suspension containing half a million cells in tissue culture salt solution (without serum) was injected subcutaneously into each of 8 seven-day-old rats which had been made tolerant to human cell antigens(2) by intravenous injections of J-111 cells on the day of birth. Five of these eight rats developed subcutaneous nodules up to 4 mm in diameter. These were removed 21 days later for histologic study. The nodules were anaplastic carcinoma resembling the tumor from which the culture originated (Fig. 2 and 3). An attempt was also made to transplant the tumor by intravenous inoculation of 1,000,000 cells into rats less than 24 hours of age, but all the recipients became cyanotic and died of asphyxia within two hours of injection, perhaps because of the large size of the cells.

Chromosomal analysis of the cultured cells. Cells of passage numbers 7, 9 and 15 were prepared for chromosomal observation by conventional techniques of colcemid and hypotonic pretreatments, acetic alcohol fixation, preparation of flame-dried slides, and acetic orecin staining. Twenty cells representing all 3 passages were counted. Numbers of chromosomes per cell were: 59, 77, 77, 78, 78, 79, 81, 81, 82, 84, 85, 86, 86, 87, 87, 92, 92, 92, 154, and 171 (Fig. 4). Thus there was no clear cut mode and the chromosome numbers were mainly in the hypotetraploid range. One hundred consecutive metaphase cells were surveyed and estimates were made of their ploidy classes. Forty-six were in the range of 75 to 95 (tetraploid range); 40 in the range of 150 to 200; and 14 in the range of 300 to 400. In all cells, including those with a count of 92 chromosomes, there was evidence of a considerable degree of structural rearrangement, resulting in new chromosomal types. Dicentrics were prevalent, ring chromosomes were found occasionally, and cells with one or more chromatid breaks or gaps were frequent. Many metaphases had very small paired acentric fragments of unknown significance.

Summary. A cell line has been established from a nodule of human giant and spindle cell carcinoma of the thyroid metastatic to a kidney. The culture has been maintained through more than 24 passages over a period of 1½ years. Its identity as a cancer cell line was established by its growth on heterotransplantation into immunologically tolerant rats with reproduction of the histologic appearance of the original tumor. Chromosomal studies showed a high degree of aneuploidy and polyploidy with numerous abnormalities of chromosomal form.

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1. Eagle, H., Science, 1955, v122, 501.
2. Southam, C., Cancer Res., 1966, v26, 2496.

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Effect of 1-Methyl-2-Mercaptoimidazole (Methimazole-Tapazole On DNA Content of the Chicken Thyroid Gland.*† (32467)

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In the determination of the thyroid hormone secretion rate (TSR) in the fowl, the goitrogen methimazole or tapazole has been used to block the recycling of I^{131} from the metabolism of L, thyroxine-I^{131}(1). As the term goitrogen indicates, it depresses the synthesis of the thyroid hormones and as a result stimulates an increased secretion of TSH which acts upon the thyroid glands to increase its weight and size to form the goiter. However, the goitrogen does not influ-

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TABLE I. Effects of 1-Methyl-2-Mercaptomidazole (Tapazole) on DNA Content of the Chicken Thyroid Gland.

Group No.	Treatment	No. of birds	Body wt (g) Initial	Body wt (g) Final	TSR (mg/100 g bw)	Wet thyroid wt (mg)*	DFFT wt (mg)	Total DNA (μg)	DNA/mg DFFT (μg)	DNA/100 g wet thyroid (μg)
1	Controls	14	2345	2345	.57	134.8 ±8.9	28.4 ±2.6	470[1] ±39	17.4 ±1.49	361.0 ±8.4
2	30 days Tapazole .5 mg/100 g bw/day	14	2205	2215	.55	224.9 ±23.2	38.4 ±3.6	805[2] ±101	22.0 ±2.58	374.0 ±38.2
3	60 days Tapazole .5 mg/100 g bw/day	10	2546	2519	.43	388.0 ±35.5	86.5 ±9.7	1161[3] ±134	14.0 ±1.40	298.0 ±19.3
4	90 days Tapazole .5 mg/100 g bw/day	10	2261	2393	.55	318.7 ±98.1	95.0 ±38.6	714[4] ±153	11.6 ±1.67	260.0 ±20.7

Student's 't' test
1 vs 2 $P < .01$ 2 vs 3 $P < .05$
1 vs 3 $P < .001$ 2 vs 4 $P < .70$
1 vs 4 $P < .200$ 3 vs 4 $P < .05$

* Mean ± standard error.
DFFT = dry fat-free-tissue

ence the release of the thyroid hormone and in a short time the colloid stored in the thyroid gland is depleted. Histological observations indicate an increase in the height of the follicular epithelium but do not indicate the extent of cell multiplication. The relationship between these two factors in weight increase may be estimated by the determination of the deoxyribonucleic acid (DNA) of the thyroid gland. Since DNA measures cell numbers, an increase in total DNA of the glands would indicate the extent of cell multiplication and the DNA per unit weight of tissue would indicate changes in cell size.

