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**The Cytological Effect of Chemicals on Ascites Sarcomas
V. Effects of Cortisone and Sarkomycin on the
MTK-Sarcoma II¹⁾**

By
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(With 3 Text-figures and 2 Plates)

Makino and his co-workers have made cytological investigations on the effects of several chemicals, such as podophyllin, CaCl_2 , AlCl_3 , H_2O_2 , podophyllotoxin, alpha- and beta-peltatin and quercetin upon tumor cells (Makino & Tanaka 1953 a, b, Makino & Cornman 1953, Makino & Nakanishi 1955, Tanaka et al. 1955). It was demonstrated that, although temporary suppression of tumor growth was maintained as a result of considerable damage to most of the tumor cells by judicious injections of these drugs, some of the tumor cells remained unaffected and became the primary source of renewed malignant growth.

Recently, the effect of cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate) upon various kinds of tumors have been studied by several investigators with various results (Sugiura et al. 1950, Higgins et al. 1950, Gottschalk & Grollman 1952, Goldie et al. 1954); some authors have reported complete or moderate inhibition of tumor growth, while some others, working with different tumors and under different experimental conditions, have obtained no inhibition at all. Sarkomycin is an antibiotic obtained from microorganisms in the soil. It has been said that this substance exerts inhibitory effect upon tumor growth (Umezawa et al. 1953). No particular attention, however, has been drawn to the cytological effect of these substances on tumor cells in any study so far carried on. The present study was undertaken in a hope to investigate the influence of cortisone and sarkomycin on growth of the ascites tumor of rats particularly with respect to the cytological features of the action of these drugs on tumor cells.

The author wishes to tender his sincere thanks to Professor Sajiro Makino for his direction and improvement of the manuscript for publication. Further cordial thanks are offered to Mrs. K. Kanô, Mr. A. Tonomura and Mr. T. Okada for their valuable advices and criticisms.

Material and methods

The rats used in the following experiments were pure inbred albinos of the Wistar

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strain, weighing 70 to 120 grams. As the tumor material, the MTK-sarcoma II was used; it was established in our laboratory during the course of the hepatoma-producing-experiments by application of azo dyes in a Wistar inbred rat by Tanaka and Kanô (1951). On the 3rd or 4th day after transfer of the ascites tumor the tumor-bearing rats received intraperitoneal injections of cortisone or sarkomycin. By that time the most active proliferation of the tumor cells had been attained in the peritoneal cavity of the animal. More than 80 tumor-bearing animals were used in the experiments as described below. For cytological observation, smear preparations were made from a droplet of the tumor ascites obtained by abdominal puncture at appropriate intervals following the application of the drugs. The slides were colored with acetic dahlia.

The numerical data in the following descriptions were calculated on the basis of about 2000 cells in every sampling of the tumor ascites.

Observations

1. *Effect of cortisone*

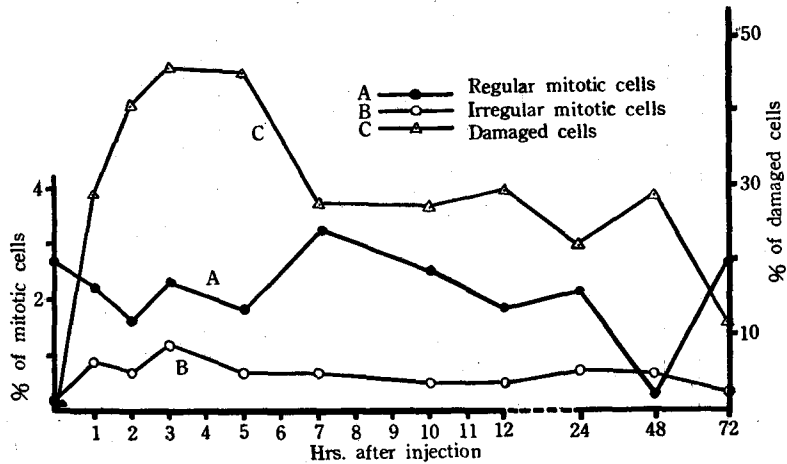
Cortisone acetate (Merck) was prepared for the use of the present investigation by suspending the substance with Ringer's solution. The suspension thus prepared in a supersaturated condition contains 25 mg of cortisone acetate in 1 cc of the Ringer's solution. A single intraperitoneal injection was given to each tumor-bearing rat on the 3rd or 4th day after transplantation at a dose level of 500 mg per 1 kg of body weight. Tumor samples were taken at various intervals following the application of the drug. At every time, the sampling was made from both the untreated control tumor-bearing rats and the treated ones.

