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CYTOLOGICAL CHANGES IN ASCITES CARCINOMA CELLS ON THE EFFECT OF NITROGEN MUSTARD, TEM, OR BCM, WITH SPECIAL REFERENCE TO CHANGES IN NUCLEAR AND NUCLEOLAR VOLUME

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In a previous communication [6] we dealt with volumetric increase in the cells, nuclei, and nucleoli, of Ehrlich ascites carcinoma cells upon the effect of colchicine and podophyllin, respectively. In the present paper the results of investigations into the same effect of nitrogen mustard, TEM, and BCM will be reported.

Material and methods

Inbred albino mice, three months old and of the same weight, were each inoculated peritoneally with the same amount (10×10^6) of Ehrlich ascites carcinoma cells and then poisoned with a single, about half-lethal, dose of 2 γ/g of nitrogen mustard (methyl-bis [β-chloroethyl] amine); or 5 γ/g of TEM (tri-ethylene melamine) or 100 γ/g of BCM (1,6-bis [β-chloroethylamine]-1,6-desoxy-D-mannite-dichlorohydrate). Two months later the experiment was repeated under identical conditions. Each drug was applied to twice two animals. With a sterile syringe ascitic fluid was drawn from the animals before treatment and 1, 3, 6, 12, 24, 48 and 72 hours after treatment. Using the methods described in our earlier paper, morphological and nuclear variation studies were carried out.

Results

Upon the action of either of the three drugs the chromatin was very soon seen to form into coarse clots, and the nuclei and nucleoli appeared conspicuously enlarged.

Nitrogen mustard caused the chromatin in the nuclei to break up into minute globules. Such globules were encountered in the cytoplasm, but also outside the cells, bare or with a narrow cytoplasmic border around them. (In Table 1 the cells suffering this type of lesion are designated as "broken-up cells".)

Characteristic of the effect of TEM was that the nuclear chromatin fell into powderlike fine particles (referred to in Table 2 as "powderlike chromatin"). The nuclear chromatin decreased in amount, the nucleus became swollen, and large-sized nucleoli formed, with two or three of them in some nuclei.

The action of BCM turned the nuclear chromatin very soon into coarse clots, and caused the nuclei and nucleoli to enlarge.

At the later stages all three drugs gave rise to polynuclear giant cells, and shortly after the administration of any one of them there was cellular disintegration; in a day or two hardly any intact cells were encountered. Based upon counts of 200 cells each, the changes in the cytological picture are listed in Tables 1, 2, and 3.

Table 1

Cytological changes on the effect of nitrogen mustard

2 γ/g

	Control	1h	3h	6h	12h	24h	48h	72h
	%	%	%	%	%	%	%	%
Normal cell	92	79	66	42	10	12	13	40
Pycnosis	8	10	10	10	20	21	15	13
Lysis	—	—	2	8	5	4	10	11
Broken-up cells	—	1	6	12	33	35	25	14
Large-sized nucleus, coarse chromatin	—	10	16	26	30	20	30	20
Several nuclei, coarse chromatin	—	—	—	—	2	8	7	10
Distorted mitosis	—	1	2	2	1	—	—	—

Table 2

Cytological changes on the effect of TEM

5 γ/g

	Control	1h	3h	6h	12h	24h	48h	72h
	%	%	%	%	%	%	%	%
Normal cell	90	87	86	70	50	10	24	45
Lysis	8	6	10	15	18	10	12	10
Pycnosis	2	2	2	2	2	1	2	5
Large-sized nucleus, coarse chromatin	—	5	2	10	16	49	36	20
Several nuclei, coarse chromatin	—	—	—	5	5	10	6	5
Powderlike chromatin	—	—	—	—	8	19	20	15
Distorted mitosis	—	—	—	1	1	—	—	—

As to our studies of nuclear variation, we have refrained from summarizing in a separate Table the changes in cell size due to the action of nitrogen mustard, TEM, and BCM. Instead, reference is made to the topmost portion of Fig. 7, which shows that on the effect of nitrogen mustard the average cell volume was