Materials and methods. Four groups of male cross-bred fowls about 1½ years of age with an average body weight of (1) 2345 g (range 1952-3405), (2) 2205 g (1952 to 3132), (3) 2546 g (2179 to 3442) and (4) 2261 g (1861 to 3178) were used in this experiment. One group served as controls whereas the other 3 groups were injected subcutaneously daily with 0.5 mg/100 g bw of tapazole for 30, 60, and 90 days. This level of tapazole has been shown to block the uptake of I^{131} by the thyroid gland(1). At the end of each period, the thyroid glands were removed and stored at −20° for later estimation of the DNA. DNA was determined by the method of Webb and Levy(2). The data were analyzed statistically using Student's 't' test(3) and Kramer's multiple range test(4).

Results. The mean wet thyroid gland weight of 14 control males weighing 2345 g was 134.8 ± 8.9 mg (Table I). After 30 days of methimazole injections, the mean wet thyroid gland weight of 14 males weighing a mean of 2215 g increase to 224.9 ± 23.2 mg (an increase of 66.8%), after 60 days to 388.0 ± 35.5 mg (an increase of 187.9%, and after 90 days to 318.7 ± 98.1 mg (an increase of 136.5%).

The mean dry fat-free thyroid tissue (DFFT) of the control group weighed 28.4 ± 2.6 mg or 21.1% of the wet weight. After 30 days the mean DFFT increased to 38.4 ± 3.6 mg or 17.1% of the wet weight. After 60 days the mean DFFT increased to 86.5 ± 9.7 mg or 22.3% of the wet weight, and after 90 days to 95.0 ± 38.6 mg or 29.8% of the wet weight.

The DNA/mg DFFT in the control group was 17.4 ± 1.49 μg. This value increased to 22.0 ± 2.58 μg after 30 days, then declined to 14.0 ± 1.40 μg after 60 days and to 11.6 ± 1.67 μg after 90 days. This would indicate that the number of cell nuclei/mg increased during the first 30 days, then declined at 60 and 90 days.

The mean total DNA is determined by multiplying the DFFT by the DNA/mg DFFT. The DNA of the control group was 470 ± 39 μg. At 30 days, the DNA was 805 ± 101 μg an increase of 71.3%, at 60 days to 1161 ± 134 μg an increase of 147% above the controls, and at 90 days to 714 ± 153 μg an increase of 52.0% above the controls.

Analysis of the data by Student's 't' test and the multiple range test of Kramer resulted in identical significance levels for comparisons of means. For this reason, only the Student's 't' levels were included in Table I.

Discussion. Methimazole or tapazole is one of a group of compounds which are classified as goitrogens because they stimulate a marked increase in thyroid gland size and weight. When administered to fowls, it has been shown to block I^{131} uptake by the thyroid gland and the synthesis of the thyroid hormones. It does not interfere with the release of thyroidal-I^{131} and as a consequence, there is complete resorption of the colloid present in the follicles. When the colloid is completely resorbed and no more hormone is present in the circulation, the secretion of TSH by the pituitary is increased which in turn stimulates an increase in weight of the thyroid glands.

The object of this study was to determine to what extent the increase in thyroid weight is due to an increase in cell number and to what extent it is due to an increase in cell size.

It has been shown in a number of species that each somatic cell including the mammary gland contains the same amount of DNA. Any increase in the total amount of DNA in a gland would indicate an increase in cell number. If it is assumed to be true of the thyroid gland of the fowl, then the present study indicated an increase in cell number of 71% after 30 days, an increase of 147% after 60 days, but a decrease to 52% above the controls after 90 days of treatment with tapazole.

In male guinea pigs fed 0.2% thiouracil in the diet, there was a 314% increase in thyroid weight, a 519% increase in total cell mass, and a 140% increase in average cell height(5).

Hendrich and Turner(6) have reported that in female rats injected subcutaneously with 400 μg/100 g bw of methimazole/day for 30 days, the mean wet thyroid gland weight increased 99%. Panda and Turner (7) reported an increase of 111%. This increase in thyroid gland weight was found to be due to an increase in cell growth of 54% and to an increase in cell size of 46% (7).

