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CHANGES IN CELL AND NUCLEAR VOLUME IN ASCITES CARCINOMA UPON THE EFFECT OF COLCHICINE AND PODOPHYLLIN

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(Received April 23, 1956)

In testing anticarcinogenic chemotherapeutic substances an ever-widening significance is being attached to Ehrlich ascites carcinoma and Yoshida ascites sarcoma. In a previous paper [7] we discussed a number of methods employed by us in attempts to disclose the changes that take place in ascites tumours upon the action of chemotherapeutic agents. The aim of the present study was the quantitative determination of changes in the size of the tumour cell, the nucleus, and the nucleoli. It needs to be mentioned that in transplanted tumours earlier authors [15] have successfully demonstrated changes in the size of the nuclei due to the action of some substances that influence division.

Material and methods

Inbred albino mice, three months old and of the same weight, were used. Having inoculated them intraperitoneally with Ehrlich ascites carcinoma cells, they were poisoned with 8 γ/g of colchicine and 20 γ/g of podophyllin. Two months later the experiment was repeated under identical conditions. Each of the two poisons was given to twice two animals. Using a sterilized syringe, ascitic fluid was drawn before the administration of the drug and 1, 3, 6, 12, 24, 48 and 72 hours after it. Some of it was used up in making smears which were then stained according to Giemsa. The rest was taken up in 96 per cent alcohol and fixed for 30 minutes; the sediment was then quickly carried through an alcohol series (the process taking in all 1 hour) and embedded, to prepare from it sections of uniformly 5 μ thickness. These were stained with haematoxylin-eosin.

The statistical method of computing nuclear variations was used in carrying out quantitative determinations. Applying 90 × 15 magnification, the cells were projected at a distance which secured a 3000fold total magnification. This method permitted a sharp tracer to be passed around the boundary lines of the nuclei and nucleoli. But as the contours of the cells were but moderately distinct, the values referring to them in the tables can by no means be regarded as exact as those pertaining to the nuclei and nucleoli. Conceiving of the cells, nuclei and nucleoli, represented in the figures as ellipsoids of revolution with a long axis, the longest diameter and the shortest perpendicular to this were measured, and the volume was determined on the basis of the formula $\frac{\pi}{6} \cdot AP^2$, where A (axis) denotes the rotational axis, and P (perpendicular) the short diameter at right angles to it. On the abscissas in the figures, the logarithmus of the volumes of the class means are represented, for according to HINTZSCHE's studies the volumes follow lognormal distribution. On the ordinates, the percentage incidence is shown. The class extensions were determined not as $\log {}^2/_4$, but as $\log {}^2/_3 = 0{,}10$. The exceedingly work-consuming grouping and the previous calculation of the geometrical mean were

dispensed with. The logarithm of 142 μ^3, log 2,15, was taken to be the fixed class mean, which agrees with JACOBJ's value for K_1. This simplified method has been described by us in earlier papers [6, 9]. The nucleoli, too, were measured independently for size. They were grouped into classes of extension log 2, without creating subclasses for them. To make the curves more perspicuous, the data for some intervals only are presented in them, but in the tables all the data are listed.

To find out whether with the technique employed by us the sections were of uniform shrinkage and thickness, the data of 800 cells were subjected to a comparative study in smears and sections. In relation to the smears the shrinkage was found to be 60 per cent, but it was approximately the same in both the treated and untreated material.

Results and discussion

By DUSTIN [5] the poisons of direct effect on mitosis are called karyoclastic substances; colchicine is the most powerful of them. According to KING and

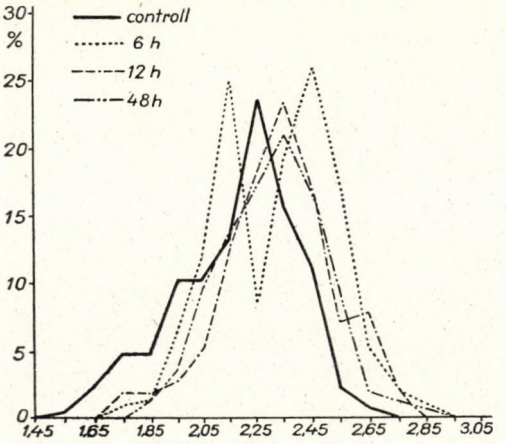

Fig. 1. Changes in nuclear volume on treatment with colchicine

SULLIVAN [11], podophyllin produces a colchicine-like effect, while CULP and KAPLAN [4] attribute its tumour-inhibiting effect to ischaemia and necrosis connected with spastic constriction of blood vessels. BRUNN [2] established a close chemical analogy between podophyllotoxin and colchicine.

