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EFFECT OF CYSTAMINE ON MAMMALIAN CELLS IN TISSUE CULTURE

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Abstract—Cystamine (0·25–1·0 mM) strongly reduced the growth-rate in monolayer cultures of human liver cells (Chang) and mouse fibroblasts (L-cells). The fibroblasts were found to be the more susceptible cell type.

In both cell types the protein content per cell was not affected by cystamine.

In the liver cells, glucose utilization and lactate production were strongly inhibited by cystamine. Also, the DNA and RNA contents per cell were reduced. In the case of the fibroblasts no similar effect of cystamine was observed.

The data suggest that cystamine may inhibit cell growth by several mechanisms.

THE disulphide cystamine is a substance of considerable interest from a radiobiological, pharmacological and biochemical point of view. In animals it reduces the deleterious effects of ionizing radiation when administered before the exposure.[1] In the doses required to obtain radioprotection it is toxic to animals.[1] *In vitro* it inhibits the growth of cells at very low concentrations.[2–7]

Cystamine is known to react readily with small molecular aliphatic mercaptanes to form mixed disulphides.[8, 9] It also reacts similarly with reactive protein sulphydryl groups[9–13] and it has been shown to inhibit certain purified sulphydryl enzymes.[13–15] Recently it has been demonstrated that cystamine and related disulphides inhibit glucose metabolism in human erythrocytes,[16] in certain rat tissues, [17]and in Yoshida hepatoma ascites cells.[18] The effect is most probably due to inhibition of hexokinase by mixed disulphide formation on its essential thiol groups.[16, 17] In view of the important role of thiol groups in biochemical processes, cystamine might be expected to have several points of attack in cellular metabolism. An attempt has therefore been made to search for other metabolic effects of cystamine, using two different types of mammalian cells.

In the present paper data are reported on the effect of cystamine on cell multiplication, protein synthesis, nucleic acid synthesis and glucose metabolism in human liver cells (Chang) and mouse fibroblasts (L-cells).

MATERIALS AND METHODS

Monolayer cultures of human liver cells (Chang) and mouse fibroblasts (strain L) were cultivated in Eagle's medium as previously described.[7] Cells from stock cultures were suspended in medium and 15-ml aliquots (about 1 million cells) were pipetted

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into 200-ml glass bottles and incubated at 37°. After 24 hr the medium was replaced by new medium containing cystamine. Pure medium was added to the control cultures. The whole procedure was carried out at 37°.

Cultures were taken for analyses at zero time and after 24 and 48 hr incubation. The glucose and lactate concentrations in the medium were determined after centrifugation. After trypsinization samples were taken for cell counts, and for determination of protein, DNA and RNA.

The cell number was determined with an automatic cell counter. Protein was determined by the Lowry method as modified for tissue culture,[19] using crystalline bovine serum albumin as the standard. After extraction of the cells,[20] DNA was determined by the indole method[21] and RNA by the orcinol method,[22] using Schwarz preparations of DNA and RNA as standards. Glucose was determined by the glucose oxidase method[23] and lactate by the method of Barker and Summerson, as modified by Ström.[24]

Glucose oxidase was obtained from C. Boehringer & Söhne, GmbH, Mannheim. All other chemicals used were commercial products of the highest purity.

RESULTS AND DISCUSSION

The effect of cystamine on cell multiplication is demonstrated in Fig. 1. In agreement with our previous results,[7] cystamine in concentrations of 0·25 and 0·50 mM exerted

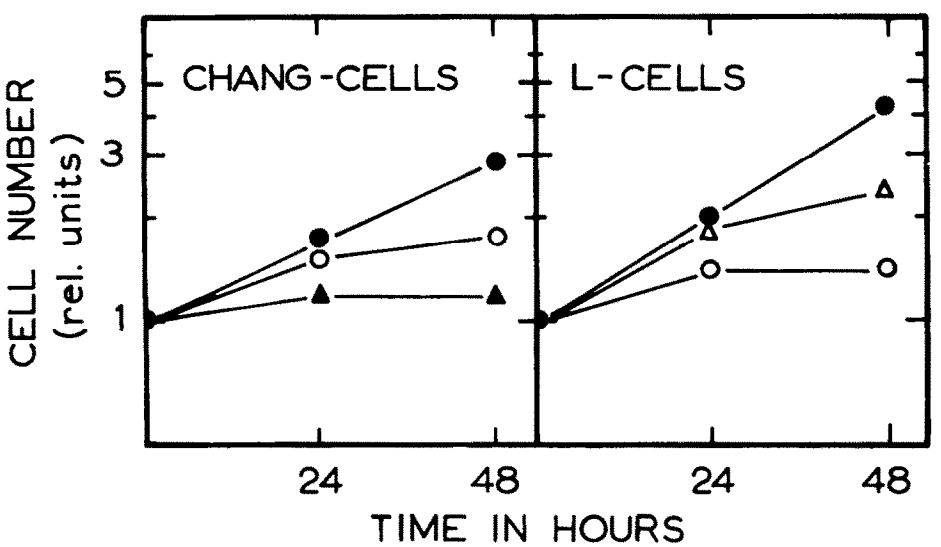

FIG. 1. The growth inhibiting effect of cystamine on human liver cells (Chang) and mouse fibroblasts (strain L). The total cell number was determined at zero time and after 24 and 48 hr incubation in the presence and absence of cystamine. The data are expressed in relative units, the cell number at zero time being set equal to 1. Each point represents the average of two independent experiments. ●——●, no cystamine; △——△, 0·25 mM cystamine; ○——○, 0·50 mM cystamine; ▲——▲ 1·0 mM cystamine.

a pronounced growth inhibiting effect on the fibroblasts. It is apparent that the liver cells were less susceptible to the toxic action of cystamine. In both cell types the

growth-inhibition increased with increasing cystamine concentration and with the length of the incubation.

