TABLE I. Cycle Length in Normal and Experimental Hamsters.

No. animals	No. cycles	Ovary position	Cycle length (days) Estrous	Pseudo-pregnant
10	59	Normal	4	9.24 ± .16*
16	85	Cheek pouch	4	9.85 ± .28

* Mean ± standard error.

characteristic morphology (Fig. 3, 4). A closer examination of the follicles produced by transplanted ovaries showed no visible evidence of morphological alteration, leading us to believe that subsequent studies on ovulation might be profitable using this technique.

It should be mentioned that there was a tendency for implants to enlarge and produce "cystic-like" follicles after several normal cycles in the pouch. Some implants have successfully regulated estrous and pseudo-pregnant cycles for as long as 6 months, only to show eventually the same loss of cyclic function due to rapid follicular enlargement.

1. Anderson, L. L., Bowerman, A. M., Melampy, R. M., in Advances in Neuroendocrinology, A. Nalbandov, ed., Univ. of Illinois Press, Urbana, 1963, 347.

2. Runner, M. N., Palm, J., Anat. Rec., 1950, v108, 537.

3. Patt, D. I., Hamdler, A. H., Lutz, B. R., ibid., 1951, v111, 170.

4. Sak, M. F., Macchi, I. A., Beaser, S. B., ibid., 1965, v152, 25.

5. McDaniel, J. W., Black, D. L., Nature, 1964, v202, 810.

6. Duby, R. T., McDaniel, J. W., Black, D. L., ibid., 1965, v205, 720.

7. Shepro, D., Kula, N., Halkett, J., J. Exp. Med., 1963, v117, 749.

8. Duby, R. T., McDaniel, J. W., 47th Endocrine Soc. Proc., 1965, 72.

Received June 16, 1966. P.S.E.B.M., 1966, v123.

Effect of 1-Methyl-2-Mercaptomidazole (Methimazole-Tapazole) on the DNA Content of the Rat Thyroid Gland.* (31541)

J. N. PANDA AND C. W. TURNER
(With the technical assistance of Mary E. Powell)
Department of Dairy Husbandry, University of Missouri, Columbia

In the estimation of thyroid hormone secretion rate (TSR), the goitrogen methimazole or tapazole has been used to block the recycling of I^{131} from the metabolism of L-thyroxine-I^{131}. This goitrogen has been used since it has been shown to have no extrathyroidal effect in comparison to other goitrogens (1). In the rat, it has been shown that 400 μg/100 g b.w./day by subcutaneous injection will block I^{131} recycling(2). After 30 days of treatment, it has been shown that the rat thyroid gland doubles in weight. Histological study of the thyroid glands indicates that the goitrogens deplete the gland follicles of all stored colloid but greatly increase the height of the follicular epithelium. Since the glands become dark red in color, it may be assumed that there is an increase in the capillary bed and blood content. It is not clear whether the doubling of the gland weight is primarily due to an increase in cell multiplication due to TSH or to an increase in cell size. If it were due to cell multiplication, such glands might be capable of greater thyroglobulin secretion when the goitrogen was withdrawn.

Matovinovic and Vickery(8) fed male guinea pigs 0.2% thiouracil in the feed for a period of 3 months. They reported 314% increase in thyroid weight, a 519% increase in total cell mass, a 140% increase in average cell height and with no change in cell population density. The DNA per unit weight of wet tissue was not changed while the DNA per unit weight of cell mass decreased 36%. The total DNA per gland increased 299%.

* Contribution Missouri Agr. Exp. Sta. Journal Series 4004. Approved by Director. This investigation was supported in part by a grant from U. S. Atomic Energy Commission Contract AT(11-1)-301-125.

The increase in cell mass with no significant change in cell population density indicated hypertrophy of thyroid cells.

Deoxyribonucleic acid (DNA) has been used as an index of the growth of the mammary glands in this laboratory(3). When cells multiply the DNA increases correspondingly, whereas when the individual cells increase in size without multiplication, the DNA per unit of dry-fat-free tissue decreases.

The present study was undertaken to determine to what extent the increase in thyroid gland weight following methimazole administration was due to cell multiplication and to what extent due to an increase in cell size.

Materials and methods. Forty adult female Sprague-Dawley-Rolfsmeyer rats with a mean body weight of 225 g were maintained at a temperature of 78 ± 1°F on Purina Lab Chow with water *ad libitum*. TSR of the rats were determined by the method previously described(7). After 14 days, the rats were divided into 2 groups with similar TSR. One group served as a control while each rat of the second group received 400 μg/100 g b.w./day of methimazole (tapazole) in 0.1 ml of saline, while the controls received 0.1 ml/100 g b.w./day saline only. Half of the rats from the experimental group were sacrificed after 20 days and the other after 30 days of treatment. The thyroid glands were collected on ice and stored in closed containers at —20°C. The DNA was then estimated in groups of 5 thyroid glands by the method used in this laboratory for estimation of the DNA in mammary glands(3).

