Membrane Potential of the Malignant Tumor Cell

With Special Interest to the Influence of Anti-cancer Agents and ^{60}Co irradiation on Ehrlich cancer cell.

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INTRODUCTION

Since Matteuci and De Bois Reymond proved the existence of difference in the electrical potential between cytoplasm and extracellular fluid of muscle fibers in 1840[5], the theory of membrane potentials, i.e, the membrane potential is caused by the permeability of the membraneous layer of cell-surface, has been generally recognized (Bernstein)[2,3].

Thereafter, the absolute values of the resting membrane potentials have been estimated through external induction from the surface of nerve and muscle fiber[12,15]. The attempt to measure such potential difference in and out of the cytoplasm membrane through a capillary micro-electrode inserting into a single cell of animal was initiated by Hodgkin and Huxley (1939),[14] Curtis and Colo (1940, 1942)[6] recorded the resting and action potentials of muscle and nerve fibers by this technique. Graham and Gerard (1946)[10] Ling and Gerard (1949)[34], Hodgkin (1951)[13], and Nastuk[40] further confirmed that the potential difference in and out of a cell with cytoplasm membrane as its border might be measured rather easily by using a microelectrode of capillary glass of less than o.5μ in diameter at its tip. Recently, the membrane potentials are studied not only on nerve and muscle fibers[13,40,50] but also on starfish egg[39] as well as of the epithelial cell of kidney, liver cell, and the cell of anterior horn of spinal cord (1952)[9]. Particularly in the field of cell physiology, many contributions have been made by use of this technique[22,23,31,39,40,41,47,50]. In 1955 Lash, Falk and Gerard[33] measured the membrane potentials of the separated cells in secreting fluid of cervical canal of the uterus by use of microeletrode, and reported that the absolute value in cancer cell was larger than that in non-cancer cell.

This paper was presented at the 20th General meeting of the Japan Cancer Association, Sendai, Oct, 22, 1961; the 21st General meeting of the Japan Cancer Association, Tokyo, Oct. 20, 1962; the 22nd General meeting of the Japan Cancer Association, Okayama, Oct. 20, 1963; and the 1st General meeting of the Japan Cancer Treatment Association, Kyoto, Dec. 6, 1963.

In 1962 Morioka[37] examined the resting membrane potential of stomach cancer cell obtained from operated human materials and also traced the diminution of the absolute value of potential as time lapsed. He reported that, in a fresh cancer cell obtained during operation, the resting membrane potential was −55 mV in average, larger than the result reported by Lash et al in terms of the absolute value, and after 80 minutes, it declined steeply to reach the final value, of −20 mV after 120 minutes.

Based on the fact above mentioned, the present author studied on the resting membrane potential of Ehrlich cancer cell implanted to mouse, and investigated the influenec of various anti-cancer drugs and ^{60}Co irradiation on the resting membrane potential of Ehrlich cancer cell. In this paper, experimental results obtained in cardiac muscle fiber, renal cortex cell, spleen cell and liver cell, as well as in the implanted Ehrlich cancer cell are reported.

METHOD

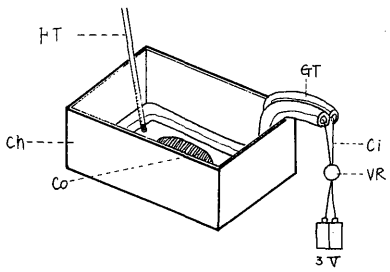
APPARATUS FOR EXPERIMENTATION

Based on Ling and Gerard's[34] micro-electrode technique, the measuring apparatus which had been used by Morioka[37] was used with some modification.

Micro-electrode: Cautiously made glass micro-electrode which had been filled with 3 mol KCl solution was used. The thickness of the tip of the electrode was calibrated by measuring the electric resistance and those which showed 30 to 50 MΩ were selected for use. The procedure for making the electrode has been described in detail elswhere[37].

Measuring chamber for test substance: The measuring chamber for test substance was consisted of a transparent box (6 × 6 × 2 cm) of synthetic resin plate of 2 mm in thickness, on the bottom of which cork plate of 2cm in diameter was cemented. A tissue piece to be tested was pinned upon it as shown in Fig. 1. In carrying out the experiment, care was taken in the thermostat device in order to keep temperature of the chamber solution constant.

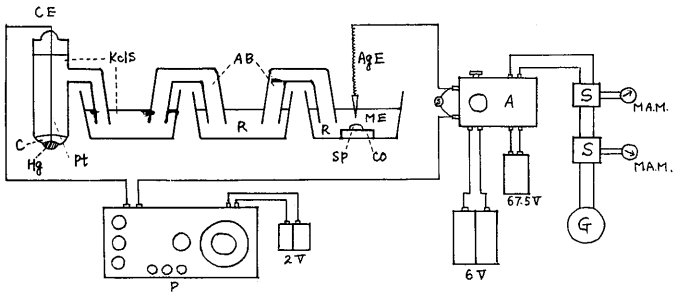
As shown in Fig. 1, a coil extended was inserted into a glass tube of 0.6 cm in diameter and sunken down to the bottom of the chamber on which the test material was to be immersed. The coil was connected to 3 volt battery and the temperature of solution in the chamber was kept constant.



FT = Fahrenheit thermometer
VR = Variable resister
Ch = Chamber Co = Cork
GT = Grass tube Ci = Coil

Fig 1. Measuring Chamber

As shown in Fig. 2, U-tubes of 1 cm in inside diameter filled with KCl saturated agar were bridged, from the inside of the measur-



CE=Calomel Electrode
Co=Cork
AB=Agar Bridge
G=Galvanometer
R=Ringer's Solution
Sp=Specimen
C=Calomel
A=Amplifier
AgE=Ag•AgCl-Electrode
ME=Micro Electrode
S=Shorting Switch
P=Potentiometer

Fig 2. Measuring Apparatus

ing chamber, over the inside of the Ringer solution tank, then to the tank filled with saturated solution of KCl. Through both tanks they finally reached to the inside of the calomel electrode, which was connected to the direct current potentiometer.

Apparatus for amplification: Trans conductance; 3100Ω. Dynamic Anode Resistance; 620Ω. Amplification Factor; An amplifier set up with Duplex-triode Tube 19.5 (12 AU7 Toshiba) was used Battery A, 6 volts in capacity and B of 67.5 volts were used for amplification.

DIRECT CURRENT PRECISION POTENTIOMETER (SHIMAZU);

An accumulator of 2 volts, the capacity of which had previously been confirmed by the standard cell, was connected to the direct current potentiometer connecting to the micro-electrode and the calomel electrode respectively. An ammeter ($50/100\times10^4$) was inserted in parallel into the circle.

