Yolk Sac Cultivation of Tumors

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INTRODUCTION

Some months ago there was published from this laboratory a brief preliminary report of a method whereby tumors could be cultivated in the yolk sacs of developing chick embryos (4). Neoplasms were first injected into the yolk sac with the idea that the tumor cells would die and disintegrate, thus releasing any virus that might be present. It was hoped that if such a release occurred the medium possibly might favor the growth and concentration of the tumor-producing principle, as Cox and others have found for the viruses of Rocky Mountain spotted fever and typhus groups (1). Since publication of the preliminary results, this technic has been used here extensively in studies involving large numbers of eggs, and it is now felt that a further and more detailed description of the method would be of value.

Special interest has been focused on the use of this medium for growing tumors because it was found possible to produce malignant neoplasms in mice with cell-free extracts of the yolk material from eggs implanted with cancer (3). The technic was hit upon in the course of investigations leading up to the discovery of this virus-like principle.

It occasioned considerable surprise when eggs so treated were opened and well developed tumors were discovered in the yolk sacs. The principles involved are essentially the same as those discovered by Murphy when he found that the chick chorioallantoic membrane and the chick itself were capable of supporting the growth of tumor tissue from the mouse and the rat (2). In the present instance the location of the growth is different, but in both methods the chick supports the cancer. In the yolk sac method the tumor does not interfere with the chick and inoculation is relatively simple.

EGGS USED

Eggs are obtained from a flock of pure bred white Leghorn hens. All our egg requirements have been satisfied from this same source for the past two years. The chickens are maintained on a well balanced diet. Egg fertility during the fall, winter, and spring seasons is over 90 per cent. The embryos are healthy and very few of them die after the fifth day of incubation has been reached successfully. However, other eggs have been used, including ordinary "yard eggs," without notable loss in utility for growing tumor material.

INCUBATION

The main incubator is a 1,200 egg cabinet fitted with a motor-driven fan and apparatus for the regulation of temperature and humidity. It holds 8 separate trays, enabling the operator to stagger the incubation periods of groups of eggs so that eggs are available for injection at spaced intervals—daily if desired. In addition, several all-metal, round, 100 egg electric machines are available for special work. The majority of the eggs are run at the normal temperature for chick-hatching—37.6° C.

TUMOR TISSUE

Both mouse and rat tumors have been cultured, either directly from the animal host or indirectly by transplants from other eggs. Several lines of spontaneous and transplanted mouse tumors, and a carcinosarcoma of the rat, Walker 256, have been grown without difficulty. No trouble has been encountered with any tumor whose growth rate is sufficient to produce a sizable mass within the incubation period of the chick. In the usual routine, tumors are used for inoculation before any appreciable necrosis has developed.

PREPARATION OF THE TUMOR FOR INJECTION

A tumor-bearing animal is lightly etherized and then decapitated. After bleeding, the body is immersed briefly in a solution of 70 per cent alcohol to which a little iodine has been added. This treatment makes it simpler to remove the tumor without contamination from the pelage. The tumor is grown in the caudal region of the animal so that it can be exposed easily by incising the skin completely around the body just posterior to the forelimbs and then rolling it backward. Until recently the excised tumor has been placed in a piece of coarse, unbleached, domestic muslin, the edges of the cloth have been folded...
over, and the tumor tissue has been squeezed through the cloth by twisting the pouch thus formed. In this manner the soft parts of the tissue can be dispersed so that they will easily pass through a 20 gauge hypodermic needle, while the tougher connective tissue elements remain behind in the sack. The paste so obtained is placed immediately in a 5 cc. syringe and then injected through the rubber stopper of a 30 cc. serum bottle (Fig. 1). Rigid asepsis is maintained at every step of the procedure.

Recently an alternative method has been used with success on both mouse and rat tumors. Wire screens of suitable mesh are placed in the barrel of a 10 ml. Luer syringe, separated by glass beads. The plunger is inserted and the syringe is dry sterilized. The tissue to be injected is removed from the host and placed in the syringe, and the mass is forced through the screens into the barrel of a second syringe without screens, in which it is measured and diluted according to requirements. The screens hold back the more resistant tissues but permit easy passage of the cancer cells. Iron wire screens with 18 meshes to the inch have been found to yield a suspension that readily passes through a 20 gauge needle. The mesh and needle size may be varied as desired.

