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Immunological studies on the membrane systems of cancer cells. II. Immunochemical specificity of the mitochondria from chemical carcinogen induced carcinoma cells

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Immunological studies on the membrane systems of cancer cells. II. Immunochemical specificity of the mitochondria from chemical carcinogen induced carcinoma cells*

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Abstract

As the results of investigating the antigenicities of various fractions from the membrane systems of cancer cells, it has been found that the remarkable cancer-specific antigenicity exists in cancer cell mitochondria. With a particular reference to this antigenicity of cancer cell mitochondria, the antigenicities of the mitochondria of various kinds of rat ascites tumors and those of tumor-bearing rat liver mitochondria have been compared with those of normal rat liver mitochondria. In addition, it has been demonstrated that a strong tumor antitransplantability is induced when the recipient rat is immunized with the tumor cell mitochondria. In order to support these experimental facts, enzymatic activities of cancer cell mitochondria have been investigated also biochemically after treating the mitochondria with the antiserum to these mitochondria. 1. The most remarkable cancer specific antigenicity exists in mitochondria among the membrane systems of cancer cells. This cancer mitochondria-specific cancer antigenicity is common to all the ascites tumor mitochondria used here. 2. The original tissue- or organ-specific antigenicities diminish or disappear at the carcinogenic transformation of cells. 3. The tumor-bearing-organ specific antigenicity appears in the organs of animals bearing tumor. 4. The tumor antitransplantability is acquired when rats are immunized with these tumor cell mitochondria. 5. The inhibition of mitochondrial ATP·ase and respiratory activities is observed when the cancer cell mitochondria are treated with the anti. serum to the mitochondria.

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IMMUNOLOGICAL STUDIES ON THE MEMBRANE SYSTEMS OF CANCER CELLS

II. IMMUNOCHEMICAL SPECIFICITY OF THE MITOCHONDRIA FROM CHEMICAL CARCINOGEN-INDUCED CARCINOMA CELLS

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The literature is replete with works on specific tumor antigen existing itself in tumors such as human cancers, spontaneous tumors of animals and artificial neoplasma induced by chemical carcinogens or viruses. At the same time, comparative studies on the antigenicity of organs or tissues of tumor-bearing animals and that of the same organs or tissues of normal animals were carried out from the aspects of immuno-specificity of the tumor-bearing body. Since it has become possible to conduct comparative studies on the antigenicity of individual fractions from the membrane systems of cells at subcellular level owing to the advent of ultracentrifugation, numerous reports have appeared, suggesting the existence of specific cancer antigenicity in cancer cell mitochondria (1—8). Subsequently many investigators (9—16) have stated about the changes of organ or tissue specific antigenicity in tumor-bearing animals.

The immunochemical analysis of membrane fractions from cancer cells conducted by this author (17) has revealed that the highest cancer-specific antigenicity exists in mitochondria in the membrane systems of AH130 rat ascites hepatoma cells. The present experiments were conducted with the purpose to compare the specific antigenicity in the mitochondria of various kinds of rat ascites tumors induced by various carcinogens and of tumor-bearing rat liver mitochondria with that of normal cell mitochondria. The results indicated that the mitochondria of every rat ascites tumor induced by chemical carcinogens have specific cancer antigenicity, and that this antigenicity of cancer cell mitochondria is common to various rat ascites tumors induced by various chemical carcinogens. In addition, a study was made to clarify whether there is tumor anti-trans-

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plantability in the rats immunized with cancer cell mitochondria.

MATERIALS AND METHODS

All rats used were inbred Donryu strain. The cancer strains used were rat ascites hepatoma (AH130, AH225A, and AH66F) and ascites form Yoshida sarcoma. AH225A, AH66F and Yoshida sarcoma were obtained through the courtesy of Drs. T. YOSHIDA and H. SATO in Sasaki Institute, 2—2 Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan.

Isolation of mitochondria: Tumor cell mitochondria, and normal and tumor-bearing rat liver mitochondria were isolated by the modification of the method of HOGEBOOM (19).

a) *Tumor cell mitochondria* (those of AH130, AH225A, AH66F and Yoshida sarcoma): Rat ascites tumor cells were harvested one week after the transplantation of ascites tumor cells to rat. In this instance, Yoshida sarcoma cells were harvested five days after the transplantation of tumor cells to rat. The ascites fluid was centrifuged at 800×g for 10 minutes, and by removing the supernatant, the residue was washed repeatedly by centrifugation with 0.25 M sucrose solution containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.6. The washed ascites tumor cells (50 g wet weight per 100 ml) were suspended in 0.25 M sucrose solution containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.6, and then 10 volumes of cold one mM $NaHCO_3$ (pH 7.6) solution was added and incubated for 20 minutes at 0°C before homogenization. A tightly-fitted teflon pestle was rotated and the glass tube was moved up and down over the pestle. Tris-bufferd one M sucrose solution (pH 7.6) was added to this suspension to make the final concentration 0.25 M. This homogenate was centrifuged at 700×g for 10 minutes. The residue as the nuclear fraction, was discarded and the supernatant was layered on an equal volume of 0.34 M sucrose solution containing 0.1 mM EDTA and 10 mM Trisl-HCl, pH 7.6, and centrifuged at 800×g for 10 minutes. The upper layer was transferred to a centrifuge tube with a pipette, and centrifuged at 5,000 ×g for 10 minutes. After discarding the supernatant, the residue (mitochondria pellet) was washed twice with 0.25 M sucrose solution containing 10 mM Tris-HCl, pH 7.6 by centrifugation at 7,000×g for 10 minutes.

b) *Normal and tumor-bearing rat liver mitochondria:* Normal and tumor-bearing rat livers were assembled, minced, and suspended in 0.25 M sucrose solution containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.6 at 0°C before centrifugation. A tightly-fitted teflon pestle of the homogenizer was rotated, and the glass tube was moved up and down over the pestle. The homogenate was centrifuged at 700 × g for 10 minutes. By discarding the residue, the supernatant was layered on an equal volume of 0.34 M sucrose solution containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.6, and centrifuged at 800×g for 10 minutes, The upper layer was transferred to a centrifuge tube with a pipette, and centrifuged at 7,000×g for 10 minutes. By removing the supernatant, the residue (mitochondria pellet) was washed twice with 0.25 M

sucrose solution containing 10 mM Tris-HCl, pH 7.6, by centrifugation at 9,000×g for 10 minutes. The other fractions were prepared by the same method of previous report (17). All the preparations were done at 0°—4°C.

White adult male rabbits weighing about 2 to 3 kg and young rats weighing about 150 to 200 g were used for the purpose of immunization.

Homogenous and heterogenous immunization were the procedures to immunize animals.