MATERIALS:

1). Pure NA 2 male mice weighing 25 gm. in average were used. Immediately after sacrificing the animal, the peritoneal cavity was opened. A specimen of cardiac muscle, liver, kidney and spleen was excised and fixed upon the cork plate with a silk sewing needle of 3cm long in the chamber which was already filled with 20 ml. of Ringer's solution at 35 to 37°C.[37]

Ehrlich ascites cancer cells (With 10000–20000 cells) were implanted subcutaneously in the gluteal region of animal. Seven to ten days after the implantation, a specimen of the Ehrlich cancer tissue which grew up in situ and formed a subcutaneous tumor was excised and served for testing.

2) Seven to ten days after implantion of Ehrlich cancer, anti-cancer agents such as Serraxin (K. C. G.) 100γ or 350γ, Mitomycin C (MM-C) 0.02 mg, Hg-Hemato-

porphyrin-Na (M. H.) 0.1 mg were injected into the abdominal cavity of mouse. Specimen of tumor tissue was obtained 24 hours after injection.

3) Remote whole body irradiation of ^{60}Co was made over the mouse 7 to 10 days after Ehrlich cancer implantation. (Toshiba Model 103C Fixed System; Source 600 curie, S. S. D. 50 cm; Irradiation Field 12 × 12 cm; Irradiation Volume per minute 20.1 r; Duration of Irradiation 24.8 minutes); total irradiation dose about 500 r. Twenty four hours after irradiation, a portion of the tumor was excised and served for the experiment. Seven to ten days after Ehrlich cancer implantation anti-cancer drugs were administered and whole body irradiation of ^{60}Co was then carried out 24 hours after injection. The specimen of the tumor was taken 24 hours after completion of both treatments. The dose of anti-cancer agent and ^{60}Co were as same as above mentioned.

4) As the control experiment, the liver of Ehrlich cancer implated mouse was excised and served for the experiment under following various conditions; untreated animal, single administration of K. C. G. 100γ, combined treatment with MH 0.1 mg and ^{60}Co 500r, and single treatment with ^{60}Co.

The dosage of anti-cancer drugs or ^{60}Co irradiation employed in this experiment was determined, as a rule, by computation so as to the single administration dosis of each drug or ^{60}Co in the experiment was to be proportionate to the total dose in a course of clinical treatmemt with each drug or ^{60}Co, arranging by body weight. In the case of M. H, however, the single administration dosis was obliged to lessen by half of the counted dosis because mice seldom survived after administration of above mentioned dosis of this drug. As to the dosage of K. C. G. one group received the dosis of which corresponded to the clinical dose for a course of treatment, and another three times as larger dose for single administration.

METHOD FOR TEST MEMBRANE POTENTIALS

After controlling the electrical charge in the amplifier set in short circuit, the balance of charging was confirmed with the aid of micromanipulator. The microelectrode was then inserted into the specimen fixed on the cork plate in the measuring chamber under a vision of binocular microscope (Olympus × 8). At the moment the micro-electrode touched to the surface of the cell, the potential was recorded from +5~6 to +15~16 mV, and when the electrode penetrated the cell membrane the potential was suddenly shifted to negative value. In addition, since the resting membrane potential of the cell varied according to the variety of individual cells, insertions were made at the ratio of about 10 to 15 times per 10 minutes and then counted the average value of potential of those measured at each time interval. Each experiment was continued for two hours period in order to observe the transition of the potential as time lapsed.

HISTOLOGICAL TEST OF THE MATERIAL

After completion of the measurement of their potentials, histological examina tion was carried out for the purpose of confirmation as to whether the cells being

stabbed by the electrode had been strictly restricted by the tumor mass. The specimen was fixed with 10% formalin solution and imbeded into paraffin. Section was made 6μ in thickness and stained by hematoxylin and eosin. In recording the cell membrane potential, such care was taken that more than 70% of the cells under the entire objective field of microscope should be recognized as those agreeable with the objective for the detection.

RESULTS

RESTING MEMBRANE POTENTIALS OF THE CELLS OF THE VARIOUS ORGANS IN NORMAL MOUSE.

The average value of potentials observed on 6 mice are shown in Table 1. The average potential values for the first 10 minutes were −71.5 mV in cardiac nuscle fiber; −62.8 mv in spleen cell; −55.9 mV in renal cortex cell; and −40.6 mV in liver cell.

Table 1. Resting Membrane Potentials of the Cells of Various Tissues of the Mouse in Normal Health.

Lapsed Times	R. M. P. (−mV) Average in 10 Minetes											
	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	120′
Renal Cortex Cell	55.9±4.4	50.7	47.6	43.9	43.0	40.0	37.9	36.2	32.8	32.3	28.2	28.0±2.8
Liver Cell	40.6±2.5	37.5	33.5	32.9	32.8	29.8	30.8	30.6	30.0	28.6	27.7±0.2	——
Cardiac Muscle Fiber	71.5±7.1	63.8	57.5	54.8	51.4	49.5	45.1	42.0	39.4	37.9	34.8	34.2±2.8
Spleen Cell	62.8±3.3	60.8	59.8	53.3	50.4	47.3	46.3	41.8	40.2	36.2	35.3	31.9±6.4

Initial and final values are presented as mean ± standard deviation.

After 120 minutes: the absolute value of potential decreased gradually showing −34.2 mV in cardiac muscle fiber; −31.9 mV in spleen cell, while in renal cortex cell the value had already fallen down to −28.2 mV after 110 minetes and in liver cell to −28.6 mV even after 100 minutes. The gradients of the absolute value of potentials measured serially until 120 minutes in the period of 10 minutes for each organ is shown in Fig. 3. Almost linear

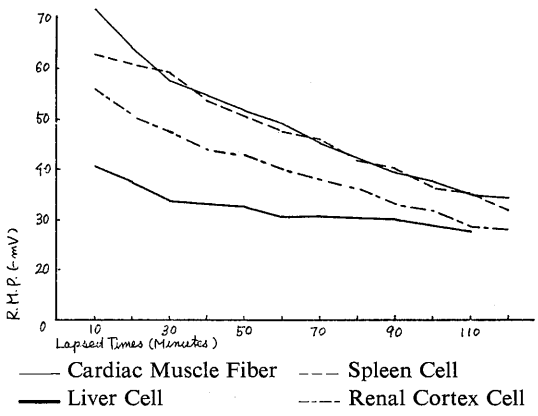


Fig 3. Resting Membrane Potentials of the Cells of Various Tissues of the Mosse in Normal Health.

diminution of the values are observed in each tissue except for the first 30 minutes. The curves for the cardiac muscle and spleen take quite similar pattern each other and stand the highest and steepest. That of the liver stands lowest with a flatt pattern. And in the renal cortex, that stands in the middle of the others.

THE RESTING POTENTIAL EHRLICH CANCER CELL.

As shown in Table 2, the average potential value of Ehrlich cancer cell in respect

Table 2. Resting Membrane Potentials of Ehrlich Cencer Cells.

