EFFECT OF CONIOPHAGE BREAKDOWN PRODUCTS ON BIOLOGICAL ACTIVITY OF FIBROBLASTS

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Cytochemical investigations showed that destroyed coniophages, phagocytosing cytotoxic and fibrogenic quartz dust, when added to the culture medium activate the synthetic function of rat fibroblasts. The use of inert talc dust under similar experimental conditions caused no appreciable changes compared with the control.

KEY WORDS: culture of fibroblasts; coniophages; quartz dust.

An important problem in the pathogenesis of silicosis is the elucidation of the character of interaction between two cells: the coniophage, directly and primarily in contact with quartz, and the fibroblast, which synthesizes scleroproteins. For a long time the importance of interaction between these cells in the development of silicosis was underestimated. There are only a few papers in the literature in which increased production of hydroxyproline by fibroblasts is described after the addition of coniophage breakdown products to the culture medium [2, 3].

The object of this investigation was to study some histochemical properties of fibroblasts when exposed to the action of coniophage breakdown products.

EXPERIMENTAL METHOD

The test material consisted of connective-tissue cells from noninbred albino rat embryos. Connective tissue films were homogenized in 0.25% trypsin solution by means of a magnetic mixer. The cells (fibroblasts) were cultured in Carrel's flasks and on sterile slides in special tubes in synthetic medium No. 199 with the addition of 10% calf serum. After 2 days, coniophages, obtained 2 days after intraperitoneal injection of 100 mg of comparatively inert talc dust or of cytotoxic and fibrogenic quartz dust into rats and disintegrated by repeated freezing and thawing, were added to the fibroblast culture. Peritoneal washings from healthy rats with sterile physiological saline were used in the series of control tests. The fibroblast culture was studied 2 days after the addition of the coniophage breakdown products to them.

Besides a general psychological investigation, cytochemical tests were carried out to detect ribonucleoproteins by Brachet's method, total protein with bromphenol blue, and activity of NAD-diaphorase and glucose-6-phosphate dehydrogenase with the aid of nitro-BT. The results of the investigation of NAD-diaphorase activity were subjected to statistical analysis (altogether 6000 fibroblasts were counted, 100 cells each time, in the control and experimental preparations).

EXPERIMENTAL RESULTS

Microscopic investigation of the fibroblasts of the control series showed that some cells were round whereas others were branched. The cytoplasm of the round cells showed marked pyroninophilia, whereas in the branched cells there was a moderate or sometimes very low content of RNP. Estimation of the total protein content also showed that it was higher in the round cells, especially in the nuclei; in the branched cells

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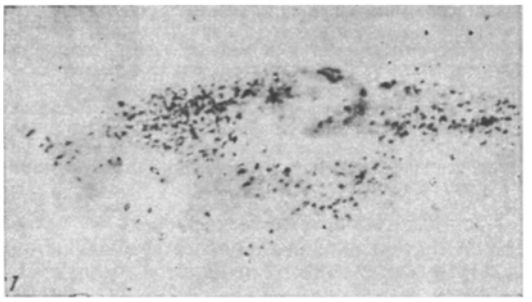

Fig. 1

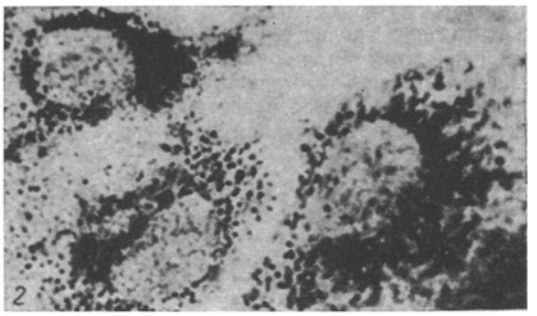

Fig. 2

Fig. 1. Fibroblasts cultured under ordinary conditions. Moderate formation of diformazan deposits in cells after staining for NAD-diaphorase. Reaction with nitro-BT, immersion.

Fig. 2. Fibroblasts grown after addition of breakdown products of coniophages which had phagocytosed quartz dust to the medium. Abundant polymorphic and diffuse deposition of diformazan in cells stained for NAD-diaphorase. Reaction with nitro-BT, immersion.

the protein content was much lower and it varied between moderate and very low.

The study of glucose-6-phosphate dehydrogenase activity in the cytoplasm of the fibroblasts revealed uniformly shaped punctate deposits of diformazan granules. The density of distribution of these granules varied from several dozens in some cells to one or two dozens in the others. Some cells had no diformazan deposits. Activity of NAD-diaphorase (Fig. 1) in the fibroblasts varied from considerable (mainly in the round cells) to moderate (mainly in branched cells).

