further investigations, since the transplant for eyelid plasty ALLOPLANT™ is a collagenous material with the characteristic architectonics.

There is also the possibility that the compounds which forming from the matrix component during technological processing of the transplant elicit a nonspecific inhibitory effect.

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Determination of the Biological Activity of Chalone-Containing Preparation from Ehrlich Ascitic Carcinoma and Its Fractions Obtained by High-Performance Liquid Chromatography in a Cell Culture Derived from This Tumor

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It is shown that cultured Ehrlich ascitic carcinoma cells are a convenient test system for the investigation of the effects of various factors on DNA synthesis in the cells of this tumor. The application of this system markedly facilitates fractionation of a chalone-containing preparation, the purpose of this fractionation being the isolation of components affecting specific phases of the mitotic cycle.

Key Words: *Ehrlich ascitic carcinoma; high-performance liquid chromatography; chalone; DNA synthesis*

Estimation of the biological activity of chalone-containing preparations (CCP) is an important task in the purification of CCP from various tissues. Each step of CCP separation requires the determination of chalone activity in separate fractions. At the present time, a variety of methods are used to determine the biological activity of CCP both *in*

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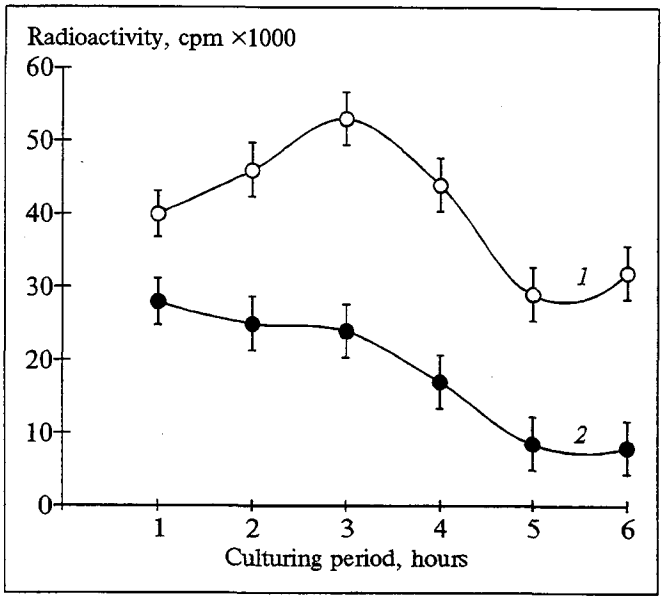

Fig. 1. Kinetics of DNA synthesis in an intact EAC culture (*1*) and after the addition of CCP (*2*).

vivo and *in vitro* [5,7,8]. The *in vivo* test is generally used for fractionation of CCP from Ehrlich ascitic carcinoma (EAC) cells [2,4,5]. This method is laborious, as it requires large numbers of animals for each experimental point. Therefore, the fractions are generally pooled, which greatly reduces the resolving capacity of such a powerful biochemical tool as high-performance liquid chromatography (HPLC).

In this study we attempted to employ an EAC cell culture as a test-system for the determination of the biological activity of CCP from EAC and CCP fractions obtained by HPLC relative to DNA synthesis. Since the effect of CCP from EAC cells on DNA synthesis in cultured EAC cells has so far not been studied, we have examined the kinetics of the effect of CCP on DNA synthesis.

TABLE 1. 3H – Thymidine Incorporation in Cultured EAC Cells under the Influence of Various CCP Fractions Obtained by HPLC ($M \pm m$)

Fraction	Protein concentration before lyophilization, μg/ml	Radioactivity, cpm per 1000 cells
Control	0	60.8±1.9
I	0.4	55.7±3.0
II	21.8	60.9±1
III	71.4	67.1±2.1
IV	87.6	70.3±2.7 (+15.6)*
V	184.3	58.6±1.8
VI	258.1	43.8±1.3 (−28.0)*
VII	53.8	44.5±2.2 (−26.8)*
VIII	131.7	42.9±0.7 (−29.4)*
IX	91.4	56.8±3.2
X	93.6	52.5±1.9 (−13.7)*
XI	138.3	54.5±0.2 (−10.4)*
XII	50.7	54.1±2.8
XIII	37.6	55.3±2.0
XIV	41.9	42.1±1.6 (−30.6)*
XV	32.3	35.9±0.9 (−41.0)*
XVI	24.2	47.9±0.4 (−21.2)*
XVII	8.1	56.3±0.3
XVIII	0.4	39.0±1.3 (35.9)*
XIX	0.4	45.6±1.0 (−25.0)*

Note. The percentage of inhibition (−) or stimulation (+) of DNA synthesis in comparison with the control is indicated in parentheses. An asterisk indicates $p<0.05$ compared with the control.