No items have been found by us in the literature that would have referred to nuclear variation statistics being employed in studies of the effect exerted by karyoclastic substances on the volume of Ehrlich ascites carcinoma cells. By haematocrit reading, RÉVÉSZ and KLEIN [17] established the average volume of these cells to be 1903 μ^3, and their average diameter 15 μ; these values agree with those obtained by us in studying smears with the nuclear variation method. BUCHER [1] plotted the nuclear variation curve for the cell nuclei of a fibroblastic culture and found that after treatment with colchicine the average volume of the nuclei increases and the distribution curve for nuclear size undergoes a

significant change. There are a few publications which do mention the increase of cellular nuclei upon the action of colchicine (MISURSKY and DOLJANSKY,

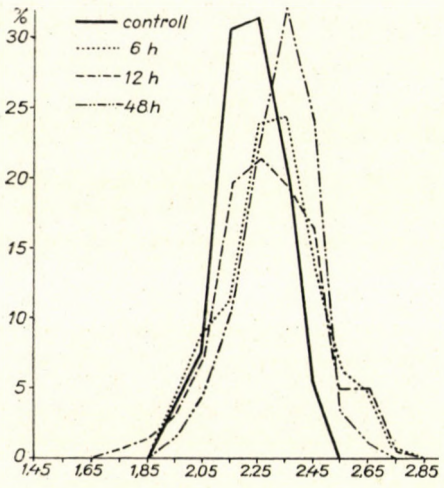

Fig. 2. Changes in nuclear volume on treatment with podophyllin

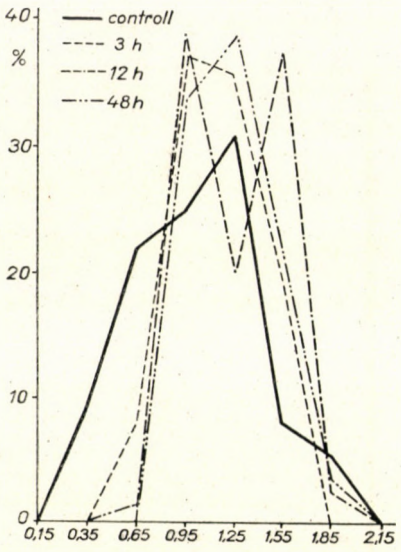

Fig. 3. Changes in nucleolar volume on treatment with colchicine

16 ; LAMBERS, 14). As regards podophyllin, reference is made to the work of MATKÓ, HOLCZINGER and KERESZTURY [15] who studied volumetric changes of the cellular nuclei in cardiac muscle, liver, and kidney. They, too, established that following treatment with colchicine or podophyllin the cellular nuclei increase in size and their distribution curve changes.

On the evidence of our own findings, after the injection of a single LD50 of colchicine or podophyllin the cytological changes can readily be read off the

Table 1

Cytological changes following treatment with colchicine
8 γ/g

	Control	30′	1h	3h	6h	12h	24h	48h
	%	%	%	%	%	%	%	%
Normal cell	90	89	87	80	47	64	73	81
Lysis	6	4	6	8	18	10	9	6
Pycnolysis	4	6	6	10	21	17	7	8
"Colchicine" cell	—	1	1	2	12	10	10	4
Distorted cell	—	—	—	—	2	2	1	1

