With lengthening of the interval between injection of SOC-HGG and reimmunization the intensity of the response increased and reached a maximum after 1 month (Fig. 3). With a further increase in this interval (to 3 months) the intensity of the secondary response remained at the same level.

The SOC-protein complex which we used to immunize the mice is easily prepared [3]. It consists of unmetabolized particles with a very large total surface area, on which single protein molecules are covalently immobilized. Immunization with this complex induces much more intensive antibody formation in animals than immunization with protein in solution or even mixed with Freund's complete adjuvant. The formation of a large AFC population makes the suggested method of immunization extremely promising for hybridoma production. The great difference between the intensity of antibody formation in mice of strains BALB/c and C57BL/6 provides wide opportunities for the analysis of the phenomenon observed.

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EFFECT OF CHOLINOMIMETICS AND ADRENOMIMETICS ON PROLIFERATION OF MOUSE B LYMPHOCYTES DURING PRIMARY IMMUNE RESPONSE TO PROTEIN ANTIGEN

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The writers showed previously that the sensitivity of mouse B lymphocytes to adrenalin changes during the primary immune response [2]. It was postulated that this is associated with proliferation and differentiation of B lymphocytes, which promote the development of precursor cells into a clone of B lymphocytes producing specific antibodies. This change in the sensitivity of B lymphocytes to neurotransmitters during development of the immune response suggests that these transmitters may influence the processes of formation of the immune response through regulation of cell proliferation. There have been only isolated studies to show the effect of cholinomimetics and adrenomimetics on the level of proliferative activity of lymphocytes [7, 8].

The aim of this investigation was to study the effect of neurotransmitters on proliferation of B lymphocytes induced by specific antigen.

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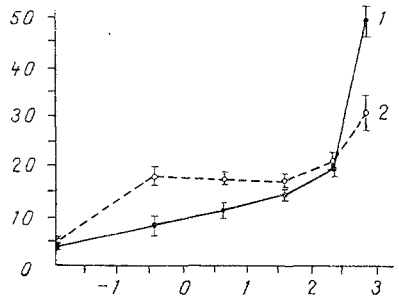

Fig. 1

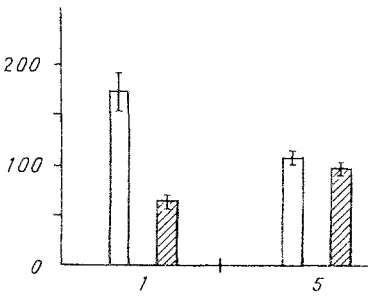

Fig. 2

Fig. 1. Effect of acetylcholine on incorporation of ^{3}H-thymidine into B lymphocytes of mice immunized with different doses of antigen, during culture. Abscissa, dose of antigen (log of dose in μg per mouse); ordinate, incorporation of ^{3}H-thymidine ($\times 10^{-3}$). 1) Background incorporation; 2) incorporation in presence of acetylcholine.

Fig. 2. Effect of acetylcholine on incorporation of ^{3}H-thymidine into mouse B lymphocytes on immunization with small and large doses of antigen at different times of culture. Abscissa, conditions of reaction: on left, incorporation during first hour of incubation; on right, during incubation for 4 h (acetylcholine injected at time 0 in both cases), ordinate: $\frac{\text{incorporation in presence of acetylcholine}}{\text{incorporation without acetylcholine}} \times 100\%$. Shaded columns, immunization with 250 μg of antigen; unshaded, immunization with 0.5 μg of antigen.

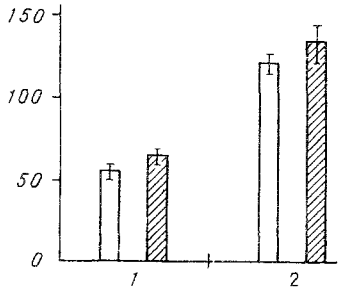

Fig. 3. Discordance of effects of adrenalin and acetylcholine on incorporation of ^{3}H-thymidine into B lymphocytes of mice immunized with different doses of ovalbumin. Abscissa, conditions of reaction: on left, effect of acetylcholine; on right, effect of adrenalin; ordinate: $\frac{\text{incoroporation in presence of acetylcholine}}{\text{incorporation without acetylcholine}} \times 100\%$. Shaded columns denote immunization with 250 μg of ovalbumin; unshaded columns, immunization with 0.5 μg of ovalbumin.

EXPERIMENTAL METHOD

Experiments were carried out on 250 female C57BL/6 mice weighing 18-20 g. The animals were sensitized by a single intraperitoneal injection of ovalbumin in doses of 0.5 to 250 μg, with 5 mg of aluminum hydroxide gel. The animals were used in the experiments 3 days after injection of the antigen — the time of maximal proliferation of splenic B lymphocytes in response to antigen [2] — and also on the 2nd and 6th days — at the time of the initial rise and subsequent fall of the proliferative response, respectively. Spleen cells were obtained by mild homogenization of the spleen in medium 199 followed by filtration through nylon gauze and centrifugation in a Ficoll—Verografin gradient [5]. To enrich the population with B lymphocytes the cells were treated with anti-Thy-serum and complement; dead cells were removed by the standard procedure of centrifugation in a Ficoll—Verografin gradient. The anti-Thy-serum was obtained by immunizing rabbits with mouse brain homogenate [6]. The serum had a titer of 1:128 and was cytotoxic in the complement-dependent cytolysis test for 90% of thymus cells and 40% of spleen cells.