The immunizing procedures: Subcutaneous inoculation method and intraperitoneal inoculation method were used.

a) *Subcutaneous inoculation method;* Each rabbit received several injections of 0.5 to 1.0 ml volumes of 30 mg of antigenic proteins incorporated into 3 ml of Freunds' complete adjuvant (Difco Freunds' Adjuvant Complete) three times subcutaneously at the interval of once a week at the middle area between both shoulder blades, and each rat received once a week several injections of 0.5 to 1.0 ml volumes of 0.25 to 0.5 mg of antigenic proteins incorporated into 3 ml of Freunds' complete adjuvant at the axilla and side neck.

b) *Intraperitoneal inoculation method;* The modified method of MUNOZ (20) was used for this experiment. Each rat was given a series of three intraperitoneal injections of antigens. Each injection consisted of one ml of adjuvant complete and 250 to 500 micrograms of antigenic protein or 250 to 500 micrograms protein as a saline suspension. Immunizations were done at the interval of once a week.

Serum preparation method: Rabbit blood samples were collected from the auricular vein on the 14th day after the last inoculation, and these samples were allowed to clot at 20°C for one hour. Then the serum was removed from erythrocytes, and heated at 56°C for 30 minutes to inactivate complement. Each serum titer was measured at once, and the rabbits of low titer serum were boostered. The serum so collected was added each time with 0.01% of methiolate, and stored at −20°C until used. The rats immunized intraperitoneally were transplanted with fresh ascites tumor cells (2×10^7) 14 to 20 days after the last inoculation, and tumor anti-transplantability in the immunized rats was observed. The serum of those rats that had a powerful antitransplantability was used for the biochemical experiments.

Methods for the determination of antigen-antibody reactions: Ouchterlony agar double diffusion method and immunoelectrophoresis in agar gel were used for the determination of antigen-antibody reactions. Difco Special Noble agar was used for double diffusion.

For the immunodiffusion in agar gel, a solution of 1.5% agar in physiological saline containing 0.01% merthiolate was prepared. This agar solution was poured into the disk about 9 cm in diameter, and allowed to harden to give a layer 3 to 5 mm thick. A central well and six circumferential wells were punched out in such a way that each surrounding well was 6 mm apart from the central well, making the center to center distance of central well to each surrounding well 20 mm (Macro Ouchterlony Method (21)).

An antiserum sample was poured into the central well and each test antigen

was poured into 6 surrounding wells. Before testing for those fractions proved to be insoluble, each test antigen was dissolved with one % sodium deoxycholate to give solubility and diffusibility in agar gel. Protein concentration of each fraction was about 2 to 3 mg protein/ml. In agar immunoelectrophoresis (22), agar powder was added to distilled water to make the final concentration 4 %. This suspension was heated to dissolve agar, then cooled, and this agar gel was cut about one cm^3 in volume, and washed in a running water for about 2 days, then dialysed 3 days or more against deionized water. Then, this purified 4 % agar gel of one cm^3 was dissolved into 0.1 M veronal buffer solution to make the final concentration 1.0 %. This agar solution poured on the glass plate with a frame of 10×7.5 cm, then allowed to harden to give a layer 2 to 3 mm thick. This agar plate was divided into three small agar plate of 2 cm width by cutting four troughs of 3 mm width in an equal distance apart. A well of 4 mm in diameter was cut at the center of each small agar plate as Fig. 1. In this well was poured the test antigen or antiserum and the electrophoresis was done. This is the macro-immunoelectrophoresis. The purification method of agar used in this experiment was the

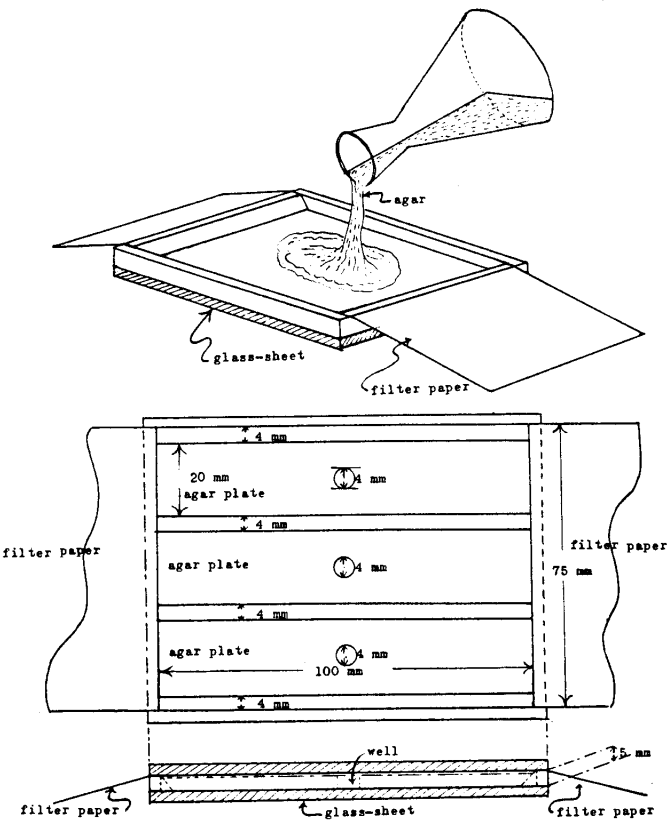

Fig. 1 Illustration of agar plate used for immunoelectrophoresis

modification of the method of TAKAYANAGI (23). In electrophoresis, the buffer solution used was 0.1 M veronal solution, and the electrode solution was 0.1 M HCl solution. The condition of electrophoresis was in 260 V, 20 mA, for six hours. After the electrophoresis, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. The samples were set at room temperature for one hour, then changing the temperature of 0° to 4°C and left overnight. Twenty-four hours, 48 hours, and 72 hours after the start of the test, they were observed and photographed. The photographs for the comparison were used at 48 hours in Ouchterlony diffusion and at 72 hours in immunoelectrophoresis.

The observations of tumor-antitransplantability : As the facts supporting the anti-transplantability to tumor in immunized rats, the phenomenon of the tumor rejection in host to transplanted tumors appeared, when the fresh ascites tumor cells were inoculated intraperitoneally to immunized rats, and the changes in cancer cell mitochondrial ATP-ase activities when the fresh cancer cell mitochondria were made to react with the rat sera immunized with cancer cell mitochondria, and the changes in cell respiration itself when cancer cells were incubated in the rat antisera immunized with cancer cell mitochondria.

ATP-ase activities were expressed in mμ moles of released Pi/mg of protein/minute at pH 7.6, measuring released Pi after the reaction by the method of TAKAHASHI (24). Oxygen consumption of cancer cells was measured by an oxymeter with rotating platinum electrode (25) in the presence of sodium succinate as the substrate.

RESULTS

The cross-matchings in agar gel : From the cross-matching in agar gel diffusion of the rabbit-antiserum to AH130 rat ascites hepatoma cell mitochondria (anti-HMt serum) against various kinds of rat ascites tumor mitochondria (mitochondria of AH130, AH225A, AH66F and Yoshida sarcoma), rat liver mitochondria, and tumor-bearing rat liver mitochondria, all kinds of tumor mitochondria including Yoshida sarcoma mitochondria reacted to this serum, forming a sharp precipitin band remarkably. Mitochondria of three different kinds of ascites hepatomas (AH130, AH225A and AH66F) and AH130-bearing rat liver mitochondria formed another sharp precipitin band just as rat liver mitochondria, suggesting it to be due to a tissue or organ specific mitochondrial antigen. In Yoshida sarcoma mitochondria, however, the common antigenicity to rat liver mitochondria was absent (Fig. 2, Photo. 1).

