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STUDIES ON LIVER CANCER. II. PRODUCTION OF α-FETOPROTEIN BY EXPERIMENTAL HEPATOMA AND ITS CHARACTERIZATION

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SUMMARY 1) The α-fetoprotein levels in pregnant rats and those after delivery were examined with a monospecific rabbit antiserum against rat α-fetoprotein. In pregnant rats, α-fetoprotein appears in the blood from the 7th day after conception. After delivery it disappears rapidly and is not present in the blood 3 days after delivery. Alpha-fetoprotein was found in the blood of newborn rats until 4 weeks after birth.

2) Ascites hepatoma AH-130 has been considered to be an " α-fetoprotein-negative " hepatoma but α-fetoprotein was found in the blood of rats transplanted with this tumor either subcutaneously or intraperitoneally. Their α-fetoprotein level gradually rose to a maximum shortly before their death.

3) The cross-reactions among rat α-fetoprotein, human α-fetoprotein and mouse α-fetoprotein were examined with a monospecific rabbit antiserum against rat α-fetoprotein by immunodiffusion, immunoelectrophoresis and immunofluorescence but positive results were not obtained.

INTRODUCTION

Abelev et al. (1963) reported the appearance of α-fetotoprotein in the blood of mice after transplantation of a hepato-cellular carcinoma and Stanislawaski-Birencwajg (1967) reported its presence in Wistar AG rat fetuses and in the blood of rats fed 4-methylaminoazobenzene (DAB). Recently, Watabe, Nishi and Hirai (1971) studied α-fetoprotein production by various transplantable strains of rat ascites hepatoma and reported that, unlike other rat ascites hepatoma strains, hepatoma AH-130 does not produce α-fetoprotein. There are many sublines of the ascites hepatoma strain, and all of them, including AH-130, have been induced by feeding DAB to Donryu strain rats. To see whether AH-130 does not actually produce α-fetoprotein, in this work the production of α-fetoprotein by AH-130 hepatoma cells was examined by immunodiffusion, immunoelectrophoresis and immunofluorescence.

Abelev (1968) reported that no cross-reaction between human α-fetoprotein and the mouse α-fetoprotein is detectable by the normal immunodiffusion technique, but that it is detectable by radioimmunodiffusion. Recently, Nishi, Watabe and Hirai (1972) using an immunodiffusion technique, detected an immunological cross-reaction between rabbit antiserum prepared by immunization with human α-fetoprotein and α-fetoprotein in fetal newborn serum of human, dog, horse, rat and rabbit. If this is correct, preparation of anti-α-fetoprotein serum for clinical use using fetal serum of these animals rather than human material would not be difficult. So, in this study the cross-reaction between α-fetoprotein in transplantable ascites hepatoma of rats and mice, which are closely related phylogenetically, was examined by immunodiffusion, immunoelectrophoresis and immunofluorescence. The cross-reactions of human α-fetoprotein with those of rats and mice were also studied.

MATERIALS AND METHODS

1. *Animals*

The rats and mice used in the study were bred in our laboratory. Donryu strain and Sprague-Dawley rats and New Zealand White stock rabbits were used. C3H mice were obtained from Shionogi Lab., Osaka and Oncians France 1 (OF1) mice from Dr. B. Coquet (IFFA-CREDO, Les Oncians, 69, Saint-Germain-sur-L'Arbresle). Both strains of rats and C3H mice were maintained by sister-brother mating.

2. *Transplantable hepatoma cells*

Ascites hepatoma AH-130 was supplied by Dr. Sato of the Sasaki Inst., Tokyo and has been maintained in our laboratory for 74 transplant generations. Rats were inoculated intraperitoneally with 1.4×10^7 AH-130 cells or subcutaneously with 1.4×10^8 AH-130 cells. The incidence of successful subcutaneous transplantations of AH-130 is said to be about 40% (Sato et al. 1971) but in our study transplantation was 100% successful. The survival time of rats with ascites hepatoma AH-130 is about 2 weeks but rats with subcutaneous tumors survived for about 35 days. Transplantation was achieved using cancer cells or cancer milk.

Mouse ascites hepatoma MH-134 was also provided by Dr. Sato and has been maintained in our own laboratory for 25 transplant generations. Mice were inoculated intraperitoneally with 7×10^6 cells of this hepatoma.

3. *Preparation of specific antiserum*

The method reported previously (Koda, Ishigami and Tanabe, 1971) was followed. The ascites, serum and cancer milk of rats and mice transplanted with ascites hepatoma and rat fetal extracts were used as starting materials. After ammonium sulfate fractionation and agar zone electrophoresis, the α-globulin region was collected and used for immunization of New Zealand White rabbits of about 2 kg body weight using incomplete Freund's adjuvant to obtain the crude antiserum. To obtain the specific antiserum this crude antiserum was adsorbed with adult mouse or rat serum which had been made water-insoluble by treatment with glutaraldehyde.

