THYROCYTE MORPHOLOGY AND FUNCTION IN ALIMENTARY OBESITY: AN ONTOGENETIC APPROACH

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Diseases whose pathogenesis is based on the alimentary factor of an excess of energy intake over consumption are widespread at the present time in economically developed countries [4, 8, 9]. An important role in the pathogenesis of obesity is played by functional and morphological disturbances of the endocrine glands, including the thyroid gland [2, 10]. However, the state of the thyroid gland has not been adequately studied in the course of overfeeding.

It was accordingly decided to undertake a morphometric study of the thyroid gland in experimental alimentary obesity, for clinical investigation precludes the possibility of its morphological study, and autopsy material does not permit one factor only (obesity) to be picked out for special study in the course of the processes taking place.

EXPERIMENTAL METHOD

To prevent any effect of the reproductive cycle [1] and of sexual dimorphism [11] on the thyroid gland, male Wistar rats were used in the model of obesity devised by Baranov et al. [3]. A special feature of this model is that overfeeding begins in the prepubertal period. Experiments were carried out in spring and summer on a population of rats (400 animals) of the same age. The experimental group consisted of 230 rats, the control group of 170. The experimental animals were kept on a mixed high-calorie diet from the age of 1.5 months. The control animals were kept all the time on the ordinary animal house diet. Observations continued until the animals were 240 days (6.5 months) old, and their body weight was measured every week. After the 98th day of life, material was taken by the dynamic observation method [6] after decapitation of the animal. Anthropometric data (body weight, weight of organs) were used in the investigation, the serum protein-bound iodine level and morphometric parameters (height of the thyrocytes, diameter of their nucleus, the cytological coefficient (CC), the ratio between the four components in the thyroid gland [5, 13]), histochemical parameters

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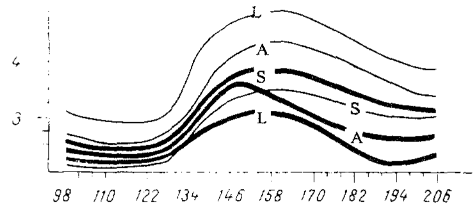

Fig. 1. Dynamics of changes in serum protein-bound iodine in groups of animals differing in body weight. Abscissa, age of animals (in days); ordinate, protein-bound iodine concentration (in μg %). Thick line – experiment, thin line – control; S) small, A) average, L) large individuals.

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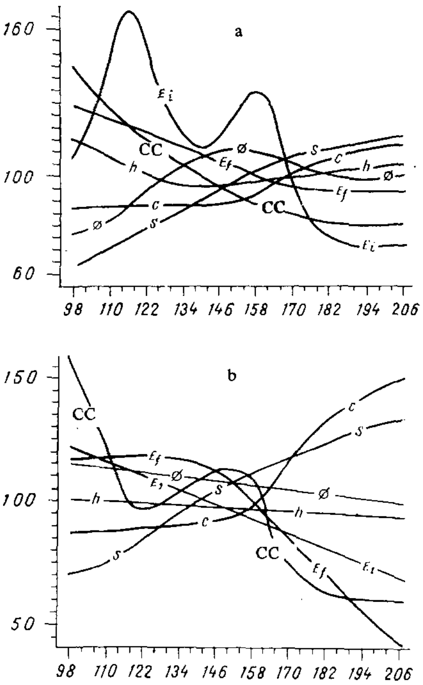

Fig. 2. Dynamics of changes in morphometric parameters of thyroid gland during ontogeny based on centered primary values. Abscissa, age of animals (in days); ordinate, ratio of actual value to mean for group (in %). E_f) relative follicular volume, E_i) relative interfollicular volume of epithelium; C) relative volume of colloid; S) relative volume of stromal component; h) height of thyrocytes; Ø) diameter of thyrocyte nucleus. a) Control, b) experiment.

[content of RNA, DNA, acid phosphatase (AcP), alkaline phosphatase (AlP), NAD, NADP, succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), peroxidase (P)], were determined, and an electron-microscopic analysis was made by the use of morphometric methods [4]. During the study of the ultrastructure of the thyroid gland attention was concentrated on organelles directly involved in the synthesis and secretion of iodine-containing hormones. The numerical data were subjected to statistical analysis by Student's test and by the method of sliding means, using centered curves [7]. The level of significance of differences was determined relative to actual values by the Wilcoxon–Mann–Whitney method.