In the cross-matching in agar gel diffusion of the rabbit-antiserum to AH225A rat ascites hepatoma cell mitochondria (anti-AMt serum) against various kinds of rat ascites tumor cell mitochondria (mitochondria of AH 130, AH225A, AH66F and Yoshida sarcoma), rat liver mitochondria,

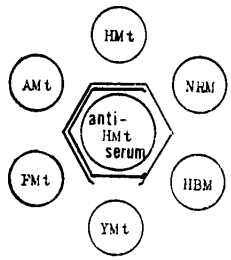

Fig. 2 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 hepatoma mitochondria. AMt: AH225A hepatoma mitochondria. FMt: AH66F hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NRM: rat liver mitochondria. HBM: AH130 bearing-rat liver mitochondria.

and tumor-bearing rat liver mitochondria, all the tumor mitochondria also reacted against anti-AMt serum, forming a sharp precipitin band remarkably. However, in this instance, it is not clear whether or not the common antigenicity to the liver mitochondria was present in cancer cell mitochondria (Fig. 3, Photo. 2).

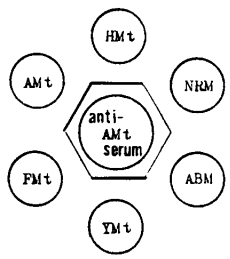

Fig. 3 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to AH225A rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 rat ascites hepatoma mitochondria. AMt: AH225A rat ascites hepatoma mitochondria. FMt: AH66F rat ascites hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NRM: rat liver mitochondria. ABM: AH225A hepatoma bearing-rat liver mitochondria.

In the case of cross-matching the rabbit-antiserum to AH66F rat ascites hepatoma cell mitochondria (anti-FMt serum) in agar gel diffusion against various kinds of rat ascites tumor cell mitochondria (mitochondria of AH130, AH225A, AH66F and YOSHIDA sarcoma), rat liver mitochondria and human liver mitochondria, all the tumor cell mitochondria reacted on this antiserum, forming a sharp precipitin band remarkably. The common antigenicity to rat liver mitochondria was traced in three different kinds of rat ascites hepatoma cell mitochondria except Yoshida sarcoma mitochondria as shown in Fig. 2 (Fig. 4).

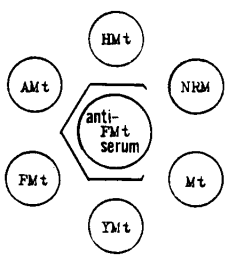

Fig. 4 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to AH66F rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 rat ascites hepatoma mitochondria. AMt: AH225A rat ascites hepatoma mitochondria. FMt: AH66F rat ascites hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NRM: rat liver mitochondria. Mt: human liver mitochondria.

From the results of the cross-matching various rat ascites hepatoma

cell mitochondria in agar gel diffusion against each antiserum after the electrophoresis, it is clear that there surely existed the immuno-specificity in the mitochondria of these four different kinds of rat ascites tumors, and this immuno-specificity was common to all the tumor cell mitochondria. Moreover, this immunospecificity was tumor mitochondria specific. One of these specific antigenicities exists in α_1 globulin position, and the other thought to be common to all the cancer cell mitochondria, in β_2 globulin position immuno-electrophoretically. Further, it was shown that rat liver mitochondria had 8 organ-specific mitochondrial antigens and such antigenicity diminished at the stage of carcinogenic transformation of cells, and on the contrary, specific cancer antigenicities of cancer cell mitochondria were potentiated remarkably (Fig. 5, Photo. 3).

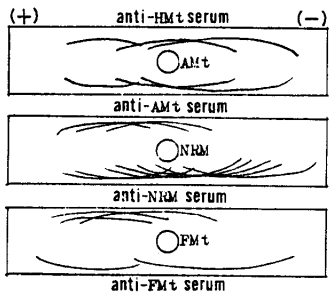

Fig. 5 The results of the immunoelectrophoresis. The condition of electrophoresis in agar gel was in 260 V, 20 mA, at 0°C, for 6 hours. After that, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. HMt: AH130 hepatoma mitochondria. AMt: AH225A hepatoma mitochondria. NRM: rat liver mitochondria. FMt: AH66F hepatoma mitochondria.

In the case of cross-matching in agar gel diffusion of the rabbit-antiserum to AH130 rat ascites hepatoma-bearing rat liver mitochondria (anti-HBM serum) against various kinds of tumor-bearing rat liver mitochondria (liver cell mitochondria of the rat bearing AH130, AH225A, AH66F, and Yoshida sercoma), rat liver mitochondria, and human liver mitochondria, these kinds of tumor-bearing rat liver mitochondria reacted on this antiserum, forming precipitin bands thought to be specific to tumor-bearing rat liver mitochondria. It is clear that AH130-bearing rat liver mitochondria have the antigenicities common to rat liver mitochondria, and that the specific antigenicity also exists in AH130-bearing rat liver mitochondria (Fig. 6).

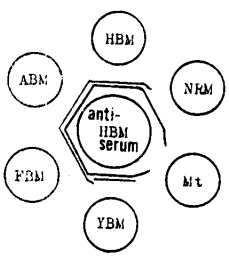

Fig. 6 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to AH130 bearing-rat liver mitochondria. Surrounding wells contain various antigens as follows: HBM: AH130 bearing-rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria. FBM: AH66F bearing-rat liver mitochondria. YBM: Yoshida sarcoma bearing-rat liver mitochondria. NRM: rat liver mitochondria. Mt: human liver mitochondria.

In the cross-matching in agar gel diffusion of the rabbit-antiserum to AH225A rat ascites hepatoma-bearing rat liver mitochondria (anti-ABM serum) against various tumor-bearing rat liver mitochondria (Fig. 7, Photo. 4), in the cross-matching of the rabbit-antiserum to AH66F rat ascites hepatoma-bearing rat liver mitochondria (anti-FBM serum) against various kinds of mitochondria (Fig. 8, Photo. 5), and in the cross-matching of the rabbit-antiserum to Yoshida sarcoma-bearing rat liver mitochondria (anti-YBM serum) against various kinds of mitochondria (Fig. 9), it has been demonstrated that each tumor-bearing rat liver mitochondria have the antigenicities common to rat liver mitochondria, and that in addition to this, there surely exists specific antigenicity common in individual tumor-bearing rat liver mitochondria. This specific antigenicity common in tumor-bearing rat liver mitochondria was not detected in cancer cell mitochondria nor in rat liver mitochondria.

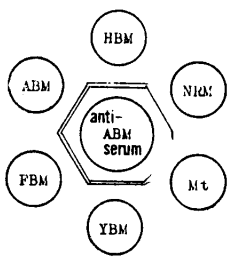

Fig. 7 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to AH225A hepatoma bearing-rat liver mitochondria. Surrounding wells contain various antigens as follows: HBM: AH130 bearing-rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria. FBM: AH66F bearing-rat liver mitochondria. YBM: Yoshida sarcoma bearing-rat liver mitochondria. NRM: rat liver mitochondria. Mt: human liver mitochondria.