When methimazole was fed at the level of 1.5 g/pound of feed to 10-day-old chicks for 7 weeks, the thyroid weight increased to 3.9 times the control thyroid weight(8).

Uotila and Kannas(9) described a quantitative histological method by which the percental proportions of epithelium colloid and stroma in the thyroid gland could be determined. In the normal guinea pig, it was estimated that the thyroid gland consisted of 63.6% epithelium, 34% colloid, and 2.4% stroma.

Assuming a similar relation in the thyroid glands of the control fowls, it is estimated that they would contain 34% colloid. The wet weight of the control glands would contain 45.8 mg of colloid and the DFFT would contain 9.7 mg of colloid. In the thyroids after 30 days of methimazole treatment, the mean wet thyroid weight increased to 224.9 mg, but it is assumed that none of this was represented by colloid. Therefore, the glands after 30 days of methimazole treatment increased by 171% in DNA, a total of 152.5 mg in weight when a constant cell size of the thyroid gland is assumed. This calculation is made by subtracting 45.8 mg (colloid) from 134.8 mg (total weight of normal thyroid) to obtain 89.0 mg of thyroid weight representing thyroid cells. If 89.0 mg of thyroid wet weight contains 470 μg DNA, then 805 μg DNA represents 152.5 mg wet weight when cell size remains constant. Since this is not the case with methimazole treatment, the additional thyroid weight (224.9 mg total minus 152.5 mg due to cell multiplication) is due to increase in cell size. Upon calculating, one finds that 152.5 ÷ 224.9 = 0.68 or that 68% of the increased thyroid gland wet weight is due to cell multiplication (hyperplasia) and that 72.5 ÷ 224.9 = 0.32 or 32% of the increase is due to enlargement of the cells (hypertrophy).

When these calculations are extended to the thyroid glands after 60 days of methimazole, the values are 57% of the increase in weight is due to cell multiplication and 43% is due to increase in cell size. Similarly, the 90 days of methimazole treatment results in a 42% increase in cell multiplication and a 58% increase attributable to an enlargement of the cells.

These observations indicate that cell multiplication is the major cause of the increase in thyroid weight (68%) after goitrogen treatment of 30 days under the stimulus of increased secretion of TSH. After 60 days, cell multiplication accounts for 57% of the increase and after 90 days for 42%.

Histological observations of thyroid glands after goitrogen treatment show the lumen of the follicles free of colloid and the height and presumably the volume of the cells increased. These data indicate that cell size increases markedly with continued goitrogen treatment.

Summary. Mature male fowls were divided into 4 groups of equal TSR. One group served as controls whereas the other 3 groups were injected daily with 0.5 mg tapazole/100 g bw/day for 30, 60 and 90 days. The mean wet thyroid gland weight of the control group was 134.8 mg. After 30 days, the thyroid weight increased 66.8%, after 60 days, the increase was 187.9%, and after 90 days the increased weight was 136.5% above the controls. The determination of the total DNA of each group of thyroid glands indicated the extent of cell multiplication involved and from these data it was possible to estimate the proportion of the weight increase which was due to cell multiplication and the increase due to cell size.

After 30 days the cell multiplication accounted for 68% and cell size for 32% of the increase, after 60 days 57% to cell multiplication and 43% to cell size and after 90 days 42% due to cell multiplication and 58% due to cell size.

1. Hendrich, C. E., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1964, v117, 218.

2. Webb, J. M., Levy, H. B., J. Biol. Chem., 1955, v213, 107.

3. Richmond, S. B., Statistical Analysis, 2nd Ed., Ronald Press Co., New York, 1964, p186.

4. Kramer, C. Y., Biometrics, 1956, v12, 307.

5. Matovinovic, J., Vickery, Austin L., Endocrinology, 1959, v64, 149.

6. Hendrich, C. E., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1965, v119, 174.

7. Panda, J. N., Turner, C. W., ibid., 1966, v123, 553.

8. Hayness, R., Glick, B., Poultry Sci., 1960, v39, 1495.

9. Uotila, Unto, Kannas, Osmo, Acta Endocrinol., 1952, v11, 49.

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Endocrine Effects of Pineal Gland and of Melatonin. (32468)

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It has been proposed recently that the pineal gland exerts an inhibitory influence on the gonads and that this inhibition is mediated by a specific neurohumor called melatonin or 5-methoxy-N-acetyl-tryptamine (1). Support for this hypothesis comes from studies which have demonstrated that the weight of the pineal gland(2), and the activ-