Within one or two hours after injection, the tumor ascites displayed a large number of damaged tumor cells. The damage to cell was maintained by a breaking down of the cytoplasm. The tumor cells at every stage were found affected. The cell bodies were first broken down, with a subsequent pycnotic disintegration of the naked nuclei, or of the chromosomes after irregular thickening (Figs. 2-5).

In order to obtain some quantitative data on the inhibitory effect of cortisone, the frequencies of the occurrence of regular mitotic cells, irregular mitotic cells and damaged cells were observed in samples obtained every one hour for 12 hours after the injection of the drug, and then at 12-hour-intervals for the following 60 hours. The results of observations are shown in Text-figure 1.

The damaged cells showed a remarkable increase in number during the first five hours after the injection of the drug, and then gradual decrease and then increase followed (Text-fig. 1, C). In the 48-hour-sample the damaged cells appeared at a high frequency, followed by a sudden decrease. The regular mitotic cells which were characterized by regular division of chromosomes with a subdiploid complex showed a considerable decrease for the first five hours showing a reverse relation to the damaged cells, and then they followed a frequency curve represented by gradual increase and then decrease during 24 hours after injection (Text-fig. 1, A). They showed a sudden decrease in the 48-hour-sample, and then a striking increase followed. The frequency of occurrence of the irregular

mitotic cells, which were characterized by irregular scattering, abnormal condensation or stickiness of chromosomes, exhibited no remarkable change after the application of the drug (Text-fig. 1, B).



Text-fig. 1. Curves showing frequencies of tumor cells after cortisone-treatment.

In a period within 24 to 48 hours after the injection of the drug, the amount of ascites was reduced to some extent. The ascites was rather viscous and characterized by containing certain fibrous elements. Then, the number of regular mitotic cells increased with the passage of time. By 72 hours after injection many tumor cells appeared in active division, regrowth of the tumor having been attained again. A series of cytological features illustrating the effect of cortisone is shown in Figures 6 to 9.

There was no induced damage of tumor cells in untreated control animals. The life spans of the treated animals showed no remarkable elongation, being rather identical with that of the control ones (Table 1). Thus it was shown that in every experimental animal, no complete regression of the tumor had been attained.

So far as the results of the present investigation are concerned, it can be concluded that the inhibitory effect on tumor growth of cortisone acetate is not very striking, and that this drug exerts its injurious influence upon the cytoplasm of both the resting and mitotic cells.

2. Effect of sarkomycin

The drug used in the following experiments was sarkomycin sodium (Meiji). Twenty percent solution of sarkomycin was prepared with Ringer's solution. A single intraperitoneal injection was made in each rat bearing the MTK-sarcoma

Table 1. Life spans of tumor-bearing animals following transplantation of MTK-sarcoma II, and after treatments with cortisone and sarkomycin.

	Sex	Body weight	Life span		Sex	Body weight	Life span
		(grams)	(days)			(grams)	(days)
Untreated	♂	70	10	Treated with sarkomycin (500mg/kg)	♂	110	11
	♀	70	7		♀	75	8
	♀	100	7		♂	120	9
	♀	70	10		♂	120	9
	♂	85	8		♂	80	8
	♂	70	8		♂	85	7
	♂	70	10		♀	75	7
	♀	100	7		♀	100	31*
	♂	120	22*		♀	85	8
	♂	90	15		♀	90	7
	♀	70	8		♀	70	7
	♂	80	10		♀	80	7
Treated with cortisone (500mg/kg)	♀	110	9	♀	90	8	
	♀	75	7	♀	70	8	
	♀	70	9	♂	80	13	
	♂	70	9	♀	70	8	
	♀	80	10	♂	100	12	
	♂	70	10	Treated with sarkomycin (2000-3500 mg/kg)	♀	85	7
	♂	70	7		♀	70	9
	♀	70	8		♂	120	8
	♂	75	5		♀	115	24*
	♂	70	8		♂	95	10
	♀	80	7		♀	80	9
	♂	80	6		♂	110	7
	♀	95	8		♀	100	9
	♂	70	8	♂	105	31*	

Animals marked with an asterisk (*) survived beyond the average life day of the tumor-bearing animal; they were sacrificed for autopsy.