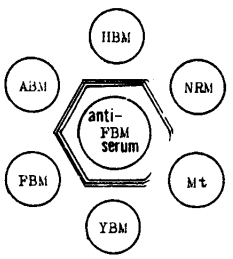

Fig. 8 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antisernm to AH66F hepatoma bearing-rat liver mitochondria. Surrounding wells contain various antigens as follows: HBM: AH130 bearing-rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria. FBM: AH66F bearing-rat liver mitochondria. YBM: Yoshida sarcoma bearing-rat liver mitochondria. NRM: rat liver mitochondria. Mt: human liver mttochondria.

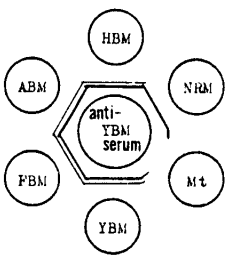

Fig. 9 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to Yoshida sarcoma bearing-rat liver mitochondria. Surrounding wells contain varions antigens as follows: HBM: AH130 bearing-rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria. FBM: AH66F bearing-rat liver mitochondria. YBM: Yoshida sarcoma bearing-rat liver mitochondria NRM: rat liver mitochondria. Mt: human liver mitochondria.

Immunoelectrophoretical analysis indicates that in tumor-bearing rat liver mitochondria the organ specific mitochondrial antigenicities were

slightly diminished and a specific antigenicity of tumor-bearing rat liver mitochondria appeared in α_1 globulin position (Figs. 10, 11, Photo. 6).

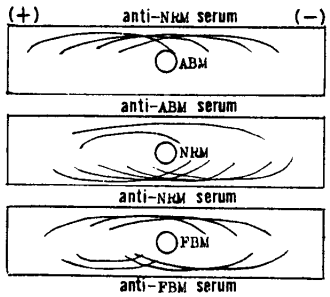

Fig. 10 The results of the immunoelectrophoresis. The condition of electrophoresis in agar gel was in 260V, 20mA, at 0°C, for 6 hours. After that, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. NRM: rat liver mitochondria. ABM: AH225A hepatoma-bearing rat liver mitochondria. FBM: AH66F hepatoma bearing-rat liver mitochondria.

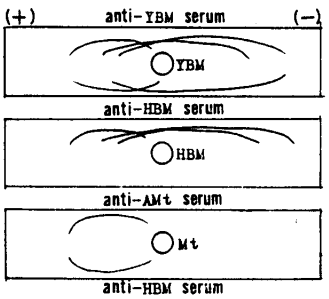

Fig. 11 The results of the immunoelectrophoresis. The condition of electrophoresis in agar gel was in 260V, 20mA, at 0°C, for 6 hours. After that, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. YBM: Yoshida sarcoma bearing-rat liver mitochondria. HBM: AH130 hepatoma bearing-rat liver mitochondria. AMt: AH-225A hepatoma mitochondria. Mt: human liver mitochondria.

The cross-matchings in agar gel of the rabbit-antiserum to rat liver mitochondria (anti-NRM serum) against various kinds of tumor mitochondria (the mitochondria of AH130, AH225A, AH66F and Yoshida sarcoma), have demonstrated that the organ specific mitochondrial antigenicities common to rat liver mitochondria are remarkably diminished in tumor cell mitochondria (Fig. 12, Photo. 7).

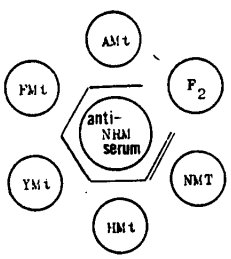

Fig. 12 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 rat ascites hepatoma mitochondria. AMt: AH225A rat ascites hepatoma mitochondria. FMt: AH66F rat ascites hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NMT: rat liver mitochondria. F_2: rat liver microsomes.

As the rabbit-antiserum to AH130 rat ascites hepatoma cell mitochondria absorbed with rat liver mitochondria respond remarkably to those four different kinds of tumor mitochondria, forming a sharp precipitin band (Fig. 13, Photo. 8), it is obvious that the tumor mitochondria-

specific antigenicity appears at the stage of carcinogenic transformation of cells.

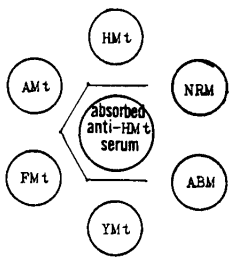

Fig. 13 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-anti-AH130 cell mitochondria serum observed with rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 rat ascites hepatoma mitochondria. AMt: AH225A rat ascites hepatoma mitochondria. FMt: AH66F rat ascites hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NRM: rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria.

In the similar cross-matchings, it is known that the organ specific mitochondrial antigenicities common to rat liver mitochondria do not diminish so remarkably in the tumor-bearing rat liver mitochondria (Fig. 14, Photo. 9). All the antigen-antibody reactions in these instances take place immunoelectrophoretically in γ-globulin region (Fig. 15, Photo. 10).

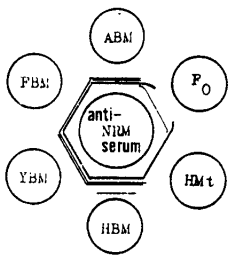

Fig. 14 The results obtained by Ouchterlony double diffusion test. Central well contains the rabbit-antiserum to rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HBM: AH130 bearing-rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria. FBM: AH66F bearing-rat liver mitochondria. YBM: Yoshida sarcoma bearing-rat liver mitochondria. HMt: AH130 bearing-rat liver mitochondria. F_0: AH130 plasma membrane fraction.

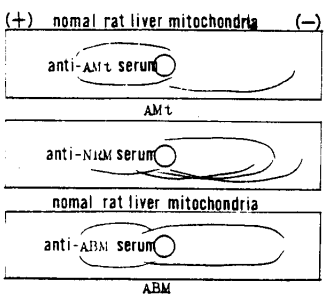

Fig. 15 The results of the immunoelectrophoresis. The condition of electrophoresis in agar gel was in 260V, 20mA, at 0°C, for 6 hours. After that, the antigens were poured into the troughs at once, then the Ouchterlony reaction was begun.
AMt : AH225A rat ascites hepatoma mitochondria.
ABM : AH225A rat ascites hepatoma bearing-rat liver mitochondria.

The observations of tumor antitransplantability : Tumor antitransplantability on the intraperitoneal transplantation of 2×10^7 fresh rat ascites hepatoma cells was tested to the rats, weighing about 150 to 200 g immunized with cancer cell mitochondria. Twenty-seven rats out of the 50 immunized with cancer cell mitochondria (54 %) rejected the transplantation of ascites hepatoma and survived (Table 1). This suggests the presence

Table 1 The tumor anti-transplantability of rat preimmunized with the tumor mitochondria

immunized with	number of rats	number of survivals	% of anti-tumor-transplantability
AH130 Mt	50	27	54
AH130 Nu	5	2	40
AH130 Mc	7	0	0
AH130 Pl. Mem.	6	0	0
none	50	0	0

Each rat received three intraperitoneal injections, at once every week of 0.5 mg protein of each antigen. The immunized rat was inoculated with AH 130 cells on the 14th day after the last immunization. Control rats were not immunized. Mt: mitochondria. Nu: nuclei. Mc: microsomes. Pl. Mem.: plasma membrane.

of specific cancer antigenicity in cancer cell mitochondria. On the contrary, in the intraperitoneal transplantation of 2×10^7 fresh ascites hepatoma cells to the control groups, non-immunized or immunized with rat liver mitochondria, and to the experimental groups immunized with the plasma membrane fraction, the nuclear fraction, or the microsomal fraction of rat ascites hepatoma cells, the tumor antitransplantability was not observed, and these rats died on the 10th to the 14th day after the transplantation of tumor cells. This indicates that the specific tumor antigenicity is remarkably present in the tumor cell mitochondria, but is not present in the plasma membrane and the microsomes of tumor cells.

