Zoology. — *A mathematical method for the determination of the state of activity of the thyroid gland.* (Preliminary note.) By J. LEVER. (Zoological Laboratory, Dept. of Endocrinology, University of Utrecht.) [1]) (Communicated by Prof. CHR. P. RAVEN.)

(Communicated at the meeting of November 27, 1948.)

It is a well-known fact that the thyrotrophic hormone induces an increase of activity of the thyroid gland. After administration of antithyroid drugs there is also an increase of activity: the formation of active thyroid hormone is prevented by these drugs, which results in a decrease of the thyroid-hormone level in blood; this stimulates the output of thyrotrophic hormone in the pituitary, thus bringing about all the histological and cytological changes found in the thyroid.

These changes, visible in fig. 1, are

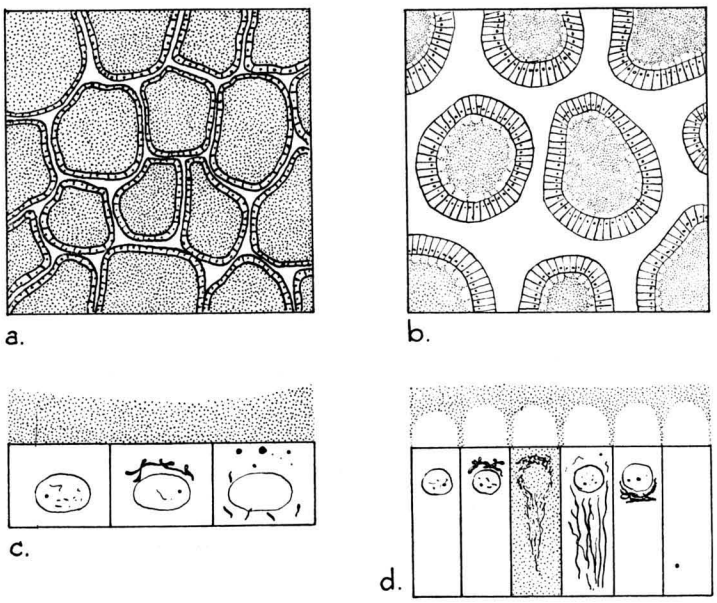

Fig. 1. Upper row: schematic drawings of sections of *a*) an inactive and *b*) an active thyroid. Bottom row: schematic drawings, showing cytological details of thyroid epithelium cells: *c*) inactive stage; *d*) active stage

1. an increase in epithelium height;
2. an absorption of colloid;

[1]) 25th Communication of the "Werkgemeenschap voor Endocrinologie", part of the "National Council for Agricultural Research T.N.O.".

3. an increase of the interfollicular spaces, caused by enlargement of the bloodvessels;
4. an increase of the number of epithelium cells (cf. SEVERINGHAUS, 1933; ELMER, 1938; PASCHKIS et al., 1945; ŘEŘÁBEK and ŘEŘÁBEK, 1947);
5. a change in the position of the nucleus from the base of the cell to its apex;
6. a change in the position of the Golgi-apparatus from the apical side of the cell to its base (COWDRY, 1922; LEVER, 1947);
7. an outgrowth of the mitochondria into long slender filaments (CRAMER and LUDFORD, 1926).

As it is easy to change the activity of the thyroid gland, is was desirable to trace the different phases phases of histological activity. Then this activity might be used as a bioassay for the active substances. Moreover, it might be useful in describing accurately the normal differences of thyroid activity. HEYL and LAQUEUR (1935) distinguished several phases of activity in the thyroid of the Guinea-pig, characterized by the form of the cells and of the nuclei, by the position of the nucleus in the cell and by the quantity of cell protoplasm.

In recent years the assay of active substances is especially based on the changes in thyroid weight. The increase in thyroid weight is generally caused by the increase in blood content of the gland (fig. 1). However, as the follicles are the internally secreting parts of the gland, only methods based on changes in the follicles can tell us something about its activity.

