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# Immunological studies on the membrane systems of cancer cells. 3. Immunospecificities of the mitochondria from virus-induced tumors by the precipitin reaction in agar gel

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# Immunological studies on the membrane systems of cancer cells. 3. Immunospecificities of the mitochondria from virus-induced tumors by the precipitin reaction in agar gel*

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**Abstract**

The mitochondrial, the microsomal, and the supernatant fractions were prepared from the cell homogenate of tumors induced by viruses, such as adenovirus type 12, SV 40, and Rous sarcoma virus, etc. and the antigenicities of these fractions were investigated. In the virus-induced tumors, there existed no antigenicity common to the mitochondrial and the microsomal fractions as in the tumors induced by chemical carcinogens, and the highest antigenicity was recognized in the mitochondrial fraction. Therefore, the properties of the tumor cell mitochondria were precisely investigated with virus-induced tumor mitochondria. 1. The mitochondria of tumors induced by viruses have clearly the specific antigenicity. 2. This specific antigenicity of virus.induced tumor mitochondria IS common to all the virus-induced tumors used in the present study. 3. This tumor mitochondria.specific antigenicity is found commonly in all the tumor mitochondria in the present experiments. 4. The specific cancer antigenicity of tumor cell mitochondria does not exist in normal organ mitochondria, but the regenerating organ mitochondria exhibit a slight antigenicity common to cancer cell mitochondria.

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# IMMUNOLOGICAL STUDIES ON THE MEMBRANE SYSTEMS OF CANCER CELLS
## III. IMMUNOSPECIFICITIES OF THE MITOCHONDRIA FROM VIRUS-INDUCED TUMORS BY THE PRECIPITIN REACTION IN AGAR GEL

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Since WITEBSKY (1) first reported about the existence of specific cancer antigens, immunological studies on the cancer specificities have begun, and numerous efforts have been made to investigate the specific cancer antigenicity. With the improvement in cell fractionation methods and immunochemical procedures, many reports have appeared suggesting the existence of specific cancer antigenicity in cancer cell mitochondria, by fractionating the cancer cells into membrane fractions at subcellular level and by comparing their antigenicities (2—9). On the other hand, the investigators looking for the cause of cancer at viruses searched for the specific cancer antigenicities to be originated in viruses by immunofluorescent technique (10—23) or ferritin antibody technique (24—30), and recently viral genome has also come to be investigated from the aspect of nucleic acids. As the specific cancer antigenicity was forturnately found in cancer cell mitochondria, and its properties were analysed pecisely in previous reports (31, 32), subcellular components of virus-induced tumor cells were fractionated by ultracentrifugation and the immunospecificities of each of these fractions were observed chiefly by immunodiffusion methods, in view of the possibility that the same methods described in the previous reports can be applied to virus-induced tumors. As the result some very interesting findings obtained are reported in this paper.

## MATERIALS AND METHODS

Inbred C3H mice, hamsters, and Donryu rats were used in this experiments. The cancer strains used were SR-RSV-induced mouse sarcoma (SR-C3H/He ascites), adenovirus type 12-induced hamster tumor, SV 40-induced hamster tumor, and AH130 rat ascites hepatoma. SR-C3H sarcoma was obtained through the courtesy of Prof. T. YAMAMOTO, Department of

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Oncology, Institute for Medical Science, University of Tokyo, Shiba-Shirokane, Tokyo (33, 34). The other experimental groups were the livers of various kinds of tumor-bearing animals, rat livers, human liver and spleen from the same person.

*Isolation of mitochondria:* Tumor mitochondria, and liver and spleen mitochondria were isolated by the modification of the method of HOGEBOOM (35).

a) *Tumor cell mitochondria of ascites form* (the mitochondria of AH130 and SR-C3H): These mitochondria were prepared by the method reported previously in Part II (32).

b) *The mitochondria of the tumor induced by adenovirus type 12 or SV 40:* Fresh hamster tumors induced by adenovirus type 12 and induced by SV 40, after elimenating necrotic tissues, were assembled, minced, and suspended in 0.25 M cold sucrose solution containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.6 at 0°C before centrifugation. The suspension was blended by homoblender for 30 seconds, transferred to a glass tube. A loosely-fitted glass pestle of the homogenizer was rotated, and the glass tube was moved up and down over the pestle, and then a tightly-fitted teflon pestle was rotated and the glass tube was moved up and down over the pestle the same as before. The homogenized suspension was filtered through tetron cloth of double layer. The filtrate was centrifuged at 700 x g for 10 minutes. By removing 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, then 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 (mitochondrial 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.

c) *Normal and tumor-bearing animal liver mitochondria:* Normal and tumor-bearing animal liver mitochondria were prepared by the same method previously reported (32).

The other fractions were prepared using the same methods previously reported (31). All the preparations were done at 0°—4°C.

White adult male rabbits weighing about 2 to 3 kg were used for the purpose of immunization,

*The immunizing procedure:* Subcutaneous inoculation method was used. Each rabbit was given 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 in the middle between both shoulder blades.

*Serum preparation method:* Rabbit blood samples were collected from the auricular vein on the 14th to 20th day after the last inoculation. 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. However, the quantity of the mitochondria of tumors induced by adenovirus type 12 and induced by SV 40, was insufficient to immunize the rabbit fully, so the pooled sera of tumor-bearing

hamster were used for the immuno-reactions.

*The determination of antigen-antibody reaction :* Ouchterlony agar double diffusion method was 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 of 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 (36)).

An antiserum was poured into the central well and each test antigen was poured into six 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. The samples were set at room temperature for one hour, then changing the temperature of 0° to 4°C and left overnight. After twenty-four hours, 48 hours and 72 hours, the reaction was observed and photographed. Only the photographs taken at 72 hours were used in this experiment for the comparison with others.