Lapsed Times	R. M. P. (−mV) Average in 10 Minutes											
	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	120′
No. 1	67.0	59.6	59.1	49.5	49.8	47.2	44.6	43.9	42.5	36.6	37.7	32.1
2	65.8	65.7	60.9	58.8	52.7	49.2	41.7	38.3	39.8	34.4	31.0	26.3
3	72.9	68.8	64.1	59.6	51.7	44.2	56.4	43.3	39.8	36.7	33.3	25.8
4	71.7	52.2	35.9	37.5	34.4	33.2	36.8	35.8	36.7	33.9	43.0	34.4
5	76.6	68.3	62.4	60.1	57.1	51.8	47.8	41.0	40.1	33.6	31.4	28.2
6	68.4	57.9	62.8	55.9	49.9	53.7	49.5	47.3	41.2	37.5	34.8	32.7
Average	70.4	62.0	57.5	53.5	48.4	47.8	46.1	41.6	40.1	35.6	35.2	32.8
S. D.	4.1	6.6	10.7	8.8	7.9	7.4	6.8	4.1	1.9	1.7	4.5	4.8
S. E.	1.7	2.8	4.5	3.7	3.3	3.1	2.8	1.7	0.8	0.7	1.9	1.9

S. D. = Standard deviation.
S. E. = Standard error.

ive 6 cases for the first 10 minutes was −70.4 mV, while the results obtained for a period of 120 minutes showed a gradual descent with the final value of −32.8 mV.

THE INFLUENCE OF ANTI-CANCER AGENTS ON EHRLICH CANCER CELL.

The resting membrane potentials of the tumor cells excised at 24 hours after administration of anti-cancer agents after Ehrlich cancer implantation are shown in Table 3. The average velue of resting membrane potential for the first 10 minutes were as follws: −70.4 mV in Ehrlich cancer cell without anti-cancer agent, in the case with K. C. G. 100γ the value showed −52.0 mV, a difference of 18.4 mV in terms of absolute value, and in the case being administered with K. C. G. 350γ was −49.6 mV a difference of 20.8 mV in terms of absolute value. In that administered with MH 0.1 mg the value was −60.8 mV, 9.6 mV less than the untreathe case in absolute value. In that with MMC 0.02 mg it was −60.1 mV, which was 10.3 mV less than the untreated case in absolute value. As shown in Table 3, variables of the absolute value of potential along with the lapsed time showed a gradual decline, each being around −30 mV at 120 minutes after initial

recording.

Table 3. Resting Membrane Potentials of Ehrlihc Cancer Cells with treatment with Anti-Cancer Agents.

Anti-Cancer Agents \ Lapsed Times	R. M. P. (−mV) Average in 10 Minutes											
	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	120′
O	70.4±4.1	62.0	57.5	53.5	48.4	47.8	46.1	41.6	40.1	35.6	35.2	32.8±4.8
K. C. G. 100 γ	52.0±5.1	46.1	44.9	46.1	42.9	41.2	38.2	39.7	37.0	35.7	32.7	29.5±2.3
K. C. G. 350 γ	49.6±3.3	46.0	45.6	42.5	40.8	39.8	37.4	36.8	34.9	32.0	29.8	27.3±1.2
M. H. 0. 1mg	60.8±5.5	54.8	50.5	48.3	46.5	43.9	41.9	38.1	37.8	35.9	34.2	31.7±2.3
MM-C 0. 02mg	60.1±3.4	56.4	54.9	48.2	46.8	46.2	41.7	41.4	38.1	35.1	32.9	29.9±1.6

Initial and final values are presented as mean ± standard deviation.

TEH INFLUENCE OF ^{60}CO UPON THE RESTING POTENTIAL OF EHRLICH CANCER CELL.

Table 4 shows the resting potential of Ehrlich cancer cell measured with material taken from implaneted tumor 24 hours after whole body irradiation with ^{60}Co. The average value for the first 10 minutes was −50.7 mV, while after 120 minutes it was −26.5 mV. Comparing this with the resting potential of the Ehrlich cancer cell without anti-cancer agent administered (Table 3), the initial absolute value

Table 4. Resting Menbrane Potentials of Ehrlich Cancer Cells with treatment with Anti-Cancer Agents and ^{60}Co.

Anti-Cancer Agents, ^{60}Co \ Lapsed Times	R. M. P. (−mV) Average in 10 Minutes											
	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	120′
^{60}Co 500 r	50.7±5.2	46.3	43.2	42.0	39.2	36.7	35.3	35.6	32.2	30.4	27.7	26.5±1.7
M. H. 0. 1mg +^{60}Co	46.9±3.0	44.5	42.9	41.7	42.0	39.0	37.9	36.7	36.0	35.6	33.8	31.0±2.4
K. C. G. 100γ +^{60}Co	48.1±4.2	44.0	41.7	39.5	36.9	36.7	35.2	35.0	32.3	31.6	30.4	28.7±2.5

Initial and final values are presented as mean ± standard deviation.

of potential was found in a decrease of 19.7 mV and increase of only 1.1 mV in comparison with the value in the case administered with K. C. G. 350 γ that showed the minimum initial level of the absolute value of potential among all cases administered with anti-cancer agents.

THE INFLUENCE OF A COMBINED USE OF ANTI-CANCER AGENT AND ^{60}CO UPON THE RESTING POTENTIAL OF EHRLICH CANCER CELL.

As shown in Table 4, the resting membrane potential as was measured for each case treated with ^{60}Co 500 r and MH 0.1 mg or K. C. G. 100 γ respectively after having been implanted with Ehrlich cancer cell, was observed lower, in terms of the absolute value, than either case of a single treatment with anti-cancer agent or with ^{60}Co irradiation. The average value for the first 10 minutes −46.9 mV in the case with MH and ^{60}Co, and −48.1 mV in that with K. C. G. and ^{60}Co. It is also noticeable that the absolute value in the case with single administration of MH was so much lower as 13.9 mV than the case with combined treatment with ^{60}Co irradiated, also in the case administered with K. C. G. 100 γ only the value was lower by 3.9 mV than that jointly treated with ^{60}Co 500 r, in both cases each showing a decrease of 3.8 mV or 2.6 mV than the case with ^{60}Co irradiation alone (Table 3 and 4).

THE RESTING POTENTIAL OF LIVER CELL IN THE RESPECTIVE CASE IRRADIATED WITH ^{60}CO OR ADMINISTERED WITH ANTI-CANCER AGENTS.

The resting potentials of liver cells after implantation of Ehrlich cancer are summarized in Table 5. The values in each group; untreated group, single administration with K. C. G. 100 γ, single treatment with ^{60}Co 500 r, and the group of

Table 5. Resting Membrane Potentials of Liver Cell in the Respective Case irradiated with ^{60}Co and or treated with Anti-Cancer Agents.