Several methods founded on this principle have been described:

1. The *cell height* of the thyroid epithelium is determined by averaging the cell height of a great number of follicles. This has been applied e.g. by ADAMS and BEEMANN (1942) and by LARSON et al. (1945). Against this method the objection may be raised that neither the colloid content of the follicle nor the mitotic activity of the follicle cells are considered. Moreover, it appears that in sections large follicles generally show a higher epithelium than small ones, as a result of unavoidable errors of observation.

2. VAN ECK (1940) determined planimetrically the relation between the colloid areas and the rest of the thyroid section. With the aid of a simple formula the percentage of *colloid content* of the gland was calculated. In the same way STEIN (1940) determined the colloid-contents in every single follicle of a human thyroid.

Here the same objection arises: very important deviations are sometimes caused by a highly developed hyperaemia of the gland.

3. DE ROBERTIS and DEL CONTE (1944) counted the number of *intracellular colloid droplets* in the thyroid epithelium after the administration of thyrotrophic hormone. GRASSO (1946) used this method for the investigation of the influence of antithyroid drugs, thus establishing a *"cytological coefficient"*. A modification of this method was published by

DVOSKIN (1947), who calculated the number of colloid droplets in the thyroid epithelium of each animal in totalling the number of droplets in 25 cross sections through the middle part of the gland.

The last two methods, however, do not take into consideration the epithelium height, the colloid content and the increase in the total number of cells. Moreover, none of the methods described above enables us to get an idea of the laws controlling the variations in activity of the gland.

We have therefore tried to find a method by which not one but three characteristics of the follicle, and also the laws of their variation are considered.

As in a circle the circumference is related to the diameter [$c = f(d)$] and in a thyroid-follicle each epithelium cell has the same size, it is easily understood that in follicles, circular in section, the number of cells must be related to the diameter. As the follicles of a given thyroid are all in the same functional phase, we can accept this relation for all follicles, large as well as small ones.

In fig. 2 such hypothetical cases are drawn for 4 follicles of different

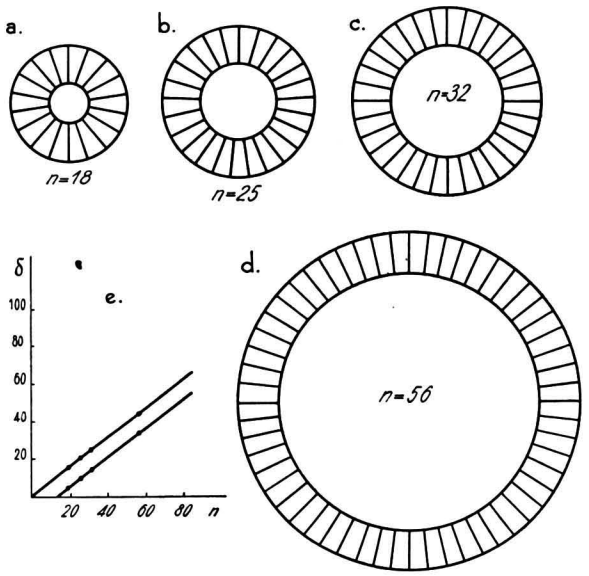

Fig. 2. *a—d:* schematic drawings of four theoretical thyroid follicles with different cell-number and cells of the same size. *e:* graph, showing regression lines in which the outer and inner diameter of the thyroid follicles are plotted against the cell numbers.

size, but in which the epithelium cells are equally large. In the graph of fig. 2 in the upper regression line the diameter of the whole follicle and in the bottom line the diameter of the follicular cavity is plotted against the number of epithelium cells. (Further on these diameters will be called outer and inner diameter respectively).

In fig. 3 similar graphs are calculated from three inactive thyroids of control cockerels (upper row) and from three thyroids of cockerels treated with antithyroid drugs (bottom row). The following details are distinctly visible:

