(since flagellin polymer is a T independent antigen [3], it must be assumed that these are B lymphocytes). Differences in the number of specific determinants on the molecule of the polymeric antigen evidently are brought to light first on contact between the substance and the precursor cell carrying fewer specific receptors than the "primed" lymphocyte, differentiated for antibody formation.

The appearance of immunodepressive properties in GLUT not found under the same conditions in POL depends mainly on the glutaraldehyde residues remaining on the GLUT molecule. Changes in the molecule caused by these residues are evidently not great enough to disturb its serologic properties, but are sufficient to change interacton between GLUT and the immunocompetent cells. This suggestion arises by analogy with the results of an investigation of intensively acetylated flagellin, which lost its ability to cause antibody formation; its ability to induce hypersensitivity of delayed type, however, was intensified [5].

A general result of this investigation is to prove that polymers with different immunologic properties can be obtained from the same protein. Evidently the concept of "polymeric antigen" in every case requires more accurate specification of the properties of the concrete preparations. The further study of polymerization and aggregation of antigen molecules is essential so that the special features which make the molecule "highly immunogenic," "tolerogenic," and "T independent" can be characterized quantitatively, so that more accurately oriented changes can be brought about in the properties of antigen.

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ROLE OF DISTANT INTERACTIONS OF LYMPHOCYTES IN THE DEVELOPMENT OF ANTIBODY FORMATION *in vitro*

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To elucidate the nature of local intercellular interactions inhibiting the proliferation of antibody-forming cells (AFC) in culture, described previously, the possibility of realization of this effect at a distance was studied. A population containing many cells was shown to be capable of inhibiting, by its action *in vitro* at a distance, an increase in the number of AFC in a cell population separated from it by Millipore membranes impermeable to cells. This effect is also transmitted through a polymethylmethacrylate film, 5-10 μ thick, which does not allow the passage of proteins with a molecular weight of 150,000 daltons ([^{125}I]IgG antibodies) and certain ions ($^{51}CrO_4$), but is permeable to other low-molecular-weight substances.

KEY WORDS: *Antibody formation; distant interaction; proliferation.*

Many investigations have been carried out to study contact inhibition of cell proliferation in a monolayer, for this deals with one of the most important mechanisms of regulation of cell division in higher animals [6, 10]. More recently similar processes have been studied in suspension cultures also [7, 11]. In particular, in the writer's laboratory the

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presence of local intercellular inhibitory interactions limiting proliferation of antibody-forming cells (AFC) in culture has been discovered [2, 8].

This paper describes an attempt to discover whether a distant effect of some cell cultures on the development of antibody formation in other cell cultures is possible. For this purpose two interacting cell populations were separated by various membranes. The possibility of distant interaction of cells through the intermediary of various humoral mediators [5, 9] and physical factors [1, 3] has been studied by several workers.

EXPERIMENTAL METHODS

Experiments were carried out on spleen cells of unimmunized C57BL/6 mice cultured as described previously in enriched Eagle's medium [2, 8]. One cell population was placed in the bottom of siliconized bottles 25 mm in diameter and 35 mm high, the other cell population in polymethylmethacrylate chambers 17 mm in diameter and 10 mm high, supported in the bottles on Plexiglas rings of the same diameter and 2 mm high, with spacers fixing the chamber exactly in the center of the bottle. The floor of the chamber was covered with the following membranes: 1) HAWP, VCWP, and VSWP Millipore filters with a pore diameter of 4500, 1000, and 250 Å respectively (from Millipore Filter Corporation, Bedford, USA), glued to the chamber with acetone, 2) a polymethylmethacrylate film 5-10 μ thick, obtained by immersing the edge of the chamber in 10% Plexiglas solution in dichloroethane followed by evaporation of the dichloroethane at room temperature. In the experiments with Millipore filters in which the same medium surrounded the cells lying both in the chamber on the filter and in the bottom of the bottle, the total volume of medium was 1.4 ml. In the experiments with polymethylmethacrylate film the volume of medium both in the bottle and in the chamber was 1 ml. Sterile sheep's red blood cells (5.10^6 cells/ml) or water-soluble antigen of sheep's red cells (50 μg/ml) was used as the antigen.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of "active" "dense" cultures (10-40 million cells) placed in chambers with a Millipore base on optimal "detector" cultures (5-6 million cells) contained in the bottom part of the bottles under the chambers was studied. To equalize the conditions of culture of the control optimal samples, chambers containing medium but without cells were also placed above them. Despite the absence of direct contact between the cultures, the "dense" cultures were found to inhibit any increase in the number of AFC in the "detector" cultures (Fig. 1). Interaction between the two cell populations separated by the membrane could take place through the passage of metabolites of varied molecular weight through it, and also through the action of various physical factors [4].

Millipore filters with pores of different sizes were used in different experiments. The results showed that a change in pore diameter from 4500 to 250 Å was not reflected in the effect studied (Table 1).