The large number of parameters studied enabled a combined analysis to be made; this gives a deeper insight into the functional morphology of the thyroid gland.

EXPERIMENTAL RESULTS

Individuals in a sufficiently large population of the same age are known to differ in size (small, average, large). In the present investigations small individuals accounted for 30%, average for 45%, and large for 25% of the total number of animals studied. Most of the methods of study mentioned above were used both on the population as a whole (control and experimental) without regard to differences in body weight, and also in groups allowing for this parameter (control and experimental).

During the 108 days of observation the body weight of the animals of both groups increased close to exponentially [12] and differed significantly ($P < 0.001$) in the control and experiment, starting from the 75th day of the animals' life and continuing until the observations ended. The difference in body weight between the control and experimental animals was 200 g or more on the 198th-206th day of life of the rats, i.e., on the 150th-160th day of special feeding.

The serum protein-bound iodine of the control and experimental animals showed wave-like fluctuations during the period of observation (Fig. 1) and fell significantly ($P < 0.001$) during the course of overfeeding.

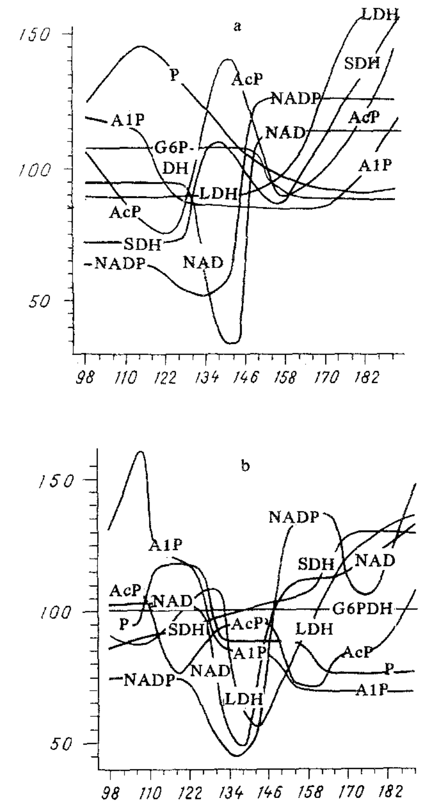

Fig. 3. Dynamics of changes in enzyme activity based on centered primary values. Legend for axes the same as in Fig. 2.

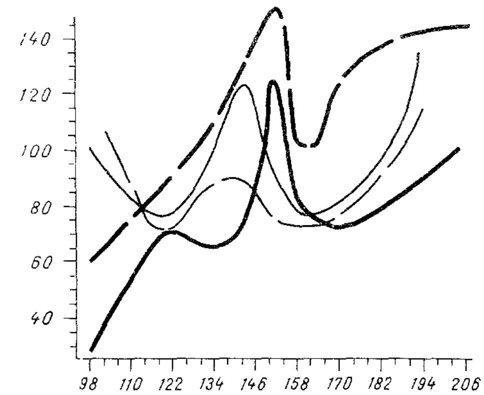

Fig. 4. Dynamics of changes in AcP activity and relative volume of lysosomes based on equalized centered values. Abscissa, age of animals (in days); ordinate, ratio of actual values to overall mean (%). Thin lines – enzyme activity, thick lines – relative volume of organelles. Broken line – experiment, continuous line – control.

This indicates depression of thyroid function in the experimental animals.

The weight of the thyroid gland increased with the animal's age; in the experimental series this parameter was significantly ($P < 0.001$) higher than in the control. However, the increase in weight of the thyroid gland in the course of overfeeding was not entirely attributable to the proportional increase in body weight.