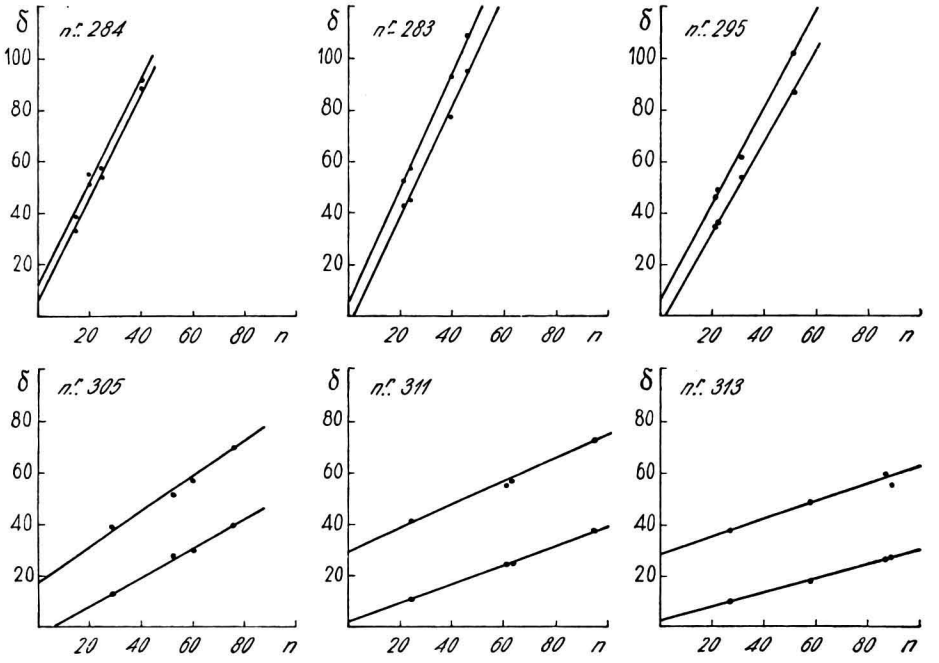

Fig. 3. Upper row: regression lines of inactive thyroids; bottom row: the same active thyroids. (Ordinates: diameters in μ, abscisses: cell numbers.)

1. In the upper row (inactive thyroids) the perpendicular distances between the almost parallel lines are very small. This distance demonstrates the difference between the outer and the inner diameter and equals twice the height of the epithelium cells. In the bottom row the distances between these 2 lines are large and demonstrate the increase in height of the epithelium cells.

2. The inclination of the regression lines in the upper and bottom graphs is very different and demonstrates the relation between the diameter of the follicles and the cell number. Comparing e.g. follicles with an outer diameter of 60 μ in the graphs 284 and 305, we see immediately that the first contains 25, the second 60 cells.

Moreover, if we presume that the height of the epithelium cells is increased by activation in only one direction, e.g. towards the lumen of the follicle, the outer diameter remaining constant, we can conclude from these graphs that the cell number in sections of follicles with a diameter op 60 μ is more than doubled. However, by measuring hundreds of follicles in inactive and active thyroids, we have found that the epithelium height

increases in both directions after activation, which became evident by studying frequency-diagrams of the outer diameters.

3. The distance between the bottom line and the abscis demonstrates the colloid content in the follicles. As in the graphs of activated thyroids the bottom line runs much more horizontally, the colloid content of the thyroid follicles decreases by activation.

Consequently the graphs resulting from our method give an insight in

1. the height of the epithelium cells of the thyroid;
2. het number of epithelium cells of the follicles, and
3. the quantity of colloid, present in the follicles.

Therefore they show simultaneously the three principal histological facts, concerning the activity of the thyroid follicle.

Our method is easy to apply as sections stained by the ordinary laboratory routine techniques can be used. The one thing required is that the sections possess the same thickness; 3 μ-sections are the best as they contain only one cell-layer. Generally it suffices to count the number of epithelium cells of four follicles, and to measure their outer and inner diameters. In the case of a follicle being more elliptic than circular, the average of the largest and smallest diameter is sufficient.