II at dose levels of 500 mg, 2000 mg and 3500 mg, per 1 kg of body weight¹⁾. Sampling of the tumor was made in the same manner as in the cortisone-experiment.

At a 500 mg/kg dose, the destructive influence of this drug on the tumor proved to be not great. The damage to tumor cells appeared about 30 minutes after the injection of the drug. It seems to first exert influence on the chromosomes of metaphasic cells. At 1 to 2 hours following injection, the division of almost all cells was blocked at metaphase, and the metaphasic chromosomes displayed a slight abnormality as shown by the extrusion of some chromosomes from the equatorial plate. The cytoplasm was observed to be affected also; it showed irregular process or atypical amoeboid protrusions resembling pseudopodia. The cells at prophase underwent no visible change, consequently the relative number of prophase cells remained unchanged. In striking contrast, there was

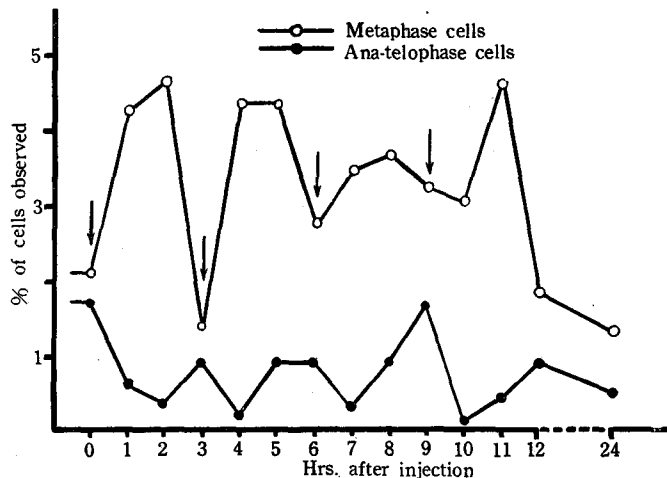
1) The dose levels are referred to as 500 mg/kg, 2000 mg/kg and 3500 mg/kg, respectively, in the following descriptions.

a pronounced decrease of the number of cells at anaphase and telophase. The data presented in Table 2 illustrate this picture clearly. By 3 to 5 hours after injection, almost all cells had attained regular growth, and the number of arrested cells had decreased gradually. The increase in number of cells at ana-telophase took place to show a similar condition to that before injection. Through the course of the above experiments, there was no remarkable degenerative change in tumor cells.

Table 2. Incidence of tumor cells at metaphase and ana-telophase on the 3rd day after transplantation of the MTK-sarcoma II. Control and sarkomycin-experiments.

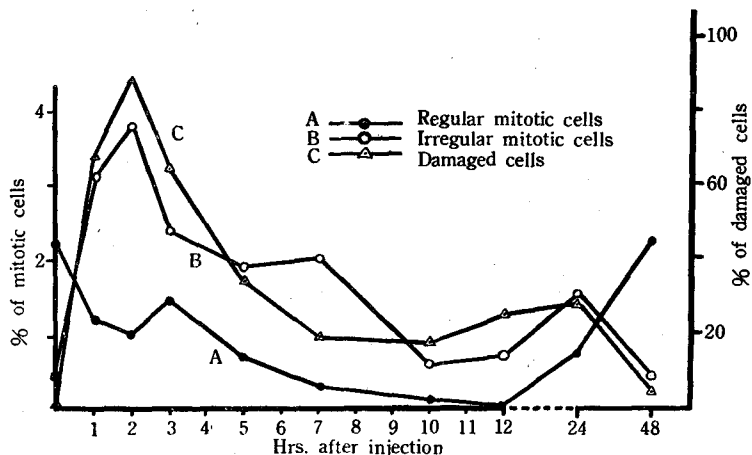
Stage	Control before injection	Time after injection (500 mg/kg sarkomycin)		
		15 min.	30 min.	1 hr.
Metaphase	222 (59,2%)	209 (83,9%)	230 (89,1%)	312 (95,1%)
Ana-telophase	153 (40,8%)	40 (16,1%)	28 (10,9%)	16 (4,9%)
Total	375	249	258	328

