From the standpoint of these discussions we can probably explain why, in some investigations, a circadian rhythm of mitosis was not found or changes in the rhythm were observed [6, 8, 9]. This phenomenon is evidently connected with the fact that the investigations were conducted at times when, in the course of natural development of the tumor, a 24-hourly rhythm had not yet appeared or it had already disappeared. Our attention is thus redirected once again to the fact that in all investigations of neoplasms, information relating to any one time cannot by any means reflect the true situation in such an actively changing biosystem.

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PRODUCTION OF ANTITUMOR CYTOSTATIC FACTORS BY INACTIVATED RESIDENT PERITONEAL MACROPHAGES OF SYRIAN HAMSTERS

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Cells of the monocytic series (Mc) are an important component of the antitumor defensive system of the body and can exert both cytolytic and cytostatic action (CSA) on tumor cells (TC). A CSA is effected by both activated [1-4] and inactivated Mc [5-7]. According to previous communications, the mechanisms of CSA of inactivated and activated Mc are different: in the case of CSA of inactivated Mc contact between effectors and targets is essential; activated Mc can exert their CSA with the aid of soluble cytostatic factors (CSF), secreted by Mc into the medium Ability to detect CSF evidently depends not only on the level of their production, but also on sensitivity of the target cells used to detect them. We previously showed that spontaneously transformed hamster epithelial cells (STHE), with a low level of malignancy, are

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TABLE 1. CSA of Mph under Supernatants on Cells of the STHE Strain and of the STHE-75/18 Variant, Selected in Vivo

Target cells	Dose of Mph	CSA of Mph		CSA of supernatants of Mph	
		$M_{av} \pm m$**	%	$M_{av} \pm m$**	%
STHE	$2{,}5 \cdot 10^5$	1 500±190	72,2	4 800±130	50,5
	$2{,}5 \cdot 10^4$	2 600±250	51,9	4 500±180	53,6
	$2{,}5 \cdot 10^3$	3 400±380	37,0	5 900±320	39,2
	—	5 400±100	0	9 700±920	0
STHE-75/18	$2{,}5 \cdot 10^5$	9 800±620	42,0	15 500±850	13,4
	$2{,}5 \cdot 10^4$	12 300±490	27,2	17 700±1200	1,1***
	$2{,}5 \cdot 10^3$	16 200±820	4,1***	17 100±610	4,5***
	—	16 900±550	0	17 900±350	0

Legend. *) Number of Mph per well on direct testing of their cytostatic activity and for obtaining supernatant. **) Incorporation of ^{3}H-thymidine into target cells. ***) Differences from control not statistically significant ($p < 0.05$).

TABLE 2. Comparison of CSA of Activated and Inactivated Syrian Hamster Mph Relative to Spontaneously Transformed Cells of the STHE Strain

Dose of Mph	CSA of Mph			
	inactivated		activated	
	$M_{av} \pm m$*	%	$M_{av} \pm m$	%
$2{,}5 \cdot 10^4$	12 300±260	68,8	15 500±1100	60,7
$2{,}5 \cdot 10^3$	25 200±1780	36,0	28 500±1600	27,7
$2{,}5 \cdot 10^2$	36 200±2660	8,1	36 700±564	6,9
—	39 400±477	0	39 400±477	0

Legend. *) Incorporation of ^{3}H-thymidine into target cells of strain STHE.

highly sensitive to the CSA of inactivated resident macrophages (Mph) from the peritoneal cavity of Syrian hamsters [8]. With the aid of these target cells, and also of less sensitive TC, we attempted to find CSF in supernatants of inactivated resident Syrian hamster Mph.