The effects of the antisera on the cancer cell mitochondrial ATP-ase activity : The ATP-ase activity of sonicated AH130 rat ascites hepatoma cell mitochondria treated with the pooled antisera of rats showing the tumor-anti-transplantability, was inhibited 38%. The rabbit-antiserum to AH130 rat ascites hepatoma cell mitochondria also inhibited the ATP-ase activity 29%. On the contrary, the ATP-ase activity of sonicated rat liver mito-

Table 2 The effects of antisera on the ATPase activity of the sonicated hepatoma cell mitochoedria. Activities are expressed as mμ moles of released Pi per mg of protein per minute at pH 7.6.

samples	in the presence of antiserum	ATP-ase activity	% inhibition
sonicated AH130 cell mitochondria	none	297	—
	rat-antiserum to AH130 mitochondria	183	38
	rabbit-antiserum to AH130 mitochondria	210	29
	rabbit-antiserum to rat liver mitochondria	248	17
sonicated rat liver mitochondria	none	258	—
	rabbit-antiserum to rat liver mitochondria	278	0

chondria treated with the rabbit-antiserum to the mitochondria was not inhibited (Table 2).

The effects of the antisera on the respiratory activity of cancer cells : When the AH130 rat ascites hepatoma cells or its homogenate were treated with the antisera to their own mitochondria, the oxygen consumption with succinate as substrate was remarkably inhibited, comparing with that of the non-treated AH130 cells or its homogenate (Table 3).

Table 3 The effects of the antiserum on the respiratory activity of whole cell and homogenate. The reaction mixture contained 0.05 M sucrose, 0.02 M KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM $MgCl_2$, 3 mM sodium succinate as a substrate, and whole cells or homogenate (total volume 2 ml). Incubation was carried out by the addition of antiserum at 37°C, and oxygen consumption was measured by a oxymeter with platinum electrode. Activities are expressed as mμ moles O_2 uptake per minute per mg of protein.

	control	antiserum to AH130 mitochondria	
		from rat	from rabbit
whole cell	4.0	1.2	0.4
cell homogenate	4.0	0.4	0

DISCUSSION

Many immunological investigations of the irreversible transformations of neoplasma have appeared with special reference to the specific cancer antigenicities, using various immunochemical techniques such as the immunodiffusion, immunoelectrophoresis, complement fixation, immunofluorescence, and immunoferritin technique, etc.. As a result there arose a question about the gain or loss of antigenicities at carcinogenic changes of cells. ZILBER (6) reported the presence of the specific cancer antigenicity in the hepatoma induced by chemical carcinogens. HIRAI (26) and others (27—30) had stated that the new antigenicity was imparted to artificial experimental neoplasma. RAPPORT and his collaborator (1954) (1) indicated the possible existence of specific cancer antigen in the mitochondria of rat lymphosarcoma ; and HORN (1955) (2, 3), (1966) (4) reported the highest antigenic fraction in Ehrlich ascites hepatoma cells to be detected in the mitochondria and the nuclear fraction. TAKEDA and his collaborators (1956) (5) stated that the rats immunized with the membrane fraction, nuclear membrane, mitochondria, or insoluble parts of the nuclei of DAB hepatoma acquired a strong anti-transplantability to this transplantable hepatoma ; KIKUCHI (1962) (7) reported similar results ; TAKAYANAGI (1964)

(31) reported that several specific cancer antigens were found in α_1 to β_2 globulin regions in his immunochemical analysis of the extracted proteins from DAB hepatomas; and HOKO (1966) (8) reported about the presence of a specific cancer antigen in DOC insoluble fraction of cancer cell mitochondria. These reports suggest the definite existence of specific cancer antigen. There are many other investigators who are of the opinions that some antigenicities originally existing in normal organ, are diminished at carcinogenic changes, resulting the simplification of antigenic components. HOGEBOOM and his collaborator (1951) (32) reported that a part of protein constituent disappeared at the carcinogenic transformation of cells in their analyses of the cancer cell mitochondrial proteins; WEILER (9) also found that the organ-specific antigens which originally existed in normal cells disappeared in rat hepatomas; KAYE (12) antigenic changes in the antigenicities of urinary epithelial cells in the patients of urinary cancer; and NAIRN (10, 11) some of the organ-specific antigenicities to disappear in the stomach or the intestine of the patients bearing cancer in digestive organs; and BELOSHAPKINA (1967) (33) and others (13—16) reported the data relating the loss of the organ-specific antigens in the organ of tumor-bearing body.

In the present experiments, it is clear that the cancer mitochondria-specific antigenicity reported previously (17) definitely exists in the four different kinds of rat ascites tumors induced by chemical carcinogens and that this specific antigenicity is common to those four tumors. These results suggest that the specific cancer antigen is present in cancer cell mitochondria. According to HORN's reports (2—4) there exist the high antigenicity in mitochondrial fraction of Ehrlich ascites hepatoma, and by TAKEDA (5) and KIKUCHI (7) the rats immunized with TCA-treated mitochondrial fraction of DAB hepatoma, acquire the tumor-antitransplantability. However, it is possible that there may be some contamination in these fractions, because these fractions from cancer cells have not been strictly fractionated in their experiments, and they did not report on the fact that only the mitochondria had the strongest specific cancer antigenicity in the membrane systems of cancer cells. As the mitochondria in the present experiments have been strictly fractionated, it is thought that the mitochondria are fully purified, and none of these investigators have reported about the cancer mitochondria-specific antigenicity as cancer specific, but only HOKO (8) has suggested that there exists specific cancer antigenicity in cancer cell mitochondria, which is absent in normal cell mitochondria, similarly as in the present report. And it is reported here that the cancer cell mitochondria-specific cancer antigenicity is

situated immunoelectrophoretically in β_2 globulin position and this specific cancer antigenicity of β_2 globulin region is common to all the tumors cell mitochondria. HOKO (8) does not refer to this respect, but there is a possibility that one of the several specific cancer antigenicities in the extracted proteins of DAB hepatoma as reported by TAKAYANAGI (31) lying in α_1 to β_2 globulin position immunoelectrophoretically, coincides with the cancer mitochondria-specific cancer antigenicity reported here. It is firmly maintained here that this cancer mitochondria-specific cancer antigenicity is common to all the tumors cell mitochondria of various kinds, but RAPPORT *et al.* (1), HORN (2—4), TAKEDA *et al.* (5), ZILBER (6), KIKUCHI (7), and HOKO (8), have not touched on this point. TAKEDA (5) and KIKUCHI (7) asserted emphatically that on the contrary, there is no specific cancer antigens common to all cancers induced by chemical carcinogens. BALDWIN and his collaborator (34) investigated specific cancer antigens in various cancers induced by chemical carcinogens and claimed that the specific cancer antigen existed in individual tumors, and it was possible to produce tumor resistance immunochemically to each tumor. However, they contend that the specific tumor antigenicity of individual tumor is not common to each other, and there exists no antigenicity common to the tumors induced by chemical carcinogens. Therefore, there are not so many reports about the presence of specific cancer antigenicity common to all the tumors except the present report. Only USUBUCHI and his collaborators (1967) (35) have reported that there might exist common antigenicity among all the malignant tumors in isogenuous mice, on the cross-immunization among various tumors of C3H mouse, and DECKER and his collaborator (1966) (36) recognized the existence of tumor specific antigens common to various tumors of rat.

