consequently, delay in their elimination from the body [3]. The study of biological equivalence — an important characteristic for the establishment of hygiene regulations for chemical compounds, also revealed quantitative dependence of the change in G-6-P activity on the intensity and duration of exposure in vivo and in vitro [6].

Changes in activity of the membrane-bound enzymes of the endoplasmic reticulum studied were accompanied by morphological and functional changes in the cells and intracellular structures. For instance, under the influence of NDMA in vivo the development of foci of necrosis and destruction of hepatocytes and changes in activity of the reticuloendothelial system were observed [1]. The results of the electron-microscopic investigation of the cell components in vitro also indicated marked changes at the level of intracellular structures. Vacuolation of the cytoplasm and the presence of numerous pinocytotic vesicles and lysosomes were observed (Fig. 1a). Changes in the mitochondria were accompanied by swelling and widening of the internal septa and disorientation of the cristae (Fig. 1b). The endoplasmic reticulum exhibited considerable polymorphism and, in some cases, the integrity of the outer membrane was disturbed.

Among the general features of manifestation of the membrane-damaging effect of NDMA both in vivo and in vitro a reduction of activity of the membrane-bound enzymes of the endoplasmic reticulum (UDP-GCT and G-6-P) may thus be included, and it is accompanied by marked changes in the intracellular structure and disturbance of the integrity of their membranes.

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EFFECT OF ASCORBIC ACID ON FREQUENCY OF COLONIES RESISTANT TO COLCHICINE AND METHOTREXATE

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Data on the mutagenic and carcinogenic action of ascorbic acid, or vitamin C, are numerous [6, 10] but highly contradictory. These contradictions are largely linked with differences in the choice of test systems, the number of animals used for in vivo tests, the doses chosen, and the schedule and site of administration of the compound [6]. Tests in vitro have shown that degradation products of vitamin C formed in aqueous solutions in the presence of Cu^{++} or Fe^{+++} ions possess mutagenic activity [13]. On the other hand, prevention of the mutagenic action of nitrosamines by ascorbic acid also has been reported [13].

Experiments to determine the carcinogenicity of vitamin C both in vivo and in vitro have been undertaken recently in many laboratories. Investigations on mice [14] and rats (cited in [14]) have shown that high pharmacological doses (over 400 mg) are not carcinogenic, and that with an increase in concentration of the substance the time of appearance of tumors in-

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TABLE 1. Results of an Experiment to Determine the Effect of Ascorbic Acid on Frequency of Colchicine- and MTX-Resistant Colonies

Dose of ascorbic acid, μg /ml	SA	Dose of SA, μg/ml	Number of colonies per dish	Average number of colonies	Frequency of colonies	RFC
150 (3 days)	—	—	223, 209, 187	206,3	1	
	colchicine	0,05	14, 1, 5	6,7	$1,6 \cdot 10^{-4}$	5,5
	MTX	0,70	2, 4, 10	5,3	$1,3 \cdot 10^{-4}$	5,6
Control	—	—	287, 318, 256	287	1	
	colchicine	0,05	0, 5, 0	1,7	$2,9 \cdot 10^{-5}$	
	MTX	0,70	1, 0, 3	1,3	$2,3 \cdot 10^{-5}$	

Legend. SA) Selective agent. Frequency of colonies determined with allowance for efficiency of colony formation by cell treated and untreated with ascorbic acid in nonselective medium. RFC) Ratio of frequency of colonies after treatment with agent to frequency in control.

