CYCLIC AMP FORMATION DURING PHAGOCYTOSIS

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The content of cyclic adenosine-3',5'-monophosphate (AMP) in phagocytic macrophages was shown to be increased especially during phagocytosis of the living microbes. The cyclic AMP formed during phagocytosis could be detected in the incubation medium, but in the cells it remained at almost the same level. The cyclic AMP concentration in cells of the intestinal mucosa and in the blood serum of germfree guinea pigs also was increased after injection of *Escherichia coli* 055 cells; this points to the participation of the adenylate cyclase system in interaction between microorganisms and the epithelium of the small intestine.

KEY WORDS: *Cyclic AMP; macrophages; phagocytosis; cells of the intestinal mucosa.*

The phagocytic activity of leukocytes is known to largely determine the resistance of the organism to infectious diseases [1, 3, 4, 10]. It is therefore of great interest to study the mechanisms of activation of the enzyme system of phagocytes. A definite role in the regulation of the enzyme activity of phagocytic cells is perhaps played by cyclic nucleotides (3',5'-AMP and 3',5'-GMP), which may be intermediaries in the cellular response to various extracellular stimuli [11].

It was decided to study the degree of participation of the adenylate cyclase system of the cells in phagocytic reactions. For this purpose the content of cyclic AMP in phagocytic macrophages and in cells of the intestinal mucosa and the blood serum of germfree animals exposed to the action of *Escherichia coli* 055 was investigated.

EXPERIMENTAL METHOD

Inbred C3H mice were used. Macrophages were obtained by irrigating the peritoneal cavity with Hank's solution 3 days after intraperitoneal injection of 2 ml nutrient broth into the mice. The cells were washed and resuspended in Hank's solution, in which the cell concentration was made up to $1 \cdot 10^{-7}$/ml. Over 80% of the cells of the exudate were macrophages.

To study the effect of microbial action on cyclic AMP formation in cells of the intestinal mucosa germfree guinea pigs obtained by hysterotomy were used [2]. A suspension of *E. coli* 055 cells containing 125 million bacterial cells in 0.25 ml of 0.9% NaCl was injected into the lumen of the upper third of the small intestine of these animals. The guinea pigs were killed immediately after the injection or 15, 30, and 60 min after contact with the bacteria. Scrappings of the intestinal mucosa were washed with 10 ml of 0.9% NaCl and a weighed sample of the scrappings was homogenized in the same solution. The cyclic AMP concentration was determined in this homogenate and also in the blood serum obtained from the guinea pigs at the same time.

Living and heat-killed *E. coli* 055 cells were used as the object of phagocytosis. A mixture of 0.25 ml of the suspension of peritoneal macrophages obtained as described above with 0.2 ml of the bacterial suspension was made. The ratio of phagocytes to bacteria in the system was 1:30. Phagocytosis took place under standard conditions during rotation of the tubes in the incubator (2 rpm). The cyclic AMP concentration was measured after incu-