EXPERIMENTAL METHOD

The cytostatic tests were carried out in 96-well planchets (Falcon). The target cells were cells of the STHE strain, which had not undergone subculture in vivo, and STHE-75/18 cells, a highly malignant variant of TC obtained from STHE after a single passage in vivo. The target cells, numbering $2 \cdot 10^4$ per well, were added to Mph or introduced into the wells 2 h before the supernatant. Activated and inactivated Mph were obtained respectively from animals with a tumor induced by subcutaneous inoculation of STHE cells, and from intact animals. Peritoneal cells [9] were applied to the plastic in fourfold and tenfold dilutions (from $5 \cdot 10^5$ to $5 \cdot 10^2$ well), and the nonadherent cells were then washed off 2 h after application. Most of the remaining cells were Mph. The number of Mph in the wells was approximately half of their number introduced initially (from $2.5 \cdot 10^5$ to $2.5 \cdot 10^2$ respectively). The CSA of the Mph and supernatant was determined by measuring incorporation of ^{3}H-thymidine into TC nuclei, as described previously [9]. The CSA of the supernatant also was tested by direct courting of target cells, removed with a solution of versene, in a Goryaev counting chamber. The percentage of CSA in this case was determined by the formula: $(N_c - N_{exp})/(N_c - N_0) \cdot 100$, where N_0 is the number of TC in the wells 2 h after introduction (before the addition of the test supernatant to other analogous wells), and N_c and N_{exp} denote the number of TC in the wells after growth for 48 h in growth medium and in the supernatant being tested for its CSA respectively. The results were subjected to statistical analysis by Student's t test. To obtain macrophagal supernatants, 24-well planchets ("Falcon") with the same density of introduction of Mph as for determination of the direct CSA of the

Mph in 96-well planchets were used. The supernatants were collected after culture of the Mph for 18 h and were centrifuged at 1000 rpm for 15 min.

EXPERIMENTAL RESULTS

Analysis of spontaneously transformed cells of the STHE strain by two methods, namely incorporation of ^{3}H-thymidine into target cell nuclei and by direct counting of the cells, revealed the presence of CSF in the 18-h supernatants of inactivated Syrian hamster resident Mph. Direct counting of the STHE cells in the wells immediately after introduction and after 48 h in culture showed that their number was approximately trebled during this time. Supernatants from wells with Mph ($2 \cdot 10^4$) inhibits the increase in the number of TC by 40-80%.

When the CSA of Mph and their supernatants was determined on STHE cells by measuring incorporation of ^{3}H-thymidine by them we observed that incorporation of the label was inhibited by 25-80%, depending on the number of Mph used in the cytostatic test and to obtain the supernatant. The level of CSA of the supernatants was about equal to that of the Mph themselves. The results of one typical experiment are shown in Table 1. Both the fact that CSF are produced by inactivated resident Mph and also that such a small number of Mph (of the order of 10^4 and 10^3 cells per well) can produce CSF at an adequate level for reacting are interesting. Since CSF have been discovered by many workers using activated Mph [1, 3], the question arises whether production of the factor by resident Syrian hamster Mph may be connected with their activated state. The criterion of the activated state of Mph, according to the generally accepted view [10-12], is their ability to produce lysis of TC Vol'pe [1] found that resident Mph of intact Syrian hamsters do not cause lysis of TC and of spontaneously transformed STHE cells, i.e., they are inactivated. There are thus grounds for asserting that, since they are inactivated, resident Syrian hamster Mph must produce CSF inhibiting proliferation of spontaneously transformed cells.

It is perfectly possible that CSF of inactivated Mph can inhibit proliferation only of spontaneously transformed cells, and not of TC used as target cells by other workers [1, 7]. Correspondingly, in our experiments cells of strain STHE-75/18, selected from STHE cells in vivo, were resident for CSF (Table 1). The difference in the sensitivity of these two strains of cells to CSF of resident Mph may be the result of several processes undergone by the cells, after primary transformation in vitro, along the path of their transformation in vivo into TC.

According to data of Zachman et al. [14], CSF may be a cytolytic factor after significant dilution. It is unlikely that the cytolytic factor and CSF in our system would be qualitatively identical and would differ only quantitatively, since activated Mph, which unlike resident have a strong cytolytic action on STHE cells [13], would have the same CSA on them as resident Mph (Table 2).

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