CULTIVATION OF HUMAN TUMORS ON THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO

D.M. Levina and V.A. Parnes

From the Department of Immunology and Malignant Tumors (Head – Active Member Acad. Med. Sci. USSR L.A. Zil'ber) of the N.F. Gamaleia Institute of Epidemiology and Microbiology (Director – Prof. S.N. Muromtsev) of the Acad. Med. Sci. USSR, Moscow

(Received June 21, 1957. Submitted by Active Member Acad. Med. Sci. USSR L.A. Zil'ber)

Numerous attempts to cultivate human tumors on the membranes of chick embryos have not, in the majority of cases, given clear results [1, 5, 6, 7, 15]. The tumors sometimes survived [5, 6, 11, 12], lasting for a few transplantations, and then without exception died [1, 3, 6, 9]. Attempts to cultivate human tumors on the membranes of preliminarily irradiated eggs [13] or after preliminary cultivation of the tumor in vitro [8] were also unsuccessful. Only after preliminary transplantation of the tumors into rats and hamsters treated with cortisone was it possible to cultivate three human tumors on the chorioallantoic membrane [4, 14]. Successful cultivation of human tumors has also been reported by A.K. Shubladze [2].

In consideration of the difficulty of cultivating human tumors on the membranes of the chick embryo we attempted to use in the cultivation the method of subculture of normal tissue. This method was proposed in 1951 by Mikhelson for cultivation of the virus of foot and mouth disease in the chick embryo.

EXPERIMENTAL METHOD

For the cultivation 28 human tumors were used, consisting of one carcinoma of the body of the uterus, four carcinomas of the cervix of the uterus, one chorionepithelioma, three fibromas of the uterus, one papillary carcinoma of the ovary, one metastasis from carcinoma of the ovary in the peritoneal cavity, twelve papillomas of the urinary bladder, one papilloma of the urinary bladder undergoing malignant change, two carcinomas of the urinary bladder, one papilloma of the urethra and one melanoma.* In addition, in parallel experiments we cultivated normal bladder tissue from human embryos.

The tissue for cultivation was used fresh (two to four hours after operation) or in a frozen condition. Freezing was carried out at −70°C in a mixture of dry ice and acetone. The frozen tissue was kept until the experiment at −20°C for 2 to 65 days.

Before being implanted in the egg the tumor material obtained at operation was washed with physiological saline and areas of necrotic and fatty tissue were removed with sterile precautions, and after cutting it into small pieces it was placed for 20-30 minutes in a solution of penicillin and streptomycin. Before the experiment the frozen tissue was thawed. Implantation was carried out on the chorioallantoic membrane of five-to ten-day-old embryos, most commonly six-to-seven-day.

The method of subculture consisted of simultaneous cultivation of a piece of tumor with a piece of normal

*We are grateful to Prof. A.P. Frumkin and Drs. L.N. Pogozheva and S.A. Smirnova for making available the operation specimens.

human embryonic tissue. For subculture we used most commonly embryonic bladder tissue which has well-marked histogenetic properties. In a series of experiments on cultivation of carcinoma of the cervix of the uterus, we subcultured pieces of tissue from the cervix of the embryonic uterus.

The subcultured tissue was brought into close contact with the implanted piece of tumor. We assumed that the subcultured normal human tissue would facilitate adaptation of the tumor tissue. Material for subculture was used both in the fresh and frozen form, usually the latter. In six experiments tumor extract, taken for transplantation, was introduced additionally onto the chorioallantoic membrane besides the tumor and normal embryonic tissue.

The eggs containing implanted pieces of tumor were incubated at 37°C for seven to ten days (until the embryos were 13-15 days old). At the end of this period they were opened for examination. If the pieces on superficial examination appeared shining and covered with vessels enveloping them with chorioallantoic membrane, then their viability was demonstrated and they were cut into three or four pieces which were implanted in other eggs. Transplantation of the pieces of tumor continued for as long as it was possible to find them on the chorioallantoic membrane at the place of implantation and their outward appearance was satisfactory.

EXPERIMENTAL RESULTS

On implantation of very small, average and large (up to 1 mm^2) pieces of tumor tissue it was found that very small pieces rapidly undergo complete lysis while the average and large pieces in some cases were preserved for a long time. Frozen tumor tissues were preserved longer and produced a more intense reaction in the chorioallantoic membrane than fresh tissues. The first passage of tumor tissue was successful in the great majority of cases: out of five to six pieces of tumor one or two and sometimes three were preserved. The pieces were enveloped in chorioallantoic membrane, and between its vessels white tumor tissue could be distinguished. On dying, the pieces appeared dull and dark in color. Such pieces were usually absorbed.