Next, four repeated injections with sarkomycin, at a 500 mg/kg dose each time, were made at three-hour-intervals. The results indicated that, as shown in Text-figure 2, metaphase cells increased in number markedly at one to two hours after every injection. On the contrary, ana-telophase cells decreased in number at the corresponding time. By 3 hours after each injection, the cells seemed to be free from mataphase-block.



Text-fig. 2. Curves showing frequencies of tumor cells at metaphase and ana-telophase after repeated injections of sarkomycin with 500 mg/kg. (Arrows indicate the time of injection).

The experiments at a dose of 3500 mg/kg revealed a striking damaging action upon the tumor, being very powerful in destructive effect. Within 15 minutes after injection, the tumor cells in the ascites were strikingly reduced in number; the sample of the tumor ascites furnished a few tumor cells (Fig. 15). It is questionable whether the tumor cells underwent damage within such a short time, since there was no debris of the damaged cells in the tumor sampling. In most probability they take shelter somewhere in the peritoneal cavity of the host. By 1 to 2 hours after injection, most cells found in the samples were in a damaged condition. As shown in Text-figure 3, B and C, more than 80 percent of the tumor cells under observation were degenerated. They showed abnormal condensation, irregular scattering, clumping and stickiness of chromosomes (Figs. 10-13). As a result of metaphase-block, affected metaphasic cells greatly increased in number, with a decrease in number of the anaphase and telophase cells. At 3 to 4 hours following injection, the affected cells showed a sudden decrease in number (Text-fig. 3, B and C). The dividing cells were low in frequency by this time (Text-fig. 3, A). The destruction of damaged cells seems to continue further, since the irregular mitotic cells and damaged cells show a decrease in number with time, though it is a rather gradual one (Text-fig. 3, B and C). By 7 to 8 hours after injection, a large number of leucocytes appeared in the tumor ascites (Fig. 16). By 10 to 12 hours after injection, the samples showed a large number of damaged cells exhibiting pycnotic aggregation of chromatin (Fig. 17). But the tumor samples obtained at 24 hours after injection contained a certain number of regular mitotic cells. They increased in number with the passage of time, with resultant regrowth of the tumor (Text-fig. 3, A). By 48 hours following injection, regrowth of the tumor had taken place to former status (Fig. 18).



Text-fig. 3. Curves showing frequencies of tumor cells after sarkomycin-treatment with 3500 mg/kg.

The life span of the experimental animals treated with sarkomycin was as shown in Table 1. There were amongst them a few animals which survived beyond the average life day of the tumor-bearing animal. At autopsy, they showed no tumorous growth in their peritoneal cavities.

With reference to the present data, it is apparent that the effect of sarkomycin is greater than that of cortisone acetate, and further that this drug exerts its destructive influence on the chromosomes at metaphase, though its influence seems not long lasting. Generally, during the period from 24 to 48 hours following the application of the drug, recovery of the tumor takes place in the treated animals, no complete regression of the tumor having been observed.

Discussion and conclusion

In the preceding papers, the cytological effects of several kinds of chemicals on ascites sarcomas have been reported by Makino and his co-workers (Makino & Tanaka 1953 a, b, Makino & Nakanishi 1955, Tanaka et al. 1955). It was concluded that the temporary regression of tumor growth was induced to a greater or less degree as a result of damage to a large number of the tumor cells. However, some of the tumor cells remained unaffected by the action of chemicals and furnished the primary source for renewed malignant growth.

The present investigation resulted in finding that the tumor damaging action of cortisone was less great than that of sarkomycin, and further that cortisone and sarkomycin exert the influence upon the tumor cells in different ways. The damage to cells by cortisone is shown first by a breakdown of the cytoplasm with subsequent disintegration of naked nuclei and chromosomes. Goldie et al. (1954) have reported that the amount of the peritoneal exudate was reduced by cortisone treatment, with a few abnormal cells in the stage of prophase. A similar situation was found in the peritoneal exudate, but a dissimilar result as to cytological effect was produced in the present experiment. The drug affected the cells at every mitotic stage by breaking down the cytoplasm.