Results. The mean thyroid gland weight of a group of 13 female control rats weighing 227 g after 20 days of 0.1 ml saline injection was 9.73 ± 0.06 mg. After 20 days of methimazole injections at a level of 400 μg/100 g b.w., the mean thyroid gland weight 16.04 ± 0.89 mg, an increase of 64.85%, and to 20.53 ± 1.1 mg after 30 days, an increase of 110.9% in wet weight (Table I).

The mean dry-fat-free tissue (DFFT) of the control group was 2.30 ± 0.06 mg or 23.6% of the wet weight.

After 20 days of methimazole treatment, the mean DFFT increased to 3.45 ± 0.18 mg, 21.5% of the wet weight, and after 30

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

Group No.	Treatment	No. of animals	Body wt Initial	Body wt Final	Thyroxine secretion rate, μg/100 g b.w.*	Thyroid gland wt (wet), mg*	DFFT, mg*	DNA, μg/mg DFFT	DNA per thyroid gland, μg	% Increase of DNA per gland
1	Control—Injected normal saline, 0.1 ml/100 g b.w. daily	13	225.0	227.0	.96 ± .008	9.73 ± .06	2.30 ± .06	15.00	34.50[1]	—
2	Treated with 0.1 ml of 400 mg% tapazole/100 g b.w. daily for 20 days	14	231.0	234.0	.95 ± .007	16.04 ± .89	3.45 ± .18	15.15	52.27[2]	51.50
3	Treated with 0.1 ml of 400 mg% tapazole/100 g b.w. daily for 30 days	13	226.0	242.0	.87 ± .009	20.53 ±1.1	3.60 ± .07	15.73	56.63[3]	64.14

* Mean ± standard error.
DFFT = Dry fat-free tissue. DNA = Deoxyribonucleic acid. b.w. = Body wt.

1 *vs* 2 = .005 > P > .001
1 *vs* 3 = .005 > P > .001

days of treatment to 3.60 ± 0.07 mg, 22.9% of the wet weight.

The DNA/mg DFFT in the 20-day control and experimental groups was 15.00 and 15.15 μg, respectively. This would indicate that the number of cell nuclei/mg was similar. After 30 days, the DNA was 15.7 μg in the experimental group/mg DFFT.

The total DNA/control thyroid gland was 34.5 μg in the control group and 52.3 μg in the goitrogen group, a significant increase of 51.50% ($0.005>P>0.001$).

The total DNA/thyroid gland after 30 days of treatment was 56.63 μg, an increase of 64.14% based on the control group ($0.005>P>0.001$).

Discussion. When a goitrogen is administered in suitable amounts to animals, it was shown that iodine uptake by the thyroid gland was blocked, thyroid hormone secretion was interfered with, the colloid present in the follicles was gradually depleted, and the weight and size of the thyroid was increased, *i.e.*, a goiter was produced. If graded levels of thyroxine or triiodothyronine were administered simultaneously, the increase in thyroid size and weight observed with the goitrogen alone was gradually decreased and at a level of thyroxine which maintained normal thyroid weight, was considered to be the equivalent of the normal thyroid hormone secretion rate (Dempsey and Astwood)(5).

The increase in thyroid size and weight under the influence of the goitrogens was suggested to be due to an increased endogenous secretion of TSH as the thyroidal colloid containing the hormone was discharged and the further secretion of thyroid hormones ceased.

That exogenous TSH would increase the size of the thyroid gland was shown by the use of this index in the early assay of TSH.

While it has long been recognized that thyroid enlargement could be induced by exogenous or endogenous TSH, the extent to which this weight increase is due to epithelial cell multiplication and to an increase in cell size (length) has not been determined.

Histological study of the thyroid gland has shown clearly that the colloid content of the gland is depleted and that the cells are elongated. This latter observation has been used in the assay of TSH. Mitotic cell division has been observed(4).

In this laboratory, the content of DNA in the mammary gland of the rat during normal pregnancy and lactation has been used as an index of growth (cell multiplication)(3). If it may be assumed that each thyroid epithelial cell contains the same amount of DNA, then the increase of DNA by the thyroid would also measure the total increase in cells stimulated by the experimental treatment. In the control thyroid glands with a mean weight of 9.73 mg, the mean DNA/gland was 34.5 μg. After 20 days of methimazole, the mean DNA/gland increased to 52.27 μg, a significant increase of 51.50%, and after 30 days of treatment to 56.63 μg, a significant increase of 64.14%. These observations indicate that much of the stimulus for cell proliferation occurs during the first 20 days but continues throughout the entire period of treatment.