Table 3

Cytological changes on the effect of BCM

100 γ/g

	Control	3h	6h	12h	24h	48h	72h
	%	%	%	%	%	%	%
Normal cell	79	35	13	11	6	3	21
Lysis	5	8	11	15	30	18	10
Pycnosis	1	—	1	3	3	7	—
Large-sized nucleus, coarse chromatin	15	8	20	31	40	35	20
Several nuclei, coarse chromatin	—	1	1	3	4	6	10
Large-sized nucleolus, coarse chromatin	—	48	54	35	14	30	38
Distorted mitosis	—	—	—	2	3	1	1

the greatest in the 12th hour, when it was about one and a half times the initial volume. On the effect of TEM it exceeded the starting volume by approximately 60 per cent, and on that of BCM by roughly 70 per cent, in 24 hours.

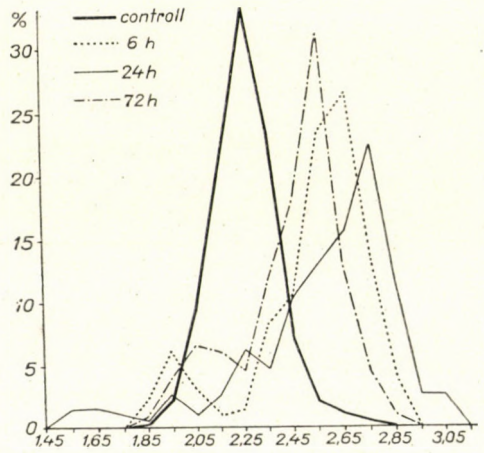

Fig. 1. Changes in nuclear volume on the effect of nitrogen mustard. Abscissa: logarithms of the volumetric value of class means. Ordinate: percentage incidence

To render more conspicuous the curves which demonstrate the volumetric changes in the nuclei and nucleoli, only the data referring to certain times of observation were included in them. On the other hand, all the intervals of observation are taken up in Fig. 7, which shows jointly the average values for the volumetric changes in the cells, nuclei, and nucleoli, on the effect of each of the three drugs.

In the curve depicting the changes in nuclear volume after treatment with nitrogen mustard there is a gradual shift to the right which becomes very marked

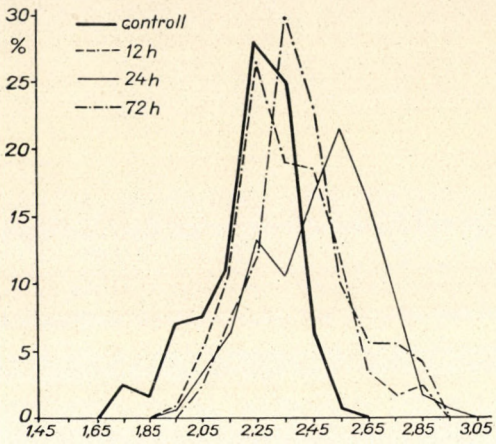

Fig. 2. Changes in nuclear volume on the effect of TEM. Abscissa: logarithms of the volumetric value of class means. Ordinate: percentage incidence

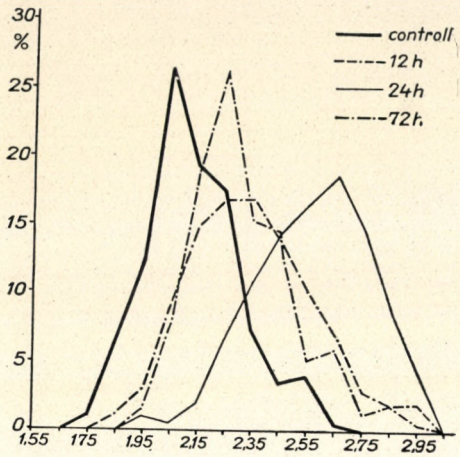

Fig. 3. Changes in nuclear volume on the effect of BCM. Abscissa: logarithms of the volumetric value of class means. Ordinate: percentage incidence