It was found that sarkomycin seems to act as a mitotic poison arresting the tumor cells at metaphase, with the subsequent pycnotic disintegration of nuclei. Makino and Tanaka (1953, a) have studied the effect of podophyllin on ascites sarcoma of rats, and reported that division of the tumor cell was strikingly blocked at metaphase and chromosomes transformed into irregular masses after condensation, with consequent great damage to the tumor cells. The application of sarkomycin seems to follow a pattern of cell-damage similar to that of podophyllin, though tumor damaging action of sarkomycin is not so severe as that of the latter.

At the dose levels tested for MTK-sarkoma II in this study with cortisone and sarkomycin, the life span of the animals has not been prolonged so far as a single injection is concerned. A few experimental tumor-bearing animals survived more than 20 days beyond the average life span. It should be mentioned,

however, that similar examples have occasionally been observed in untreated control animals (Table 1).

After the use of each of the drugs, it was revealed that, though there occurred a temporary reduction of growth of the tumor to a greater or less degree as a result of the damage to most of the tumor cells, there was no complete recovery from the tumor, and renewed growth of the tumor took place in the treated animals. It seems highly probable that the renewed growth of the tumor is caused by multiplication of a certain number of stem-cells which remained alive and unaffected by the drug, being in a similar pattern as shown by Makino and Tanaka with some chemicals (1953 a, b). The physiological nature of these resistant stem-cells remains entirely unknown, and it is a matter of great importance to investigate them in relation to cancer chemotherapy.

Summary

This study deals with the effects of cortisone acetate and sarkomycin on tumor cells of the MTK-sarcoma II, an ascites tumor of rats, and their influences on the growth of tumors. Intraperitoneal injection was given to each tumor-bearing rat at the dose levels of 500 mg/kg for cortisone, and 500 mg/kg, 2000 mg/kg and 3500 mg/kg for sarkomycin.

It was found that cortisone exerted a damaging influence first on the cytoplasm of the tumor cells, with subsequent disintegration of the nuclei and chromosomes. The inhibitory effect of this drug on the growth of the tumor was not striking.

Sarkomycin damages the tumor cells by blocking them at metaphase. The destructive action of this drug is greater than that of cortisone.

By the application of these drugs, the temporary retardation of growth of the tumor was induced in greater or less degree, but no complete inhibition of the tumor growth has been attained. No remarkable prolongation of the life span resulted in the treated animals at the dose levels tested in the present study.

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Explanation of Plate XX

Photomicrographs of tumor cells of MTK-sarcoma II, from smear preparations stained with acetic dahlia.

Fig. 1. Tumor cells on the 3rd day after transplantation, just before injection. (Control). $\times 300$.

Figs. 2-5. Disintegration of chromosomes of tumor cells following cortisone-treatment. $\times 800$. Fig. 2, abnormal cells at prophase, 44 hours after injection. Fig. 3, thickening of metaphase chromosomes, 3 hours after injection. Fig. 4, clumping of metaphase chromosomes, 3 hours after injection. Fig. 5, irregularly condensed chromosomes aggregated into pycnotic masses, 48 hours after injection.

Figs. 6-9. Damage to tumor cells following cortisone-treatment. $\times 300$. Figs. 6-7, breakdown of the cytoplasm, 6 hours and 12 hours, respectively, after treatment. Fig. 8, disintegration of tumor cells with pycnotic nuclei, 48 hours after injection. Fig. 9, 72 hours after injection, showing regrowth of the tumor.