Lapsed Times	R. M. P. (−mV) Average in 10 Minutes											
	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	′120′
E. C.	44.8±1.0	40.7	36.2	35.6	34.6	32.0	30.8	28.3	29.6	26.6	26.1	24.5±0
E. C. K. C. G. 100γ	44.7±1.8	40.8	41.4	39.9	36.8	34.6	31.1	28.7	28.7	25.7	26.3	24.8±0
E. C. ^{60}Co 500r	42.1±0	39.0	36.4	36.7	34.7	33.5	32.4	31.2	30.8	26.5	28.6	24.6±0.6
E. C. MH 0.1mg ^{60}Co 500r	40.0±0.6	38.6	38.0	35.3	32.5	28.9	24.3	26.6	24.4	25.1	23.7	21.8±0

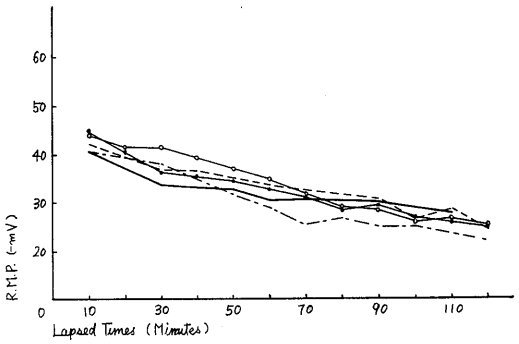
E. C.=Ehrlich Cancer
Initial and final values are presented as mean ± standard deviation

combined treatment with MH 0.1 mg and ^{60}Co 500 r, showed small difference each other; −44.8 mV, −44.7 mV, −42.1 mV and −40.6 mV respectively.

There was observed, in three of these four cases, an increased absolute value by 4.2 mV, 4.1 mV and 1.5 mV, in comparison with the resting potential of the liver cell in normal mouse, with an exception only in the case of combined treatment with MH 0.1 mg and ^{60}Co 500 r in which the value was equal to that of the liver in normal mouse. The resting potentials in these four cases after 120 minutes were −24.5 mV; −24.8 mV; −24.6 mV; and −21.8 mV respectively, showing a gradual declining curve in absolute values as shown in Fig. 7.

DISCUSSION

As to the resting potential caused by the micr-electrode, Hodgkin and associates[13] assumed that the resting potential of the cell came out through a mechanism in which the potential was created by potassium ion stored in high concentration in the cell and was determined by the permeability of the cytoplasm membrane. Other than this, many investigators have made their great efforts to elucidate the mechaism involved in the membrane potential in connection with membrane permeability[4,6,8,16,27,28,35,37,40].



—— Normal (Liver Cell)
∘—∘ E. C. C. impl. +K. C. G. 100γ
–·–· E. C. C. impl. +M, H. 0.1mg +^{60}Co 500r.
•—• Liver Cell of after. E. C. C. implantation.
---- E. C. C. impl. +^{60}Co 500r.

Fig 7. Resting Membrane Potential of Liver Cell in the Respective Case irradiated with ^{60}Co and or administered with Anti-Cancer Agents.

The difference in resting potentials was found in the cells in various organs and tissues of normal mouse in this experiment. The most conspicuous difference in it was demonstrated between cardiac muscle fiber and liver cell; 30.9 mV in absolute value, as shown in Table 1. The considerable explanation of this difference has so far been made on the basis of the difference of concentration gradients of K^+ in and out of each cell bordered by the cell membrane[13]. The difference of membrane potentials between the cells was also reported by several investigators as shown in Table 6. The membrane potential of Ehrlich ascites cancer cell, reported by Sekiya in 1961[45], is described as -16.9 ± 0.5 mV, in average, which is far from the date obtained in the present experiment. However, there was a considerable lapse of time between initial accumulation of the ascites cells and the final imbedding in agar before mesurement of the potential in his experiment.

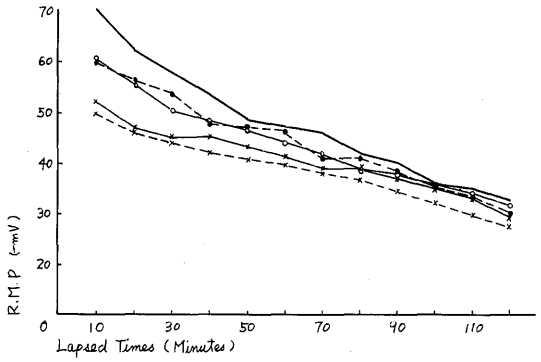
Table 6. Resting Membrane Potentials of other Tissues

Tissues	R. M. P. (−mV)	
Frog Muscle Fieber	88	Hodgkin[13]
Frog Nerve Fieber	71	〃 〃
Guinea-Pig Liver Cell	36	Li-Choh-Lu & Mcllwain[7]
Cat Liver Cell	34	〃 〃
Guinea-Pig Kidney Cell	35	〃 〃

This time lapse might be responsible for the discrepancy between the two results with regard to the inevitable degeneration of the cell itself during this period. Therefore, it is hard to compare his figure with the resting potential for the fresh cells shortly after taking out from the living body as we did.

As to the diminution gradients of the absolute values of potential following the sequence of time passing, there is a quite similarity between Ehrlich cancer cell and cardiac muscle fiber, as shown in Figs 3 and 4.

Meanwhile,it seems to be noteworthy that the diminution pattern as to the Ehrlich cancer cell shows almost linear pattern as likely as that of cardiac muscle fiber and no such peculiarity was demonstrated as shown in the case of stomach cancer cell in which the diminution pattern of absolute values of potential exhibited an acute and remarkable decline at about 80 minutes as reported by Morioka[37]. Such difference in the pattern of curves might be explained by the difference of the outer environment of the devolopment of both cells.



—— Cont. (E. C. C.) •---• MM-C 0.02mg
×---× K. C. G. 350γ o—o MH 0.1mg
×—× K.C. G. 100γ

Fig 4. Resting Membrane Potentials of the Influence of Anti-Cancer Agents on Ehrlich Cancer Cell.

As the results of the measurements of resting potetials following the lapsed time, after administration with anti-cancer agent it was observed that, as shown in Fig. 4, marked difference is seen in the patterns of gradients as well as the initial value of potential among each group. The most interesing feature observed in this figure is that the gradients of these curves are clearly divided into three groups from the basis of the level of initial potentinl and the gradient along with lapse of time : 1) control group of Ehrlich cancer cell, 2) cases with MH and MM-C, 3) cases with K. C. G. 100 γ and 350 γ. In the group 2 and 3, the similarity is observed not only in the initial levels of potential but in the pattern of each curve.

Concerning the initial values of potential, the difference in each group is quite outstanding by way of step like fall from group 1 to group 3 with a difference of 10 mV in absolute value of potential. For the convenience in further discussion, two experimental groups such as group 2 and group 3 in above classification are named as group A and group B. So far as the action mechanism of these anti-cancer substances is concerned, MH is said that though this substance dose not invade directly upon cancer cell but detoxicating effect against the toxic sub-

stances released from malignant tumor and stimulation to the liver are act as if it were carcinostatic drug. On the other hand, according to the evidence that porphyrin has a strong affinity with cell and it increases the sensibility to radiation ray, it seems that MH accelerate the effect of radiation ray to inhibit or restrain the growth of malignant tumor[1,11,24,25,26,36,43,53,].

