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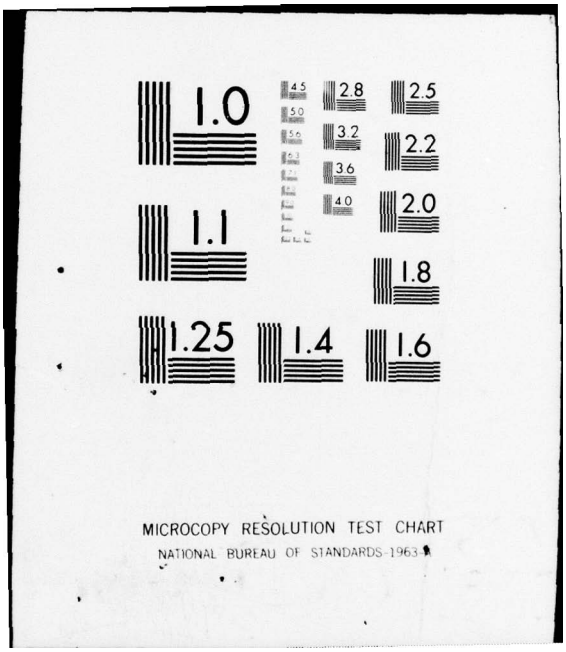
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DETERMINATION OF THE ACTIVE COMPONENT(S) OF HEPARIN ASSOCIATED WITH WOUND HEALING AFTER SEVERE BURN: STRUCTURAL ANALYSIS AND BIOLOGICAL ACTIVITY

Howard M. Jenkin

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Heparin and casein are two potential burn healing compounds. Their effect on growth of mammalian cells cultivated *in vitro* has been investigated. Heparin appears to have some growth promoting effect on prepuce cells, while it showed little effect on growth of Novikoff hepatoma, monkey kidney and baby hamster kidney cells. Heparin also reversed the inhibitory effect of prednisolone-21-sodium-succinate of growth of prepuce and baby hamster kidney cells.
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20. Partially hydrolyzed casein having an amino N/total N ratio of 0.09 exhibited growth-stimulating activity for prepuce cells which resembles activity of newborn calf serum. This activity was abolished when casein was more fully hydrolyzed with trypsin.

DETERMINATION OF THE ACTIVE COMPONENT(S) OF HEPARIN ASSOCIATED WITH WOUND HEALING AFTER SEVERE BURN: STRUCTURAL ANALYSIS AND BIOLOGICAL ACTIVITY

Much of the efforts during the last year have been focused on studying another potential burn healing compound, partially hydrolyzed casein (PHCI). Casein is a phosphoprotein, precipitated from milk by dilute acid. PHCI was prepared by hydrolysis of casein with trypsin at 46°C for varying periods of time in which varying amino N/total N ratios were obtained. The use of PHCI in the local treatment of burns was found to accelerate burn repair in guinea pigs and humans (unpublished report by Kraft Company). The mechanism of how PHCI accelerates burn repair is still not well understood. It was, therefore, of interest to investigate whether PHCI can stimulate multiplication of human skin diploid and other cell types *in vitro* which might be related to the mode of action of the burn healing process and be used to estimate cell growth-stimulation activity.

METHOD

Human skin diploid (prepuce) cells were cultivated in Eagle's minimum essential medium containing 0% (MEM_0), 4% (MEM_4), or 10% (MEM_{10}) newborn calf serum in the presence of 0.4 or 1.6 mg/ml PHCI. The medium was buffered with 20 mM HEPES and supplemented with 100 µg/ml of streptomycin and 100 units/ml of penicillin. The cells were cultivated in MEM_{10} for the first 24 hr when the effect of PHCI was studied on cells grown in MEM_0 or MEM_4. The medium was discarded and cells rinsed with Hanks' balanced solution (BSS). Fresh medium containing PHCI was added to the cells. The experiments were carried out in 25 cm^2 polystyrene cell culture flasks containing 4 ml of medium using an initial cell density of 2.0 to 2.0 x 10^5 cells/flask. The cells were incubated at 37°C for 7-8 days and enumerated at varying intervals of time after trypsinization with the aid of a Coulter counter or Biophysics cytograph.

RESULTS

The graph presented in Fig. 1 shows the effect of partially hydrolyzed casein I (PHCI) (having an amino N/total N ratio of 0.09) on the growth of prepuce cells cultivated in MEM_{10} medium. The addition of 0.4 to 1.6 mg/ml PHCI to the growth medium resulted in increases of 50% and 113% on day 5 and 35% and 64% on day 7 in cell populations over that of control cells.

The growth-stimulating activity of PHCI for prepuce cells grown in medium containing 0 or 4% newborn calf serum was similar

to that in medium containing 10% newborn calf serum (Fig. 2). Cell populations in MEM_4 supplemented with 1.6 mg/ml PHCI had increases of 41%, 47% and 31% over that of cells grown in MEM_4 alone on day 3, 6 and 8, respectively. Cells grown in medium without serum and no PHCI supplement did not multiply over the entire incubation period. Addition of 1.6 mg/ml PHCI to the medium containing no serum resulted in a 250% increase of cells after 8 days of incubation at 37°C.

Partially hydrolyzed casein II and III (PHCII and PHCIII having amino N/total N ratios of 0.36 and 0.70, respectively) did not appear to exert much effect on growth of prepuce cells (Figs. 3 and 4). Human kidney, swine testis and rabbit epidermal cells showed little or no stimulation of growth in the presence of PHCI (Table I) (see attached manuscript for details).

DISCUSSION

Spieker-Polet (1) and Polet (2) identified albumin as the serum factor essential for the growth of activated human lymphocytes. Dulak and Temin (3) have isolated and purified polypeptide from spent culture medium of rat liver cells with growth-stimulating activity for chicken and rat embryo fibroblasts.

The results of the present experiments show that PHCI actively enhanced the growth of prepuce cells cultivated in MEM_{10} (Fig. 1). It was found that prepuce cells could multiply to some extent in medium without a serum supplement when PHCI was added to the medium (Fig. 2). The growth curve in Fig. 2 also showed that the cell population in medium supplemented with 4% newborn calf serum and PHCI was higher than that of cells grown in medium containing 10% newborn calf serum alone. These observations suggest that PHCI contains stimulatory activity for growth of prepuce cells which resembles activity of newborn calf serum.

Spieker-Polet (1) and Polet (2) demonstrated that growth-promoting activity of albumin for growth of activated lymphocytes was completely abolished by pepsin digestion. Similar results were observed in this study. When casein was almost completely hydrolyzed with trypsin having amino N/total N ratio of 0.36 or 0.70, growth stimulation of prepuce cells did not occur (Figs. 3 and 4). It is suggested that the growth-stimulating activity of casein is attributed to the casein molecule itself, optimal concentrations of amino acids and/or polypeptide fractions.

PHCI alone appears to stimulate cell growth. It may have application for growth of cells in vitro in a serum-free medium. Chemical definition of active growth factors in the hydrolyzed casein should be performed to make a clean synthetic supplement. Casein is sometimes hard to handle and must be sterilized with ethylene oxide while in a dry state.

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