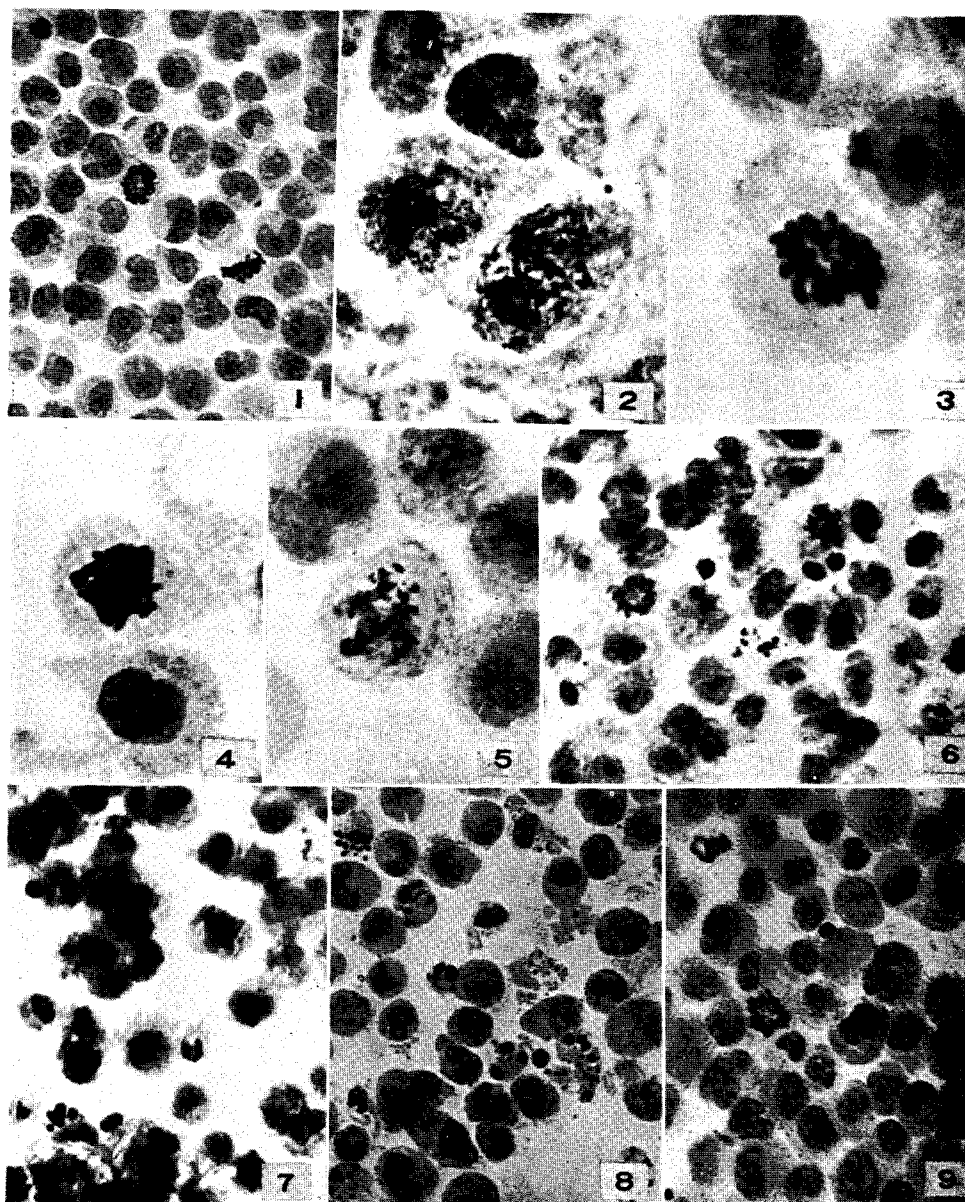
Explanation of Plate XXI

Photomicrographs of tumor cells of MTK-sarcoma II, from smear preparations stained with acetic dahlia.

