THE SUCCESSFUL PRODUCTION OF A MAMMALIAN TUMOR WITH A VIRUS-LIKE PRINCIPLE

A METHOD has been developed whereby a powerful tumor-producing principle can be obtained from tumor tissue. Concentrations of a virus-like Berkefeld-passing factor have been produced which when injected subdermally in mice gave rise to tumors, the inception and growth of which were more rapid than that of implants of the donor cancer tissue.

The original tumor material consisted of mammary carcinoma tissue which had passed through more than 30 generations of transplants in dba mice where it initially appeared spontaneously. The tumors induced by the filterable factor were transplantable, rapid-growing and histologically were made up of malignant cells of carcinoma and sarcoma types.

Tumor development took place at the site of the injected material. Additional smaller growths have been found in the liver and in the visceral peritoneum of the digestive tract. There was, as would be expected, considerable variation in the rate of growth of the tumor in individual mice.

This investigation was begun about a year ago, at which time large numbers of eggs were being used in a study concerned with the effects of hypervitaminosis of some of the B vitamins on the growth and development of the chick embryo.[1] It occurred to the author that the yolk sac of the chick embryo might prove of value in demonstrating the possible existence of a virus-like principle as the immediate cause of tumors in general with particular reference to mammalian neoplasm. The successful growth of so many viruses in this medium was, of course, the basis for the idea.

In the meantime some thousands of eggs and more than 300 mice have been utilized on this problem.

Very early in the study, it was found that cell-free filtrates of yolk from chick embryo yolk sacs which had received an injection of tumor tissue a few days previously, contained a substance capable of inducing varying degrees of tissue hyperplasia when injected into a mouse. These growths developed rapidly, but after attaining 1.0 to 1.5 cm in diameter became stationary and then gradually regressed.

Similar growth stimulants could be obtained by injecting ground tumor cells and cell-free extracts of fresh tumor tissue into yolk sacs of chick embryos. It was found that yolk from eggs so treated could be passed by injection from yolk sac to yolk sac for many generations of chick embryos and still the yolk contained tissue growth stimulating substances.

Some months ago it was discovered that tumor tissue grows readily in the yolk sac of the chick embryo.[2] As more attention was given to this method of producing tumor tissue, it was noted that relatively large cancers, several grams in weight, occasionally occurred. Berkefeld filtrates of the yolk surrounding these large tumors contained the virus-like tumor-producing principle to a high degree. The evidence indicates that the tumor cells constantly gave off the virus substance which was caught and preserved by the surrounding yolk. Whether the tumor factor is able to grow independently in the yolk medium has not been definitely determined.

Briefly summarized, the method which proved successful is as follows: The yolk sacs of chick embryos were implanted, as previously described,[2] on the fifth day of incubation with saline suspensions of fresh tumor tissue. The eggs were then incubated for another 12 days, after which the yolk sacs were examined and yolk was collected from those bearing comparatively large tumors (a gram or more). Since the yolk at this time is very thick and viscous, saline solution was added in the portions of 1 to 1. The material was then centrifuged and the supernatant liquid passed through an N-size Berkefeld filter. Care was taken to keep all these operations aseptic. Dba mice were given subdermal injections ($\frac{1}{3}$ cc per mouse) of the filtrate so obtained.

The implications of the successful production of a mammalian tumor with a virus-like cell free product of tumor tissue will be obvious to workers in the cancer field. Certainly the hypothesis held by so many for so long that a virus-like principle is the primary cause of tumorous growths receives support.

There are grounds for hoping that the yolk sac method will prove useful in demonstrating the presence of a virus-like principle in other mammalian tumors and also in the many fowl tumors which have to date proved refractory in this respect. By utilizing eggs of fowls which have relatively long hatching period, it is hoped that this method of attack may be applied to human neoplasms.

A detailed report of methods and results will be given at an early date.

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[1] A. Taylor, H. K. Mitchell and M. A. Pollack, Univ. of Texas Pub. 4137, 67, 1941.

[2] A. Taylor, J. Thacker, D. Pennington, SCIENCE, 96: 342, 1942.