4. *Induction of hepatoma be feeding 4-methylaminoazobenzene (DAB)*

The rats were reared from 8 weeks after birth on MF Feed from Oriental Yeast Co., Tokyo supplemented with 0.06% DAB.

5. *Detection and quantitative determination of α-fetoprotein*

Immunodiffusion, immunoelectrophoresis and single radial immunodiffusion were performed as described previously (Koda et al. 1971).

6. *Immunofluorescent studies*

The method reported previously (Koda et al. 1971) was followed.

RESULTS

1. *Alpha-fetoprotein in Donryu strain rats*

To study the production of α-fetoprotein in hepatoma bearing rats, it is necessary to examine the production of α-fetoprotein in normal, non-tumor bearing Donryu strain rats.
1) Alpha-fetoprotein in pregnant Donryu strain rats

As showin in Fig. 1, α-fetoprotein was detected by immunodiffusion in the serum of rats from the 7th day of pregnancy. During pregnancy it gradually rose to a maximum just

FIGURE 1. *Immunodiffusion picture of serum α-fetoprotein in pregnant and postpartum rats. Serum on 1, day 7; 2, day 8; 3, day 9; 4, day 12; 5, day 19 of pregnancy and 6, day 1; 7, day 2 and day 3 after delivery.*

before delivery, declined rapidly after delivery. Alpha-fetoprotein could no longer be detected in the serum 3 days after delivery.

2) Alpha-fetoprotein in newborn Donryu strain rats

Alpha-fetoprotein was still detectable by immunodiffusion in the serum of rats 28 days after birth (Fig. 2). However, it was no longer detectable on the 35th day after birth. On the 28th day, the mean value of α-fetoprotein determined by single radial immunodiffusion was 550 mg/dl.

So, experiments on cancer induction and transplantation were carried out using rats of more than 35 days of age.

2. *Detection of α-fetoprotein in the sera of Donryu strain rats bearing an AH-130 hepatoma*

When 1.4×10^7 AH-130 cells were transplanted intraperitioneally into Donryu strain rats α-fetoprotein could be detected in the serum by single radial immunodiffusion from the 4th day after inoculation at a mean concn of 9.5 mg/dl. The volume of ascites increased thereafter and the rats died on the 14th day. During this period, the quantity of α-fetoprotein in the serum was almost constant, having a mean value of 400 mg/dl on the 14th day, just before death.

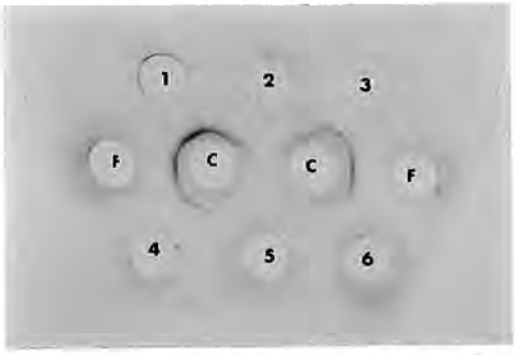


FIGURE 2. *Immunodiffusion test on serum α-fetoprotein in the new born rat serum 1, 1 week; 2, 2 weeks; 3, 3 weeks; 4, 4 weeks; 5, 5 weeks and 6, 6 weeks after birth. F, Fetal extract; C, Purified antiserum against α-fetoprotein rat fetal extract.*

Fig. 3 shows findings on rats inoculated subcutaneously with 1.4×10^8 AH-130 cells. A weak precipitation line was observed on the 5th day and α-fetoprotein was clearly detected on the 8th day. On the 12th day after inoculation, the mean serum α-fetoprotein level was 600 mg/dl and on the 35th day, the mean α-fetoprotein level in cancer milk was 860 mg/dl.

3. *Comparative studies on α-fetoprotein of rats bearing ascitic AH-130 hepatoma and DAB induced hepatoma*

Immunodiffusion patterns are shown in Fig. 4. Specific antiserum of rabbits immunized

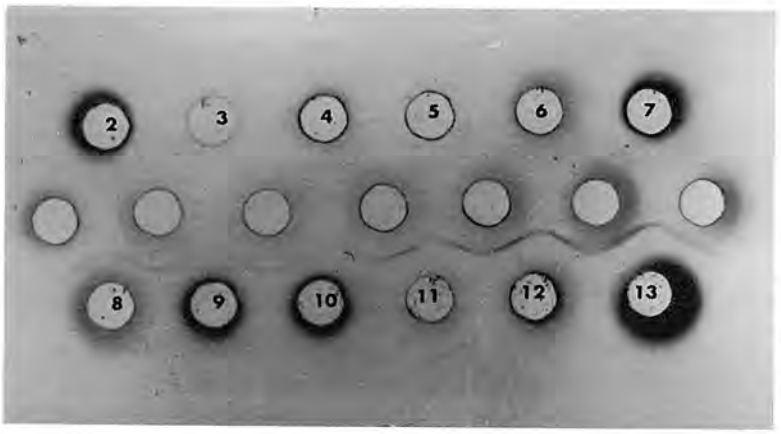


FIGURE 3.
Immunodiffusion test on serum α-fetoprotein of rat bearing AH-130 (solid form). Numbers show days after transplantation.