MM-C exerts influence on lactic acid dehydrogenase in the cancer cell and prevents the selective permeability of the protoplasm membrane. It is said that the area between cytochrome D anb C is attacked and the utilization of glutamic acid is hindered by this drug. Thus the reducion of protein and nucleic synthesis results the destruction of Ehrlich cancer cells. The morphological evidence revealed that there appeared the restraining of mitosis elicited by the application of this drug and disturbance of cellular activity of the resting nucleus took place subsidiarily. Kobayashi and his associates reported that MM-C gave rise to a morphological change in the nucleus of tumor cell and simultaneously the chromosomes lose their stainability followed by abnormal deformation and mutation. The nucleolus also changed remarkblly in its shape and stainability and finally the nucleolus substance flew out to the cell substance[29,44,49,51,52,]. K. C. G. which is chiefly composed of bacterial polysaccharide, is a substance having anti-tumor effect. Generelly the bacterial polysaccharides, acting specifically on the tumor cell, is said to have a tendency to elicite haemorrhagic necrosis in tumor cell. In fact, histology revealed following changes in tumor cells after administration of K. C. G. in this experiment, necrosis and edema-like change of the tumor cell, swelling of nucleus, dissolution of nucleoplasm, thinning of nuclear membrane and its destruction. These changes are thought to be due to the retention of protein-like substance in the endoplasmic reticulum and it is assumed that such findings as a distinguished formation of blister in cell body, marked appearance of small vacuoles in centrodesmus are the changes caused by the swelling of mitochondria or thread granules[17,18,19,30,54,55,56,].

In view of the origin of cell membrane potential which is said to be created by the distribution equilibrium of the ions in and out of cytoplasm as well as the consideration of location of nucleus and cytoplasm in a cell, the different feature observed in the gradients of resting membrane potentials between group A and B is quite agreeable with the difference in action mechanism between two different groups of anti-cancer drugs. In other words, an assumption might be able to made that the drugs belonged in group A such as M. H. and M. M. C. acts upon nucleus and nucleolus chiefly, while that belonged in group B such as K. C. G. chiefly on cytoplasm probably. And this assumption might be compatible with the previous comments about the site of effect of these drugs reported by several investgators as mentioned above.

However, as described above, the action mechanism of such anti-cancer agent has not yet been perfectly made clear, explaining it still being unable without

standing on hypothetical supposition.

The averege value of the resting potential of Ehrlich cancer cell after whole body irradiation with ^{60}Co 500 r was -50.7 mV for the first 10 minutes. To compare this value with the results of those groups being administered with anti-cancer agents, the value stands approximately middle point between the values obtained in the case being administered with K. C. G. 100 γ and in that with K. C. G. 350γ.

The transition of the resting potential for a period of 120 minutes, as shown in Fig. 5, appeared as a declining curve quite similar to that of the group B previously described. In the biological changes of a cell caused by the irradiation of radioactive rays, the protoplasm, which is in the outer circumstance of the cell, is most likely to be affected by such rays. However, different from the nucleus, the protoplasm is said to be able to recover itself quite soon even if it is influenced by the irradiation beceuse of it's protective ability or adaptability[20]. The stoppage of the cell division is one of the earliest signs of such changes[38,42], which is known as Bergonie and Tribondeau's law.

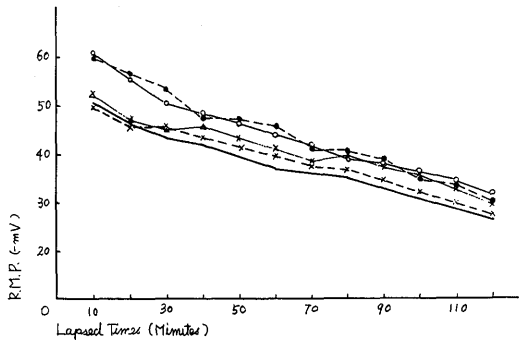


Fig 5. Resting Membrane Potentials of the Influence of Anti-Cancer Agents and ^{60}Co on Ehrlich Cancer Cell.

The cancer cell has a strong sensibility to radiation. In general, in the cell of high radiation sensibility the metabolism is mainly going on by means of the process of glycogenolysis of the nucleus, it is shown, suggesting that a powerful oxide produced by the effect of irradiation will excert an influence over the system in which the anaerobic metabolism is carrying on in the nucleus of a cell in normal condition[46]. Other than this, investigations have been made on abnormalities of chromosome and so on in refference to the effect of irradiation[32]. Otto Warburg reported in 1955 that the anti-cancer effect of X-ray was caused by its inhabitory effect on the respiration of cells.

Although many facts still remain unknown about anti-cancer effect of X-ray, it is assumed that from the results obtained in this experiment the irradiation influences in stronger degree upon the protoplasm than the nucleus, since we made irradiation of ^{60}Co in a short period consequentely, so far as the anti-cancer effect of ^{60}Co is concerned, it is reasonable to presume that there may be some difference in the action mechanism between anti-cancer drugs belonged in the group A and ^{60}Co irradiation from the point of view based on the resting potential study.

In examining the effect of a combined treatment with anti-cancer agents and ^{60}Co irradiation, MH which is said to have sensibility to radiation ray was selected from the group A, and K. C. G. was taken up from the group B, and the resting potentials of the Ehrlich cancer cell were measured in combination of 0.1 mg of MH or 100 γ of K. C. G. and ^{60}Co 500 r. As shown in Table 4 and Fig 6, in three cases which being treated by ^{60}Co irradiation alone, by the combination of MH and ^{60}Co and by K. C. G. and ^{60}Co, the initial potentials and the features of diminution curves in absolute value are quite similar in all three, contrary to the results obtained in the cases administered with anti-cancer agent alone. It is of very much interest that the difference in absolute values of potentials in the cases between MH group and K. C. G. group decreased significantly by means of adding of ^{60}Co irradiation.

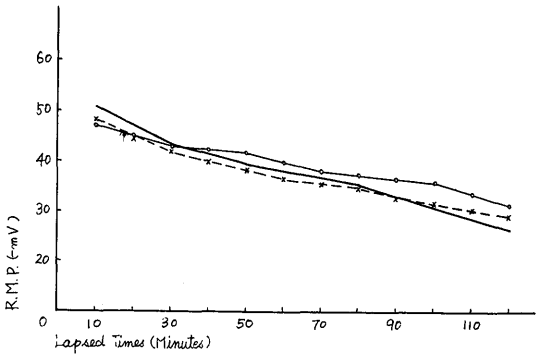


Fig 6. Resting Membrane Potentials of the Influence of ^{60}Co and combined use of Anti-Cancer Ageant and ^{60}Co upon Ehrlich Cancer Cell.