TABLE 2. Increase in Frequency of Cells Resistant to Colchicine and MTX Induced by Ascorbic Acid

Dose of ascorbic acid, μg /ml	Number of experiments	Survival rate of cells after treatment with ascorbic acid, %	Increase in number of colonies	
			colchicine-resistant	MTX-resistant
150	4	75—93	5,5—8,6	2,9—5,6
200	2	60	14,3—19,8	—

duced by known carcinogens is lengthened, and the number of tumors is reduced. There is evidence of the effective use of ascorbic acid to prevent the carcinogenic action of nitrosamine [13], dimethylbenz(a)anthracene (DMBA) [10, 15], etc. Vitamin C reduces the degree of binding of some carcinogens with DNA and also modifies the activity of microsomal mono-oxygenases, which play a key role in active xenobiotic metabolism [1]. The inhibitory effect of ascorbic acid also has been observed in mice with transplantable sarcoma 180 [6]. Epidemiological studies have given a statistically significant picture of negative correlation between vitamin C consumption and the frequency of carcinoma of the cervix uteri [8]. Meanwhile the more rapid development of carcinoma of the urinary bladder has been observed [9] in mice, and of transplantable sarcomas 39 and 180, and Watts' sarcoma in rats. There is evidence [13] of induction of carcinoma of the thyroid gland and of other hormone-dependent tumors by ascorbic acid. The possibility cannot be ruled out that in these cases a promotor effect of the compound is exhibited. In fact, high doses of sodium ascorbate have a promotor effect on the formation of preneoplastic changes, induced by methylnitrosourea [12], in the upper part of the stomach and in the epithelium of the urinary bladder in rats.

The writer showed previously [2] that chemical substances with promotor activity (DMBA, methylcholanthrene, aflatoxin B_1, etc.) cause gene amplification in somatic cells of the Djungarian hamster in vitro. The aim of this investigation was to determine whether ascorbic acid, which may possibly possess promotor activity, can induce gene amplification.

EXPERIMENTAL METHOD

The following substances were used: colchicine, from Merck, West Germany; methotrexate (MTX) from Lederle, USA; ascorbic acid, of USSR origin.

Experiments were carried out on Djungarian hamster DM-15 cells [3]. The cells were cultured in medium RPMI 1640 containing 10% bovine serum and 100 U/ml of monomycin.

The effect of vitamin C on the development of gene amplification was judged by its effect on the frequency of appearance of variants resistant to colchicine and MTX in the population.

Resistance to each of these selective agents arises as a rule as a result of amplification of certain regions of DNA [5, 7, 11, 17]. Investigation in both systems meant that the question of whether vitamin C induces gene amplification could be settled more confidently. After treatment of the cells for 3 days with subtoxic doses of ascorbic acid (determined from the curve of survival of DM-15 cells), namely 150-200 μg/ml, the cell populations were seeded into selective medium containing 0.05 μg/ml colchicine or 0.5-0.7 μg/ml MTX. The method of determination of the frequency of cell colonies resistant to colchicine and MTX was described previously by Kopnin et al. [4].

EXPERIMENTAL RESULTS

There were altogether six experiments. Table 1 gives the results of one experiment to determine the effect of vitamin C on the frequency of appearance of resistant colonies. After treatment of the cells with ascorbic acid the number of resistant colonies in both selective systems increased about fivefold. The pooled results of six experiments are given in Table 2. The degree of induction of resistant colonies in the colchicine system varied from 5.5 to 19.8, and in the MTX system from 2.9 to 5.6; moreover (according to the colchicine test), the degree of induction of resistant colonies was observed to depend on the ascorbic acid concentration in the medium. According to data in the literature [9, 13], ascorbic acid when tested in vivo is a promotor of tumour growth. In our previous experiments two promotors (mezerein and Tween-80), like all the complete carcinogens, with promotor activity (DMBA, etc.), increased the frequency of cell colonies resistant to colchicine and MTX by about the same number of times (from 6.8 to 56.3 and from 1.2 to 22.5, respectively) as did ascorbic acid. These similar results of induction are in agreement with the relationship found previously between promoting activity and induction of gene amplification.

The possible genetic effect of ascorbic acid in vitro may be evidence of the risk of using high doses of this substance for prophylactic and therapeutic purposes. We know that the same agent can induce amplification of different genes [5, 16], including, evidently, oncogenes. However, for final proof of the induction of gene amplification by ascorbic acid it will be necessary to isolate resistant colonies and to hybridize their DNA with cloned genes responsible for resistance. Further investigations in this direction must help to shed light on the genetic risk attached to high doses of ascorbic acid.

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