Trauma to muscle was shown to stimulate mitosis with an increase in MI_c (by 36%) in CE. By performing four identical experiments with injury to the submandibular salivary gland it was shown that the phenomenon of stimulation of proliferation in CE after trauma is reliably reproduced.

Acute injury to epithelial, muscle, and gland tissues had a stimulating action on proliferation of CE with the participation of distant mitogenic substances, for contact between CE cells and products produced by tissue trauma was effected entirely by the humoral route. This effect probably does not depend on the ability of individual tissues to undergo post-traumatic proliferation, established during evolution [9]. However, the fact that stimulation of MI_c varied from 155 to 240% is evidence of the action of other, as yet unstudied factors, on proliferation. It can be tentatively suggested that the phenomenon studied is connected with mediators of the autonomic nervous system, whose concentration rises rapidly after any kind of trauma, and which participate in the mechanism of action of growth stimulators [5]. This is shown by the nonspecificity of the response: In the chosen tissues both the number of mitoses after trauma and the times of maximal increase in the level of proliferation differed.

The results as a whole are evidence that proliferation in CE is stimulated by trauma to rapidly and slowly proliferating tissues, and this is evidently connected with the production of remotely acting substances.

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EFFECT OF EXOGENOUS CALMODULIN ON LYMPHOCYTE PROLIFERATION IN NORMAL SUBJECTS

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KEY WORDS: proliferation; lymphocytes; calmodulin.

Calmodulin (CM) is a low-molecular-weight calcium-binding protein which mediates the effect of Ca^{++} on several metabolic processes (synthesis and breakdown of cyclic nucleotides, glycogenolysis, lipolysis, etc.), and also its effect on membrane permeability (activation of Ca—ATPase of human erythrocytes, increased permeability of synaptic vesicles for mediators, and so on) [6]. This protein is found in virtually all animal tissues. Its polyfunctionality suggests that it is one of the principal intracellular receptors for Ca^{++} ions.

Ca-binding proteins are known to possess Ca-ionophore properties, i.e., they can increase the passive permeability of membranes for Ca^{++}. Lymphocytes react by an increase in blast transformation to many agents, including those which increase membrane permeability for Ca^{++} ions [5].

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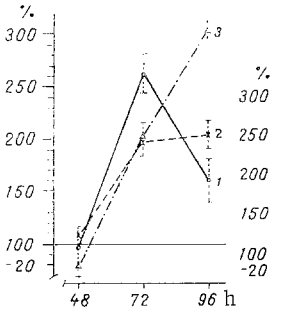

Fig. 1

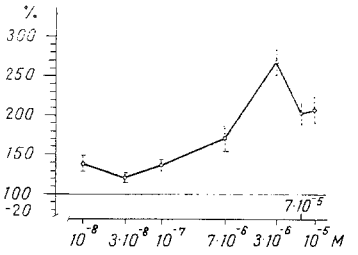

Fig. 2

Fig. 1. Action of CM on incorporation of [^{3}H]-thymidine into lymphocytes. Abscissa, time of incubation of lymphocytes with CM (in h); ordinate, [^{3}H]thymidine incorporation (in %). 1) 50 μg, 2) 100 μg, 3) 150 μg CM/ml sample.

Fig. 2. Dose dependence of stimulating action of CM on [^{3}H]thymidine incorporation after incubation of lymphocytes for 72 h. Abscissa, CM concentration (in M); ordinate, [^{3}H]thymidine incorporation (in %).

The aim of this investigation was to study the effect of exogenous CM on blast transformation of normal human lymphocytes.