Table 1 reveals the interesting fact that cystamine had a strikingly different effect on glucose metabolism in the two cell types studied. While the glucose utilization and

TABLE 1. EFFECT OF CYSTAMINE ON GLUCOSE UTILIZATION AND LACTATE PRODUCTION OF HUMAN LIVER CELLS (CHANG) AND MOUSE FIBROBLASTS (STRAIN L)

Strain	Observation period (hr)	Cystamine concentration (mM)	Glucose utilization* (μg/10^6 cells per hour)	Lactate production* (μg/10^6 cells per hour)
Chang-cells	0–24	0·0	53	51
		0·5	42	43
		1·0	22	23
	24–48	0·0	49	41
		0·5	30	28
		1·0	16	20
L-cells	0–24	0·00	41	40
		0·25	50	33
		0·50	47	33
	24–48	0·00	23	13
		0·25	28	14
		0·50	27	10

* Glucose and lactate were determined on aliquots of the medium. The values reported (the average of two independent experiments) were obtained by dividing the total glucose utilization or lactate production for the respective periods by the mean number of cells per hour, obtained by integrating the growth function between the time limits.

TABLE 2. EFFECT OF CYSTAMINE ON CELLULAR CONTENT OF PROTEIN, DNA AND RNA*

Strain	Cystamine concentration (mM)	Amount per cell in per cent of that of untreated cells					
		Protein after		DNA after		RNA after	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Chang-cells	0·5	93	110	84	78	85	76
	1·0	100	95	87	24†	88	24†
L-cells	0·25	92	111	95	110	95	96
	0·50	97	104	92	83	93	100

* The total cell number and the protein, DNA and RNA contents were determined in the cultures after 24 and 48 hr of incubation in the presence and absence of cystamine. The cellular contents of protein, DNA, and RNA were calculated in μg per cell and expressed in per cent of that of the corresponding control cultures. The values reported represent the average of two independent experiments.

† Cells showed pronounced morphological signs of degeneration.

lactate production of the Chang-cells were markedly inhibited by cystamine, no similar effect was obtained in the case of the L-cells. The finding in preliminary experiments[25] that cystamine increased the lactic acid production of L-cells could not be

confirmed. The effect of cystamine on the carbohydrate metabolism of the Chang-cells is similar to that previously observed on other cells and tissues,[16–18] and might well be caused by an inhibition of hexokinase as suggested by these authors.

The different behaviour of the two cell types is also reflected in the data shown in Table 2. In the treated liver cells the DNA and RNA contents per cell were definitely lower than in the untreated cultures, indicating that cystamine somehow interfered with their nucleic acid metabolism. Whether this effect is associated with the concurrent effect on the carbohydrate metabolism, or whether it is an independent effect cannot be decided by the present data. It should be noticed that the strong reduction in the DNA and RNA contents found after treatment with 1 mM cystamine for 48 hr was associated with marked morphological signs of degeneration. Only 60–70% of the cell population appeared normal as judged by phase microscopy.

The reason for the different response of the two cell types to cystamine is not revealed by the present data. However, animal tissues are capable of reducing disulphides by the glutathione reductase system[26] and by a recently discovered system localized in mitochondria.[27] Since different tissues differ considerably in their capacity to reduce disulphides,[17] the apparent failure of cystamine to affect the glucose and nucleic acid metabolism of the L-cells could possibly be due to a more rapid reduction of cystamine to cysteamine in these cells. It should be realized that the effects observed upon incubation with cystamine could in part be due to the action of cysteamine formed within the cells. Previous attempts to measure the growth inhibiting effect of cysteamine on L-cells were unsuccessful, as the cysteamine suffered rapid spontaneous oxidation in the growth medium.[7] However, the related thiol, mercaptoethylguanidine, was found to be far more toxic to the L-cells than was cystamine.[7]

The present finding that L-cells are strongly inhibited by cystamine in the absence of gross effects on protein, nucleic acid or carbohydrate metabolism, suggests that cystamine or its metabolic products may inhibit cellular multiplication by interfering with other processes. One obvious possibility is that cystamine or cysteamine may block mitosis by interacting respectively with the thiol and disulphide bonds of the spindle proteins.[28–30]

The present experiments provide no indication as to the mechanism of the growth inhibiting effect of cystamine on the L-cells. However, the results seem to warrant the conclusion that different cells may respond differently to the action of cystamine and that their growth may possibly be inhibited by different mechanisms.

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