## RESULTS

As the result of the cross-metching the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria (anti-AHMt serum) against the mitochondria, microsomes, cell homogenate, and the supernatant (supernatant of 105, 000 x g, 30 min.) of SR-C3H mouse ascites sarcoma, and the mitochondria and the microsomes of SR-C3H sarcoma-bearing mouse liver cells in agar gel diffusion, it became evident that this antiserum reacted only of SR-C3H sarcoma mitochondria, but it did not react on the microsomes, the supernatant of SR-C3H, and the mitochondria and the microsomes of SR-C3H-bearing mouse liver cells (Fig. 1 & Photo. 1)

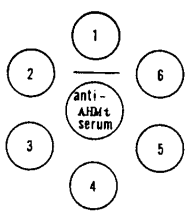


Fig. 1 The result of Ouchterlony central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. 1 : SR-C3H sarcoma mitochondria. 2 : SR-C3H sarcoma microsomes. 3 : SR-C3H cell homogenate. 4 : the supernatant of 105,000 × g, 30 min. of SR-C3H sarcoma. 5 : SR-C3H sarcoma-bearing mouse liver mitochondria. 6 : SR-C3H sarcoma-bearing mouse liver microsomes.

1

In the cross-matchings in agar gel diffusion of the rabbit-antiserum to SR-C3H mouse ascites sarcoma cell mitochondria (anti-SRC3HMt

serum) against the mitochondria, microsomes, cell homogenate, and the supernatant (sup. of 105,000×g, 30 min.) of SR-C3H sarcoma cells, and the mitochondria and the microsomes of SR-C3H sarcoma-bearing mouse liver cells, it was also clear that only the mitochondria of the mouse sarcoma cells react on this antiserum, forming the clear sharp precipitin band, and other fractions did not respond at all. But the cell homogenate of SR-C3H sarcoma cells slightly reacted on this serum, forming a weak precipitin band (Fig. 2 & Photo. 2).

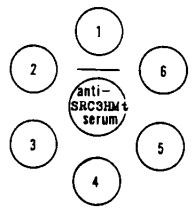


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Fig. 2 The result of Ouchterlony central well contains the rabbit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. 1: SR-C3H sarcoma mitochondria. 2: SR-C3H sarcoma microsomes. 3: SR-C3H cell homogenate. 4: the supernatant of 105,000×g, 30 min. of SR-C3H sarcoma, 5: SR-C3H sarcoma-bearing mouse liver mitochondria. 6: SR-C3H sarcoma-bearing mouse liver microsomes.

By cross-matching the pooled sera of hamsters bearing tumor induced by adenovirus type 12 to the mitochondria, microsomes, cell homogenate, and the supernatant (sup. of 105,000 x g, 30 min.) of SR-C3H mouse ascites sarcoma cells, and the mitochondria and the microsomes of liver cells of mouse bearing SR-C3H ascites sarcoma in agar gel diffusion, it became clear as shown in Fig. 2 that only the mitochondria of this sarcoma reacted on this pooled serum, forming a clear sharp precipitin band, and none of the other fractions reacted at all. But the homogenate of SR-C3H ascites sarcoma cells slightly reacted on this serum due to the existence of the mitochondria in cell homogenate (Fig. 3).

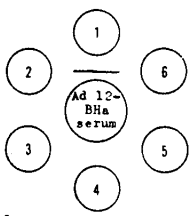


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Fig. 3 The result of Ouchterlony central well contains the pooled sera of hamsters bearing tumor induced by adenovirus type 12. Surrounding wells contain various antigens as illustrated below. 1: SR-C3H sarcoma mitochondria. 2: SR-C3H sarcoma microsomes. 3: SR-C3H cell homogenate. 4: the supernatant of 105,000×g, 30 min. of SR-C3H sarcoma. 5: SR-C3H-bearing mouse liver mitochondria. 6: SR-C3H-bearing mouse liver microsomes.

In the cross-matchings in agar gel diffusion of the pooled sera of hamsters bearing tumor induced by SV 40 virus to the mitochondria, microsomes, cell homogenate, and the supernatant (sup. of 105,000×g, 30 min.) of SR-C3H mouse ascites sarcoma cells, and the mitochondria and the microsomes of liver cells of mouse bearing SR-C3H ascites sarcoma, it became evident as Figs. 2 and 3 that only the mitochondria of this

sarcoma cell reacted (Fig. 4).

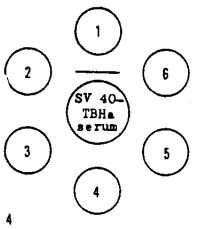


Fig. 4 The result of Ouchterlony central well contains the pooled sera of hamsters bearing tumor induced by SV 40. Surrounding wells contain various antigens as illustrated below. 1: SR-C3H sarcoma mitochondria. 2: SR-C3H sarcoma microsomes. 3: SR-C3H cell homogenate. 4: the supernatant of 105,000 x g, 30 min. of SR-C3H sarcoma. 5: SR-C3H sarcoma-bearing mouse liver mitochondria. 6: SR-C3H sarcoma-bearing mouse liver microsomes.

From Figs. 1, 2, 3, and 4, specific tumor antigenicity is contained in the tumor cell mitochondria, which is common to various kinds of tumors induced artificially in animals, but it has no connection with the kinds of viruses as the carcinogens in precipitin reactions. This specific antigenicity of the mitochondria of tumor cells induced by viruses seems to be the same as that existed in the mitochondria of tumors induced by chemical carcinogens. So this tumor-specific mitochondrial antigenicity can possibly be analysed more precisely in agar gel diffusion.