Table 2

Cytological changes following treatment with podophyllin

20 γ/g

	Control	30′	1h	3h	6h	12h	24h	48h
	%	%	%	%	%	%	%	%
Normal cell	86	78	76	80	47	41	59	74
Lysis	8	10	10	8	20	15	10	10
Pycnolysis	5	6	5	10	18	19	20	8
"Podophyllin" cell	—	—	3	2	10	24	10	8
Distorted cell	—	—	1	1	2	1	1	—

sections [12, 18, 19, 20]. There soon appeared the so-called colchicine and podophyllin cells, respectively [10, 8, 14, 3]. Upon the effect of colchicine about half

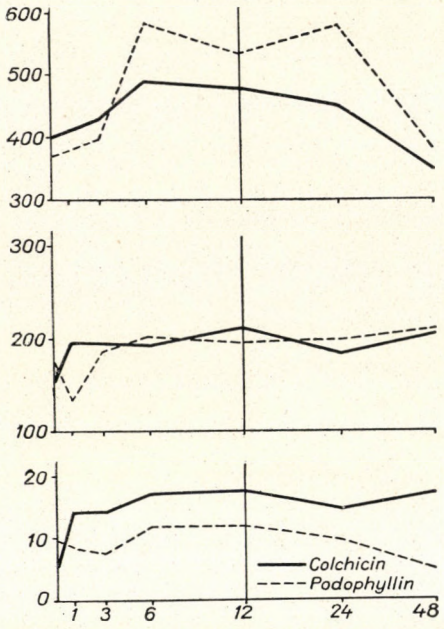

Fig. 4. Average volumetric changes in cells, nuclei, and nucleoli, on the effect of colchicine and podophyllin, respectively. On the abscissa the intervals are shown in hours at which studies were made; on the ordinate the volumetric values are presented in μ^3

the cells suffered lesions in 6 to 12 hours, and upon that of podophyllin approximately 60 per cent in 12 to 24 hours. Based upon counts of 200 cells each, the alterations in the cytological picture are presented in Tables 1 and 2.

The topmost part of figure 4 shows that the increase in cell size is about 25 per cent after the administration of colchicine, and roughly 50 per cent after that of podophyllin, both lasting for from 6 to 24 hours.

In the curve depicting volumetric changes in the nuclei, a shift to the right can be seen to have taken place as early as 1 hour after the treatment with

Table 3

Changes in nuclear volume on treatment with colchicine

	1,45	1,55	1,65	1,75	1,85	1,95	2,05	2,15	2,25	2,35	2,45	2,55	2,65	2,75	2,85
Before treatment ...	1	5	5	5	5	10	10	13	24	15	11	2	1	—	—
1	—	—	1	1	2	2	7	12	22	26	25	23	1	—	—
3	—	—	—	1	1	1	8	9	23	29	17	7	5	1	—
6	—	—	—	1	8	6	12	25	9	20	26	16	6	2	1
12	—	—	—	1	2	3	5	13	20	24	18	8	8	2	1
24	—	—	—	1	2	6	12	17^5	17	22	18	8	2	1	0^5
48	—	—	—	—	1^5	4	10	15	14	21	18	9	2	1	0^5

Horizontal top row = logarithm of volume of class means.
Incidence expressed in per cent

Table 4

Changes in nuclear volume on treatment with podophyllin

	1,55	1,65	1,75	1,85	1,95	2,05	2,15	2,25	2,35	2,45	2,55	2,65	2,75	2,85
Before treatment	—	—	—	—	4	7,5	32	33	24	5,5	—	—	—	—
1	—	—	—	0,5	3	3	28	27	23	9	1	—	—	—
3	—	—	—	—	5	14	17	25	18	15	10	1	—	—
6	—	—	—	—	5	9	11	29	24^5	16	9m	6	1	—
12	—	—	—	1,5	3	7,5	20,5	22,5	19,5	8,5	5	5	1	—
24	—	—	—	—	2	6	43	45	22	10,5	8,5	3	—	—
48	—	—	—	—	1^5	4^5	11	24	40	28	3^5	1	—	—

Horizontal top row = logarithm of volume of class means.
Incidence expressed in per cent

colchicine ; this was more marked in the 6th hour, with the curve becoming bicuspid, to attain its peak in the 12th hour; even in the 48th hour there was still a displacement to the right (Fig. 1, Table 3). On an average, in the 12th hour the nuclei were about one and a half times their initial size, and in the 48th hour they were still seen to be enlarged (Fig. 4).