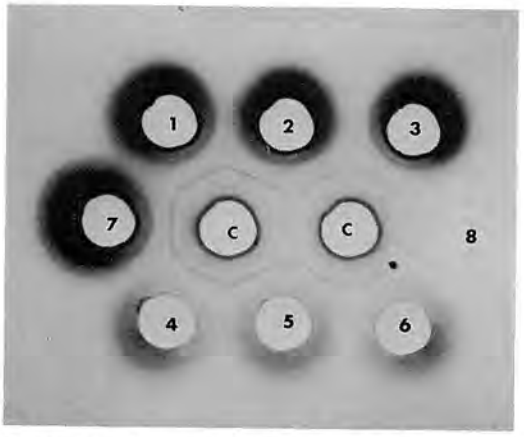


FIGURE 4. *Immunodiffusion test on various α-fetoproteins. 1, 4, Serum and ascites of rat with hepatoma induced by DAB; 2, 5, Serum and cancer milk of rat bearing AH-130 (solid form); 3, 6, Serum and ascites of rat bearing AH-130 (ascitic form); 7, Fetal extract of rat; 8, Normal rat serum; C, Purified antiserum against α-fetoprotein of rat fetal extract.*

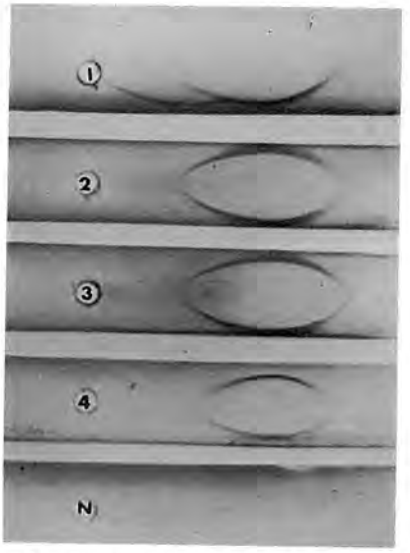


FIGURE 5. *Immunoelectrophoresis of various α-fetoproteins. 1, Fetal extract of rat; 2, Serum of rat with hepatoma induced by DAB feeding; 3, Serum of rat bearing AH-130 (solid form); 4, Serum of rat bearing AH-130 (ascitic form); N, Normal rat serum; Purified antiserum was prepared against α-fetoprotein of rat fetal extract.*

with rat fetal extract was placed in the center well (C). Serum and ascites of a Donryu strain rat with a hepatoma induced by DAB, were placed in wells 1 and 4, respectively. Serum and cancer milk of a rat bearing a solid tumor were placed in wells 2 and 5. Serum and ascites of a rat with a transplanted ascites hepatoma AH-130 were placed in wells 3 and 6, respectively. The fetal extract which had been used as immunogen to obtain antiserum was placed in well 7 and the serum of a healthy mature Donryu strain rat was placed in well 8. Materials in all except well 8 gave a precipitation line indicating an identical reaction and no spur formation was observed. The immunoelectrophoretic patterns of the rat fetal extract and the sera of the DAB-fed rat, the rats bearing solid and ascitic forms of AH-130 hepatoma

and the normal rat are shown in Fig. 5. In this experiment antiserum used was prepared with rat fetal extract. The extract, which showed a single precipitation line by immunodiffusion, gave an additional precipitation line by immunoelectrophoresis. This additional line extended to the β-glubulin region and fused with the main precipitation line appearing in the α-globulin region with spur formation. Therefore, it was necessary to clarify whether this difference in the appearance of the precipitation line was due to a difference between the specific antisera prepared using fetal extract and AH-130 ascites as immunogens. So, the immunoelectrophoretic patterns of the sntisera against the two immunogens were compared. The results are given in Fig. 6. Regardless of the immunogen the specific antisera showed an additional line extending to the β-globulin region against fetal extract. This line fused with the precipitation line in the α-globulin region and showed spur formation with the precipitation line in the β-globulin region. With AH-130 ascites, however, only a precipitation line in the α-globulin region was observed, regardless of the specific antiserum used. In this experiment, the fetal extract used had been kept for 3 months at 4 C. To test whether this affected the results, immuno-