As the result of cross-matching in agar gel diffusion of the rabbit-antiserum to AH130 rat ascites hepatoma cell mitochondria (anti-HMt serum) against AH130 hepatoma mitochondria, SR-C3H sarcoma mitochondria, the mitochondria of hamster tumor induced by adenovirus type 12, the liver mitochondria of rat bearing AH130 hepatoma, of hamster bearing tumor induced by adenovirus type 12, and of mouse bearing SR-C3H sarcoma, this serum reacted on AH130 mitochondria most remarkably, SR-C3H sarcoma mitochondria slightly, and the mitochondria of hamster tumor induced by adenovirus type 12 remarkably, but did not react on the liver mitochondria of these tumor-bearing body (Fig. 5 & Photo. 3). It

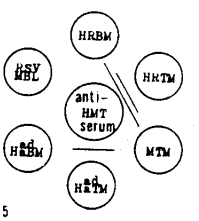


Fig. 5 The result of Ouchterlony central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various a ntigens as illustrated below. HRTM: AH130 hepatoma mitochondria. MTM: SR-C3H sarcoma mitochondria. adHaTM: adenovirus type 12-induced hamster tumor mitochondria. HRBM: AH130 hepatoma-bearing rat liver mitochondria. SRVMBL: SR-C3H sarcoma-beariug mouse liver mitochondria. adHaBM: adenovirus type 12-iuduced tumor-bearing hamster liver mitochondria.

is considered that specific tumor antigenicity of tumor mitochondria does not exist in the organ cell mitochondria of tumor-bearing body.

By the cross-reactions in agar gel diffusion of the rabbit-antiserum to AH130 rat ascites hepatoma cell mitochondria (anti-HMt serum) against human liver mitochondria, beef heart mitochondria, and rat liver mitochondria, this antiserum reacted only slightly on rat liver mitochondria,

forming the precipitin band faintly, but both human liver mitochondria and beef heart mitochondria had no reaction to this serum. This shows that the organ mitochondria-specific antigenicities remain slightly in the mitochondria of AH130 rat ascites hepatoma (Fig. 6 & Photo. 4).

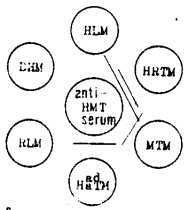


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Fig. 6 result of Ouchterlony central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HRTM: AH130 hepatoma mitochondria. MTM: SR-C3H sarcoma mitochondria. ad HaTM: adenovirus type 12-induced hamster tumor mitochondria. HLM: human liver mitochondria. BHM: beef heart mitochondria. RLM: rat liver mitochondria.

By the cross-matchings in agar gel diffusion of the rabbit-antiserum to SR-C3H mouse ascites sarcoma cell mitochondria (anti-SRC3HMt serum) against AH130 hepatoma mitochondria, SR-C3H sercoma mitochondria, the mitochondria of hamster tumor induced by adenovirus type 12, rat liver mitochondria, liver mitochondria of the mouse bearing SR-C3H sarcoma, and of the hamster bearing tumor induced by adenovirus type 12, this antiserum reacted remarkably on SR-C3H sarcoma mitochondria, AH130 hepatoma mitochondria and the mitochondria of hamster tumor induced by adenovirus type 12, forming a clear sharp remarkable precipitin band to those mitochondria. This fact shows that some tumor mitochondria-specific tumor antigenicity common to chemical carcinogen-induced tumor, RNA virus-induced tumor, and DNA virus-induced tumor, would surely exist in tumor cell mitochondria (Fig. 7 & Photo. 5).

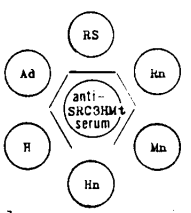


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Fig. 7 The result of Ouchterlony central well contains the rabbit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. RS: SR-C3H sarcoma mitochondria. Ad: adenovirus type 12-induced hamster tumor mitochondria. H: AH130 rat ascites hepatoma mitochondria. Rn: AH130 hepatoma-bearing rat liver mitochondria. Mn: SR-C3H sarcoma-bearing mouse liver mitochondria. Hn: adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

From the results of cross-matching in agar gel of the rabbit-antiserum to SR-C3H sarcoma mitochondria (anti-SRC3HMt serum) against the liver mitochondria of rat bearing AH130 hepatoma, of mouse bearing SR-C3H sarcoma and of hamster bearing tumor induced by adenovirus type 12, neither AH130 bearing-rat liver mitochondria nor adenovirus type 12-induced tumor-bearing hamster liver mitochondria reacted on this serum, but only the liver mitochondria of SR-C3H sarcoma-bearing mouse dis-

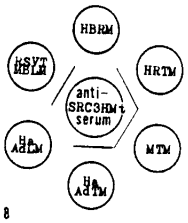


Fig. 8 The result of Ouchterlony central well coutains the rabbit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. HaAdTM : adenovirus type 12-induced hamster tumor mitochondria. HBRM : AH130 hepatoma-bearing rat liver mitochondria. RSVTBMLM : SR-C3H sarcoma-bearing mouse liver mitochondria. HaAdLM : adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

tinctly reacted on this serum (Fig. 8 & Photo. 6). This fact is thought to be natural because the organ- or tissue-specific antigens of mouse remain in SR-C3H sarcoma.

