values of the short circuit current obtained by 1.31 is thus perfectly adequate when the use of manual compensators of serial resistance changing in the course of the experiments is ineffective, and a clamp with automatic compensation of resistance is complex and only a single one of its kind may be in existence.

The method can be used both in experiments to study mechanisms of active transport through various epithelial tissues (intestine, urinary and gall bladders, amphibian skin, retina) and also under clinical conditions to assess the functional state of resected segments of intestine.

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COMPARISON OF THE IMMUNODEPRESSANT ACTION OF MICROBIAL DEAMINASE FROM DIFFERENT SOURCES

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KEY WORDS: microbial deaminases; immunodepressant action.

Asparaginase is an enzyme used in the treatment of several human diseases connected with malignant transformation of lymphocytes [8]. It was initially held that asparaginase acts only on malignant cells, growth of which is dependent on asparagine. Clinical studies have shown that asparaginase also inhibits proliferation of normal tissue cells and has an immunodepressant action, inhibiting rejection of grafted organs and tissues [7, 10]. The enzyme also inhibits blast transformation of lymphocytes stimulated by phytohemagglutinin (PHA) [4]. However, the mechanism of the immunodepressant action of asparaginase has not yet been completely explained. The most widely held explanation of this phenomenon is the ability of the enzyme to catalyze hydrolysis of asparagine, thus making it in short supply for lymphocytes [11]. This view is supported by data showing that asparagine, added to the medium, prevents the inhibitory action of asparaginase on blast transformation of lymphocytes [11]. However, glutamine [6] also has a similar action. It has also been shown that asparaginase from *Erwinia carotorova*, which has higher glutaminase activity than the the enzyme from *Escherichia coli*, is a more powerful immunodepressant [5]. To study the role of glutaminase activity in

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TABLE 1. Inhibition of Blast Transformation of Lymphocytes by Microbial Deaminases

Source of enzyme	Enzyme activity, IU/ml	Number of proliferating lymphocytes, percent
Escherichia coli	0,01	53,2
	0,10	26,3
Mycobacterium album	0,01	3,8
	0,10	0,4
Pseudomonas fluorescens	0,01	8,7
	0,10	3,2

depression of immunity by microbial asparaginases, it was decided to compare the immunodepressant action of deaminases with different ratios between their asparaginase and glutaminase activities. Previously the writers isolated in a homogeneous state deaminases of asparagine and glutamine from *Pseudomonas fluorescens* AG and *Mycobacterium album*, which possess antitumor activity [1, 2].

The object of this investigation was to compare the immunodepressant activity of these enzymes with the action of asparaginase from *Escherichia coli*.

EXPERIMENTAL METHOD

Deaminases were purified to a homogeneous state as described previously [1]. Asparaginase EC-2 from *E. coli* was obtained from Bayer, West Germany. To obtain lymphocytes, heparinized blood from donors was layered on Ficoll-Paque, and then centrifuged in accordance with the recommendations of the manufacturer (Pharmacia Fine Chemicals, Sweden). The lymphocytes were cultured in medium 199 with Hanks' salt solution, containing 20% blood serum from the same donor. The volume of the incubated samples was 5 ml and they contained 2 million lymphocytes in 1 ml. PHA was added in a final concentration of 15 μg/ml. During investigation of the ability of the enzymes to inhibit blast transformation of lymphocytes they were added to the incubation mixture 30 min before addition of PHA. The cultures were taken for determination of the number of proliferating lymphocytes after 72 h.

EXPERIMENTAL RESULTS

Under the influence of PHA the lymphocyte culture was transformed into various kinds of cells. The toxicity of all three enzyme preparations may be considered to be low, for the number of lysed cells did not exceed 15%. Blast transformation in control samples not containing PHA took place with only 0.8% of lymphocytes. Stimulation of blast formation by PHA increased this proportion, to yield on average 69.8%.

The results of the study of the effect of these deaminases and of asparaginase on blast transformation of lymphocytes stimulated by PHA are given in Table 1.

As Table 1 shows the deaminases studied had a marked ability to inhibit mitogen-induced blast transformation. The inhibitory activity of deaminases from *Ps. fluorescens* and *M. album* was similar and was an order of magnitude higher than activity of asparaginase EC-2 from *E. coli*. Comparison of the substrate specificity of the three enzymes studied revealed correlation between the inhibitory action of these enzymes and their activity toward glutamine. If the rate of hydrolysis of asparagine is taken as 100%, the rate of hydrolysis of glutamine by asparaginase EC-2 from *E. coli* is only 2% [10], whereas for deaminases from *Ps. fluorescens* and *M. album* the corresponding values are 150 and 130% [1]. Asparaginases with no glutaminase activity, like asparaginase from agouti [3] or guinea pig [13] blood serum, and purified asparaginase from *Myobacterium bovis* [15], inhibit blast formation in lymphocytes. Meanwhile, the inhibitory action of the bifunctional deaminases is prevented by glutamine, but not by asparagine [6]. Asparaginase from *Vibrio succinogenes*, which has virtually no glutaminase activity, has no immunodepressant action [9], and on this basis the authors cited conclude that glutaminase activity plays a role in inhibition of blast transformation of lymphocytes. Inhibition of blast transformation by glutamine antagonists [12], by highly specific glutaminase, or by an enzyme with very low asparaginase activity [14] also indicate the important role of glutamine in this phenomenon. The results now obtained, showing the effect of various microbial deaminases on blast transformation, are evidence in support of a role for glutaminase activity in this process.

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ANALYSIS OF LYMPHOCYTE CELL MEMBRANE CYCLASE ACTIVITY AFTER STIMULATION BY A MITOGENIC POLYANION

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KEY WORDS: polyanion; lymphocyte membrane; cyclase enzymes.

The molecular mechanism of lymphocyte stimulation by ligands with different chemical structure and properties is not yet explained. However, there are now serious grounds for considering that the key systems concerned in the formation of the signal at the plasma membrane level are as follows: 1) the system of cyclase enzymes of the membrane, 2) the membrane ionic transport system, and 3) the system of the lipid matrix of the membrane, which must be regarded along with membrane enzymes as controlling the chemical and physicochemical properties of the matrix [7, 8].

We have studied the molecular mechanisms of triggering the response of the lymphocytes by polyelectrolytes and, in particular, by the mitogenic polyanion polyacrylic acid (PAA) [1, 2, 6, 8]. Most attention was paid to the state of the above-mentioned "key" systems of the plasma membrane. PAA has already been shown to have a strong modulating action on permeability of the plasma membrane and on activity of ion-transporting ATPase [1, 2].

The aim of this investigation was to study activity of adenylate and guanylate cyclases in the plasma membrane of lymphocytes before and after activation by mitogenic doses of PAA. Since it was shown previously that PAA activates division of B lymphocytes [9], the action of PAA was compared with that of another B-cell mitogen, namely the lipopolysaccharide (LPS) from *Escherichia coli*.

EXPERIMENTAL METHOD

Cultures of splenic lymphocytes from (CBA × C57BL)F_1 mice were prepared in accordance with recommendations described previously [5, 7]. Division of B lymphocytes was activated by PAA according to the optimal "pulsed" scheme [6]. A preparation of PAA with molecular weight of 80,000 daltons, which was added to the lymphocyte suspension for 10 min in a final concentration of 20-40 μg/ml, was used. The lymphocytes were washed free from the polyanion by triple centrifugation, after which the cells were resuspended in enriched culture medium. LPS of *E. coli* (from Sigma, USA) activated B-cell proliferation in a final concentration of

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