To remove CIC two factors must probably be present: either components of C, which dissolve CIC, or active phagocytes, but since burns are characterized by dysfunction of phagocytosis, and solubilizing and opsonizing components of C bind with necrotic tissues [7], these circumstances prevent degradation of CIC and lead to their accumulation in the circulating blood.

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IMMUNOMODULATING ACTIVITY OF p-HYDROXYPHENYL-LACTIC AND ASCORBIC ACIDS

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p-Hydroxyphenyl-lactic acid (PHPLA) is formed from p-hydroxyphenylpyruvic acid which, in turn, is a transamination reaction product of tyrosine. PHPLA is converted into homogentesic acid in the presence of ascorbic acid (AA). In vitamin C deficiency and also in patients with hemoblastoses the urinary PHPLA level rises sharply [3]. PHPLA has been shown to exhibit the properties of an endogenous carcinogen and, on systemic administration to animals, it induces increased formation of leukemias and hepatomas [1, 2].

In the investigation described below the effect of PHPLA and AA on cell proliferation was studied in a one-way mixed lymphocyte culture (MLC) from healthy blood donors and patients with carcinoma of the large intestine, who as a rule have AA deficiency [5].

EXPERIMENTAL METHOD

PHPLA in the culture medium and lymphocytes was determined on an MAT-311A chromatomass-spectrometer (Varian, West Germany), connected through an interface with a 3700 chromatograph (West Germany) [3]. AA was measured by a spectrophotometric method with phenylhydrazine. The cell proliferation index of MLC and in the blast transformation reaction to polyclonal mitogens was determined by the method described previously [3]. Splenocytes were obtained from mice with avitaminoses B_6 and A [6, 8].

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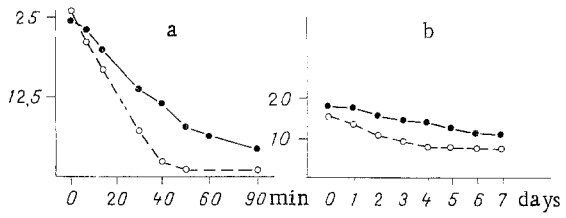

Fig. 1

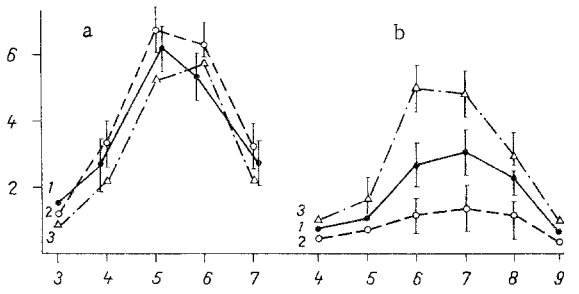

Fig. 2

Fig. 1. Kinetics of AA (a) and PHPLA (b) concentrations in culture medium. Abscissa, duration of cultures *in vitro*; ordinate, concentration of acids (in μg/ml). 1) Culture medium without cells; 2) MLC of healthy donors ($3 \cdot 10^6$ cells/ml).

Fig. 2. Effect of PHPLA and AA on proliferative response in MLC from healthy donors (a) and patients with carcinoma of the large intestine (b). Abscissa, days of incubation *in vitro*; ordinate, proliferation index. 1) control MLC; 2 and 3) MLC in presence of $5 \cdot 10^{-5}$ M PHPLA and $2 \cdot 10^{-6}$ M AA, added every 12 h to culture. Mean values ($\bar{X} \pm t\bar{X}$ at P = 0.05) from five or six parallel experiments are given.