By cross-reactions carried in agar gel diffusion with the pooled sera of hamsters bearing tumor induced by adenovirus type 12 (ad-12BHa serum) to AH130 hepatoma mitochondria, SR-C3H sarcoma mitochondria, the mitochondria of hamster tumor induced by adenovirus type 12, and the liver mitochondria of the rat bearing AH66F ascites hepatoma, of the mouse bearing SR-C3H sarcoma, and of the hamster bearing tumor induced by adenovirus type 12, it was clear that this serum reacted on AH130 hepatoma mitochondria, SR-C3H sarcoma mitochondria, and adenovirus type 12-induced hamster tumor mitochondria most remarkably, and reacted on the liver mitochondria of the hamster bearing tumor induced by adenovirus type 12 very slightly, making a clear sharp precipitin band remarkably, but did not react at all on the liver mitochondria of the mouse bearing SR-C3H sarcoma (Fig. 9 & Photo. 7). These findings

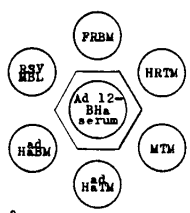


Fig. 9 The result of Ouchterlony central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 rat ascites hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. adHaTM : adenovirus type 12-induced hamster tumor mitochondria. FRBM : AH66F hepatoma-bearing rat liver mitochondria. RSVMBL : SR-C3H sarcoma-bearing mouse liver mitochondria. adHaBM : adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

demonstrate the presence of tumor mitochondria-specific tumor antigenicity common to tumors of various kinds of various strains.

As the result of cross-matching in agar gel diffusion the ad-12BHa serum to rat liver mitochondria, mouse liver mitochondria, human liver mitochondria, the spleen mitochondria of the same human, and the liver mitochondria of hamster bearing tumor induced by SV 40 and of mouse bearing SR-C3H sarcoma, it became clear that these organ mitochondria of various kinds and the liver mitochondria of hamster bearing tumor

induced by SV 40 did not react at all on such pooled sera of hamsters bearing tumor induced by adenovirus type 12 (Fig. 10 & Photo. 8). However, it was obvious that the liver mitochondria started to react on this serum, when the precipitin reaction in agar gel set for 120 hours or more (Fig. 11 & Photo. 9). This result suggests a possible existance of the cancer

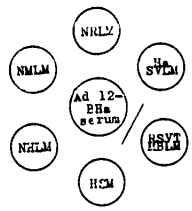

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Fig. 10 The result of Ouchterlony central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. NRLM: rat liver mitochondria. NMLM: mouse liver mitochondria. NHLM: human liver mitochondria. HSM: human spleen mitochondria. HaSVLM: SV 40-induced tumor-bearing hamster liver mitochondria. RSVTBMLM: SR-C3H sarcoma-bearing mouse liver mitochondria.

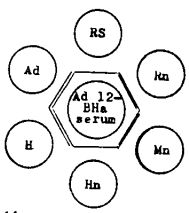

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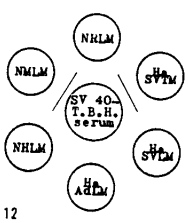
Fig. 11 The result of Ouchterlony central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. RS: SR-C3H sarcoma mitochondria. Ad: adenovirus type 12-induced hamster tumor mitochondria. H: AH130 hepatoma mitochondria. Rn: rat liver mitochondria. Mn: mouse liver mitochondria. Hn: hamster liver mitochondria.

mitochondria-specific cancer antigen-like substance in organ mitochondria, or a possible disappearance of organ specific antigenicities in organ mitochondria of tumor-bearing body.

By the cross-matchings in agar gel diffusion of the pooled sera of hamsters bearing tumor induced by SV 40 (SV 40-TBHa serum) to rat liver mitochondria, mouse liver mitochondria, human liver mitochondria, SV 40-induced hamster tumor mitochondria, and the liver mitochondria of hamsters bearing tumor induced by SV 40 and of hamsters bearing tumor induced by adenovirus type 12, it became evident that SV 40-tumor mitochondria reacted on this serum, forming a clear sharp precipitin band remarkably, and other antigens did not react at all (Fig. 12 & Photo. 10). In this instance, it is natural that SV40-tumor mitochondria react on this

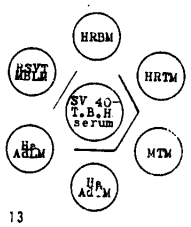


12

Fig. 12 The result of Ouchterlony central well contains pooled sera of SV 40-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. NRLM: rat liver mitochondria. NMLM: mouse liver mitochondria. NHLM: human liver mitochondria. HaSVTM: SV 40-induced hamster tumor mitochondria. HaSVLM: SV 40-induced tumor-bearing hamster liver mitochondria. HaAdLM: adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

serum, but a question still remains unsolved why mouse liver mitochondria react on this serum.

As the result of cross-matchings in agar gel diffusion the pooled sera of hamsters bearing tumor induced by SV 40 to AH130 rat ascites hepatoma mitochondria, SR-C3H ascites sarcoma mitochondria, the mitochondria of hamster tumor induced by adenovirus type 12, and the liver mitochondria of rat bearing AH130 hepatoma, of mouse bearing SR-C3H sarcoma, and hamster bearing tumor induced by adenovirus type 12, it was clear that the tumor mitochondria of these kinds reacted on such serum remarkably, forming a clear sharp precipitin band most remarkably. This facts show that cancer-specific common antigenicity exists in cancer mitochondria of various kinds as shown in Figs. 7, 8, 9, and 11. In this instance, however, a question still remains unsolved why the liver mitochondria of mouse bearing SR-C3H sarcoma react on this serum (Fig. 13 & Photo. 11).



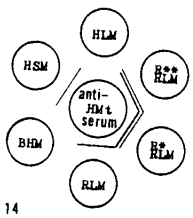

13

Fig. 13 The result of Ouchterlony central well contains pooled sera of SV 40-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 rat ascites hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. HaAdTM : adenovirus type 12-induced hamster tumor mitochondria. HRBM : AH130 hepatoma-bearing rat liver mitochondria. RSVTBLM : SR-C3H sarcoma-bearing hamster liver mitochondria. HaAdLM : adenovirus type 12-induced tumor-bearingahmster liver mitochondria.