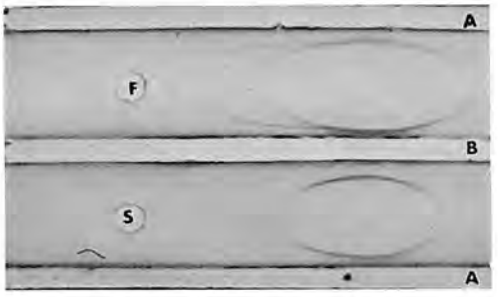
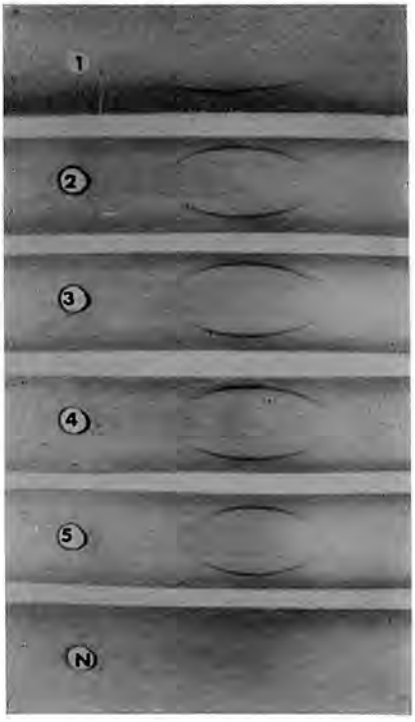


FIGURE 6. *Comparison of rat α-fetoproteins. F, Fetal extract of rat; S. Ascites of rat bearing AH-130; A, Purified antiserum against fetal extract of rat; B, Purified antiserum against AH-130 ascites.*



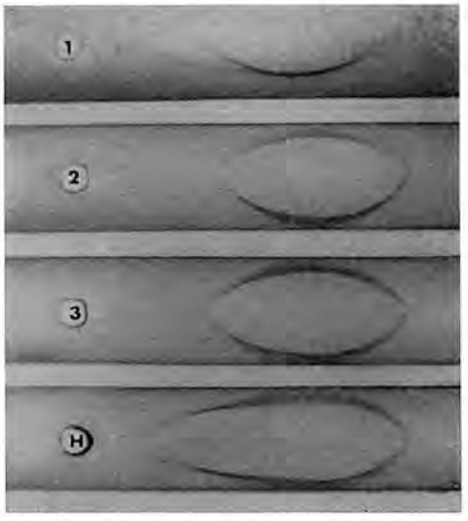


FIGURE 7. *Immunoelectrophoresis of fetal extract of rat (A) and α-fetoprotein of pregnant rat (B). 1, Aged fetal extract of rat; 2, Fresh fetal extract of rat; 3, Fresh fetal extract of rat without liver; 4, Fetal serum of rat in 19th day of pregnancy; 5, Serum of rat in 19th day of pregnancy; N, Normal rat serum; H, Fetal extract heated at 56 C for 30 min. Purified antiserum was prepared against α-fetoprotein of rat fetal extract.*

electrophoresis was carried out with aged and fresh fetal extract, extracts with and without liver and extract which had been heated at 56 C, for 30 min. The results are shown in Figs. 7A and 7B. It can be seen that there is a precipitation line in the α-globulin region with freshly prepared fetal extract, regardless of the presence or absence of liver. After heating at 56 C the β-globulin region became extended but there was no spur formation. When α-fetoprotein of AH-130 ascites was heated, no extension of the line was observed. This phenomenon was not observed with human α-fetoprotein.

Since it was shown that α-fetoprotein was produced by AH-130 cells, the urine of tumor-bearing rats was examined. By immunodiffusion, a single precipitation line, indicating an identical reaction, was obtained with serum, ascites and urine (Fig. 8). Quantitative determination of α-fetoprotein in the serum, ascites and urine by single radial immunodiffusion gave mean values of 110 mg/dl for serum, 221 mg/dl for ascites and 2.2 mg/dl for urine.

4. *Alpha-fetoprotein production by ascites hepatoma AH-130 cells in Sprague-Dawley rats and OF1 mice*

Rat ascites hepatoma AH-130 is usually maintained in Donryu strain rats. To ascertain whether this hepatoma could be transplanted into other stocks and if, so to see whether α-fetoprotein was produced in the latter, 1.4×10^7 AH-130 cells were inoculated intraperitoneally into Sprague-Dawley rats. Tumors grew in these animals as in Donryu strain rats, and animals died after 14 days, and moreover α-fetoprotein was detected in their serum and ascites by immunodiffusion (Fig. 9). Thus ascites hepatoma AH-130 can be maintained not only in the Donryu strain rats but also in Sprague-Dawley rats, where it has been maintained for 19 transplant generations. Furthermore α-fetoprotein is still being produced in these animals.

