was extremely small when the substances were injected simultaneously with and 2 days after immunization. This heterogeneity of changes in the functional affinity of the antibodies, determined by the times of administration of the alkylating compounds and the duration of their action, was reflected in corresponding changes in the secondary manifestations of the antigen–antibody reaction such as are observed, for example, under the influence of cyclophosphamide [2].

Changes in functional affinity of antibodies taking place under the influence of the different compounds, despite their specificity, nevertheless cannot be ascribed to a strictly definite substance. All compounds, although differing in the degree of the changes produced in individual indices, sharply reduced the heterogeneity index. Heterogenization of antibodies was observed at all times during the determinations, and for nearly all times of injection of the compounds. This fact suggests that heterogenization of antibodies is a feature of the action of alkylating agents on antibody formation. The types of distributions of antibody affinity that were observed corresponded to types described previously [16]: heterogeneous and bimodal. Changes in the heterogeneity index toward a decrease also point to the mitostatic action of EI and its derivatives, and to inhibition of antibody formation. Meanwhile the different changes in the indices of the standard free energy and mean effective association constant reflect differences in the action of the various compounds used. DEI and PEI, which have a strong heterogenizing effect on antibody synthesis, give rise to a marked decrease in the mean effective association constant and the standard free energy, accompanied by negligible changes in the concentration of hapten-binding sites. The fact that the reduction in the indices of functional affinity of the antibodies assumes such different forms, and their heterogenization in immunized rabbits under the influence of EI and its derivatives are evidence both that these compounds have different points of application in the mechanism of antibody synthesis and that the inhibition sites are polyfunctional.

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