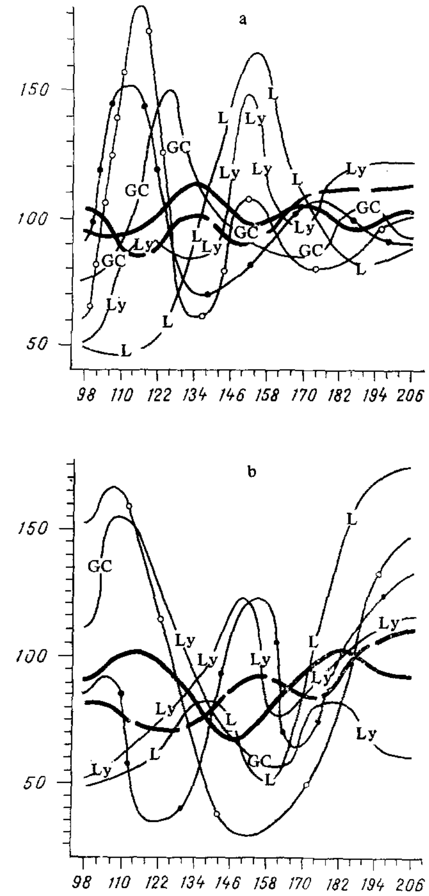

Fig. 5. Dynamics of changes in relative volume of organelles based on centered primary values. Continuous lines – mitochondria, broken lines – endoplasmic reticulum. Empty circles – electron-transparent granules; filled circles – electron-dense granules, Ly) lysosomes; L) lipid droplets; GC) Golgi complex. a) Control, b) experiment. Legend for axes the same as in Fig. 2.

Under the light microscope the thyroid gland of the obese and control animals showed no distinct demonstrable differences. To reveal any functional or morphological differences in the thyroid gland during prolonged overfeeding, morphometry was used. Analysis of each parameter (Fig. 2) separately enabled its time course during the period of observation to be determined. Analysis of the combined values of the morphometric parameters (comparison of areas) showed the overall variability of the morphological features of the thyroid gland and enabled the character of these changes to be assessed. The area of the positive values in the experimental series was 3% less than the corresponding value in the control (the conventional zero level was taken to be 100), whereas the area of negative values was 12% greater than in the control. This indicates a lowering of thyroid activity as reflected in morphometric criteria. Similar results also were obtained by the use of histochemical methods (Fig. 3).

Electron microscopy showed that qualitatively speaking the ultrastructure of the thyrocytes showed no particular features in the course of overfeeding and it was represented by the same organelles as in the control. However, the cytoplasm of the thyrocytes, as morphometric examination showed, was significantly more saturated ($P < 0.05$) with lysosomes, mitochondria, apical granules, and the hypertrophied Golgi complex. Comparison of the time course of changes in enzyme activity and the relative volume of the organelles (Fig. 4) revealed the clear effect of overfeeding, which was manifested differently in different enzyme–structural complexes (morphological structures containing or producing enzymes). Analysis of the relative volume of the combined organelles (Fig. 5) in the control showed a tendency for the scatter of the values to be reached with

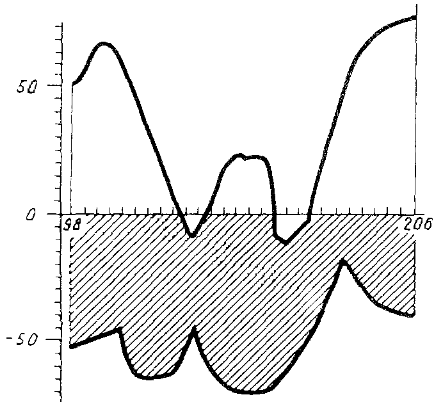

Fig. 6. Combined morphometric values for thyroid organelles. Shaded part — combined morphometric values of deficient (negative) values (experiment). Legend for axes the same as in Fig. 2.

age compared with the experimental series. The area of the relative volume of the combined organelles was 1.36 times greater in the course of overfeeding than in the control. The area of the positive values in the experimental series was 1.9% greater, whereas that of the negative values was 75.8% greater in the experimental series (Fig. 6). Changes in the relative volume of the organelles were not synchronized in the experimental and control animals, and their extrema often corresponded to different ages of the animals.

Comparison of the combined values of different parameters of rats in the prepubertal and pubertal period and in the course of overfeeding demonstrated cyclic changes with an interval of 32-36 days. Periods of combined values, detectable ultrastructurally, precede the combined values found at the light-optical level a little, i.e., ultrastructural changes precede histological. The higher saturation of the thyrocytes with organelles found in obesity, compared with the control, and the decrease in the concentration of iodine-containing fractions found in the peripheral blood, combined with the decrease in activity of the principal thyrocyte enzymes are evidence of a disturbance of hormone production in the thyroid gland in alimentary obesity; this disturbance is probably connected with the reduced formation of enzymes and hormones, or such enzymes and hormones which are produced are insufficiently active biologically. It is also very characteristic that individuals of a population of the same age differ in body weight and in the weight of their viscera, and that large individuals have the greatest degree of risk of becoming overweight in the course of prolonged overfeeding in the prepubertal and pubertal periods.

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