To examine the species specificity of hepatoma AH-130, OF1 mice were also inoculated intraperitoneally with 7×10^6 rat ascites hepatoma AH-130 cells. Pronounced stagnation

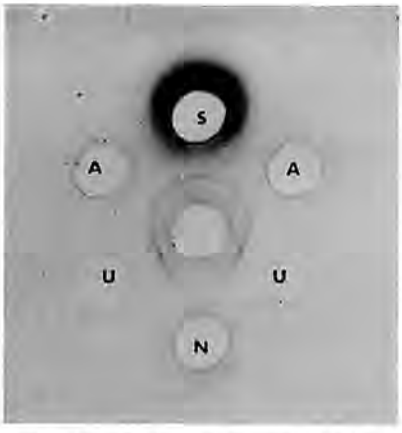


FIGURE 8. *Comparison of immunodiffusion pictures of α-fetoprotein in serum, ascites and urine of rat bearing AH-130 (ascitic form). S, Serum; A, Ascites; U, Urine; N, Normal rat serum; C, Purified anti-serum against α-fetoprotein of AH-130 ascites.*

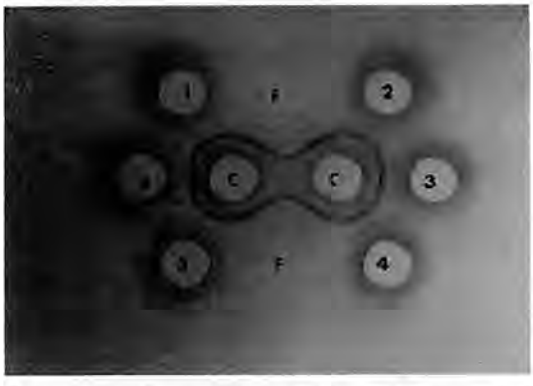


FIGURE 9. *Immunodiffusion picture of serum, and ascites α-fetoprotein of rat and mouse bearing AH-130 (ascitic form). 1, 6, Serum and ascites of Donryu strain rat with transplanted AH-130; 2, 3, Serum and ascites of Sprague-dawley rat with transplanted AH-130; 4, 5, Serum and ascites of OF1 mouse with transplanted AH-130; F, Fatal extract of Donryu strain rat; C, Purified anti-serum against α-fetoprotein of rat fetal extract.*

of ascites was observed in 90% (18/20) of the animals and 5% (1/20) died about 3 weeks after inoculation. In the survived animals, the ascites gradually decreased thereafter. An attempt was made to pass the tumor to the next generation using ascites collected on the 21st day, but the ascites was clear and contained few cells so that passage of the tumor was not possible. The immunodiffusion patterns between the specific antiserum against rat α-fetoprotein and the serum and ascites (23 days after inoculation) of a mouse with a first generation transplant is shown in Fig. 10. Alpha-fetoprotein was detected, but antibody was not found.

5. *Alpha-fetoprotein production by mouse ascites hepatoma MH-134 in mice and rats*

The mouse ascites hepatoma MH-134, is similar to rat ascites hepatoma AH-130, but is not strain specific. It was found to be transplantable and could be maintained in OF1 mice as well as C3H mice. When 7×10^6 MH-134 cells from a C3H mouse were inoculated intraperitoneally into OF1 mice, the tumor grew in them as in C3H mice and animals died with ascites hepatoma formation 10 days after transplantation. The ascites hepatoma could be passed to the next generation of OF1 mice and was maintained for 17 transplant generations in OF1 mice. Intraperitoneal inoculation of 1.4×10^8 MH-134 cells into a Donryu strain rat resulted in death of the animal 10 days after transplantation but the second transplant generation of Donryu strain rats survived for 35 days after transplantation showing an unsuccessful third passage.

No precipitation line could be detected by immunodiffusion between specific antiserum against rat α-fetoprotein and fetal extract of C3H mice, or serum and ascites of a C3H mouse bearing MH-134. Similarly no cross-reaction could be observed with serum and ascites of an OF1 mouse bearing MH-134. The serum of the first generation Donryu strain rat inoculated with mouse ascites hepatoma MH-134 did not give a precipitation line with specific antiserum against rat α-fetoprotein, as shown in Fig. 10. In addition, no precipitation line was observed between serum from a Donryu strain rat with a transplanted MH-134 tumor and that from an OF1 mouse with a transplanted AH-130 tumor.