The DNA/mg DFFT is also of considerable significance. This value indicates the number of cells present in a unit weight of thyroid tissue. If the cells are small, *i.e.*, are associated with a small amount of cellular protoplasm, the DNA/mg DFFT would be high due to increased number of cells in a unit weight of tissue.

In the control glands, the DNA/mg of DFFT was 15.0 μg and after 20 and 30 days of methimazole treatment was 15.15 and 15.73 μg, respectively. Thus, the DNA per unit weight of thyroid gland remained constant. How may this observation be explained when it has been shown histologically that the epithelial cells increase in length (volume) and therefore, fewer cell nuclei would be present in a unit of weight?

The explanation is believed to be due to the decline in colloid present in the gland equal to the increase in cell size. Uotila and Kannas(6) described a quantitative histological method by which the percental proportions of epithelium, colloid and stroma in the thyroid could be determined. In the control guinea pig, it was estimated that the thyroid gland consisted of 63.6% epithelium, 34.0% colloid, and 2.4% stroma. Based upon an estimated 34% colloid in the rat thyroid gland, the wet weight of the control glands would contain 3.30 mg, and the DFFT

0.78 mg of colloid. In the thyroids after 30 days of methimazole treatment, the mean wet thyroid weight increased to 20.53 mg, but it is assumed that they lost 3.30 mg of colloid present in the control glands. This difference of 17.23 mg represents the increase in weight due to cell multiplication and cell size. The increase in cell multiplication measured by the increase of 64.14% in DNA multiplied by the difference in wet weight (17.23 mg) indicated an increase of 11.05 mg due to cell multiplication. The increase in weight due to cell multiplication is therefore 54% and to cell size 46%. These values do not take into consideration any alteration in the stroma.

Summary. Mature female rats were injected with 400 μg/100 g b.w. of methimazole/day for 20 and 30 days. In 20 days, the mean thyroid gland weight increased from 9.73 mg for the controls to 16.04 mg, an increase of 65% and after 30 days to 20.53 mg, an increase of 111%. To determine to what extent the increase in thyroid gland weight was due to cell multiplication and to an increase in cell size, the total DNA/gland and the DNA/mg DFFT was determined. After 20 days the DNA/gland increased 52% and after 30 days, 64%, whereas the DNA/mg DFFT remained constant in the 3 groups. It has been calculated that the increase in thyroid gland weight after 30 days of goitrogen treatment represents a 54% increase in cell growth (multiplication) and a 46% increase in cell size.

1. Grosvenor, C. E., Endocrinology, 1962, v70, 934.
2. Hendrich, C. E., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1965, v119, 174.
3. Damm, H. C., Turner, C. W., ibid., 1957, v95, 466.
4. Scholchaert, J. A., Arch. Internat. de Pharmacodyn et de Therap., 1931, v41, 23.
5. Dempsey, E. W., Astwood, E. B., Endocrinology, 1943, v32, 309.
6. Uotila, Unto, Kannas, Osmo, Acta Endocrinol., 1952, v11, 49.
7. Djojosoebagio, S., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1964, v116, 1099.
8. Matovinovic, J., Vickery, Austin L., Endocrinology, 1959, v64, 149.

Received June 16, 1966. P.S.E.B.M., 1966, v123.

Acceleration of Wound Healing with Heterologous Cartilage. Immunological Considerations.* (31542)

EVAN R. WOLARSKY,† STANLEY R. FINKE,‡ AND JOHN F. PRUDDEN
Departments of Surgery and Medicine, Columbia University College of Physicians and Surgeons, New York, N. Y.

Investigation of the process of wound healing has long been one of the prime interests in surgical laboratories. The most interesting facet of this effort has always been the hope of accelerating this biological process so as to shorten the healing time. Many substances are capable of producing relevant phenomena *in vitro*, but they do not increase the tensile strength of experimental wounds in the slightest(1). Only one substance has been demonstrated to produce a consistent acceleration in wound healing and this material is cartilage(2,3,4,5,6,7). Extracts of the material will produce an accelerator effect(8) as will local application of the material. These biological effects have been demonstrated with cartilage from a variety of species in wounds in a number of different animals(9). Furthermore, recent work from this laboratory demonstrates conclusively that cartilage preparations also accelerate human wound healing(10).

As the cartilage preparations have been of heterologous origin (cow, shark) and hence contain foreign substances, the problem of the antigenicity of such compounds must necessarily be considered.

* Supported in part by Nat. Inst. of Arthritis & Metab Dis. (AM-02581).

† School of Med., Univ. of Pennsylvania.
‡ Current address: Institute of Allergy, Roosevelt Hospital, New York.