When the tumor for injection is taken from a previously implanted yolk sac it is not necessary to squeeze the material through the cloth. Yolk sac-grown tumors are much softer than those grown in animals so that the tissue can be placed immediately in the syringe. It may then be forced without difficulty through a 20 gauge needle into the serum bottle.

The practice has been to suspend 1 volume of tumor paste in 4 volumes of an 0.85 per cent saline solution and to use 0.5 cc. of the resultant mixture, containing 0.1 cc. of tumor for each egg injected. However, individual tumors may require different treatment in this respect.

Fig. 1.—Serum bottle for reception of dispersed tumor tissue.

Fig. 2.—Diagrammatic representation of the egg after 5 days' incubation. Hypodermic needle is in place for yolk sac inoculation.
Fig. 5.—The dia transplantable carcinoma that has been used in the greater part of the egg work. Mag. × 1,000.

Fig. 6.—The tumor shown in Fig. 5 as it appears when growing in the yolk sac of the chick embryos. Mag. × 1,000.
Yolk Sac Implantation

In preparation for injection, eggs that have been incubated for about 5 days are allowed to remain at least 30 minutes without turning. Since the eggs are incubated while resting in a horizontal rather than an upright position, as is the practice followed in some experimental work, the embryo floats to the top of the yolk just above and posterior to the air space. Taking care not to change the position the egg has maintained for the previous half hour or so, the operator sterilizes the surface of the shell covering the air space and taps a small hole to permit entrance of the syringe needle. One point of a medium sized scissors may be used for this operation. The shell is pierced, but not the inner shell membrane.

The saline suspension of tumor is injected into the yolk by means of a 1/4 inch 20 gauge hypodermic needle. The yolk is so situated that almost immediately past the air space, the needle enters the yolk sac (Fig. 2).

Many variations in the technic of implanting have been and are being tried. For example, injections have been made through the side of the egg with good results, for although a certain amount of the white oozes out the subsequent development of the embryo seems normal.

Fig. 2 illustrates the general procedure involved in the inoculation of the yolk sac. After injection, the small hole in the blunt end of the shell is sealed with cellulose tape.

Yolk Sac Tumors

The difficulty of maintaining rigid asepsis throughout the several steps necessary in transferring tumor from the mouse to the yolk sac causes the loss of a small percentage of the eggs. If massive infection occurs it is immediately evident and kills the embryos in a very short time. Light contamination may not appear for several days after implantation. Some infective organisms take considerable time to develop in the incubating egg so that as much as a week or 10 days may elapse before the death of the embryo is brought about.

When the inoculated egg is opened, at the end of 17 or 18 days of incubation, the tumor in the yolk sac tends to resemble in gross appearance those grown from the same donor tissue in host animals (Figs. 3 and 4). Histological examination discloses that the yolk sac tumor has a supporting stroma supplied by chick tissue, otherwise the general characteristics found in the donor material are preserved (Figs. 5 and 6). Usually there is one main aggregation of neoplastic tissue situated in that region of the yolk sac known as the yolk sac umbilicus. This area, in the 17 to 18 day chick, is connected with the albumen sac. Occasionally smaller nodules of tumor are found in other regions of the yolk sac.

The chick embryo will usually live on to the termination of the incubation time even when the yolk sac contains a tumor weighing as much as 5 gm. Often the larger tumors are hemorrhagic and, as a result, the yolk sac may be filled with partially hemolyzed blood. When this happens the chick, although it may be undersized, will be fairly vigorous. Occasionally a large tumor may cause the death of the embryo a day or two before the termination of the experiment.

When the method of inoculation used is consistent for a series of eggs the resulting yolk sac tumors show distinctly less variation in size than tumors produced by implants in mice. This is to be expected when it is recalled that the mice may be resistant to a varying degree, according to their heredity, whereas the chick presumably does not contain any factors that resist heterologous cancer tissue.

The eggs of some types of fowl have a longer incubation period than hen's eggs, and the extended hatching period should make them suitable for the cultivation of comparatively slow-growing tumors. The egg of the Muscovy duck, for example, hatches after about 35 days of incubation. A few experiments with duck eggs have shown that tumors will grow in the yolk sac of these embryos.

SUMMARY

A method is described whereby tumors from the mouse and rat can be cultivated in the yolk sac of the developing chick embryo.

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