It seems that cancer mitochondria-specific cancer antigenicity existing in the mitochondria of tumor induced by chemical carcinogens, would also exist in the mitochondria of tumors induced by viruses. These findings indicate that it is necessary to investigate what relation the antigenicity of cancer cell mitochondria would have with the mitochondria of those relatively immatured and fast dividing cells. But as it was very difficult to prepare the immature germ cells, the mitochondria of regenerated rat livers on the second and the 14th day after partial hepatectomia were used for this purpose.

From the result of cross-matching in agar gel diffusion the rabbit-antiserum to AH130 hepatoma mitochondria (anti-HMt serum) against human liver mitochondria, the spleen mitochondria of the same human, beef heart mitochondria, rat liver mitochondria, and the mitochondria of regenerated rat liver on the second and the 14th day after partial hepatectomia, the regenerated rat liver mitochondria reacted slightly on this serum, forming a slight weak precipitin band. It is natural that the regenerated rat liver mitochondria have the strong antigenicity common to rat

liver mitochondria, but it is very interesting that the regenerated rad liver mitochondria show the slight antigenicity common to AH130 rat ascites hepatoma mitochondria (Fig. 14 & Photo. 12). This is a question which requires further investigations.




14

Fig. 14 The result of Ouchterlony central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HLM: human liver mitochondria. HSM: human spleen mitochondria. BHM: beef heart mitochondria. RLM: rat liver mitochondria. R*RLM: the mitochondria of regenerated rat liver on the 2nd day after partial hepatectomia. R**RLM: the mitochondria of regenerated rat liver on the 14th day after partial hepatectomia.

## DISCUSSION

There are very many reports suggesting that the specific cancer antigenicity exists definitely in cancer. Recently, ZILBER (7) reported that the specific cancer antigens exist in carcinomas induced by chemical carcinogens as proven by the anaphylaxic reactions, and PREHN (1960) (37), (1962) (38), and others (39, 40) reported that the artificial neoplasma acquired new antigenicities. TAKAYANAGI (1964) (41), (1966) (42) reported that several specific cancer antigens were found in $\alpha_1$ to $\beta_2$ globulin position by immunoelectrophoretical analyses of the extracted proteins of DAB-hepatoma, and DECKER and his collaborator (1966) (43) also reported the existence of the specific cancer antigens in rat hepatomas by immunodiffusional and immunoelectrophoretical analyses of various tumors, and their results of immunoelectrophoresis were similar to those of TAKAYANAGI (41, 42). HIRAI (1966) (44) found the specific cancer antigen in one of the rat ascites hepatomas and succeeded in crystallizing this antigen.

With a greater attention focused on the intracellular localization of specific cancer antigens, RAPPORT *et al.* (2), HORN (3—5), TAKEDA *et al.* (6), KIKUCHI (8), and HOKO (9) reported that the cancer cell mitochondria were the strongest antigenic constituent in the cancer cell. Further, with an idea that viruses might be one of the causes of cancers, immunofluorescent and electron microscopic studies have gained the momentum to look for in cancer the antigens originating from a virus. Especially, since the T-antigen was found to be associated with viruses, the investigations in this field were intensified. HUEBNER and his collaborators (11) reported that adenovirus specific antigen existed in the nucleus of virus free hamster tumor and rat tumor by immunofluorescent techniques; BLACE and his collaborators (13) proved the existence of T-antigen originating from SV

40 in tumor cell induced by SV 40, by immunofluorescent technique; GILDON and his collaborators (19) stated that in SV 40-induced tumors, T-antigen existed in both nucleus and cytoplasm immunofluorescently; POPE and his collaborators (15) also reported that T-antigen originated from SV 40 exists in SV 40-transformed cell; RAPP and his collaborators (17) investigated precisely the T-antigen of SV 40 virus and of adenovirus by the immunofluorescent method; and FOGEL and his collaborator (23) proved the existence of T-antigen originated from polyoma virus in tumor cell induced by polyoma virus. Moreover, the movements became active to investigate T-antigen more precisely at subcellular level. MORGAN and his collaborators (24) proved electron microscopically the presence of intracellular viral antigen; BLACK and his collaborator (25) the induction of T-antigen by SV 40-DNA; KALNINS and his collaborators (26, 27) reported a possible existence of virus-associated-antigen originated from adenovirus type 12; and investigated electron microscopically the localization of T-antigen in adenovirus type 12-induced tumor cell precisely; LEVINTHAL and his collaborators (28, 29) investigated in view of the T-antigen of adenovirus and of SV 40 by ferritin antibody technique; and OSHIRO and his collaborators (30) the localization of SV 40 viral antigen in tumor cell induced by SV 40 by the ferritin antibody technique precisely. Now, those investigators working on viral tumors, would only search for the conditions under which the viral genome as carcinogen might be incorporated into cell, while those investigators working on the tumors induced by chemical carcinogens do not take the participation of viruses into consideration. It will be one way to acertain the characteristics of cancer, to investigate what immunochemical changes take place in cancer cell regardless of whether the cause of cancer is a virus, chemical substance, or physical stimulant.

The author holds the opinion that the changes of the cell itself, the cell transformation to neoplasma, woud occur irrespective of the kinds of carcinogens, and almost the same changes will be expected immunochemically to all tumors. If the tumors are different in the immunochemical nature by differences of carcinogenesis, it may be no exaggeration to say that we cannot open the way to destroy all the cancers with the same way. Fortunately, in the previous report (31), it is stated that the highest antigenic constituent in membrane systems of cancer cell was the mitochondria; and in another report (32), this cancer mitochondria-specific cancer antigenicity is common to four different kinds of ascites tumors induced by chemical carcinogens and the rat, immunized with the mitochondria of rat ascites hepatoma, rejects the transplantation of the fresh hepatoma

cells. From these facts, it may be thought that a malignant transformed cell was already changing to the cell which was made by a mould so-called "cancer" and which had the same antigenic substances partially common to each other, if its cause to cell transformation is a virus. In the basis of this, an attention is drawn to the mitochondria of the tumors induced by viruses in the present report as well in previous reports.