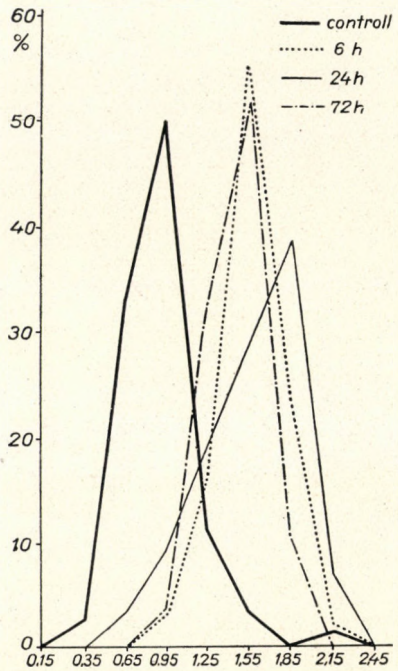

Fig. 4. Changes in nucleolar volume on the effect of nitrogen mustard

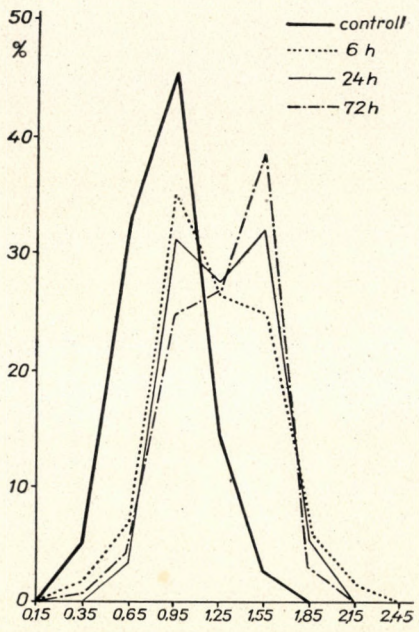

Fig. 5. Changes in nucleolar volume on the effect of TEM

at the 24-hour point, whereafter it slowly abates. The average value for the volume of the nucleus in the tumour cell exceeds the initial value by 80 per cent in 6 hours, by more than 100 per cent in 24 hours, and is still on about a 150-per cent level in 72 hours (Fig. 1).

In the curve illustrating the TEM effect displacement to the right begins in 12 hours, gradually intensifies in 24 hours, the curve becoming bicuspid, and

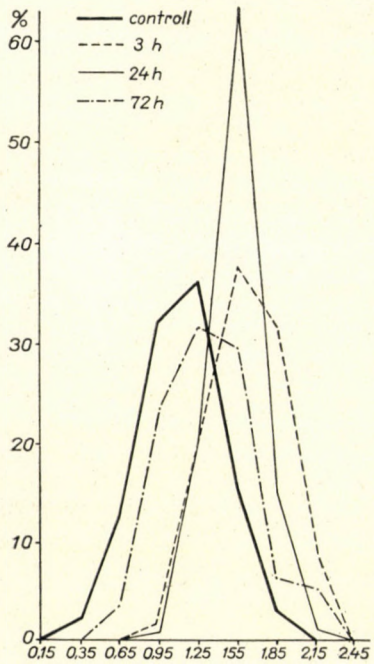

Fig. 6. Changes in nucleolar volume on the effect of BCM

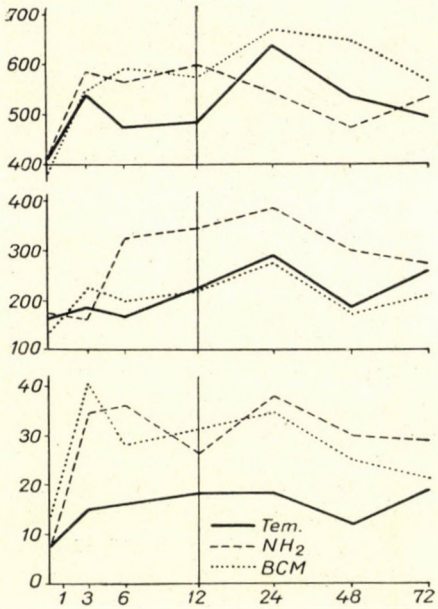

Fig. 7. Changes in the average cellular nuclear, and nucleolar, volume on the effect of nitrogen mustard, TEM, and BCM, respectively. Abscissa: time intervals. Ordinate: volumes in μ^3

is of almost the same rate even 72 hours after treatment. Accordingly, the average value for nuclear volume rises gradually from the starting value, is doubled in 24 hours, and almost doubled as late as in the 72nd hour (Fig. 2).