MATERIALS AND METHODS

EAC cells were cultured as described elsewhere [1]. Chalone-containing preparation was obtained by alcohol fractionation [6]. The protein concentration in the initial solution was measured by the method of Lowry [10]. The CCP was fractionated by HPLC on a TSK-Gel G-2000 S column (LKB, Sweden) [4]. Protein in the eluate was detected at 226, 260, and 280 nm using a flow cuvette and a flow spectrophotometer with a 1040A diode matrix (Hewlett-Packard, USA). The fractions (1 ml) were pooled according to the elution profile, lyophilized, and dissolved in distilled water to a volume equal to 12% of the initial volume. The biological activity of the concentrated fractions was estimated by their effect on DNA synthesis in cultured EAC cells. For this purpose 100 μl of the sample was added to the culture. ^{3}H-Thymidine (1.5 μCi/ml) was added 1 h prior to the radioactivity measurement. DNA was precipitated on nitrocellulose filters (pore diameter 2.5 μ), and radioactivity was measured in a Rackbeta scintillation counter (LKB, Sweden) [9]. The significance of differences was evaluated using Student's t test ($p<0.05$).

RESULTS

The initial CCP (alcohol precipitate) elicited a pronounced inhibitory effect on DNA synthesis in the EAC culture, indicating that the preparation contains components that affect the G1 and/or S phase of mitosis. As seen from Fig. 1, the inhibitory effect increased during the 6-h period of culturing: after 1 h of culturing DNA synthesis was reduced by 30.4%, after 2 h - by 46.2%, 3 h - by 55.2%, 4 h - by 62%, 5 h - by 70.3%, and 6 h - by 74.3%, the difference in comparison with the control was significant in all cases ($p<0.01$). Since the maximum inhibitory effect of the CCP was observed only after the 5th hour, this time