Table 5

Changes in nucleolar volume on treatment with colchicine

	0,35	0,65	0,95	1,25	1,55	1,85	2,15	lg V
Before treatment	12	22	25	31	8	5	1	
1	0,3	0,5	50	30	20	—	—	
3	—	7	38	36	20	—	—	
6	—	—	33	27	37	—	—	
12	—	1	39	20	37	2	—	
24	1	7	30	42	20	3	—	
48	—	1	31	49	30	4	—	

Horizontal top row: logarithm of volume of class means.
Incidence expressed in per cent.

After treatment with podophyllin, the shift to the right was most pronounced in the 6th and the 48th hour (Fig. 2, Table 4), but, in relation to the initial size, nuclear enlargement was less than it was on colchicine treatment, being only about 20 per cent (Fig. 4).

As regards changes in the volume of the nucleoli, the curve representing them shows a displacement to the right in 3 hours, attaining the peak in 12 hours, by which time it is definitely bicuspid. In 48 hours the shift to the right is still there (Fig. 3, Table 5). The average nucleolar volume is about three times the initial volume in 1 hour, almost four times that in 12 hours, remaining as much even 48 hours after treatment with colchicine (Fig. 4).

There was no significant displacement observable on treatment with podophyllin.

Summary

The effect of a single LD50 of colchicine or podophyllin on the volume of the nuclei and nucleoli in Ehrlich ascites carcinoma cells has been studied. It has been established that colchicine significantly enlarges the cells, the nuclei, and the nucleoli, the increase in volume reaching its peak in 12 hours, and with the augmentative effect lasting for as much as 48 hours. Treatment with podophyllin increases essentially the cellular volume, but enlarges nuclear volume in a lesser degree than colchicine, and is of no appreciable effect on the volume of nucleoli. The increase in volume attains its maximum in 6 hours, but even after 48 hours the effect can still be observed.

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ИЗМЕНЕНИЕ ОБЪЕМА КЛЕТОК И ЯДЕР ПРИ АСЦИТИЧЕСКОМ РАКЕ НА ДЕЙСТВИЕ КОЛЬХИЦИНА И ПОДОФИЛЛИНА

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Исследовалось действие однократной полусмертельной дозы кольхицина и подофиллина на объемы ядер и ядрышек асцитических раковых клеток Эрлиха. Было установлено, что после подачи кольхицина проявляется характерное увеличение объема клеток, ядер и ядрышек, достигающее после 12 часов своего максимума. Это действие доказуемо и по истечении 48 часов. После подачи подофиллина увеличивается объем клеток, однако, объем ядер увеличивается в меньшей мере, чем в случае подачи кольхицина, в то время как объем ядрышек не проявляет значительного изменения. Увеличение достигает своей максимальной величины после 6 часов. Это действие доказуемо также еще по истечении 48 часов.

ZELLEN- UND KERNVOLUMENVERÄNDERUNGEN BEI ASZITESKARZINOM AUF WIRKUNG VON COLCHICIN UND PODOPHYLLIN

É. GÁTI, G. INKE und M. PALKOVITS

Die Wirkung von einmaligen halbtödlichen Dosen von Colchicin und Podophyllin auf das Kern- und Nukleolen-Volumen von Ehrlichschen Aszitеskarzinomzellen wurde untersucht. Es wurde festgestellt, daß nach Verabfolgung von Colchicin eine signifikante Vergrößerung des Zellen-, Kern- und Nukleolen-Volumens erfolgt, die nach 12 Stunden ihr Maximum erreicht. Die Wirkung ist auch nach 48 Stunden nachweisbar. Nach Verabreichung von Podophyllin vergrößert sich das Zellvolumen signifikant, wogegen das Kernvolumen sich in geringerem Maße als im Falle von Colchicin vergrößert, während das Nukleolen-Volumen keine bedeutenden Veränderungen aufweist. Die Vergrößerung erreicht nach 6 Stunden ihr Maximum. Die Wirkung ist auch noch nach. 48 Stunden nachweisbar.

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