Heterologous Tumor Transplantation by Intravenous Inoculation of the Chick Embryo[1]

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In general, tissue transplanted from one species to another fails to grow. Whether donor tissue is normal or neoplastic, the antagonistic response of the recipient usually precludes survival of heterologous transplants.

Explantation of tumor tissue with survival for varying periods has been accomplished by tissue culture technics; by inoculation of neoplastic cells into the anterior chamber of the guinea pig or rabbit eye; and by inoculation of such cells onto the chorioallantoic membrane or into the yolk sac of the chick embryo.

There is no evidence to indicate that the chick embryo forms antibodies before the 18th day of incubation. The fact that many types of tissue from various sources have been grown for short periods on the chorioallantoic membrane may well be related to the absence of antibody formation.

Previous experience (*1*) with intravenous inoculation of chick embryos with suspensions of *Myco. tuberculosis* suggested that this method might be useful in establishing heterologous tumor growth within the embryo.

Sterile tumor tissue was obtained from patients at operation, from tumor-bearing rats and mice, and from tissue culture. The tumor tissue was collected under aseptic precautions and placed in sterile physiological saline solution. It was then forced through a 70-mesh Monel wire screen and a cell suspension prepared in physiological saline solution. It has been determined previously that cell suspensions produced in this manner contain viable tumor cells (*2*).

Chick embryos incubated for 11 days were prepared for intravenous injection by removal of the shell over the air sac and exposure of the allantoic veins by reflection of a portion of the shell membrane. The technic has been reported previously in detail (*1*). An inoculum of 0.05 cc of cell suspension was injected intravenously into each embryo. A total of 278 embryos were injected in a series of 17 experiments. Approximately 50% of the embryos survived and were opened for examination on the 20th day of incubation.

In the experiments in which human tumor tissue was used there were 4 embryos (10% of survivors) which showed tumor "takes" upon histologic examination. The transplantation of the C57 strain mouse sarcoma (#241) was more successful. Twenty per cent of surviving embryos injected with a cell suspension prepared from this mouse sarcoma have shown tumor growth. In the entire series of experiments there was evidence of tumor growth in the brain or liver of 13% of surviving embryos.

The neoplastic cells maintained their histologic character in the embryo and closely resembled the parent tumors. This morphologic evidence suggests the probable identity of the transplants with the parent tumors.

[1] This work was carried out in the laboratories provided by the Henry Phipps Institute of the University of Pennsylvania. Equipment was supplied by the Heyden Chemical Corporation.

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Diffuse and Nodular Hyperplasia of the Thyroid Gland in Thiouracil-treated Rats[1]

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In 1927, Wegelin (*10*) reported thyroid adenomata in rats on stock and experimental diets in endemic goiter regions in Switzerland and observed a malignant spindle-cell sarcoma of the thyroid with metastases to the lungs, myocardium, and pericardium in an old rat. The occurrence of adenomata in the thyroid glands of aged rats has been infrequently reported (*3, 6, 8, 9, 10*). Hellwig (*5*) observed thyroid adenomata in albino rats receiving a calcium-rich goitrogenic diet for 140 days. Bielschowsky (*1*) produced benign and malignant tumors of the thyroid in rats by the simultaneous or successive administration of 2-acetyl-amino-fluorene and allyl-thiourea for long periods of time; however, neither drug alone produced neoplastic growths in the thyroids. Griesbach, Kennedy, and Purves (*4*) noted that diets containing 45% *Brassica* seeds could produce multiple thyroid adenomata in rats and that the seeds contained chemical substances related to thiouracil and sulfonamides. Purves and Griesbach (*7*) observed adenomata of the thyroid gland in a high percentage of animals treated with thiourea for 12 months or more, and such neoplasms had a tendency to become malignant when the administration of thiourea was extended to 20 months or longer. In two animals with malignant tumors metastases to the lungs were observed. Donald and Dunlop (*2*) reported "extreme parenchymatous hyperplasia and an almost complete absence of colloid" in a patient given thiouracil for 5 months.

The purpose of our report is to indicate the high incidence of diffuse and nodular hyperplasia of the thyroid gland induced by prolonged administration of thiouracil. This is a part of a general study showing that thiouracil-induced hypothyroidism is accompanied by increased severity in rat polyarthritis induced by pleuropneumonia-like organisms (L_4 strain). The details of this study will be reported separately.