Viewed from their influence of several kinds of anti-cancer agent (including ^{60}Co) used in this experiment upon the resting potential of the Ehrlich cancer cell, it is obvious that they can be divided into two groups —A and B. And, so far as the resting potential is concered, there seems to be an obvious difference in their action mechanisms between the groups A and B, although the action mechanism of each anti-cancer agent still remain obscure in many aspects. It is suggested that such a conspicuous difference as shown above in the cases of combined treatment is not due to the mere fact that MH has a special affinity to the radiation ray, but the combined use of these drugs which belong to the group A or B each having a different action mechanism makes it possible to obtain far better anti-cancer effect than the single administration with any of each drug.

That is to say, a larger effect is obtained by a combined use of MH (Group A) and ^{60}Co (Group B) than by use of K. C. G. and ^{60}Co both belonging to the group B. The quite agreeable report was published by Kayama[30] and others that they obtained far better anti-cancer effect by the combined use of K. C. G. and MM-C, Tespamin, Nitromin etc. than the case of a single administration with any of each. As a mean to study whether it acts upon the cancer cell selectively or not, and or what changes come out in other organs of a living organism when an anti-cancer agent or ^{60}Co is applied to a cancer-bearing animal, the liver was selected as the organ

suitable for such test as it seemed to be relatively more sensitive to these substances than other tissues. Then measurment of the resting potentials was made on the specimen of the liver under the same condition with implantation of Ehrlich cancer. The results, in short, the absolute value of potential in the liver after implantation with Ehrlich cancer was larger than that of the normal liver, regardless of the treatment with anti-cancer agents or ^{60}Co irradiation, except for the case treated with the combination of M. H. and ^{60}Co in which the initial potentials were as same as in the normal liver.

The larger absolute value of potential of the liver after implantation with Ehrlich cancer than that of the normal liver seems to be due to the metastasis of Ehrlich cancer, which has been confirmed through detection of tissue preparation. It appears that the absolute value of potential which increased through this metastasis would approach to the value of resting potential of the normal liver, decreasing along with the changing of cancer cells caused by the administration of the anti-caneer agent and ^{60}Co. However, there is some difference between the resting potentials of Ehrlich cancer cells and those of the liver cells administered with anti-cancer agent and ^{60}Co, described herewith. This seems to come from the difference in the action mechanism of the anti-cancer agent to the tumor cell and the liver cell, for instance, K. C. G. has an effect on the liver to stimulate the function of it. Furthermor, in view of the fact that the cells of the treated liver with anti-cancer agents and ^{60}Co did not show lower value of the resting potential that of the cells of the untreated normal liver it might be tought that the anti-cancer agent and ^{60}Co would act on the cancer cell more selectively.

SUMMARY

The resting potential of the cells of the liver, renal cortex, spleen and cardiac muscle fiber of normal mice were measured by the glass micro-electrode method, which resulted in the following values respectively: -40.6 mV, -55.9 mV. -62.8 mV and -71.5 mV. These potentials were also pursued as time lapsed until two hours from the first recordimg. As a result, in so far as the potentials of the cardiac muscle fiber, a gradient decline first steeply until 30 minutes after and then further went down rather gradually when they were expressed as the absolute values, and reached at the final value of -34.2 mV. In the other tissues, however, the gradients appeared less steeply and the final levels were as high as approximately -30 mV.

In the measurement conduced for the resting potential of implanted Ehrlich cancer cells at the time of seven to ten days after the implantation, the average potential for the first 10 minutes was -70.4 mV and increased to -32.8 mV at 120 minutes period.

Whole body irradiation of ^{60}Co or intraabdominal administration of various anti-cancer agents was made 24 hours after subcutaneous implantation of Ehrlich cancer cells. Seven to ten days after the implantation, the resting potential of tumor cells was recorded with results of -50.7 mV in ^{60}Co group, -60.8 mV in MH, -60.1 mV in MM-C, -52.0 mV in small dose of K. C. G. and -49.6 mV in large dose of K. C. G. in average for the first 10 minutes. In comparison with the resting potential of Ehrlich cancer cell without treatmeant, the absolute values of potentials of treated cases were clearly divided into two groups. The one, those being administered with MH and MM-C each showing a decrease of about 10 mV, and another, those which being treated with K. C. G. or irradiated with ^{60}Co, each showing a downfall of about 20 mV in absolute values of potentials. The action mechanism of these drugs or radiation ray upon the membrane permeability of cancer cell is supposed to be difierent between these two groups. The potentials of these cases were also traced for a period of two hours. An obvious difference was observed in the gradients of each case until around 80 minutes after the measurement was started, while thereafter no significant difference could be noted in all cases.

In the group being subjected with combined treatment of ^{60}Co irradiation and any of anti-cancer agents, the average value of the resting potentials for the first 10 minutes was -46.9 mV in cases of $^{60}Co+MH$ and -48.1 mV in $^{60}Co+K.$ C. G. In both of them the absolute values of potentials were seen lower than that observed in the cases of a single administration of MH, K. C. G. or ^{60}Co. In particular, it is interesting to note that the absolute value of potential in the case of combined use of $^{60}Co+MH$ came to be lower than the case with $^{60}Co+K.$ C. G.. This relation was appeared inverse in cases of single use of these drugs; i. e. the absolute value of potential in single MH group was higher than that of the group with K. C. G.. From this result, it is surmised that the combined use of the drugs or radiation ray belongs to different group, the action mechanism of which seems to be different from each other is more effective than the combined use of the drug of the same group.

The resting potentials of liver cells at the time of a week after the implantation of Ehrlich cancer were also measured on four groups of untreated and treated animals with anti-cancer drugs and ^{60}Co. The results showed that in the group with combined treatment of ^{60}Co and MH the resting potential was equal to that of the normal liver, while in animals received K. C. G. or ^{60}Co irradiation separately, as well as untreated cancer-implanted animal, the absolute values of potentials were more or less larger than that of normal one.