It is reported here that, when the rats are immunized with cancer cell mitochondria, these rats acquire the tumor antitransplantability. RAPPORT *et al.* (1), TAKEDA *et al.* (5), AIGAWA *et al.* (37), and KIKUCHI (7) agree on this point, but their antitransplantability induced by tumor mitochondria was not so strong and remarkable as that reported here. It may be pointed out that the biochemical investigations were made in the present experiment to analyse by what mechanism the anti-transplantability to tumor is acquired in an organism, while available reports do not deal with this problem.

This mechanism may be explained as the resistance to transplantation of cancer cells is imparted by the inhibitory effect of the antibody formed in the recipients immunized with cancer cell mitochondria, resulting in the inhibition of the mitochondrial ATP-ase and respiratory activities so

that the cancer cells implanted can no longer proliferate.

By isolating and purifing the mitochondrial nucleic acids of various tumors and analysing of their properties, it is possible to assume that the cancer mitochondria-specific cancer antigen is found in cancer cell mitochondria if the cancer-specific DNA exists in cancer cell mitochondria, and it follows then that the appearance of tumor resistance will take place by immunization of cancer cell mitochondria in animals. On the basis of these and referring to the appearance of the tumor-resistance induced by viruses as reported by HABEL (38), SACKS (39), SJOEGREN (40), and KOCH (41), this problem will be dealt with in part III, how the cancer mitochondria-specific cancer antigenicity would relate to virus-induced tumors.

A discussion was made on the diminution of the original organ specific antigenicities at carcinogenic changes of cells in the liver cell mitochondria, and it was reported that the liver mitochondria-specific antigenicity of tumor-bearing body exists in the liver mitochondria of animals bearing tumor, which is not present in normal animal liver mitochondria. Concerning the diminution or the disappearance of organ specific antigenicities at carcinogenic transformation of cells in animals bearing tumor, there are many reports by WEILER (9), who proved by immunofluorescent technique that organ specific antigen of liver cell diminished in hepatoma cell; by NAIRN *et al.* (11); KAYE *et al* (12); and NELKEN (13) who proved that the organ specific antigenicities disappeared in animals bearing tumor; and by DAO *et at.* (14); AMOS *et at.* (15); and HUR *et al.* (16) proved that heterogenous skin graft was possible to the animals bearing tumor. They reported the changes of antigenicities in animals bearing tumor, but did not report the existence of tumor-bearing organ specific antigenicity which did not exist in the same organ of normal animals. There are found few reports dealing with antigenicities of both tumor and tumor-bearing organs.

SUMMARY

As the results of investigating the antigenicities of various fractions from the membrane systems of cancer cells, it has been found that the remarkable cancer-specific antigenicity exists in cancer cell mitochondria. With a particular reference to this antigenicity of cancer cell mitochondria, the antigenicities of the mitochondria of various kinds of rat ascites tumors and those of tumor-bearing rat liver mitochondria have been compared with those of normal rat liver mitochondria. In addition, it has

been demonstrated that a strong tumor antitransplantability is induced when the recipient rat is immunized with the tumor cell mitochondria. In order to support these experimental facts, enzymatic activities of cancer cell mitochondria have been investigated also biochemically after treating the mitochondria with the antiserum to these mitochondria.

1. The most remarkable cancer specific antigenicity exists in mitochondria among the membrane systems of cancer cells. This cancer mitochondria-specific cancer antigenicity is common to all the ascites tumor mitochondria used here.

2. The original tissue- or organ-specific antigenicities diminish or disappear at the carcinogenic transformation of cells.

3. The tumor-bearing-organ specific antigenicity appears in the organs of animals bearing tumor.

4. The tumor antitransplantability is acquired when rats are immunized with these tumor cell mitochondria.

5. The inhibition of mitochondrial ATP-ase and respiratory activities is observed when the cancer cell mitochondria are treated with the antiserum to the mitochondria.

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REFERENCES

1. RAPPORT, M. M. and GRAF, L.: Extraction of complement fixing lipid antigens from mitochondria of Murphy-Sturn lymphosarcoma. *Proc. Amer. Ass. Cancer Res.* **1**, 39, 1954
2. HORN, E. C.: *In vivo* effect of nucleoprotein from Ehrlich ascites tumor cells. *Biochim. et Biophys. Acta* **16**, 440, 1955
3. HORN, E. C.: Ascites tumor development I. An analysis of *in vivo* effect of nucleoprotein from Ehrlich ascites cells. *Cancer Res.* **15**, 663, 1955
4. HORN, E. C.: Cytotoxicity of various antisera prepared against Ehrlich ascites tumor cell component. *Cancer Res.* **16**, 595, 1956
5. TAKEDA, K., YOSHIO, H. and SAKATA, Y.: Immunopathological studies on the specificity of the tumor antigen of rat treated with TCA. *Gann* **47**, 549, 1956
6. ZILBER, L. A.: Specific tumor antigens. *Advance Cancer Res.* **5**, 291, 1958
7. KIKUCHI, K.: The intracellular localizations of tumor specific antigens of rat tumor. *Gann no Rinsho* **8**, 639, 1962
8. HOKO, T.: Immunochemical analysis of mitochondrial fraction from cancer cells. *Juzen Igaku Zasshi* **74**, (No. 1), 30, 1966
9. WEILER, E.: Die Anderung der serologischen Organspezifitat beim Buttergelb-tumor der ratte im vergleichzu normaler leber. *Z. Naturforsch.* **7**, 324, 1952