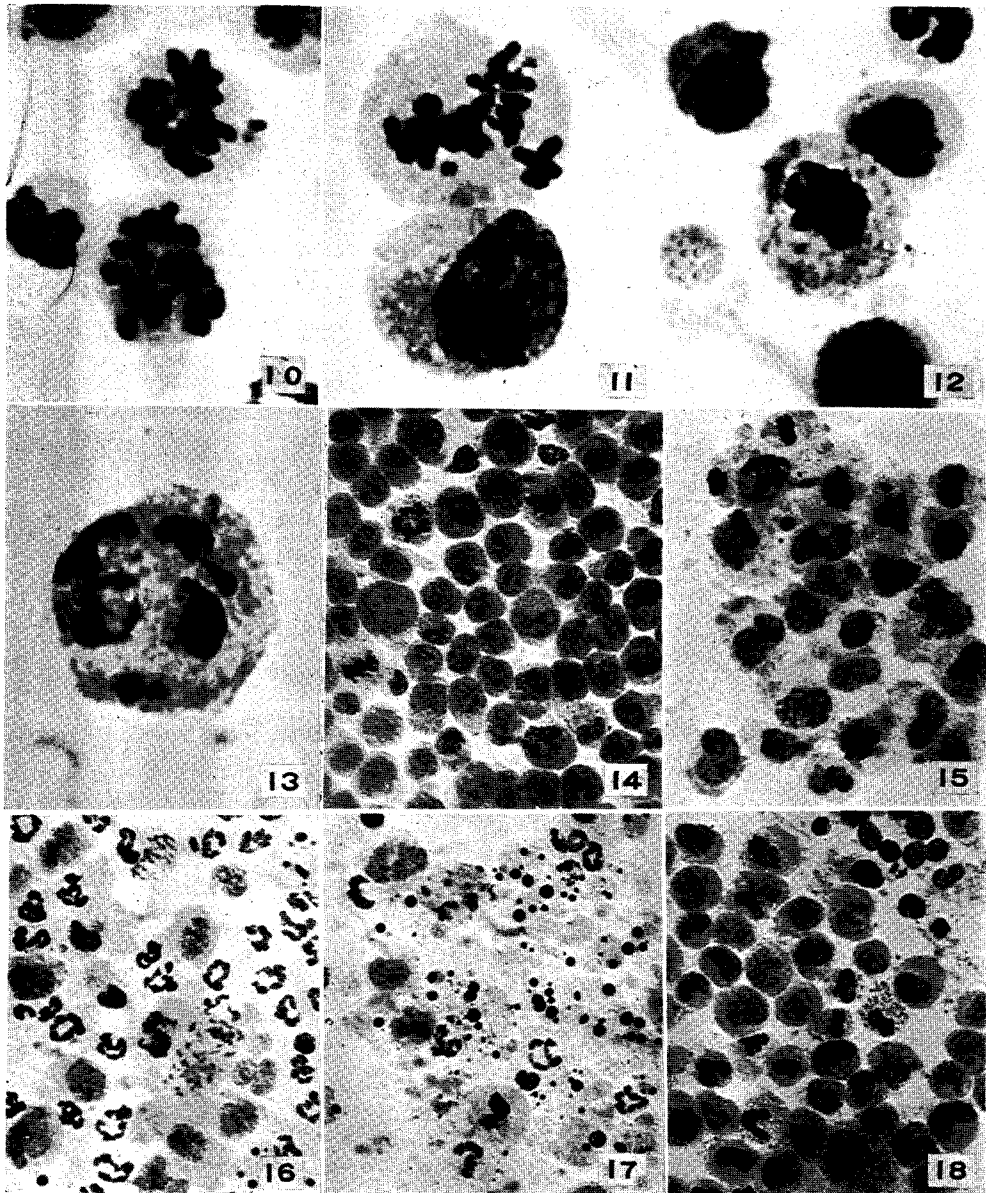
Figs. 10-13. Disintegration of chromosomes of tumor cells following sarkomycin-treatment (3500 mg/kg). $\times 1200$. Fig. 10, irregular swelling of chromosomes, 3 hours after injection. Fig. 11, extrusion of several chromosomes from the equatorial plate, 1.5 hours after treatment. Fig. 12, clumping of chromosomes, 5 hours after injection. Fig. 13, irregularly condensed chromosomes aggregated into pycnotic masses, 5 hours after injection.

Fig. 14. Tumor cells on the 3rd day after transplantation, just before injection. (Control). $\times 300$.

Figs. 15-18. Damage to tumor cells following sarkomycin-treatment (3500 mg/kg). $\times 300$. Fig. 15, showing the tumor ascites furnishing a few tumor cells, 30 minutes after injection. Fig. 16, leucocytes in the tumor ascites, 7 hours after injection. Fig. 17, disintegration of tumor cells with pycnotic nuclei, 10 hours after injection. Fig. 18, 48 hours after injection, showing regrowth of the tumor.



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