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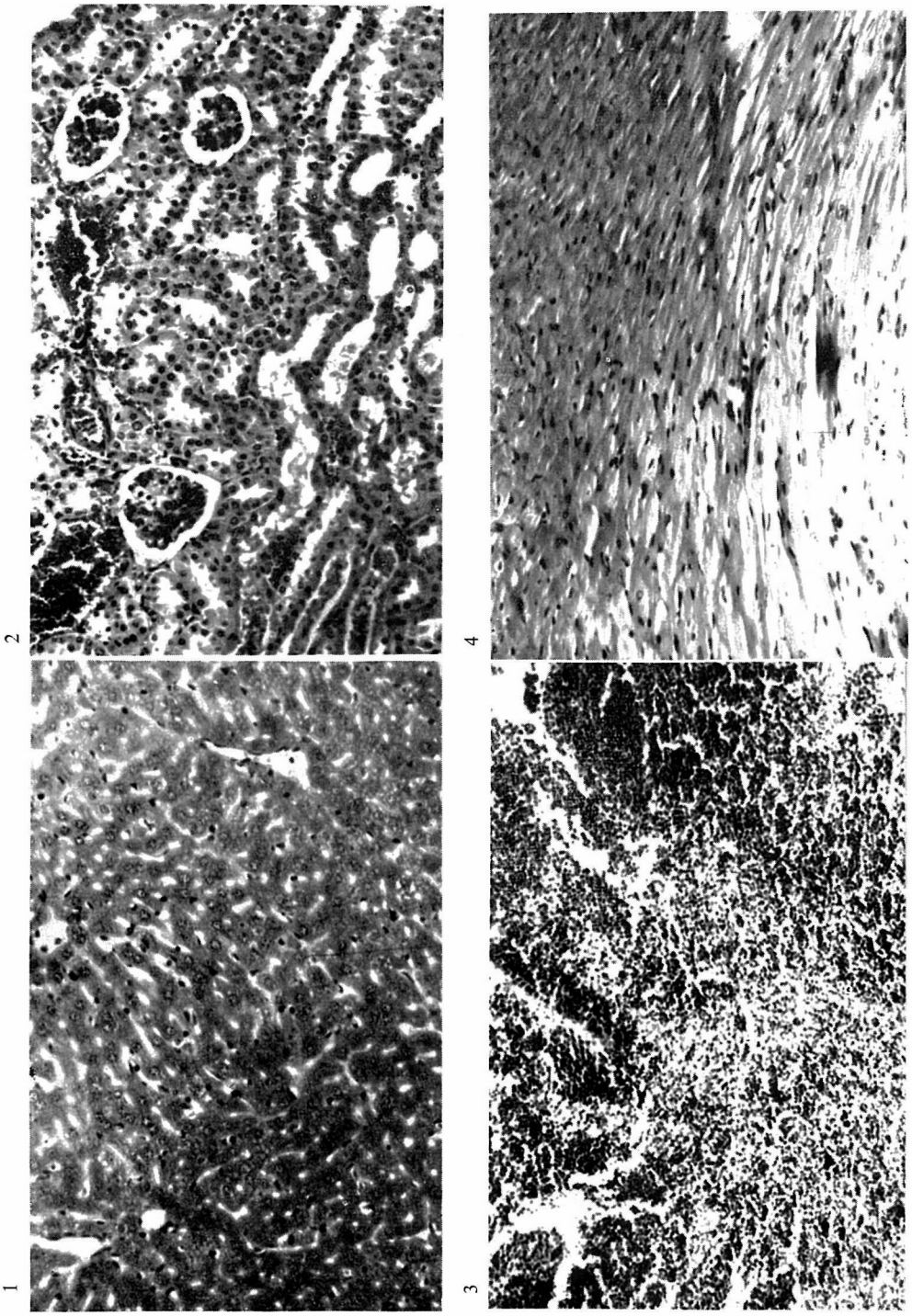
Explantation of Plate XIV

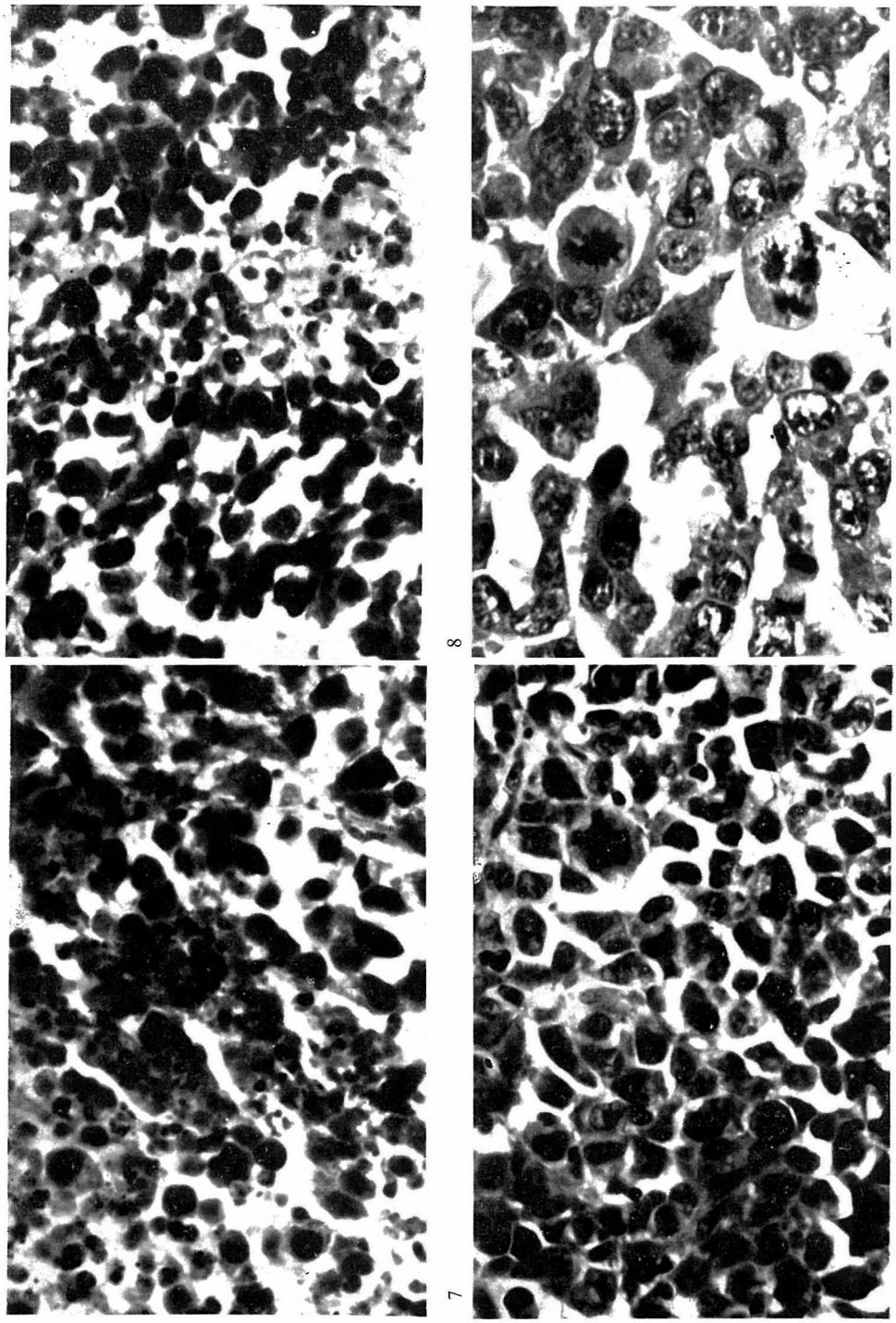
phot. 1. normal liver. H. E. (stain) × 100
phot. 2. normal renal cortex. H. E. (stain) × 100
phot. 3. normal spleen. H. E. (stain) × 100
phot. 4. normal cardiac muscle. H. E. (stain) × 100
phot. 5. Ehrlich cancer cell subcutaneously implanted into the gluteal region of the mouse : Numerous cancer cells are seen. In the upper right portion irregular mode of cell division is recognized. H. E. (stain) × 400
phot. 6. Ehrlich cancer cell administered with K. C. G. 350 γ : Pyknosis, karyorrhexis and degeneration of the cancer cell with infiltration of leukocytes are observed. H. E. (stain) × 400
Phot. 7. Ehrlich cancer cell administered with MH 0.1 mg : Focal severe necrosis (coagulation necrosis) and karyorrhexis of the cancer cells are seen. H. E. (stain) × 400
phot. 8. Ehrlich cancer cell administered with MM-C 0.02 mg : Individual cancer cell is swollen, and small vacuoles in the cytoplasm and formation of small processes are recognized. Generally, the nucleus is swollen with condensation of chromation. Considerable number of mitotic figure are seen .H. E. (stain) × 400
phot. 9. Ehrlich cancer cell irradiated with ^{60}Co 500 r : Cancer cell become bubble-like with indistinct cellular border. The cytoplasm is clear and the nucleus is moderatelly pyknotic. H. E. (stain) × 400
phot. 10. Ehrlich cancer cell of a combined use of MH 0.1 mg and ^{60}Co 500 r. : Individual cancer cell is separated. Cancer cells are atrophic and the nucleus shows pyknosis and karyorrhexis. The findings observed both in phot. 7 and phot. 9 are recognized in this picture. H. E. (stain) × 400
phot. 11. Ehrlich cancer cell of a combined use of K. C. G. 100 γ and ^{60}Co 500 r. : At the left, necrosis of cancer cells are seen.
Other cancer cells are separated and the nucleus is swollen and deeply stained. H. E. (stain) × 100
phot. 12. High magnification of phot. 11.
The nucleus shows deformity and pleomorphism, and is deeply stained. H. E. (stain)