The curve recording the BCM effect runs a course similar to that of the TEM curve, but displays a much more pronounced shift to the right. This shift increases after 24, but lessens after 72, hours. The average nuclear volume is about 150 per cent of the initial volume in 3, and approximately 200 per cent in 24, hours, to fall back to the neighbourhood of the starting point in 72 hours (Fig. 3).

The curve illustrating the changes in the volume of the nucleoli after treatment with nitrogen mustard begins the shift to the right as early as in 3 hours, with the maximum displacement in 24 hours. A shift to the right is

still visible in 72 hours. In 6 and 24 hours the average nucleolar volume is about four times the original volume (Fig. 4).

The effect of TEM is similar, but with a lesser shift to the right. The average nucleolar volume is about twice the initial volume, even 72 hours after treatment (Fig. 5).

On the action of BCM the curve shows a very marked displacement in 3 hours; the rate of the shift begins to decrease in 72 hours (Fig. 6).

Discussion

Nitrogen mustard and its derivatives are being widely used in the treatment of malignant tumours. Many authors have devoted attention to experiments concerned with the effect and the mechanism of the action of this drug; among them are GILLMAN and PHILLIPS [7], KARNOFSKY and GRAEF [13], SUGÁR and KELLNER [18], KELLNER [15], NÉMETH, SELLEI and MAYER [16], BICHEL [2], and HEILMEYER [9]. The agent gives rise to marked lesions in the nucleus, but attacks the cytoplasm as well. Like X-ray, it acts not only on cells in division but also on those in interphase.

BESTIAN [1] was the first to call attention to it that the ethylenimine derivatives used in the textile industry are nucleophilic, like nitrogen mustard is. Among these derivatives TEM (triethylene melamine) has been found to be the most efficaceous. Its experimental effect and mechanism of action have been studied by a host of research workers (HADDOW [8]; HENDRY, HOMER, ROSE and WALPOLE [10]; PHILIPS and TIERS [5]). In its effect on the cells it resembles nitrogen mustard, but is of higher toxicity.

BCM (1,6-bis[β-chloroethylamine]-1,6-desoxy-D-mannite-dichlorohydrate) is a mustard derivative recently synthetized by VARGHA [21] in Hungary; its cytostatic action and therapeutic value were tested in our Institute (KELLNER, NÉMETH, SELLEI [14]). Its effect was found to be similar to that of nitrogen mustard, but less toxic.

There are many communications dealing with the effect nitrogen mustard exerts on Ehrlich ascites carcinoma cells (LETTRÉ [17]; SUGIURA and STOCK, [20]; SUGÁR and VIGH [19]). In agreement with data in the literature, we, too, have found that this effect is easy to read. In our experiments about 90 per cent of the cells were observed to have suffered lesions in 12 to 24 hours after administration of the drug.

Decreases in the amount of nuclear chromatin, oedematous swelling and enlargement of nuclei and nucleoli due to the action of TEM have been described by BUCKLEY, STOCK, CROSLEY, and RHOADS [3] in solid tumours. On the evidence of our own investigations about 90 per cent of the cells display pathological lesions in 24 hours.

The morphological effect of BCM on Guérin tumours has been discussed in detail by KELLNER [15]. Half a lethal dose of the drug was found to injure 94 per cent of the Ehrlich ascites carcinoma cells in 24 hours.

Concerning the effect of nitrogen mustard on the size of cell nuclei, there are several data available in the literature. According to KARNOFSKY [12] yeast cells damaged *in vitro* increase in size in 8 to 12 hours, to regain later their original dimensions.