In spite of the fact that some tumor implantates could be kept in an outwardly viable condition through many passages, the tumor cells gradually died away. Vascularization of the pieces was incomplete and the true size of the tumor implantate and mitoses could not be found (except one case). The reaction of the chorioallantoic membrane itself in many cases was so violent that a hollow impression was created where the implanted tumor was growing.

The summarized results of the experiments on cultivation of pieces of tumor tissue are shown in the table.

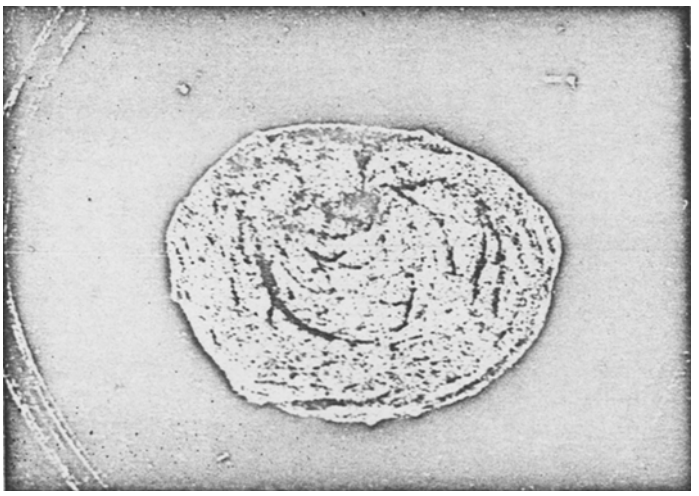

Fig. 1. Cultivation on the chorioallantoic membrane of the chick embryo of tissue from a metastasis of carcinoma in the peritoneal cavity. 1st passage, egg No. 320.

Cultivation of Human Tumors and Embryonic Tissues on the Chorioallantoic Membrane of the Developing Chick Embryo

Expt. No.	Character of tumor or tissue	Situation	Number of successive transplantations without subcultures	Number of successive transplantations with subculture	Total number of successive transplantations of pieces of tissue with and without subcultures
I	Carcinoma	Cervix of the uterus	3	5	8
II	"	Urinary bladder	4	4	8
III	"	Body of the uterus	1	3	4
IV	Multiple fibroma	Uterus	1	2	3
V	Carcinoma	Cervix of the uterus	1	4	5
VI	"	" "	1	0	1
VII	Fibroma	Uterus	1	3	4
VIII	Carcinoma	Cervix of the uterus	1	0	1
XI	Fibroma	Uterus	2	1	3
XII	Carcinoma of the ovary*	Metastasis in the peritoneal cavity	1	0	1
XVI	Papillomas*	Urinary bladder	1	0	1
XVII	Carcinoma*	" "	1	0	1
XVIII	Chorionepithelioma	Uterus	2	0	2
XIX	Chorionepithelioma*	"	3	0	3
XXII	Papillary carcinoma*	Ovary	3	0	3
XXIII	Melanoma	—	6	0	6
XXX	Papillomas	Urinary bladder	10	0	10
XXVIII	"	" "	8	0	8
XXXV	"	" "	1	0	1
XXXV	"	" "	1	0	1
XL	Papillomas with malignant transformation	" "	1	0	1
XLIII	Papillomas	" "	3	0	3
XLV	"	" "	2	0	2
XLVIII	"	" "	4	0	4
XLVIII	"	" "	2	0	2
LIII	"	Urethra	2	0	2
LV	"	Urinary bladder	1	0	1
LVI	"	" "	1	0	1
LVII	"	" "	1	0	1
IX	Embryonic tissue	" "	1	0	1
X	" "	" "	1	0	1
XIV	Fragment of chick embryo chorioallantoic membrane	—	1	0	1
XXI	Embryonic tissue	Urinary bladder	4	0	4

*Tumor extract was introduced onto the chorioallantoic membrane.

The normal embryonic urinary bladder tissue used for subculture was itself able, as shown in experiment XXI to survive and preserve its viable condition throughout several transplantations.