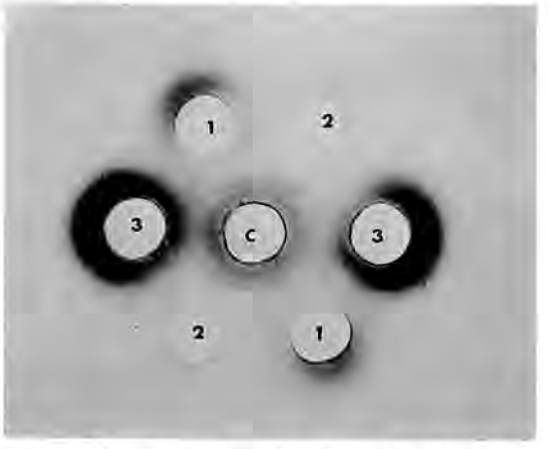
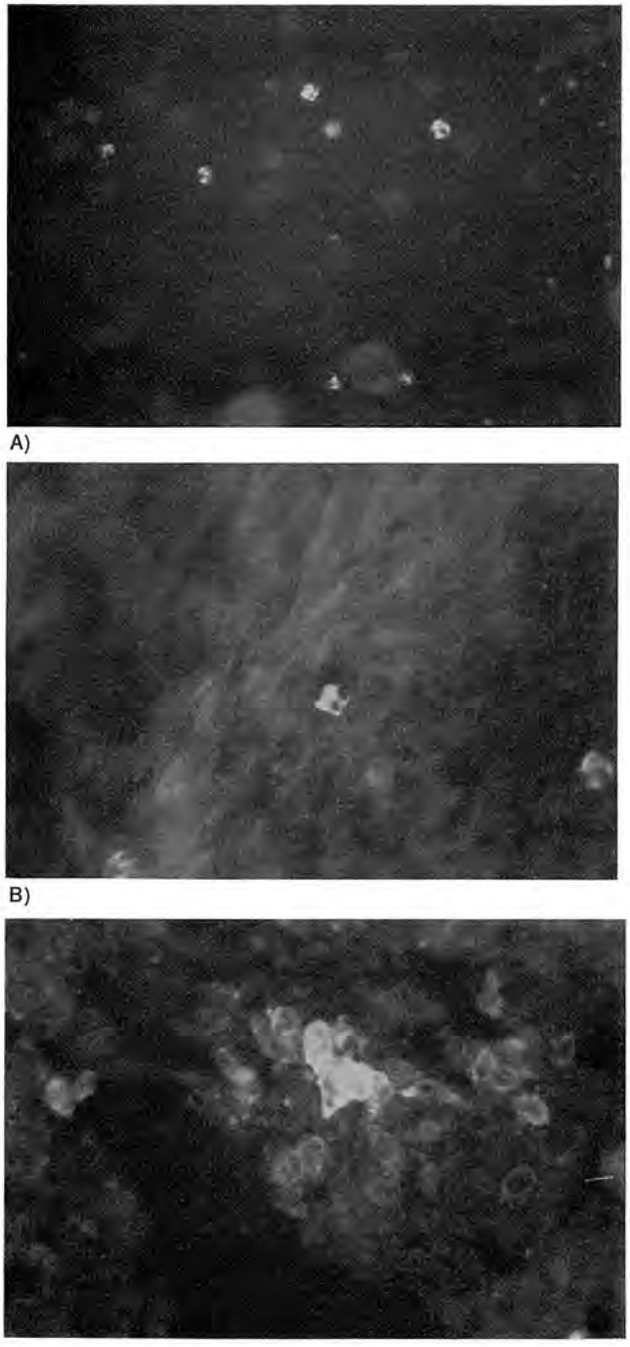


FIGURE 10. *Comparison of α-fetoprotein of rat bearing AH-130 (ascitic form) and mouse bearing MH 134 (ascitic form). 1, Serum of Donryu strain rat with transplanted AH-130; 2, Serum of OF1 mouse with transplanted AH-130; 3, Serum of Donryu strain rat with transplanted MH 134; C, Purified antiserum against α-fetoprotein of AH-130 ascites.*

6. *Immunofluorescent studies on α-fetoprotein in AH-130 heaptoma cells*

As shown in Figs. 11A, B and C, specific fluorescence similar to that observed in patients with primary liver cancer was found in the ascitic and subcutaneous tumor cells of hepatoma AH-130. Tumor cells of a hepatoma induced by DAB also showed specific fluorescence by a monospecific rabbit antiserum against rat α-fetoprotein, as shown in Fig. 12B. These tissues were examined by the direct immunofluorescent method using a fluorescence-labeled specific anti human α-fetoprotein antibody. However, no specific fluorescence was observed. Moreover no specific fluorescence was observed when human liver cancer cells were tested with a fluorescence-labeled anti-rat α-fetoprotein antibody. It was also noted that

on blocking by pretreatment with hetero-specific antiserum followed by treatment with the respective corresponding fluorescent antibody, liver cancer cells showed a specific fluorescence. These findings also suggest the absence of a cross-reaction between rat and human α-fetoproteins.