Summarizing the results of our cytological and nuclear variation studies, it may be stated that upon the action of any of the three drugs the increase in the average volume of cells and nuclei is the greatest 24 hours after treatment, i. e., at the time when about 90 per cent of the cells appear to be affected with morphological lesions, and when the scatter of the values for nuclear volume is the most pronounced. Nucleoli, on the other hand, attain their maximum volume much earlier (in 1 to 3 hours). It would appear that their enlargement is a delicate early indicator of the functional and morphological changes which upon the action of these agents are about to take place in the tumour cells. The fundamental work of CASPERSSON [4] and numerous other publications register the fact that while the nucleolus, nucleus and cytoplasm of the perishing tumour cell are increasing in volume, there is practically no longer any protein or nucleic acid forming ; the amount of nucleic acid is decreasing in the nucleus as well. HUTH [11] observed this phenomenon in aged or dying Ehrlich ascites carcinoma cells. Upon the action of nitrogen mustard, or TEM, or BCM, these changes develop rapidly and intensively ; this has an adverse effect on cellular metabolism and points to the impending death of the tumour cell.

Evaluating the joint cytological and nuclear-variation picture, the conclusion seems permissible that it is some chemical agent that acts upon the Ehrlich ascites carcinoma cells. The present investigations allow the inference that the single dose of colchicine or podophyllin described in our previous communication gave rise to morphological and quantitative changes that were lesser and took a more rapid course than those induced by nitrogen mustard, TEM, or BCM.

Summary

The effect has been studied which half a lethal dose of nitrogen mustard, TEM, or BCM, exerts on Ehrlich ascites carcinoma cells and the volume of the nuclei and nucleoli in them. Upon the action of any of the three drugs about 90 per cent of the cells were found to have suffered lesions in 24 hours, by which time they and their nuclei were enlarged, while the nucleoli had increased in size earlier, in 1 to 3 hours after treatment. To a limited extent, the drug effect was still seen in 72 hours.

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ЦИТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ АСЦИТИЧЕСКИХ РАКОВЫХ КЛЕТОК НА ДЕЙСТВИЕ ГОРЧИЧНОГО АЗОТА, ТЕМ И БКМ, ПРИНИМАЯ ВО ВНИМАНИЕ ИЗМЕНЕНИЯ ОБЪЕМОВ ЯДЕР И ЯДРЫШЕК

Э. ГАТИ, Г. ИНКЕ, А. БАЙТАИ и Е. ДЬЯРФАШ

Авторы исследовали действие однократной полусмертельной дозы горчичного азота, ТЕМ и БКМ на морфологическую картину асцитических раковых клеток Эрлиха, как и на объем клеток, ядер и ядрышек. Было установлено, что на действие всех трех вышеупомянутых веществ по истечении 24 часов прибл. 90% клеток были повреждены, причем наблюдалось выраженное увеличение клеток и ядер, в то время как увеличение ядрышек произошло уже после 1—3 часов. Одинаковое действие наблюдалось, хотя и в меньшей мере, даже и по истечении 72 часов.

ZYTOLOGISCHE VERÄNDERUNGEN VON ASZITESKARZINOMZELLEN AUF WIRKUNG VON SENFNITROGEN, TEM UND BCM, IM HINBLICK AUF DIE VERÄNDERUNG DES KERN- UND NUKLEOLENVOLUMENS

E. GÁTI, G. INKE, A. BAJTAI und J. GYÁRFÁS

Die Wirkung von einmaligen halbtödlichen Dosen von Senfnitrogen, TEM und BCM auf das morphologische Bild der Ehrlichschen Asziteskarzinomzellen, als auch auf das Zellen-, Kern- und Nukleolenvolumen wurde untersucht. Es wurde festgestellt, daß auf die Wirkung der obigen Stoffe nach 24 Stunden ca 90% der Zellen geschädigt wurden mit einer ausgesprochenen Vergrößerung der Zellen und Kerne. Die Vergrößerung der Nukleolen erfolgte schon zu einem früheren Zeitpunkt — nach 1—3 Stunden. Dieselbe Wirkung wurde in geringerem Grade auch noch nach 72 Stunden beobachtet.

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