[1] This work was done under contract with the Office of Naval Research.

Albino rats weighing approximately 100 gm. were grouped according to sex and placed on a diet containing

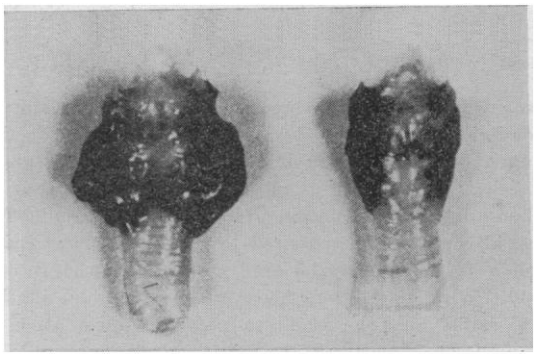

FIG. 1. Marked thyroid hypertrophy (left) in a thiouracil-medicated rat compared with the thyroid (right) of a control unmedicated rat.

0.1% thiouracil. The diet was prepared by grinding the thiouracil in a mortar and blending it for 30 min in a mechanical mixer with the basic diet of ground Purina Dog Checkers. Control animals received the basic diet. All animals were permitted unrestricted access to both food and water.

fection of the microorganisms had any effect on the thyroid gland.

Histologically, the thyroid glands from animals which had received no thiouracil were normal. Glands of those animals which were removed from the thiouracil diet after 34 or 51 days and placed on the control diet failed to show hyperplastic changes. The thyroid glands from all animals on the thiouracil diets for 120 days or longer were distinctly hyperplastic with areas of nodular hyperplasia. In animals which had received thiouracil for 120 and 142 days, the numbers of discrete hyperplastic nodules were 2 and 3, respectively. Fifteen nodules were found in the thyroids of 7 of the 24 animals which had received thiouracil for 233 days. These areas of nodular hyperplasia were occasionally demarcated from the adjacent hyperplastic thyroid parenchyma by a zone of condensed fibrous tissue. The thyroid epithelium within the areas of nodular hyperplasia was distinctly different from the adjacent tissue in that the cells were high cuboidal rather than columnar and the nuclei less vesicular and more deeply stained with nuclear stains. Colloid with peripheral vacuoles frequently filled the acini.

From these experiments it is concluded that thiouracil, administered to normal albino rats for prolonged periods of time, induces diffuse and nodular hyperplasia of the thyroid gland. The frequency of nodular hyperplasia is related to the total amount of thiouracil ingested.

TABLE 1

INFLUENCE OF THIOURACIL ON WEIGHT OF THE RAT THYROID

Total experimental period (days)	Thiouracil medication (days)	Females			Males		
		No. of animals	Avg. wt. of thyroid (mg/100 gm body wt.)	Increase above normal wt. (%)	No. of animals	Avg. wt. of thyroid (mg/100 gm body wt.)	Increase above normal wt. (%)
120	0	9	8.3	0	5	8.4	0
	34–51	7	12.5	50.6	4	14.2	69.1
	120	8	31.8	283.	6	39.8	378.
142	0	7	8.2	0	....	....	....
	142	9	33.6	309	....	....	....
233	0	3	8.4	0	3	7.5	0
	233	13	49.3	487	11	44.2	489.3

Since this experiment was designed to determine the effects of induced hypothyroidism on the course of experimental polyarthritis in rats, all animals were inoculated intraperitoneally after varying periods of time (34, 51, or 85 days, respectively) with 2 ml of an 18-hr broth culture of the L_4 strain of pleuropneumonia-like organisms. In some experiments, the basic diet was substituted for the thiouracil diet at the time of inoculation.

At the end of the experimental period the animals were killed, and their thyroid glands weighed and examined histologically. The extreme thyroid gland hypertrophy in those animals which had received thiouracil for long periods of time was evident on gross inspection (Fig. 1), and this was further substantiated by the gland weights. The results are presented in Table 1, in which the animals are grouped according to the total experimental period and the time they were on the thiouracil diet. There was no evidence that the time of inoculation or in-

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