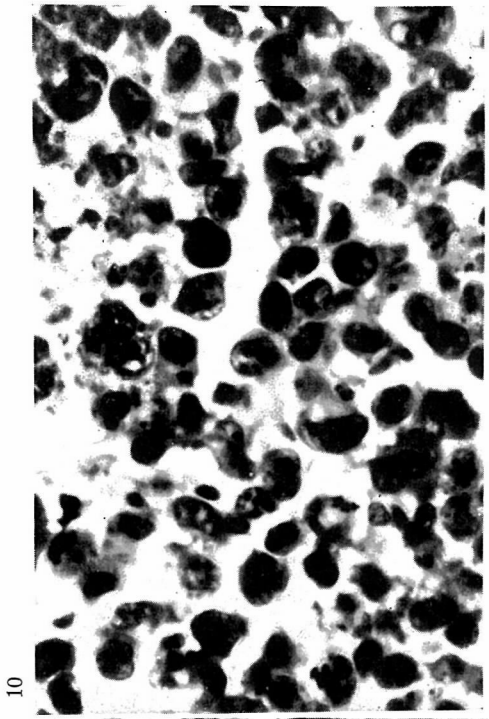
× 400

phot. 13. Liver adminstered with K. C. G. 100 γ after implantation of Ehrlich cancer cells in the gluteal region of the mouse : metastasis is recognized in the central zone. Cancer cell shows degenerative changes with swelling of the cytoplasm, pyknosis and karyorrhexis. H. E. (stain) × 400

phot 14. Low magnification of phot. 13. H. E. (stain) × 100







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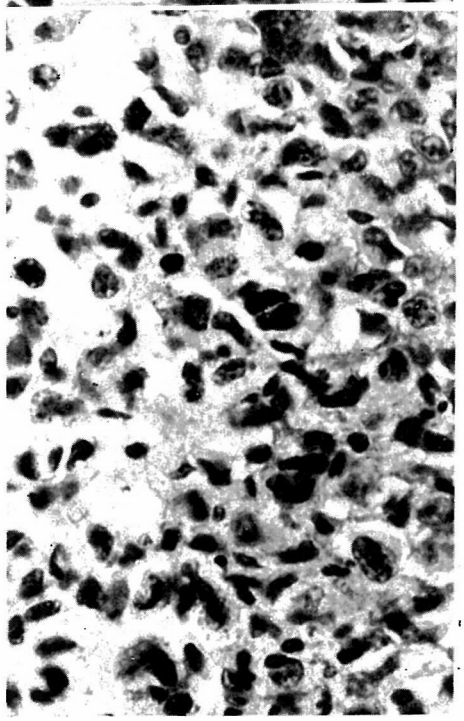

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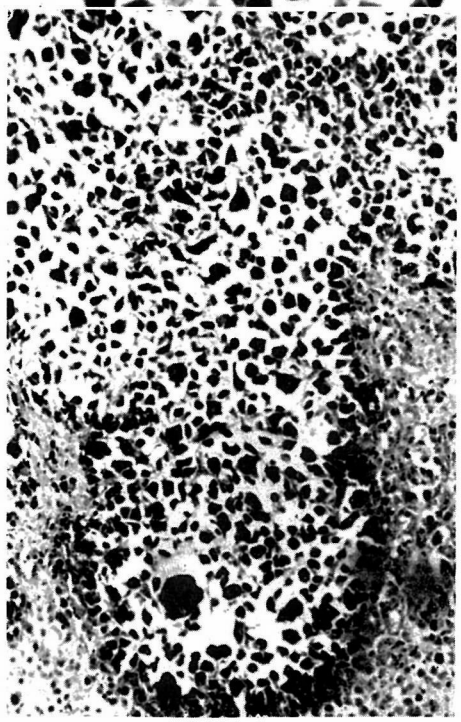

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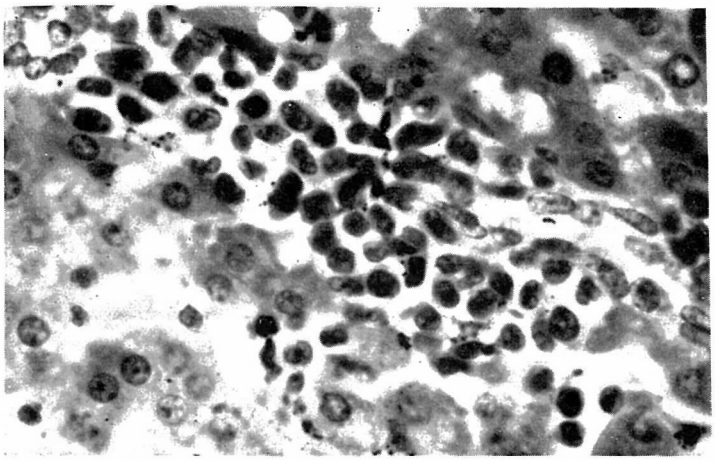

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