EXPERIMENTAL METHOD

CM were isolated from bovine brain by the method in [4, 7] with modifications [1]. The protein thus obtained was homogeneous on disk electrophoresis in the presence of sodium dodecylsulfate. The protein was able to activate the Ca-dependent form of phosphodiesterase in the presence of Ca^{++} ions. The Ca-binding and other physicochemical properties of this CM preparation were described previously [2]. Experiments were carried out on normal human peripheral blood lymphocytes isolated in a Ficoll-Urografin density gradient (ρ = 1.078) by the method in [3]. The cells thus obtained were resuspended in incubation medium consisting of 80% medium 199, 20% inactivated group AB (IV) serum, with 100 Units/ml each of penicillin and streptomycin. The final cell concentration was 2×10^6/ml medium. CM was added in doses of 10^{-8} to 10^{-5} M and the cells were incubated at 37°C for 48, 72, and 96 h. [^{3}H]thymidine in a dose of 2 μCi per sample was added 2 h before the end of culture to the flasks in order to assess the stimulating action of CM on lymphocytes. After incubation the cells were washed three times with cold physiological saline and centrifuged for 10 min at 1200 rpm. The residue was then covered with 5% TCA and allowed to stand for 30 min in a refrigerator. Acid-insoluble material was collected on millipore filters ("Rufs" 2.5 μ). The filters were washed with 50 ml of 5% TCA and 10 ml of 96% ethyl alcohol. The filters were then dried and radioactivity measured on a Mark 2 liquid scintillation counter (Nuclear Chicago, USA) in toluene scintillator. The results were expressed as percentages of incorporation of label into the control samples, taken as 100%, and were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results showed that homogeneous CM in doses of 50 to 150 μg/ml incubation medium stimulated proliferation of normal human lymphocytes (Fig. 1). The level of incorporation of [^{3}H]thymidine into lymphocytes 72 h after the beginning of incubation of the cells with CM, in doses of 50 and 100 μg CM was increased by 2-2.6 times compared with the control, but in a dose of 150 μg/ml incorporation reached a maximum (three times greater than in the control) after incubation for 96 h.

Since proliferation of lymphocytes was maximal after culture of the cells in CM for 72 h, doses of CM between 10^{-8} and 10^{-5} M were tested at that time. The results of these experiments showed that all doses of CM tested stimulate [^{3}H]thymidine incorporation; the stimulating effect of CM, moreover, increased with an increase in its concentration in the incubation

medium (Fig. 2). Dependence of [^{3}H]thymidine incorporation on the CM concentration was biphasic in character. In low CM concentrations (10^{-8}-10^{-7} M) an effect of 20-30% was observed, whereas in a concentration of 10^{-6}-10^{-5} M, additional enhancement of proliferation was observed. This may mean that there are two lymphocyte populations with different affinity for CM or that there are two pathways of action of CM on proliferation: one operates in low CM concentrations, the other in high concentrations.

The effect of CM on lymphocyte proliferation thus revealed may be associated with increased membrane permeability for Ca^{++} ions. A similar effect of CM was found on synaptic vesicles in which CM, acting through Ca-dependent protein kinase, stimulates neurotransmitter secretion. More than 10 enzymes which are regulated by CM are known, but all these enzymes are located either in the cytoplasm or on the inside of the plasma membrane. Nevertheless, the possibility cannot be ruled out that CM, on penetrating into lymphocytes, can exert its effect on cell metabolism, thus stimulating proliferation. Further research is required to elucidate the mechanism of action of CM on [^{3}H]thymidine incorporation.

CM is known to be present in all animal tissues. However, it has not been found in blood plasma. It can be tentatively suggested that CM, secreted from foci of injury or inflammation of the tissues, may act as a factor stimulating lymphocyte proliferation in this focus.

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NUMBER OF STROMAL PRECURSOR CELLS IN THE BONE MARROW OF YOUNG AND OLD CBA MICE

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KEY WORDS: bone marrow; monolayer cultures; stromal precursor cells; aging.

The *in vitro* cloning method enables the number of cells forming fibroblast clone colonies (FCC) in the hematopoietic and lymphoid organs of certain species of mammals to be counted. FCC belong to the category of stromal precursor cells, the progenies of which are responsible for the main functions of the hematopoietic microenvironment [4]. The possible role of the microenvironment of the hematopoietic and lymphoid organs in age changes in hematopoiesis, especially lymphoid, has not been determined. To investigate this problem information is needed on the basic characteristics of the stromal tissue of the hematopoietic organs of old animals.

In the investigation described below the number of FCC was compared in the bone marrow of young and old CBA mice.

EXPERIMENTAL METHOD

The donors of the bone marrow cells were young (1-4 months) and old (24-30 months) female CBA mice. Explantation of the cells into monolayer cultures and subsequent culture were car-

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