As in obvious from the Figs. 1, 2, 3, and 4, each mitochondrion of rat ascites hepatoma induced by chemical carcinogens, of hamster tumors induced by adenovirus and SV 40, and of mouse ascites sarcoma induced by Rous sarcoma virus, has a strong tumor specificity and this tumor mitochondria-specific tumor antigenicity is common to all the tumors of various kinds. Up to date, no investigators have accepted the existence of tumor-specific common antigen in tumors, except USUBUCHI *et at.* (45), reporting that the existence of tumorspecific common antigen might be thought in order to understand the tumor immunity; and DECKER *et al.* (43), about the presence of specific common cancer antigen in various kinds of rat hepatomas. On the basis of the findings obtained in the present experiment, it may be reasonable to assert the existence of common antigenicity in various kinds of tumors cell mitochondria. It was reported in a previous report that the organ mitochondria-specific antigenicity of tumor-bearing body was shown in the mitochondria. Thorough-going investigations of the organ mitochondria-specific antigenicity of animals bearing tumor induced by viruses or chemical carcinogens have revealed that the liver mitochondria of AH130 hepatoma-bearing rat show the antigenicity common to AH130 hepatoma mitochondria slightly but no antigenicity common to the liver mitochondria of tumor-bearing body of other strain, and SR-C3H sarcoma-bearing mouse liver mitochondria exhibit the antigenicity common to SR-C3H sarcoma mitochondria but no antigenicity common to the liver mitochondria of tumor-bearing body of other strain, and adenovirus type 12-induced tumor-bearing hamster liver mitochondria also show the antigenicity common to the mitochondria of hamster tumor induced by adenovirus type 12 slightly but no antigenicity common to the liver mitochondria of tumor-bearing body of other strain, and SV 40-induced tumor-bearing hamster liver mitochondria also show the antigenicity common to SV 40-induced hamster tumor mitochondria but no antigenicity common to the liver mitochondria of tumor-bearing body of other strain. It is obscure whether the same antigen to cancer cell mitochondria exists in organ mitochondria of tumor-bearing body, or the ascites tumor cells infiltrate into the liver because of situated the same abdominal cavity, judging from the facts that the liver mitochondria of

mouse bearing SR-C3H sarcoma often show the antigenicity common to other cancer cell mitochondria and the common antigenicity is chiefly found in the liver of ascites tumor-bearing body, or whether cancerous changes would take place in the organs of tumor-bearing body by metastasis or cancerous transformation in the organ itself. In this field, DECKER and his collaborator (43) and others (46—53) reported about the antigenicities of the organs of tumor-bearing body. All of them only stated that the original antigenicities of organ itself diminished at the cancerous transformation of cells, and there are few precise reports dealing with the antigenicities of tumors induced by chemical carcinogens as well as of the tumors induced by viruses especially in mitochondria.

If the antigenic differesnces between normal and cancerous tissue do not exist, it will not be possible to take adequate measures to cancer immunochemically. It is very significant that specific tumor antigens are present and this is the fact that many researchers have searched for them. In the present series of studies, any specific cancer mitochondrial antigenicity has not yet been demonstrated in normal cell mitochondria. It is recognized that the cancer cell itself has already lost organ-antigenicity at malignant transformation, and the specific tumor antigenicity has replaced the original antigenicity. Since the mitochondria of tumors induced by viruses show the strong antigenicity common to chemical carcinogen-induced tumor mitochondria, it may be thought that the tumor mitochondria-specific antigenicity common to all the tumor mitochondria will exist over the strains of animals, but it is a question requiring the further investigations if this specific antigenicity of tumor mitochondria might exist commonly in the mitochondria of undifferentiated immature cells.

## SUMMARY

The mitochondrial, the microsomal, and the supernatant fractions were prepared from the cell homogenate of tumors induced by viruses, such as adenovirus type 12, SV 40, and Rous sarcoma virus, etc. and the antigenicities of these fractions were investigated. In the virus-induced tumors, there existed no antigenicity common to the mitochondrial and the microsomal fractions as in the tumors induced by chemical carcinogens, and the highest antigenicity was recognized in the mitochondrial fraction. Therefore, the properties of the tumor cell mitochondria were precisely investigated with virus-induced tumor mitochondria.

1. The mitochondria of tumors induced by viruses have clearly the specific antigenicity.

2. This specific antigenicity of virus-induced tumor mitochondria is common to all the virus-induced tumors used in the present study.

3. This tumor mitochondria-specific antigenicity is found commonly in all the tumor mitochondria in the present experiments.

4. The specific cancer antigenicity of tumor cell mitochondria does not exist in normal organ mitochondria, but the regenerating organ mitochondria exhibit a slight antigenicity common to cancer cell mitochondria.

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## LEGENDS FOR PHOTOGRAPHS

The photographs are the results obtained by Ouchterlony agar double diffusion test.

Photo. 1 Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. 1 : SR-C3H sarcoma mitochondria. 2 : SR-C3H sarcoma microsomes. 3 : SR-C3H cell homogenate. 4 : the supernatant of SR-C3H sarcoma. 5 : SR-C3H sarcoma-bearing mouse liver mitochondria. 6 : SR-C3H sarcoma-bearing mouse liver microsomes.

Photo. 2 Central well contains the rabdit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. 1 : SR-C3H sarcoma mitochondria. 2 : SR-C3H sarcoma microsomes. 3 : SR-C3H cell homogenate. 4 : the supernatant of 105,000 x g, 30 min. of SR-C3H sarcoma. 5 : SR-C3H-bearing mouse liver mitochondria. 6 : SR-C3H-bearing mouse liver microsomes.