DISCUSSION

In disagreement with the report of Watabe et al. (1971) we found that AH-130 cells do produce α-fetoprotein. Our finding seems reasonable since asc ites hepatoma AH-130 was induced in rats by feeding DAB, and most hepatoma cells induced by DAB produce α-fetoprotein. We found by immunodiffusion that the α-fetoprotein in the serum and ascites of rats with hepatomas induced by DAB was identical to the α-fetoprotein in rats with transplanted AH-130 tumors. The discrepancy between our results and those of Watabe et al. may be due to a difference in the quality of specific antiserum used. In our experiment, Donryu strain rats were inoculated with 1.4×10^7 AH-130 cells and all the animals died on the 14th day and α-fetoprotein was found in the serum from the 6th day. Watabe et al. (1971) may have examined the serum before the appearance of α-fetoprotein in the blood. Immunofluorescent studies also showed that among the AH-130 cells only a few show specific fluorescence indicating α-fetoprotein production and Watabe et al. (1971) may have inocul-

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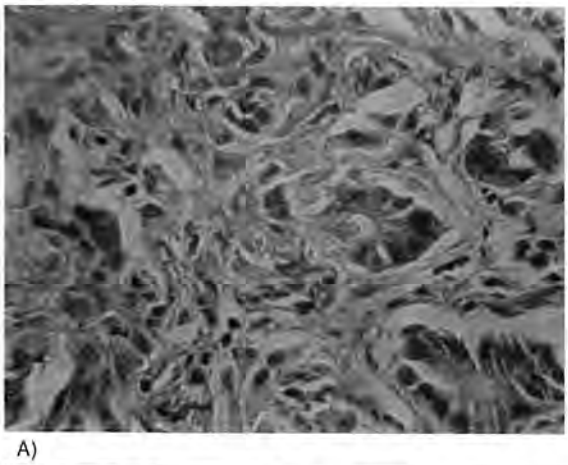
FIGURE 11. *Immunofluorescent picture of AH-130 cells. A, Cells in ascites (magnification × 200); B, Cells in ascites (magnification × 400); C, Cells in solid tumor (magnification × 400).*

▶

FIGURE 12. *Liver of rat after 90 days of DAB administration. A, Histological findings (magnification × 400); B, Immunofluorescent picture (magnification × 400).*

ated too few cells to be able to detect α-fetoprotein.

As shown in Fig. 4, no additional line or spur formation was observed by immunodiffusion between rat fetal α-fetoprotein and either specific antiserum prepared with fetal extract or with AH-130 ascites. But, by immunoelectrophoresis, a precipitation line extending to the β-globulin region and spur formation from the main precipitation line in the α-globulin region were observed. This phenomenon was not observed with fresh fetal extract and, therefore, cannot be explained on the basis of the report of Wise, Ballard and Ezekiel (1963) that rat α-fetoprotein shows two bands on starch gel electrophoresis. A precipitation line occurs in the β-globulin region with carcinoembryonic antigen (CEA) in colon cancer, but the precipitation line seen in our experiments in the β-globulin region may not correspond to this, because 1) the antiserum in this experiment was obtained from the α-globulin region separated by zone electrophoresis, 2) the phenomenon was observed between fetal extract and specific antiserum prepared from α-fetoprotein from AH-130 ascites or fetal extract, and 3) the precipitation line in the β-globulin region fused with the precipitation line in the α-globulin region. These findings suggest that its appearance may be related to storage of the rat fetal extract in the cold. Thus it seems likely that the cause of spur formation from the main precipitation line in the α-globulin region to the precipitation in the β-globulin region may be clarified by immunodiffusion or immunoelectrophoretic analysis of the break-down products of α-fetoprotein produced by various proteases and studies on this are in progress.



A)

B)

When freshly prepared fetal extract had been heated at 56 C for 30 min, its precipitation line extended to the β-globulin region without spur formation. The α-fetoprotein in the serum of pregnant rats and mothers for 1 to 2 days after delivery, which has been transported through the placenta, gave a precipitation line only in the α-globulin region. However, on heating the serum the line extended to the β-globulin region. This agrees with the finding of extension of the precipitation line of α-fetoprotein of Wistar AG rat fetuses into the β-globulin region reported by Stanislawski-Birencwajg (1967). However, with α-fetoprotein of AH-130 cell origin no extension into the β-globulin region was observed. It is impossible to decide yet whether this is related to the number of antigen determinants in fetal or hepatoma α-fetoprotein.

When AH-130 cells maintained in Donryu strain rats were inoculated into Sprague-Dawley rats, there was no rejection, indicative of allogenicity of the cells, and it was possible to passage the cells without loss of

their ability to produce α-fetoprotein.