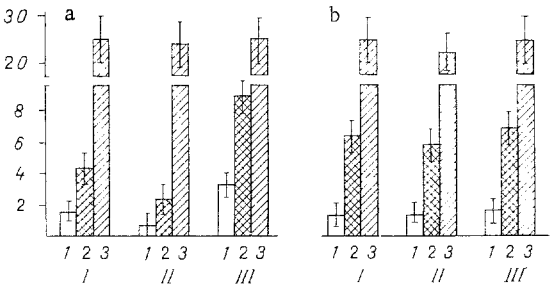

Fig. 3. Effect of PHPLA and AA on proliferative response of lymphocytes from patients with carcinoma of the large intestine, stimulated *in vitro* by Con A (a) and PWM (b). a: 1, 2, 3) Con A used in concentrations of 1, 5, and 15 μg/ml respectively; b: 1, 2, 3) PWM in concentrations of 0.2, 2, and 20 μl/ml respectively. I) Control cultures; II and III) $5 \cdot 10^{-5}$ PHPLA and $2 \cdot 10^{-6}$ M AA added to culture every 12 h.

EXPERIMENTAL RESULTS

The concentration of AA added to the culture *in vitro* fell sharply, especially in the presence of proliferating cells, to reach 10-20% of its initial level 90 min after the experiment began (Fig. 1). A high AA concentration was maintained in lymphocytes for 3-6 h, when it fell gradually. The PHPLA level in the culture medium remained virtually unchanged in the presence of lymphocytes during incubation for 5-7 days (Fig. 1).

AA and PHPLA did not affect cell proliferation in MLC from healthy donors (Fig. 2). In separate experiments only negligible stimulation of lymphocytes was observed in the presence of PHPLA. AA in optimal concentration significantly intensified the proliferative response of lymphocytes from patients with carcinoma of the large intestine to alloantigens (Fig. 2) and to suboptimal concentrations of T-cell mitogen Con A but did not affect proliferation of cells stimulated by PWM (Fig. 3). In the presence of PHPLA inhibition of cell proliferation was observed in MLC from patients with carcinoma of the large intestine and in the blast-transformation reaction in response to suboptimal concentrations of Con A (Figs. 2 and 3). No immunomodulating action of PHPLA and AA was observed when lymphocytes transformed by optimal concentrations of mitogens were used. Delafuente and Panush [7] reported previously that AA and dihydro-AA, over a wide range of concentrations (0.1-100 μg/ml) stimulated the proliferative response of lymphocytes from healthy blood donors to Con A *in vitro*. In the present experiments increased incorporation of ^{3}H-thymidine into lymphoblast DNA was observed only with leukocytes from patients with carcinoma of the large intestine.

The effect of PHPLA on proliferation and formation of cytotoxic T-lymphocytes also was studied in MLC from spleens of allogeneic intact mice and mice with avitaminoses. Splenocytes were obtained from animals kept for 4 days on an artificial diet without pyridoxine or vitamin A [6, 8]. The retinoid level in liver tissue [6] and the pyridoxal-5'-phosphate level in the liver and spleen of animals with avitaminosis as a rule were depressed by 40-50%. PHPLA in a concentration of 10^{-4}-10^{-6} M did not affect incorporation of ^{3}H-thymidine into lymphoblast DNA or T-killer formation in MLC from control mice, and in a concentration of 10^{-4}-10^{-5} M it inhibited proliferative activity of lymphocytes in MLC of spleens obtained from animals with avitaminoses A and B_6 by 50-60%. PHPLA did not affect induction of cytotoxic T lymphocytes in MLC *in vitro*.

It can be concluded from these results that PHPLA as a weak but distinct immunodepressive action on cellular immunity reactions and may play a definite role in the development of the secondary immunodeficiency state in cancer patients. The writer showed previously that urinary excretion of hydroxyphenolic acids is increased in patients with carcinoma of the large intesine [5] and that proliferative activity of lymphocytes in response to alloantigens and mitogens is disturbed [4]. Considering that T-lymphocyte function *in vitro* can be stimulated by optimal doses of AA, this suggests that vitamin C might be successfully used to correct the biochemical and immunologic parameters in cancer patients.

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