Photo. 3 Central well contains the rabbit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. RS : SR-C3H sarcoma mitochondria. Ad : adenovirus type 12-induced hamster tumor mitochondria. H : AH130 rat ascites hepatoma mitochondria. Rn : AH130 hepatoma-bearing rat liver mitochondria. Mn : SR-C3H sarcoma-bearing mouse liver mitochondria. Hn : adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

Photo. 4 Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. adHaTM : adenovirus type 12-induced hamster tumor mitochondria. HRBM : AH130 hepatoma-bearing rat liver mitiochondria. SRVMBL : SR-C3H sarcoma-bearing mouse liver mitochondria. adHaBM : adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

Photo. 5 Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. adHaTM : adenovirus type 12-induced hamster tumor mitochondria. HLM : human liver mitochondria. BHM : beef heart mitochondria. RLM : rat liver mitochondria.

Photo. 6 Central well contains the rabbit-antiserum to SR-C3H sarcoma mitochondria. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 hepatoma mitochondria. MTM : SR-C3H sarcoma mitochondria. HaAdTM : adenovirus type 12-induced hamster tumor mitochondria. HBRM : AH130 hepatoma-bearing rat liver mitochondria. RSVTBMLM : SR-C3H sarcoma-bearing mouse liver mitochondria. HaAdLM : adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

Photo. 7 Central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. HRTM : AH130 hepatoma mitochondria. MTM : SR-C3H sarcoma mitochendria. adHaTM : adenovirus type 12-induced hamster tumor mitochondria. FRBM : AH66F hepatoma-bearing rat liver mitochondria. RSVMBL : SR-C3H sarcoma-bearing mouse liver mitochondria. adHaBM : adenovirus type 12-induced tumor-bearing hamster liver mitochonbria.

Photo. 8 Central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. NRLM : rat liver mitochondria. NMLM : mouse liver mitochondria. NHLM : human liver mitochondria. HSM : human spleen mitochondria. HaSVLM : SV 40-induced

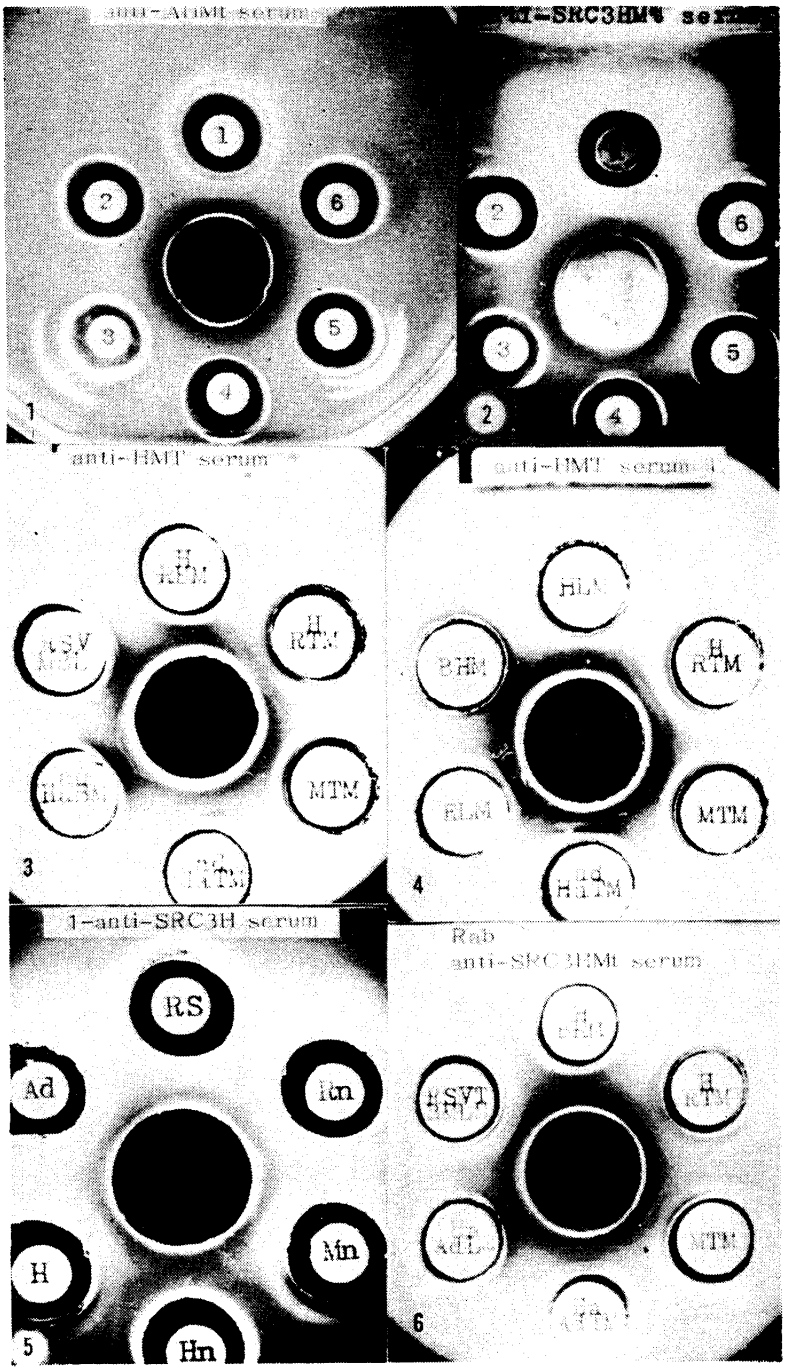
tumor-bearing hamster liver mitochondria. RSVTBMLM: SR-C3H sarcoma-bearing mouse liver mitochondria.