10. NAIRN, R. C., RICHMOND, H. G. and FOTHERGILL, J. E.: Defferences in staining of normal and malignant cell by non-immune fluorescent protein conjugates. *Brit. Med. J.* **2**, 1341, 1960
11. NAIRN, R. C., FOTHERGILL, J. E. and MCENTEGART, M. G.: Loss of gastrointestinal specific antigens in neoplasma. *Brit. Med. J.* **1**, 1791, 1962
12. KAYE, H. E. M. and WAILACE, D. M.: A and B antigens of tumors arising from urinary epithelium. *J. Nat. Cancer Inst.* **26**, 1349, 1961
13. NELKEN, D.: Loss of leucocyte individual specific antigens in disease of acute leukemia. *Vox. Sang.* **8**, 638, 1963
14. DAO, T. L., TANAKA, Y. and GAWLAK, D.: Effects of polycyclic hydrocarbons on mammary homograft survival and tumorigenesis in rats. *J. Nat. Cancer Inst.* **33**, 963, 1964
15. AMOS, D. B., HATTLER, B. G. and SHINGLETON, W. W.: Prolonged survival of skin graft from cancer patients on normal recipients. *Lanet* **1**, 414, 1965
16. HUR, N. B., SCHNEIDER, W. C. and ROBINSON, E.: Comparison of the survival of skin grafts from normal mice and mice with Ehrlich ascites tumor. *Transplantation* **4**, 205, 1966
17. WAKABAYASHI, A.: Immunological studies on the membrane systems of cancer cells; I. Immunochemical analysis of membrane fractions from cancer cells. *Acta Med. Okayama* 22, 1968
18. WAKABAYASHI, A. and ODA, T.: Immunological studies on the membrane systems of cancer cells. II. Immunological specificities of cancer cell mitochondria. Proc. of Jap. Can. Ass. 26th Annual Meeting p. 237, 1967 (Nagoya)
19. HOGEBOOM, G. E.: Fractionation of cell components of animal tissues. *Methods in Enzymology*, Vol. 1, p. 16, Academic Press, New York, 1955
20. MUNOZ, J.: Production in mice of large volumes of ascites fluid containing antibodies. *Proc. Soc. Exp. Bio. Med.* **95**, 757, 1957
21. KABAT. E. A. and MAYER. M. M.: Precipitin reaction. *Experimental Immunochemistry*, 2nd edition, p. 22, CHARLES C THOMAS. publisher, Springfield, Illinois, U. S. A., 1964
22. KABAT, E. A. and MAYER, M. M.: Electrophoresis and immunoelectrophoresis. Experimental Immunochemistry, 2nd edition, p. 622, CHARLES C THOMAS. publisher, Springfield, Illinois, U. S. A., 1964
23. TAKAYANAGI, N.: Analysis of pleural and ascites fluids by immunoelectrophoretic methods. *Rinsho Byori*, **10** (No. 7), 353, 1962
24. TAKAHASHI, T.: "The experimental methods from phosphorous metabolism" Hirokawa Publish Co., Tokyo, p. 29, 1958
25. UTSUMI, K. and YAMAMOTO, G.: An apparatus for simultaneous measurement of 90° light-scattering fluorescence intensity of reduced pyridine nucleotides and oxygen consumption of mitochondria. *Acta Med. Okayama* **18**, 111, 1964
26. HIRAI, H.: Specific antigenic proteins of rat ascites hepatoma. *Specific Tumor Antigen*, Vol. 1, p. 42, A symposium organized by the International Union against Cancer and the U. S. S. R. Academy of Medical Science, 1966
27. PREHN, R. T.: Tumor-specific immunity to trans-planted dibenzanthracene-induced sarcoma. *Cancer Res.* **20**, 1614, 1960
28. PREHN, R. T.: Specific isoantigenicities among chemically induced tumors. *Ann. N. Y. Acad. Sci.* **101**, 107, 1962
29. HADDOW, A.: Immnnology of the cancer cell: Tumor specific antigens. *Brit. Med. Bull.* **21**, 133, 1965
30. GORDON, J.: Isoantigenicity of tumor induced by an Azo dye. *Brit. J. Cancer* **19**, 387, 1965
31. TAKAYANAGI, N.: Study on the extracts from DAB-hepatoma of rat by immunodiffusion methods. *The Jahresbericht des Kurashiki-Zentralhospitals*, Jg. 33, Nr. 2, p. 190, 1964

32. HOGEBOOM, G. H. and SCHNEIDER, W. C.: Protein of liver and hepatoma mitochondria. *Science*, **113**. 355, 1951
33. BELOSHAPKINA, T. and KHRANKOVA, N.: Organospecificity of liver cell surface antigens and its loss by ascitic hepatoma. *Nature*, **214**, 1366, 1967
34. BALDWIN, R. W. and BARKER, C. R.: Tumor-specific antigenicity of aminoazo-dye-induced rat hepatomas. *International J. Cancer*, **2** (No. 4), 355. 1967
35. USUBUCHI, I., SOBAJIMA, Y., KUDO, H., HONGO, T. and SUGAWARA, M.: Suppression in autotransplantation of mouse mammarian carcinoma by cross-immunization. *Igaku no ayumi*, **61** (No. 11), 629, 1967
36. DECKER, C. and DECKERS-Passaue, LILIANE (Belgium): Specific tumor antigens in rat hepatomas and leucosarcomas. *Specific Tumor Antigens* vol. 1, p. 34, A symposium organized by the International Union against Cancer and the U. S. S. R. Academy of Medical Sciences, 1966
37. AIGAWA, K., SUYAMA, T. and IRIE, M.: Anti-transplantabilities of the rats immunized against formalin and TCA. *Gann*, **47**, 550, 1956
38. HABEL, K.: Resistance of polyoma virus immune animals to transplanted polyoma tumors (26453). *Proc. Soc. Exp. Biol. Med.*, **106**, 722. 1961
39. SACKS, L.: Tumor transplantation in mice inoculated with polyoma virus. *Exp. Cell Res.* **24**, 185, 1961
40. SJOEGREN, H. O., HELLSTROEM, I. and KLEIN, G.: Resistance of polyoma virus immunized mice to transplantation of established polyoma tumors. *Exp. Cell Res.*, **23**, 204, 1961
41. KOCH, M. A. and SABIN, A. B.: Specificity of virus-induced resistance to transplantation of polyoma and SV 40 tumors in hamsters (28260). *Proc. Soc. Exp. Biol. Med.* **113**, 4. 1963

LEGENDS FOR PHOTOGRAPHS

The photographs are the results obtained by Ouchterlony double diffusion tests and immunoelectrophoresis.

Photo. 1 Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 hepatoma mitochondria. AMt: AH225A hepatoma mitochondria. FMt: AH66F hepatoma mitochondria. YMt: YOSHIDA sarcoma mitochondria. NRM: rat liver mitochondria. HBM: AH130 bearing-rat liver mitochondria.

Photo. 2 Central well contains the rabbit-antiserum to AH225A rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HMt: AH130 hepatoma mitochondria. AMt: AH225A hepatoma mitochondria. FMt: AH66F hepatoma mitochondria. YMt: Yoshida sarcoma mitochondria. NRM: rat liver mitochondria. ABM: AH225A bearing-rat liver mitochondria.

Photo. 3 The condition of electrophoresis in agar gel was in 260 V, 20 mA, at 0°C, for 6 hours. After that, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. HMt: AH130 rat ascites hepatoma mitochondria. AMt: AH225A rat ascites hepatoma mitochondria. NRM: rat liver mitochondrla. FMt: AH66F rat ascites hepatoma mitochondria.