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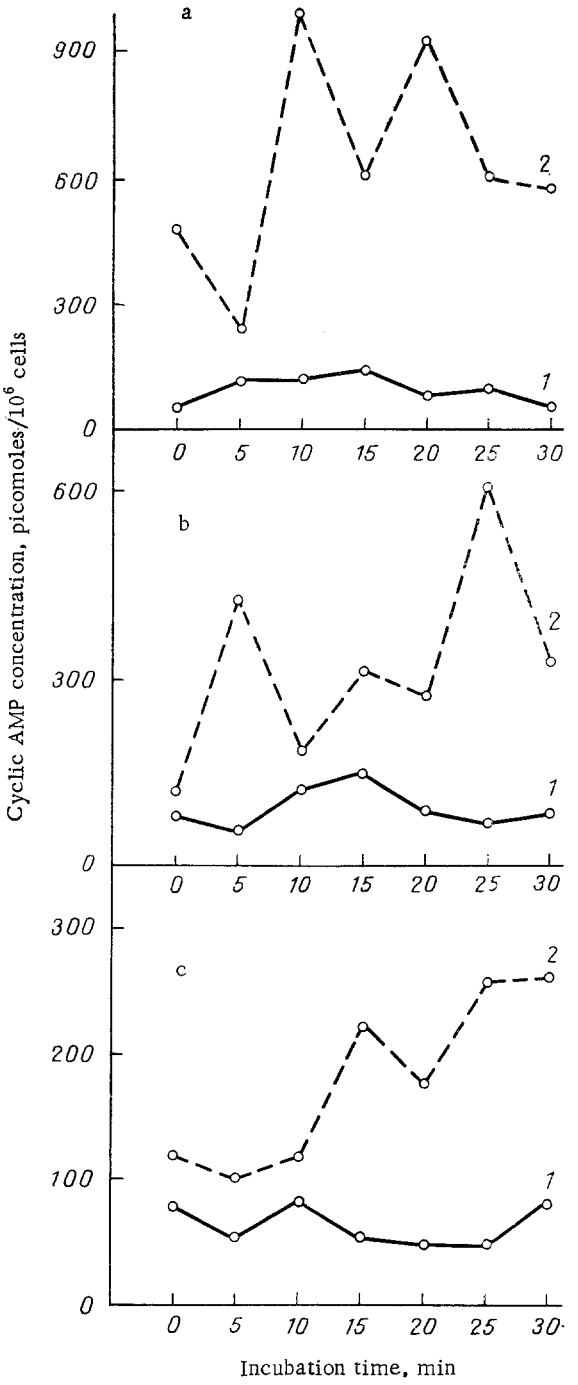

Fig. 1. Formation of cyclic AMP during phagocytosis of *E. coli* 055: a) phagocytosis of living microbes; b) of heat-killed microbes; c) incubation of macrophages in 0.9% NaCl solution. Curves show: 1) cyclic AMP content in cells (macrophages); 2) cyclic AMP content in incubation medium. Mean results of 3 experiments given.

bation for 5-60 min both in the cell residue and in the supernatant (S_1) obtained after centrifugation of the incubation mixture for 3 min at 600g. To 0.1 ml of S_1 and to the whole volume of the residue 1 ml of 5% TCA was added, after which the cells were homogenized for 2 min at 700-800g in a glass homogenizer. The homogenates and S_1 were treated with 0.1 ml of 1 N HCl, then washed 3 times with 2 volumes of ethyl ether, and centrifuged at 1000g for

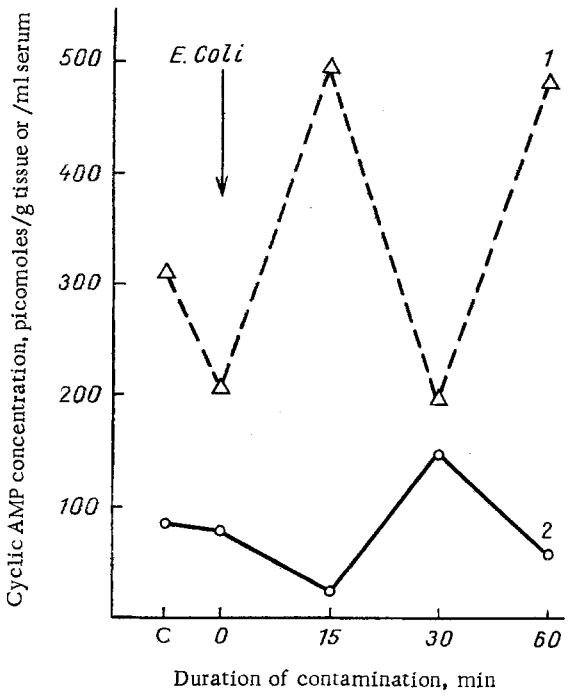

Fig. 2. Cyclic AMP content in cells of intestinal mucosa (1) and in blood serum (2) of germfree guinea pigs after microbial contamination. C) Control. Mean results of 3 experiments given. Arrow indicates beginning of microbial action.