To estimate proliferative activity, lymphocytes enriched with B cells were incubated in medium 199 for 2 h at 37°C in a dose of $2 \cdot 10^6$-$5 \cdot 10^6$ cells with 2 μCi of ^{3}H-(methyl)-thymidine;

TABLE 1. Incorporation of ^{3}H-Thymidine into Splenic B Lymphocytes of Mice Immunized with Low and High Doses of Antigen, during Culture

Culture time, h	Incorporation of label (in cpm) during 1 h	
	0.5 μg of antigen	250 μg of antigen
1	11 625±1 280	56 185±1 106
5	17 147±1 720*	42 667±1 230**

Legend. Here and in Tables 2 and 3: *P < 0.05, **P < 0.01.

TABLE 2. Action of Substances Changing Intracellular Cyclic Nucleotide Level on Intensity of Incorporation of ^{3}H-Thymidine into B Lymphocytes of Immunized Mice in Early and Late Stages of Immunization

Days after immunization	Substance	Intensity of incorporation of label, % of incorporation without substance	
		5 μg of ovalbumin	250 μg of ovalbumin
2-	Carbachol	192,6±33,5*	266,7±20,9*
	Adrenalin	75,8±3,3*	95,1±26,3
6-	Carbachol	70,0±8,2*	68,0±6,0**
	Adrenalin	128,0±27,0	143,3±30,0

TABLE 3. Action of Substances Changing Intracellular Cyclic Nucleotide Level on Incorporation of ^{3}H-Thymidine into B Lymphocytes of Intact (I) and Immunized (II) Mice

Substance	Intensity of incorporation of ^{3}H-thymidine into B lymphocytes of intact and immunized mice, % of incorporation without subs.	
	I	II
Carbachol (1 μM)	117±11	201±18**
cGMP (1 mM)	106±3	348±92*
Adrenalin (1 μM)	88±12	80±7*
cAMP (1 mM)	83±7	88±5*

Legend. Immunized animals used on 3rd day of immunization with 5 μg of ovalbumin.

the cells were then washed to remove label by centrifugation twice in physiological saline and once in 10% TCA, and incorporation of the radioactive label into the acid-insoluble fraction (DNA) was determined on a Mark III liquid scintillation counter. Incubation was carried out in the presence of various concentrations of carbachol, adrenalin, 1 mM cAMP, and 1 mM cGMP.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

As the writers showed previously [2] sensitization of mice with ovalbumin caused an increase in proliferative activity of the spleen cells of these animals; the peak of incorporation of the labeled DNA precursor (^{3}H-thymidine), moreover, occurred 3 days after injection of the antigen. The effect of carbachol on the intensity of DNA synthesis by B lymphocytes was studied at this time (Fig.1). The effect was found to vary depending on the dose of anti-

gen used. In a concentration of 1 μM (the dose inducing the maximal response) carbachol increased the incorporation of radioactive label into DNA of B lymphocytes of mice immunized with low doses of antigen, and inhibited proliferation if high doses of antigen were used. Both these effects — stimulation and inhibition — were exhibited after incubation of the cells for only 1 h with the preparation, and they disappeared if incorporation of label was studied 4 h after addition of carbachol (Fig.2). Investigation of incorporation of radioactive thymidine for 30 min showed that on injection of the doses of antigen during the period of culture lymphocyte proliferation was increased, whereas if high doses of antigen were used it decreased (Table 1). Carbachol thus acted in the same manner as natural changes in the trend of proliferation of B lymphocytes. When the effect of carbachol was studied 6 days after injection of the antigen [2], acetylcholine was found to reduce incorporation of the label for all doses of antigen. Meanwhile, in the early stages of the immune response, namely 2 days after injection of the antigen, carbachol increased thymidine incorporation in response to all doses of antigen (Table 2). Adrenalin had the opposite action to carbachol (Fig. 3), cAMP acted in the same way as adrenalin, and cGMP in the same way as carbachol; an effect was observed, moreover, only for lymphocytes obtained from immunized, and not from intact animals (Table 3).

The neurotransmitters studied were thus able to exert a direct influence on proliferation of splenic B lymphocytes of mice during immunization. The direction of the change in proliferation caused by the neurotransmitters was similar to the natural direction of the change in B lymphocyte proliferation in the course of the immune response. Since lymphocytes in all phases of the cell cycle were present simultaneously in the spleen of the sensitized mice, the ratio between the number of cells starting to proliferate (the G_1/S transition) and the number emerging from the stage of DNA synthesis (the S/G_2 transition) must determine the direction of the change in incorporation of the label. Predominance of cells in the G_1 phase under these circumstances must be accompanied by an increase in incorporation of label with time, whereas predominance of cells in the S phase must be accompanied by a decrease. In both cases acetylcholine potentiated the process, i.e., it evidently accelerated the natural course of the cell cycle of the B lymphocytes. Adrenalin, on the contrary, reduced the rate of passage of the B lymphocytes through the cell cycle.

The effect of both transmitters was determined by their effect on the cyclic nucleotide system, because it could be induced by exogenous cAMP and cGMP. Acetylcholine is known to be able to raise the cGMP level [7], and adrenalin the cAMP level [3]. There are also indications of stimulation of the immune response by cGMP and its inhibition by cAMP [4, 9].

Incidentally, intact cells did not respond by a change in incorporation of label to injection of cyclic nucleotides or neurotransmitters. This could not be the result of absence of receptors for neurotransmitters on resting cells: it has been shown that acetylcholine and adrenalin can change the cyclic nucleotide level and induce different types of responses, such as mobility, in intact B lymphocytes [1]. Proliferating lymphocytes are evidently in a state which differs qualitatively from the state of the resting cells as regards reactivity to cyclic nucleotides and to substances changing their level in the cell.

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