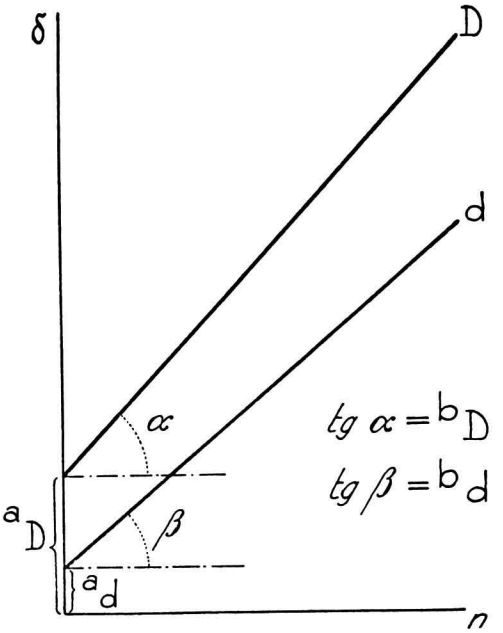

Fig. 4. Graph, showing the coefficients a_d, a_D, b_d and b_D (cf. text).

The lines of the graphs, delineated above, may be described by the general formula $y = a + bx$ (fig. 4). In this formula is y the ordinate, x

the abscis, a the part of the ordinate cut off by the regression line and b the tangens of the angle of inclination.

As each thyroid is represented by 2 regression lines, relating to the outer and inner diameters, D and d respectively, the 2 formulae:

$$y_D = a_D + b_D x \quad \text{and} \quad y_d = a_d + b_d x$$

represent the whole gland.

The distance between both lines — i.e. the difference between the outer and inner diameter of the follicle, or twice the epithelium height — in follicles of different sizes is the same in inactive thyroids only. In these b_d and b_D are practically equal.

Therefore in this case we can derive from these 2 lines one with the formula:

$$y_{dD} = A + Bx$$

in which $A = a_D - a_d$ and $B = \frac{b_D + b_d}{2}$, A being related to the epithelium height (cf. fig. 4) and B to the number of cells dependent on the diameter of the follicle.

Consequently, if the two lines run nearly parallel, we can use in stead of two factors A and B only one coefficient

$$\frac{A}{B} = \frac{2(a_D - a_d)}{(b_D + b_d)}.$$

In practice we have found that it is possible to simplify the method still further. As a matter of fact the value of a_d always fluctuates around the graph's zero, suggesting that in reality in a thyroid the value $\frac{d}{n}$ is the same in follicles of all sizes. This quotient is generally the same as b_d, but it is much easier to calculate. Moreover, it was experimentally stated that the coefficient $\frac{d}{n}$ is the best in expressing thyroid activity, for by activation the numerator (d) diminishes and the denominator (n) increases. Consequently the lower the value of $\frac{d}{n}$, the more active the thyroid is. In practice we have found that in chickens $\frac{d}{n}$ varies between $\pm$ 2.7 and $\pm$ 0.2.

We will give here only one example:

In 2 groups of 9 cockerels which were treated with thyroid activating substances it was not possible, when applying one of the above mentioned methods based only on epithelium height, on colloid content or on a- and b-factors, to demonstrate a distinct difference between these 2 active groups. But when using for both groups the values of $\frac{d}{n}$ for every observed follicle and the average value $\frac{\overline{d}}{n}$, we could easily separate them (cf. table);

this quotient is directly related to the colloid content and indirectly to the height of the epithelium cells, as the inner diameter decreases with the increase of the epithelium height.

Table, showing values of $\frac{d}{n}$ and $\frac{\bar{d}}{n}$ for two groups of cockerels, treated with thyroid activating substances. Group 1—9 is more active than group 10—18.

Number of animal	$\frac{d}{n}$	$\frac{\bar{d}}{n}$	Number of animal	$\frac{d}{n}$	$\frac{\bar{d}}{n}$
1	0.6, 0.5, 0.4, 0.5	0.5	10	1.4, 1.3, 1.4, 1.2	1.3
2	0.5, 0.6, 0.5, 0.4	0.5	11	0.9, 0.9, 0.8, 0.8	0.9
3	0.6, 0.6, 0.7, 0.7	0.7	12	0.9, 1.0, 0.9, 0.9	0.9
4	0.7, 0.7, 0.6, 0.7	0.7	13	0.8, 1.1, 0.9, 0.9	0.9
5	0.7, 0.7, 0.7, 0.7	0.7	14	0.8, 0.9, 0.9, 0.9	0.9
6	0.7, 0.8, 0.7, 0.6	0.7	15	1.0, 1.1, 0.9, 0.9	1.0
7	0.7, 0.8, 0.7, 0.7	0.7	16	1.0, 0.9, 0.8, 1.0	0.9
8	0.8, 0.7, 0.7, 0.8	0.8	17	1.0, 1.2, 0.9, 1.4	1.1
9	0.7, 0.7, 0.7, 0.7	0.7	18	0.9, 0.8, 1.0, 0.9	0.9