20 min. The supernatant (S_2) was collected, 1 ml of Na–acetate buffer, pH 4.0, was added, and it was kept at –20°C. Homogenates of the mucosal cells and blood serum were treated in the same way.

Cyclic AMP was determined in S_2 by a modified Gilman's method [7].

Radioactivity was measured in a Mark II scintillation counter in toluene scintillator. The cyclic AMP concentration was expressed in picomoles/10^6 cells/g mucous membrane or ml blood serum.

EXPERIMENTAL RESULTS

Separate analysis of the cell residue and the supernatant showed that practically all the cyclic AMP formed during phagocytosis passed into the supernatant (Fig. 1). The concentration in the cells remained at almost the same level. On incubation of the intact macrophages, comparatively little cyclic AMP was synthesized (Fig. 1b), but the rate of synthesis increased considerably during phagocytosis of killed microorganisms, synthesis of cyclic AMP began practically at once without the lag period that is observed during phagocytosis of living microorganisms.

Having discovered the increased formation of cyclic AMP in the macrophages during phagocytosis of *E. coli*, the next step was to study the effect of microbial action on the cyclic AMP content in other cells, such as cells of the intestinal mucosa. These cells are usually in continuous contact with the microflora and they are an important receptor–effector component which is responsible for local immune protection against intestinal infections.

After contamination of the germfree animals with *E coli* 055 the cyclic AMP content at the beginning of microbial action was found to be reduced both in the mucosal cells and in the blood serum, but its concentration then began to rise, more especially in the intestinal mucosa (Fig. 2). Intensification of cyclic AMP synthesis in the cells of the intestinal mucosa also was observed 1 h after contamination of the animals, whereas the cyclic AMP concentration in the blood serum had then fallen to its initial (control) level. These changes in the cyclic AMP concentration were perhaps the result of interaction between the microorganisms and the epithelium of the small intestine. It can tentatively be suggested that

changes of this sort in the cyclic AMP concentration taking place during the first minutes of contact with the microorganisms must have some effect on the host organism as a whole. Cyclic AMP formed in the cells of the intestine may play the role of intermediary (or catalyst) in the development of the diverse secondary responses of the body to microbial contamination, which is known to be followed in some cases by rapidly developing reactions resembling shock in type [8]. The physiological role of the cyclic AMP formed under such conditions in the intestine likewise is unknown, but it may perhaps participate in the early stages of formation of the immune response [5].

The increase discovered in the cyclic AMP content in the macrophages during phagocytosis of bacteria is in agreement with the observations of Park et al. [8, 9], who demonstrated increased synthesis of cyclic AMP during phagocytosis of inert (latex) particles. These processes are evidently autonomous and regulatory in character. Increased liberation of cyclic AMP during phagocytosis may perhaps function as a special type of stop signal, preventing excessive ingestion of microorganisms by phagocytes [6].

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EFFECT OF HYDROCORTISONE ON COMPOSITION OF ACID MUCOPOLYSACCHARIDE FRACTIONS OF THE AORTA

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Five fractions of acid mucopolysaccharides were identified in the rabbit aorta: hyaluronic acid, heparitin sulfate, and chondroitin sulfates, A, C, and B. During prolonged administration of hydrocortisone the concentration of hyaluronic acid rose but that of heparitin sulfate fell. The relative percentages of chondroitin sulfate A, C, and B were lowered 15 days after administration of the hormone ceased.

KEY WORDS: *Mucopolysaccharides; aorta; corticosteroids.*

Previous investigations have shown that adrenal insufficiency developing after cessation of prolonged administration of hydrocortisone changes the state of the mucopolysaccharide filter of the vascular wall, so that its permeability is disturbed and lipids are deposited in it [1, 2]. Information on the composition of the acid mucopolysaccharide (AMPS) of the aorta is limited in amount and contradictory in nature [5-7].

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