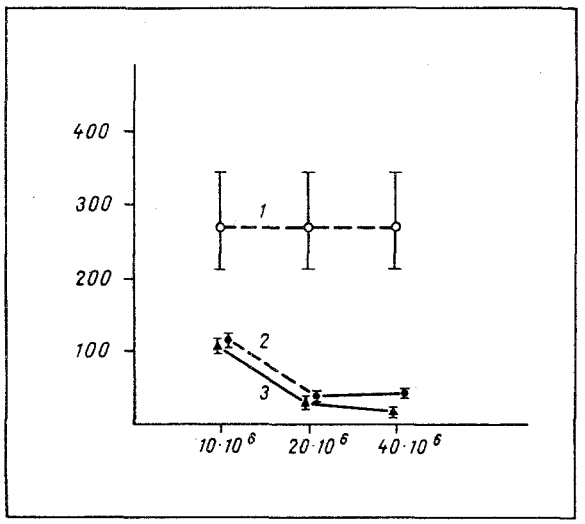

Fig. 1. Effect of different numbers of cells acting through Millipore membrane on AFC formation in "detector" cultures. Abscissa) number of cells of "dense" culture; ordinate) number of AFC formed per 10^6 living cells in control (1), "detector" (2), and "dense" (3) cultures (geometric mean number of AFC and interval of standard error).

TABLE 1. Effect of "Dense" Cultures on "Detector" Cultures through Millipore Filters of Different Pore Size

Diameter of pores, Å	Number of AFC per 10^6 living cells		
	control culture	"detector" culture	acting culture
4500	266 (228—309)	62 (47—80)	13
1000	363 (286—462)	77 (66—89)	13
250	349 (279—437)	44 (37—53)	13

Experiments in which polymethylmethacrylate film 5-10 μ thick was used as the separating membrane were particularly interesting. These experiments showed that under these experimental conditions also the "dense" cultures had a significant inhibitory action on the increase in the number of AFC in the "detector" cultures. This action also was potentiated by an increase in the number of "acting" cells (Fig. 2) and it was comparable in magnitude with the action observed in experiments with Millipore filters as the membrane separating the two cell populations (Fig. 3). "Acting" cultures contained in chambers made of Teflon or quartz had a similar effect.

The "acting" cells may exert their effect on the "detector" culture through a common atmosphere. To test this hypothesis experiments were carried out in which the bottom of the chamber with the "dense" culture was 4 mm above the level of the medium in the compartment with the "detector" cells. Under these experimental conditions, despite the retention of a common atmosphere, the effect of the "acting" cells was abolished (Fig. 3b).

To study what substances can penetrate through the polymethylmethacrylate film used in these experiments, various methods were applied. First, the the beginning of the experiment labeled protein with a molecular weight of 150,000 daltons (a commercial preparation of [^{121}I]IgG antibodies, from Izotop, USSR) was added to the suspension of "acting" cells in the chamber. Determination of the label at the end of incubation on both sides of the membrane showed that the membrane was impermeable to this compound (Table 2). The membrane was also found to be impermeable for the action of the salt Na_2CrO_3, labeled with the radioactive iso-

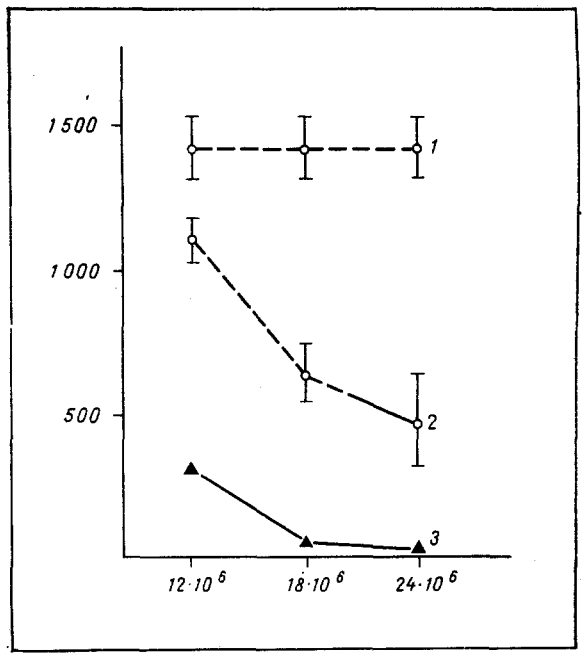

Fig. 2. Effect of different numbers of cells acting through polymethylmethacrylate film on AFC formation in "detector" cultures. Legend as in Fig. 1.