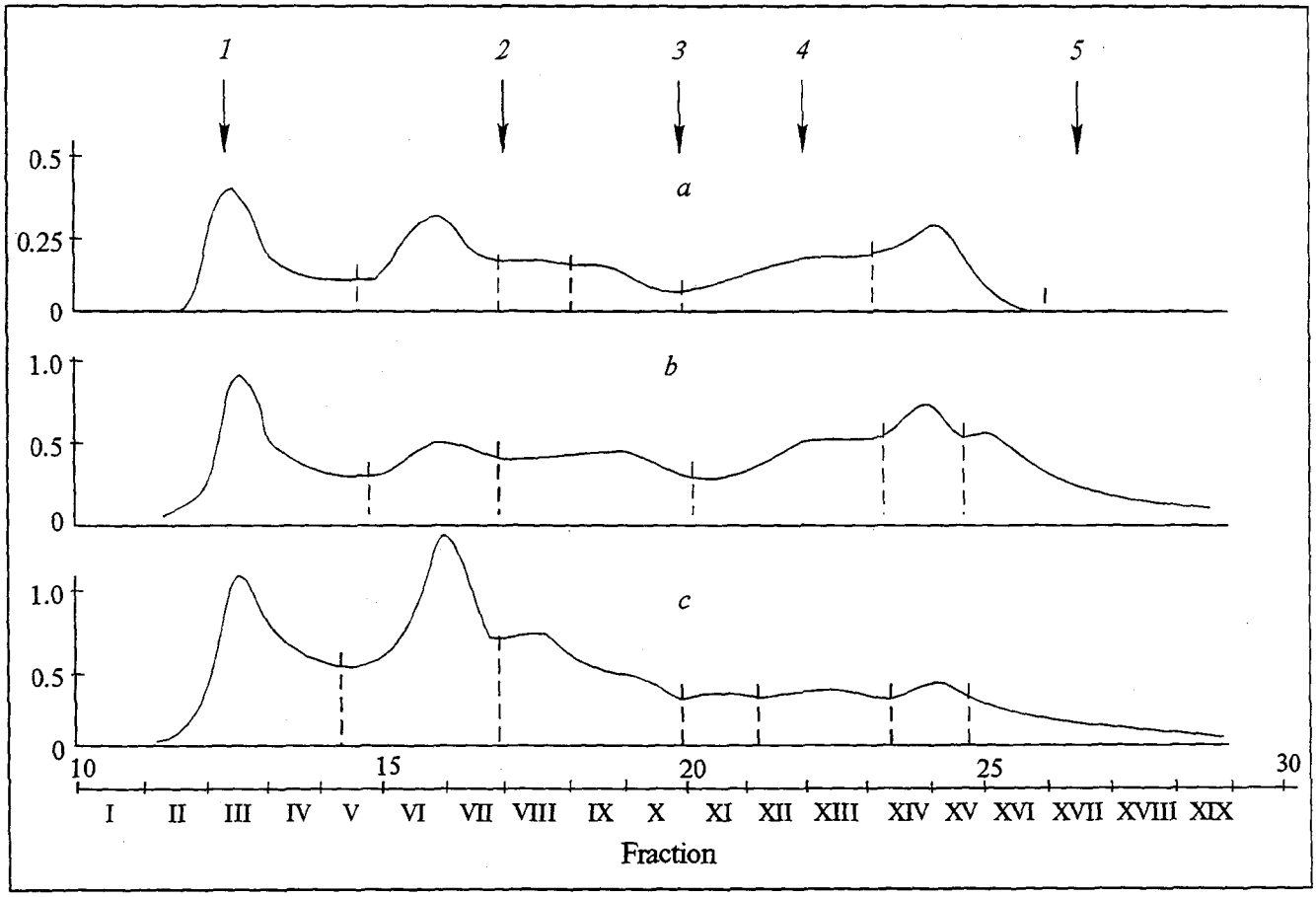

Fig. 2. Fractionation of CCP by HPLC. Abscissa: volume of eluate (ml); ordinate: absorbance, optical density units at 280 nm (*a*), 260 nm (*b*), and 226 nm (*c*). Arrows indicate the compounds used for column calibration: *1*) Dextran blue; *2*) ovalbumin; *3*) chymotrypsinogen; *4*) cytochrome C; *5*) ferricyanide K.

period was chosen for the evaluation of the G1-chalone activity of the CCP fraction.

Figure 2 shows fractionation curves (HPLC) for CCP (alcohol precipitate). It can be seen from Table 1 that the preparation is not homogeneous with respect to its activity vis-a-vis DNA synthesis. Fractions I, II, III, V, IX, XII, XIII, and XVII had no effect on it. Fraction IV stimulated, while fractions VI, VII, VIII, X, XI, XIV, XV, XVI, XVIII, and XIX exerted a statistically significant inhibitory effect on DNA synthesis in EAC cells. It can be concluded that the preparation contains compounds that both inhibit and stimulate DNA synthesis, the stimulating activity being confined to high-molecular-weight fractions. This and the G1-chalone activity of fractions VI, VII, and VIII are in agreement with our previous results [3,4]. It should be mentioned that faction XV exhibited the maximum chalone activity: DNA synthesis was decreased by 41%. The same activity was also present in fractions XVIII and XIX (low-molecular-weight fractions with the lowest protein content). This fact may be explained by the ability of low-molecular proteins to form complexes with high-molecular protein carriers. Assuming that this holds true for G1-chalone, fractions XVIII and XIX may contain free chalone, whereas the other fractions which inhibit DNA synthesis contain chalone complexed with various protein carriers. Depending on the conditions of CCP isolation, period of CCP storage, and other factors, the ratio between free and complexed chalone is changed, which alters the results of CCP fractionation. Another explanation for the elution profile obtained in this study may be that the CCP contains individual chalones that inhibit cells from entering the S phase (G1-chalone proper) and the movement of cells in the S phase (S-chalone). Since previously we failed to separate G1- and S-chalone activities [2,4], the first explanation seems more plausible.

The results obtained indicate that a temporary culture of EAC cells is a simple and convenient tool for the investigation of the effects of CCP and

its fractions on DNA synthesis in these cells. It is noteworthy that in addition to compounds with chalone activity, the preparation contains compounds that stimulate DNA synthesis.

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EXPERIMENTAL GENETICS

Intracellular Ceruloplasmin-Like Protein of Mammals

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Pulse-chase experiments show that, in addition to the secreted molecular forms of ceruloplasmin, an intracellular ceruloplasmin-like protein is synthesized in rat hepatocytes. Radioimmunochemical assay demonstrates that a nonserum isoform of ceruloplasmin is bound to the Golgi membranes.

Key Words: *protein biosynthesis; copper metabolism; ceruloplasmin; molecular forms; intracellular localization*

A family of ceruloplasmin-like proteins play an important role in the transport, redistribution, and excretion of copper in the mammalian organism [6,8,10]. The least studied stage of copper metabolism is its intracellular transport to the sites where copper-containing enzyme-proteins are synthesized. In this connection we tried to discover a non-secretory, intracellular molecular form of ceruloplasmin (CP).

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MATERIALS AND METHODS

Biosynthesis and subsequent migration of immunoreactive, *de novo* synthesized CP were studied in pulse-chase experiments. Hundred-milligram aliquots of adult rat hepatic tissue, minced with scissors, were incubated under automatic stirring in 1 ml of Hanks' solution containing 10 mM glucose, 1%

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