In exceptional cases it is possible to combine also the last factor with the formula by using the quotient $\frac{\frac{E}{d}}{n} = \frac{nE}{d}$, in which E is the epithelium height. As mentioned above this is only allowed in those cases in which the epithelium has the same height in all follicles, i.e. in inactive thyroid glands.

This method was successfully applied in sectioned thyroids of fishes (*Scylliorhinus canicula, Onos mustela, Callionymus lyra* and *Orthagoriscus mola*), of *Rana esculenta*, of the fowl and of mammals (Guinea pig, rabbit, pig, cattle, blue and sperm-whales).

Summary.

The principles of a new mathematical method in determining the state of activity of the thyroid gland are described, based on the relations between the cell-number, the outer and inner diameter and the height of the epithelium cells of the follicle. In applying this method not only the laws which govern the histological changes of thyroid activity, but also the activity of several antithyroid drugs can be studied.

Acknowledgements.

I wish to thank Prof. G. J. VAN OORDT and Prof. CHR. P. RAVEN for their kind advice and encouragement during this investigation.

REFERENCES.

ADAMS, A. E. and E. A. BEEMAN. The reaction of the chick thyroid to frog and mouse anterior pituitaries. Endocrinology, **31**, 128 (1942).

COWDRY, E. V. The reticular material as an indicator of physiologic reversal in secretory polarity in the thyroid cells of the guinea-pig. Am. Journ. of Anat., **30**, 25 (1922).

CRAMER, E. and R. J. LUDFORD. Cellular activity and cellular structure as studied in the thyroid gland. Journ. of Physiol., **61**, 398 (1926).

DVOSKIN, S. Intracellular colloid droplets as a basis for thyrotrophic hormone assay in the chick. Endocrinology, **41**, 220 (1947).

ECK, W. F. VAN. Een experimenteel onderzoek over eenige werkingen van het thyreotrope hormoon. Thesis. Amsterdam (1940).

ELMER, A. W. Iodine metabolism and thyroid function. London (1938).

GRASSO, R. The action of thiourea on the intracellular colloid of the thyroid gland. Anat. Rec., **95**, 365 (1946).

HEYL, J. G. and E. LAQUEUR. Zur quantitativen Bestimmung der thyreotropen Wirkung von Hypophysenvorderlappenpräparaten und die Einheit des thyreotropen Hormons. Arch. int. de Pharm. et de Thér., **49**, 338 (1935).

LARSON, R. A., F. R. KEATING Jr., W. PEACOCK and R. W. RAWSON. A comparison of thiouracil and of injected thyrotropic hormone on the collection of radioactive iodine and the anatomic changes induced in the thyroid of the chick. Endocrinology, **36**, 149 (1945).

LEVER, J. On the position of the Golgi-apparatus in the thyroid cell under normal and experimental conditions. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **50**, 1365 (1947).

PASCHKIS, K. E., A. CANTAROW, A. E. RAKOFF and M. S. ROTHENBERG. Mitosis stimulation in the thyroid gland induced by thiouracil. Endocrinology, **37**, 133 (1945).

ŘEŘABEK, J. and E. ŘEŘABEK. Nucleic acids and cytological changes in the thyroid gland after thiouracil. Acta Physiol. Scand., vol. **14**, 276 (1947).

ROBERTIS, E. DE and E. DEL CONTE. Método citólogico para determinatión de la hormona tireotropa de la hipófisis. Rec. Soc. Arg. Biol., **20**, 88 (1944).

SEVERINGHAUS, A. L. Cytological observations on secretion in normal and activated thyroids. Z. für Zellforsch. u. mikr. Anat., **19**, 653 (1933).

STEIN, H. B. The volume of the colloid of a normal human (Bantu) thyorid gland, with a note on the staining reactions of the colloid. Am. J. of Anat., **66**, 197 (1940).