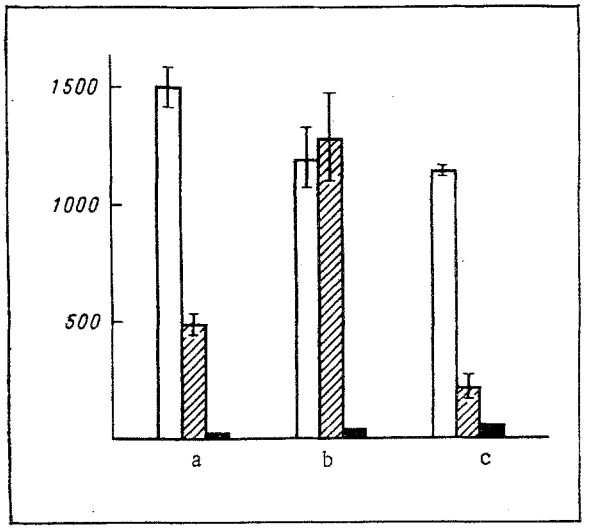

Fig. 3. Inhibitory effect of "dense" cultures on "detector" cultures through polymethylmethacrylate film (a) and Millipore filter (c) and absence of this effect through a common atmosphere (b). Ordinate) number of AFC formed per 10^6 living cells in control (unshaded columns), "detector" (obliquely shaded), and "dense" (black columns) cultures (mean geometric number of AFC and interval of standard error).

tope ^{51}Cr (from Izotop, USSR). The absence of pores formed during preparation of the film also was demonstrated by measuring the electrical conductivity of the film, which agreed with the standard value given in the tables for polymethylmethacrylate.* However, experiments with [^{3}H]uridine and [^{3}H]leucine (both from Izotop, USSR) gave different results. From 0.2 to 0.5% of the label passed through the membrane (Table 2). The difference between the permeability of the film for chromium and for the tritium-labeled compounds was perhaps due to differences in their charges. Another possible explanation might be that in the second case it was not the uridine and leucine which passed through the membrane, but tritium present in these preparations as an impurity.

These experiments showed that intercellular interactions inhibiting antibody formation are not dependent on direct contact between the interacting cells. The writers showed previously that they cannot be transmitted from one culture to the other by the humoral route.

*The authors are grateful to L. V. Chernomordik, of the Institute of Electrochemistry, Academy of Sciences of the USSR, for kindly undertaking this measurement.

TABLE 2. Permeability of the Polymethylmethacrylate Used for Labeled Compounds

Labeled compound	Radioactivity, cpm		
	sample from chamber	sample from bottle	background
IgG-^{125}I	2 591	56	64
	2 779	88	80
$^{51}CrO_4$	1 415 000	81	60
	1 426 000	69	77
[^{3}H] Leucine	80 304	330	67
	89 960	428	57
[^{3}H] Uridine	85 482	256	53
	84 215	296	50

Consequently, the distant effect observed in the experiments described above can only be dependent on low-molecular-weight shortliving metabolites or on certain physical factors.

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EFFECT OF MOUSE ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS ON ACCUMULATION OF ROSETTE-FORMING AND ANTIBODY-FORMING CELLS IN MICE IMMUNIZED WITH SHEEP'S RED BLOOD CELLS

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Data are given on the effect of mouse antiserum against isologous aggregated immunoglobulins (MAAS) on the kinetics of rosette-forming (RFC) and antibody-forming cells (AFC) in mice immunized with sheep's red blood cells (SRBC). The effect of MAAS in the experiments *in vivo* was assessed by injecting this serum for 5 days into CBA mice, the first injecting being combined with injection of 5.10^7 SRBC. Injection of MAAS into mice immunized with SRBC was shown to cause a marked decrease in the number of RFC in the spleen on the 5th and 9th days after immunization. MAAS has no appreciable effect at these same times on proliferation of AFC producing IgM hemagglutinins. Meanwhile MAAS intensified proliferation of IgG-AFC during the period when the number of these cells of the spleen in the immunized mice was maximal. After adsorption of MAAS with immune complexes formed by mouse IgG antibodies this serum was shown to lose much of its ability to block RFC *in vivo*. It is postulated on the basis of these results that the property of MAAS of influencing the accumulation of RFC and AFC producing IgG hemagglutinins is due to a factor which reacts with the immune complex formed by mouse IgG antibodies. This factor may perhaps be antibodies against aggregated immunoglobulins of this class.

KEY WORDS: *Humoral immune response; rosette-forming and antibody-forming cells.*

The mechanism of specific inhibition of the humoral immune response by antibodies has not yet been explained [1]. It was recently suggested that an important role in this mechanism is played by antigen–antibody complexes, which are fixed to the FC receptors of B lymphocytes and depress their antigen-dependent proliferation and transformation into antibody-producing cells [7]. This hypothesis is of considerable interest in the light of the previously established fact that aggregated antibodies are present on rosette-forming B cells which appear in the spleen of mice after antigenic stimulation [2, 4]. These cells lost their ability to interact with antigen after treatment with rabbit antibodies specifically

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