The Role of Cellular Fractions in the Transplantation of the Walker Rat Carcinoma 256

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Stasney, Paschkis, and Cantarow (6) have reported that hepatomas may develop in rats that have received intrahepatic injections of a cell-free mitochondria fraction obtained from 2-acetaminofluorene-induced rat hepatomas. They found two hepatomas at 29 and 35 days, respectively, in 36 rats injected with this mitochondria fraction. Intrahepatic injection of intact tumor cells or crystals of 2-acetaminofluorene suspended in serum produced no tumors in 48 rats. The present investigation was designed to determine whether cellular fractions from a tumor not chemically induced would incite malignancy when injected as a cell-free inoculum.

MATERIALS AND METHODS

The transplantable Walker rat carcinoma 256 was used in this study. This tumor originally arose as a spontaneous breast carcinoma and has been consecutively transplanted in rats for many years. Histologically, it has the appearance of an undifferentiated carcinoma with some sarcomatous characteristics. Ordinarily, 95–100 per cent of rats receiving subcutaneous injections of cell suspensions develop the tumor. It grows rapidly, metastasizes to regional lymph nodes relatively late, and kills usually within 30 days after injection.

The method of Hogeboom et al. (3), with sucrose as a dispersing medium, was employed to obtain various cellular fractions. Under sterile conditions, 5.5 and 10.5 gm. of tumor in the first and second experiments, respectively, were homogenized in 9 volumes of cold 0.25 M sucrose immediately after aseptic dissection from three donor rats. (In the first experiment 20,000 units of crystalline penicillin G [Lilly] were added to the homogenate.) All but 5.0 ml. were centrifuged in the cold room at 3°C. at 1,500 g for 10 minutes to precipitate the nuclei, together with small numbers of intact cells which were not disrupted during homogenization. The nuclei were washed twice for 10 minutes with 3.0 ml. of sucrose solution. The washings and supernatant solution of the nuclei were combined and centrifuged at 10,800 g for 15 minutes and washed twice with 3.0 ml. of sucrose solution for 10 minutes to sediment the mitochondria. Centrifugation at 21,000 g for 1 hour of the resulting supernatant solution, together with the washings of mitochondria, yielded a precipitate of submicroscopic material (the “Microsomes” of Claude [2]). The microsomes were washed once with 3.0 ml. of sucrose solution and centrifuged at 21,000 g for 1 hour. The final supernatant solution and washing were saved for injection.

The efficacy of the separation procedure has been shown for liver tissue (2, 3, 5); however, it is important to demonstrate the reliability of the method for this tumor. Smears of the various resuspended fractions were stained with Wright’s stain and Janus green B. The nuclei fraction contained intact cells. The exact proportion was difficult to estimate accurately because of the presence of many nuclei with variable amounts of adherent cytoplasm. The mitochondria fraction contained a number of nuclei, but no intact cells were seen. The microsome fraction was found to be free from mitochondria.

The supernatant solution of the microsomes doubtless contained particulate material which could be sedimented at higher centrifugal speeds; Chantrenne (1) stated that the microsomes range in size from very small units of nearly pure ribonucleo-protein to relatively large aggregates of nucleo-proteins and other proteins.

A total of 90 Sprague-Dawley rats (45 males and 45 females), weighing 250–350 gm., were divided into groups of 10, each group consisting of 5 males and 5 females. The hair over the back of each rat was clipped, the skin was painted with Merthiolate 8, and under ether anesthesia the various fractions were injected subcutaneously.
The volumes of each fraction given per injection per group of ten rats in the first experiment were: 0.25 ml. of 10 per cent homogenate, 0.25 ml. of mitochondria suspension (mitochondria residue suspended in 6.0 ml. of 0.25 M sucrose solution), 0.5 ml. of microsome suspension (residue suspended in 6.0 ml. of sucrose solution), and 2.0 ml. of the final supernatant (70 ml.). In the second experiment, the volumes of each fraction injected were: 0.25 ml. of homogenate, 0.5 ml. of nuclei suspension (nuclei residue suspended in 6.0 ml. of sucrose solution), 0.5 ml. of mitochondria suspension, 0.5 ml. of microsome suspension and 10.0 ml. of final supernatant (140 ml.). The rats were checked weekly for tumors. Observations were continued for 6 weeks.

RESULTS

Tumors developed in six of the ten injection sites in the first experiment and in nine of the ten injection sites in the second experiment, in the groups receiving the tumor homogenate. Tumors also developed in eight of the ten injection sites in the second experiment in the group receiving the nuclei suspension which was known to contain intact cells.

The second experiment was designed to increase the concentration of the suspension and the amount of suspension injected. Since all the residues were resuspended in 6 ml. of isotonic sucrose solution, there was a twofold increase in residue concentration. The volume per injection was increased twofold for the mitochondria suspension and fivefold for the final supernatant solution. In spite of the increase in concentration of the suspensions and the increase of volume per injection, no tumors developed in the groups receiving mitochondria suspension containing nuclei, microsome suspension, or final supernatant solution.

DISCUSSION

The only successful transplantations occurred with the crude homogenate and the nuclei fraction, both of which contained intact cells. Although no attempt was made to prepare nuclei free of intact cells, evidence in these studies indicates that nuclei are not sufficient for the successful transplantation of the Walker rat carcinoma 256. Since the mitochondria fraction which contained both nuclei and mitochondria failed to produce "takes," neither of these is sufficient for successful transplantation. If this fraction had produced tumors it would be impossible, of course, to tell which was responsible, and further refinement of the technic of separation would have been necessary.

While it is theoretically possible to transplant a tumor by injection of one intact cell into a susceptible animal, it has been our experience with this particular tumor that a large number of cells must be injected to insure success of the transplant. Hence, no significance is attached to the difference in incidence of tumors in rats injected with the homogenate in the first experiment (60 per cent) as compared to the higher incidence (90 per cent) in the second experiment.

We have found that bacterial contamination of the inoculum may interfere with the transplantation of this tumor. To control this factor, penicillin was added to the homogenate in the first experiment, and sterile precautions were employed in both experiments. Since no infections developed in any of the 90 injection sites, there was no evidence of bacterial contamination of the cellular fractions.

With the possible exception of certain tumors of virus etiology (4), successful transplantation of neoplasms is probably due to proliferation of the cells inoculated into the recipient. The cells of the recipient animal do not take part in the production of the tumor mass except to provide vascularity and a supporting stroma. The view that this is the mechanism involved in the successful transplantation of the Walker rat carcinoma 256 is supported by our data.

Cell-free mitochondria fractions from chemically induced hepatomas appear, from the results of Stasney et al. (6), to have the ability to induce malignancy. In view of the fact that transplantability to other animals of the same strain is a fairly generally accepted criterion of malignancy of tumors of mice and rats, it is surprising that Stasney and co-workers did not obtain tumors in the rats injected with intact tumor cells.

SUMMARY

Five- and 10-gm. portions of a Walker rat carcinoma 256 were fractionated by ultra-centrifugation into subcellular components immediately after dissection from three donor rats. Only the fractions containing intact cells produced tumors when injected subcutaneously into rats. The mitochondria fraction which was known to contain nuclei, the microsome fraction, and the supernatant of the microsomes produced no tumors over a 6-week observation period.

REFERENCES


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