After addition of breakdown products of coniophages which had phagocytosed talc dust to the culture, no appreciable differences were found from the picture observed in the control tests.

Morphological examination of fibroblast cultures to which breakdown products of coniophages which had phagocytosed quartz dust were added revealed the same two types of cells – round and branched. After staining by Brachet's method, marked pyroninophilia was found in the round cells, whereas in the branched cells the pyroninophilia was mainly moderate, and only less frequently increased.

Investigation of the total protein content revealed marked staining of the round cells with bromphenol blue. The total protein content of most of the branched cells was very low.

The majority of the round and branched cells had moderate glucose-6-phosphate dehydrogenase (G6PD) activity, as reflected in the formation of not very many (a few dozen) monomorphic diformazan granules in their cytoplasm. Cells with low or high G6PD activity also were found.

The study of NAD-diaphorase activity revealed numerous polymorphic and diffuse diformazan deposits in the cytoplasm of the round cells and also in most of the branched cells (Fig. 2).

Quantitative analysis of the material showed that the number of fibroblasts with marked NAD-diaphorase activity in cultures treated with breakdown products of coniophages which had phagocytosed quartz dust was more than 3.5 times greater than their number in the control cultures (30.4% compared with 8.05 ± 0.78%). In a series of experiments with talc dust the number of fibroblasts with marked NAD-diaphorase activity was not significantly increased (to 9.0 ± 0.62%) compared with the control.

These experiments thus showed that disintegrated coniophages which had phagocytosed cytotoxic and fibrogenic quartz dust (but not the comparatively inert talc dust), when added to the culture medium, activate the synthetic function of rat fibroblasts. This was reflected in intensification of the pentose cycle (as shown by increased G6PD activity), a raised RNP level, intensification of oxidoreductive reactions (activation of NAD-diaphorase), and more rapid excretion of protein at times characteristic of fibroblasts in the active phase [1, 4, 5]. These results are in agreement with those of biochemical investigations [2, 3].

In the light of these facts, good results are to be expected from investigations aimed at identifying coniophage breakdown products that are responsible for stimulating fibroblasts, for this may lead to the possible correction of the pathological process in silicosis by removal of these substances with the aid of specific hydrolysis or the formation of inert complexes.

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EFFECT OF QUERCITRIN ON STRUCTURAL CHANGES IN THE LARGE AND SMALL INTESTINE IN EXPERIMENTAL ENTEROCOLITIS

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Sensitization of rats with homologous antigen of large intestine together with Freund's adjuvant causes marked immunological changes in the large intestine of rats with manifestations of enteritis in the small intestine. Oral administration of the flavonoid quercitrin in a dose of 25 or 100 mg/kg daily for 10 days leads to a decrease of the focal lymphohistiomonocytic infiltration and eosinophilic exudation in the mucous membrane and of the sclerotic changes in the stroma of the large intestine. Meanwhile the structural disturbances in the small intestine are repaired. The effect of quercitrin on morphological changes in the epithelium of the mucosa of the large and small intestine was very slight. It is postulated that the therapeutic action of quercitrin may be due to restoration of the local circulation when disturbed by sensitization of the animals.

KEY WORDS: allergic enterocolitis; quercitrin.

Focal perivascular infiltration, edema, and disturbance of the local homeostasis and microcirculation arising in the intestinal mucosa during the antigen–antibody reaction [1, 7] lead to a disturbance of its specific function [4, 9]. For a long time now, to depress the autoallergic reaction in enterocolitis steroid hormones [5, 11, 14], immunodepressants [13], and antihistamine [3, 10] drugs have been given. However, these substances have side effects [6, 11, 15] and they are not always effective [6], especially in the late stages of sensitization [2]. During the development of a pathogenetic therapy of enterocolitis, especially in the period of allergic manifestations, substances regulating vascular permeability might prove effective.

The object of this investigation was to study the possibility of using the flavonoid quercitrin in order to depress immunomorphological changes arising in the intestinal mucosa during autosensitization of animals with homologous antigen of the large intestine.

EXPERIMENTAL METHOD

An allergic model of enterocolitis was produced by sensitizing animals with homologous antigen of the large intestine together with Freund's adjuvant. Experiments were carried out on 60 male albino rats weighing 170-190 g. Four injections of antigen were given at intervals of 7 days. The animals were divided into four groups: 1) intact rats, 2) sensitized but untreated animals (control), 3) sensitized animals receiving

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