However, it was impossible to pass rat AH-130 to xenogenic mice, indicating the strict species specificity of this tumor. Similar findings were obtained with mouse ascites hepatoma MH-134. No antibody against the respective mouse and rat α-fetoprotein was found in surviving mice and rats. It is possible to prepare immune serum in rabbits with rat or mouse α-fetoprotein, so it should be possible to produce antibody by immunizing rats and mice with α-fetoprotein with the exception of hepatoma cells from AH-130 ascites. In the presence of hepatoma cells antibody production could not be observed. This may be attributed to the fact that when ascites fluid, containing heterogenous hepatoma cells, is repeatedly inoculated into animals intraperitoneally, symptoms of graft-versus-host disease develop and no animals survive more than 4 inoculation. However, it is possible that heterogeneous hepatoma cells are involved in the antibody production, so experiments are in progress on the influence of the number of cells transplanted on antibody production.

Since Abelev et al. (1963) first reported an interspecies cross-reaction between human and mouse α-fetoprotein, numerous papers has been published on this subject. Abelev (1968) stated that this phenomenon could not be detected by the usual immunodiffusion method but was detectable by radioimmunodiffusion. Recently, Nishi et al. (1972) reported that a cross-reaction can be demonstrated between rabbit antiserum against human fetal α-fetoprotein and fetal newborn serum of human, dog, horse, rat and rabbit by immunodiffusion.

In the present study, no cross-reaction between rat α-fetoprotein and human α-fetoprotein or mouse α-fetoprotein was observed by immunodiffusion, immunoelectrophoresis or immunofluorescence using specific antiserum against human α-fetoprotein or specific antiserum against rat α-fetoprotein. This discrepancy may be due to the quality of the specific antiserum used. The new technique of radioimmunoassay is very sensitive, so it is now possible to detect α-fetoprotein, not only in primary liver cancer but also in maternal serum in pregnancy. If the cross-reaction described by Nishi et al. (1972) actually occurs the results of clinical diagnostic test must be very complex and the detection of α-fetoprotein may become meaningless. The major factor causing this complexity would be whether a monospecific antiserum had been used. It is known that some serum proteins produce antibodies which react with heterogeneous serum proteins when hyperimmunization is induced. But the present findings clearly indicate that antiserum completely free from cross-reactive antibody must be used. Serum albumin (Gitlin and Bossman, 1966; 1967), acute phase α_2-glycoprotein (Menninger, Esber and Bogden, 1970) and carcinoembryonic antigen in particular must be considered as possible contaminants when antiserum of fetal extracts is used.

ACKNOWLEDGEMENTS

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REFERENCES

Abelev, G. I. 1968. Production of embryonal serum α-globulin by hepatomas: review of experimental and clinical data. Cancer Res. 28: 1344–1350.

Abelev, G. I., S. Perova, N. Khramkova, Z. Postnikova, and Y. Irlin. 1963. Production of embryonal α-globulin by transplantable mouse hepatomas. Transplantation 1: 174–180.

Gitlin, D., and M. Boesman. 1966. Serum α-fetoprotein, albumin and γG-globulin in the human conceptus. J. Clin. Invest. 45: 1826–1838.

Gitlin, D., and M. Boesman. 1967. Sites of serum α-fetoprotein synthesis in the human and in the rat. J. Clin. Invest. 46: 1010–1016.

Koda, T., S. Ishigami, and S. Tanabe. 1971. Studies

on liver cancer. I. Immunofluorescent studies on the localization of α-fetoprotein in patients with primary liver cancer. Biken J. 14: 369–377.

Menninger, F. F., H. J. Esber, and A. E. Bogden. 1970. Isolation of an acute phase α_2-glycoprotein from rat inflammatory serum. Clin. Chim. Acta 27: 385–394.

Nishi, S., H. Watabe, and H. Hirai. 1972. Production of antibody to homologous α-fetoprotein in rabbits, rats and horses with human α-fetoprotein. J. Immunol. 109: 957–960.

Sato, H., H. Sato, H. Kobayashi, I. Hirono, and Y. Hashimoto. 1971. Annual Report of the co-operative Research Ministry of Education-cancer-suppl. Table of transplantable tumor maintained in Japan. p. 18. [In Japanese]

Stanislawski-Birencwajg, M. 1967. Specific antigens of rat embryonic serum. Cancer Res. 27: 1982–1989.

Stanislawski-Birencwajg, M., J. Uriel, and P. Grabar. 1967. Association of embryonic antigens with experimentally induced hepatic lesions in the rat. Cancer Res. 27: 1990–1997.

Watabe, H., H. Hirai, and H. Satoh. 1972. α-Fetoprotein in rats transplanted with ascites hepatoma. GANN 63: 189–199.

Wise, R. W., F. J. Ballard, and E. Ezekiel. 1963. Developmental changes in the palsma protein pattern of the rat. Comp. Biochem. Physiol. 9: 22–30.