Photo. 4 Central well contains the rabbit-antiserum to AH225A hepatoma-bearing rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HBM: AH130 hepatoma bearing-rat liver mitochondria. ABM: AH225A hepatoma bearing-rat liver mitochondria. FBM: AH66F hepatoma-bearing rat liver mitochondria. YBM: YOSHIDA sarcoma bearing-rat liver mitochondria. NRM: rat liver mitochondria. Mt: human liver mitochondria.

Photo. 5 Central well contains the rabbit-antiserum to AH66F hepatoma bearing-rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HBM : AH130 hepatoma bearing-rat liver mitochondria . ABM : AH225A hepatoma-bearing rat liver mitochondria. FBM : AH66F hepatoma bearing-rat liver mitochondria. YBM : Yoshida sarcoma bearing-rat liver mitochondria. NRM : rat liver mitochondria. Mt : human liver mitochondria.

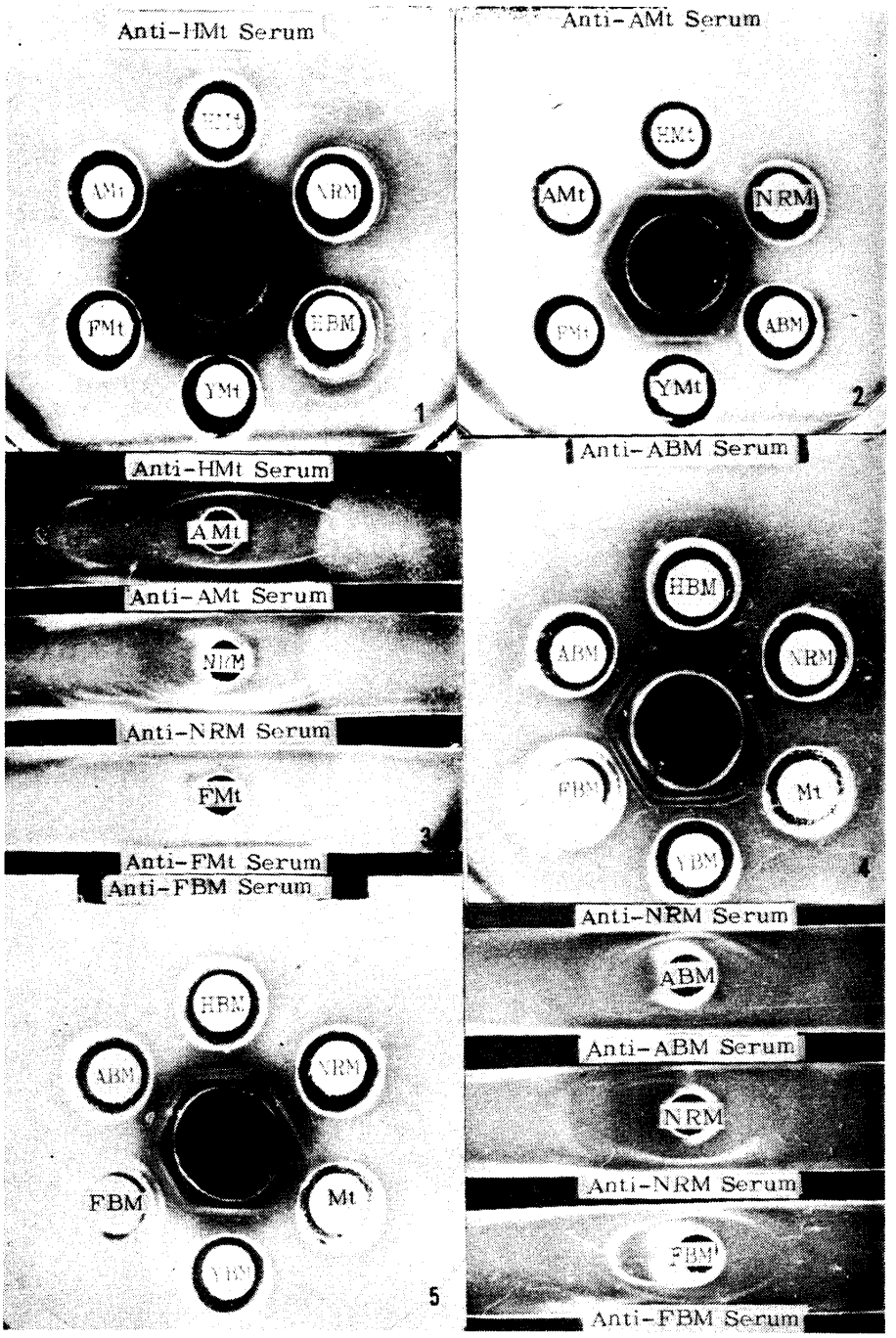
Photo. 6 The condition of electrophoresis in agar gel was in 260 V, 20 mA, at 0°C, for 6 hours. After that, the antisera were poured into the troughs at once, then the Ouchterlony reactions were begun. NRM : rat liver mitochondria. ABM : AH225A hepatoma bearing-rat liver mitochondria. FBM : AH66F hepatoma bearing-rat liver mitochondria.

Photo. 7 Central well contains the rabbit antiserum to rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HMt : AH130 rat ascites hepatoma mitochondria. AMt : AH225A rat ascites hepatoma mitochondria. FMt : AH66F rat ascites hepatoma mitochondria. YMt : Yoshida sarcoma mitochondria. NMT : rat liver mitochondria. F_2 : rat liver microsomes.

Photo. 8 Central well contains the rabbit-anti-AH130 cell mitochondria serum absorbed with rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HMt : AH130 rat ascites hepatoma mitochondria. AMt : AH225A rat ascites hepatoma mitochondria. FMt : AH66F rat ascites hepatoma mitochondria. YMt : Yoshida sarcoma mitochondria. NRM : rat liver mitochondria. ABM : AH225A hepatoma bearing-rat liver mitochondria.

Photo. 9 Central well contains the rabbit-antiserum to rat liver mitochondria. Surrounding wells contain various antigens as illustrated below. HBM : AH130 hepatoma bearing-rat liver mitochondria. ABM : AH225A hepatoma bearing-rat liver mitochondria. FBM : AH66F hepatoma-bearing rat liver mitochondria. YBM : Yoshida sarcoma bearing-rat liver mitochondria. HMt : AH130 rat ascites hepatoma mitochondria. F_0 : AH130 plasma membrane fraction.

Photo. 10 The condition of electrophoresis in agar gel was in 260 V, 20 mA, at 0°C, for 6 hours. After that, the antigens were poured into the tronghs at once, then the Ouchterlony reactions were begun. AMt : AH225A rat ascites hepatoma mitochondria. ABM : AH225A hepatoma bearing-rat liver mitochondria.


Anti-HMt Serum
AMt
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1
Anti-AMt Serum
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Anti-NRM s
AMt
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F2
YMt
HMt
Anti-AHMt Serum
absorbed with NRM
HMt
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ABM
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YBM
HMt
HBM
Normal Rat Liver Mitochondria
Anti-AMt Serum
AH225A Cell Mitochondria
Anti-NRM Serum
Normal Rat Liver Mitochondria
Anti-ABM Serum
AH225A Bearing Rat Liv. Mt.