Photo. 9 Central well contains pooled sera of adenovirus type 12-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. RS: SR-C3H sarcoma mitochondria. Ad: adenovirus type 12-induced hamster tumor mitochondria. H: AH130 hepatoma mitochondria. Rn: rat liver mitochondria. Mn: mouse liver mitochondria. Hn: hamster liver mitochondria.

Photo. 10 Central well contains pooled sera of SV 40-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. NRLM: rat liver mitochondria. NMLM: mouse liver mitochondria. NHLM: human liver mitochondria. HaSVTM: SV 40-induced hamster tumor mitochondria. HaSVLM: SV 40-induced tumor-bearing hamster liver mitochondria. HaAdLM: adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

Photo. 11 Central well contains pooled sera of SV 40-induced tumor-bearing hamsters. Surrounding wells contain various antigens as illustrated below. HRTM: AH130 hepatoma mitochondria. MTM: SR-C3H sarcoma mitochondria. HaAdTM: adenovirus type 12-induced hamster tumor mitochondria. HRBM: AH130 hepatoma-bearing rat liver mitochondria. SRVTBLM: SR-C3H sarcoma-bearing mouse liver mitochondria. HaAdLM: adenovirus type 12-induced tumor-bearing hamster liver mitochondria.

Photo. 12 Central well contains the rabbit-antiserum to AH130 rat ascites hepatoma mitochondria. Surrounding wells contain various antigens as illustrated below. HLM: human liver mitochondria. HSM: human spleen mitochondria. BHM: beef heart mitochondria. RLM: rat liver mitochondria. R*RLM: the mitochondria of regenerated rat liver on the 2nd day after partial hepatectomia. R**RLM: the mitochondria of regenerated rat liver on the 14th day after partial hepatectomia.


anti-AHMT serum
1
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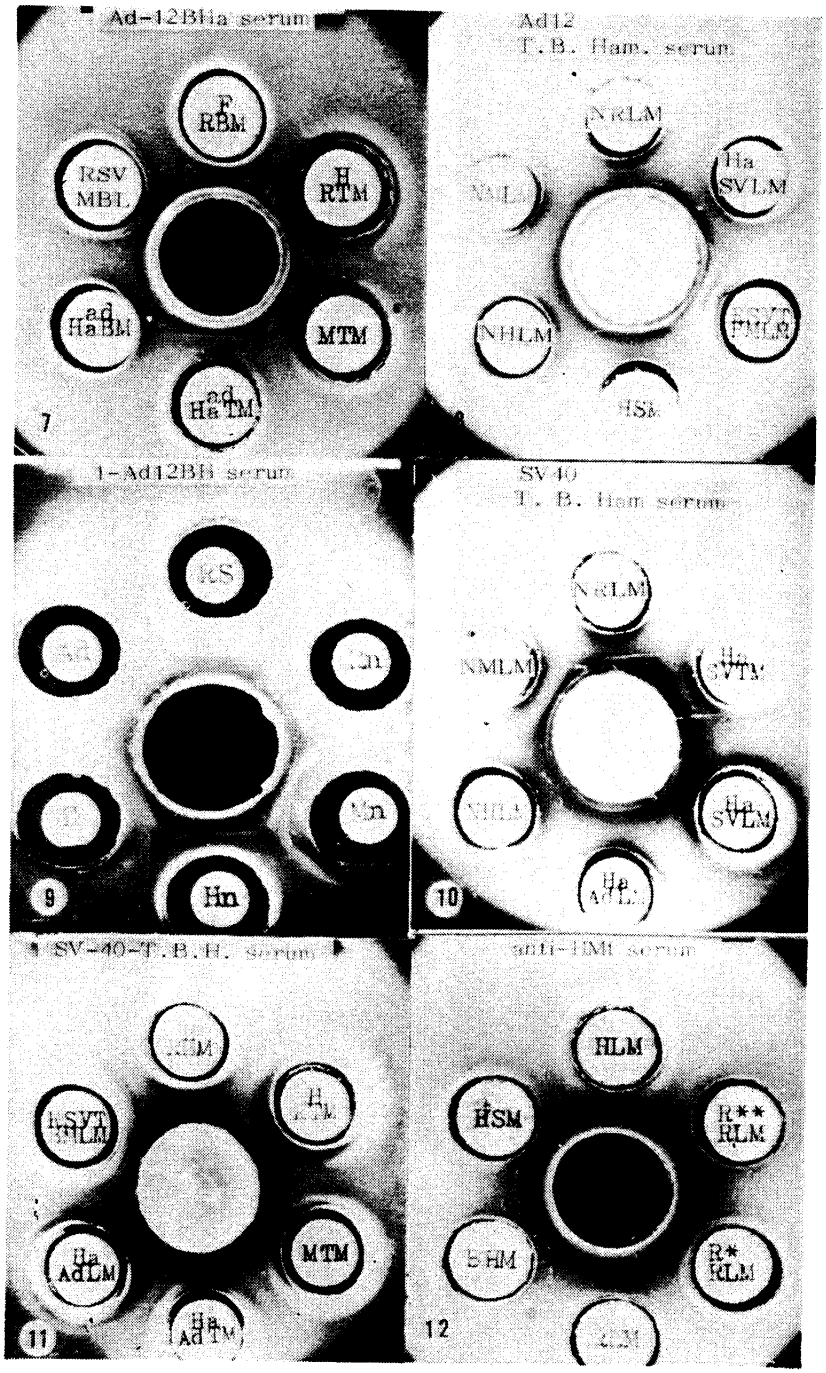
Ad-12BHa serum
RSV MBL
MTM
7
Ad12 T.B. Ham. serum
NRLM
Ha SVLM
NHLM
HSA
1-Ad12BH serum
Hn
9
SV40 T.B. Ham serum
NRLM
NMLM
10
SV-40-T.B.H. serum
MTM
11
HLM
HSM
R** RLM
R* RLM
12