On histological examination, carried out by V.V. Suntsova, true growth of the implanted tumor tissue or the occurrence of mitoses (with the exception of one case with subculture) could not be established. Evidently, the survival of the tumor cells was only temporary.

The reaction of the chorioallantoic membrane to transplantation of the tumor was expressed in the form of marked edema of the membrane and by chronic inflammation with hyperplasia of all the layers of the membrane, and by hemorrhages. In Figures 1 and 2 are shown, in their natural size, pieces of tumor at the site of implantation on the embryonic membrane. Their dimensions considerably exceed those of the piece originally implanted (1 x 1 mm), but this is not true growth of the implanted tumor but the result of the reaction to it of the tissue of the egg.

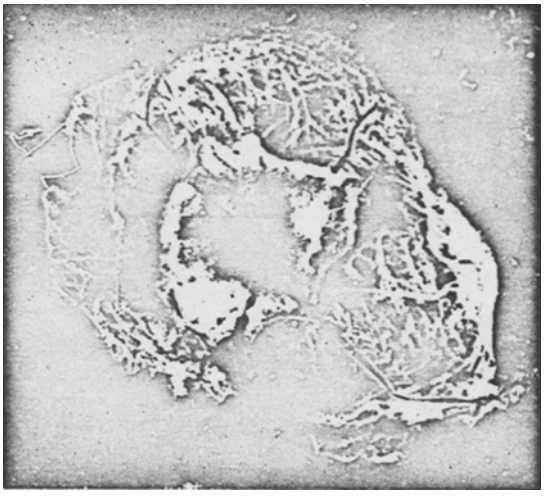

Fig. 2. Cultivation on the chorioallantoic membrane of the chick embryo of a chorionepithelioma, 3rd passage, egg No. 425.

Nonspecific nodular proliferations, as we know, may appear on the chorioallantoic membrane even from the action of physiological saline, broth and emulsion of normal chorioallantoic membrane [1, 10]. Human tumor tissue is evidently an even stronger stimulant of the membranes of the chick embryo.

SUMMARY

Twenty-eight human tumors of various localization and structure were cultivated on chorioallantoic membrane of developing chick embryo. True growth of the tumors could not be obtained. Small parts of tumors survive for a short time and cause turbulent reaction in the chorioallantoic membrane. These pieces may be retransplanted, but later they are subjected to destruction and resolution. When normal human embryonic tissue was introduced – this only promoted a more prolonged preservation of the transplantate. However, it did not result in the true growth of transplanted tumors. Small pieces of papilloma of the urinary bladder appeared to be the most viable and withstood nine transplantations.

LITERATURE CITED

[1] Z.I. Merkalova, Voprosy Onkol. 3, 2, 174-179 (1957).

[2] A.K. Shubladze, Zhur. Mikrobiol., Epidemiol. i Immunobiol. 11, 36-47 (1955).

[3] L.Ia. Iablonskaia, Voprosy Onkol. 2, 4, 432-439 (1956).

[4] C.P. Dagg, D.A. Karnofsky et al., Proc. Am. assoc. 2, 1, 11-12 (1955).

[5] E.W. Goodpasture, B. Douglas and K. Anderson, J. exp. Med. 68, 891-904 (1938).

[6] E.W. Hurst, B. Cooke and G.C. McClennan, Austr. J. exp. Biol. 17, 215-224 (1939).

[7] D.A. Karnofsky, L.M. Ridgeway and P.A. Patterson, Ann. N.Y. Acad. Sci. 55, 313-329 (1952).

[8] N. Kaufman et al., Am. J. Path. 28, 5, 561-562 (1952).

[9] E. Melrowsky, L.W. Freeman and J.S. Woodard, J. Invest. Derm. 22, 5, 417-429 (1954).

[10] D. Metcalf, Brit. J. of Cancer 9, 1, 222-228 (1955).

[11] E.A. Mirand and J.G. Hoffman, Exp. med. and surg. 11, 1, 63-72 (1953).

[12] J.B. Murphy, J. Am. med. assoc. 58, 874-875 (1912).

[13] S.C. Sommers, B.A. Sullivan and S. Warren, Cancer research 12, 12, 915-917 (1952).

[14] H.W. Toolman, Proc. Am. assoc. Cancer Research 1, 2, 49 (1954).

[15] E.C. Wynne, J.O. Norman and W.O. Russel, Proc. Amer. Assoc. Cancer Research 2, 1, 56 (1955).