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Contribution to the Knowledge of the Enzymatic Activities in the Thyroid Gland of Pigs in the Connection with the Pathological Changes of the Gland

ABSTRACT

The activity of alkaline phosphatase (AP), acid phosphatase (SP), nonspecific esterases (NE), α -glycerophosphate (α -GPDH)-, glutamic (GIDH)-, lactic (LDH)-, succinic (SDH)-, β -hydroxybutyric (β -OH-BDH)- and glucose-6-phosphate (G-6-PDH)-dehydrogenases as well as nictotinamide-adenine-dinucleotide- and nictotinamide-adenine-dinucleotide-phosphate (NADH₂- and NADPH₂-DH)-diaphorases was investigated on 53 thyroid glands of pigs of both sexes, aged 8-10 months, divided on the basis of the pathohistological changes into three groups: hyperplastic, cystic and adenomatose goitre glands.

INTRODUCTION

There are numerous researches of the histochemical activities of the thyroid gland in connection with the pathological changes. The available data show a certain degree of disagreement between the authors as to the production of the hormones and the activities of the examined enzymes. As the role of the thyroid gland in the metabolic activities, especially its influence on the degree of the

oxidative processes is very significant, so an explaining of the connection between the enzymatic activities and the pathological condition of the thyroid gland would be of great interest. Therefore, we tried to find out, examined the thyroid glands of young pigs, whether the kind and the degree of the pathological changes of the gland are followed by the differences of the intensity of the activities of certain oxidative and hydrolitic enzymes.

Tremblay and Pearse (1960), examining a series of oxidative enzymes, like nicotinamide-adenine-dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate-diaphorases, DPH-isocitric-, succinic-, glucose-6-phosphate-, -hydroxybutyric-, malic-dehydrogenases and citochrom oxidase on the human thyroid gland, found out that there was no essential differences in the enzymatic activity of the follicular cells considering the kind of the goitre. Tellkää et al. (1960) found an increase of the succinic dehydrogenase activity on the thyroid gland of rats stimulated with thiouracil. Pakdaman et al. (1961) examined the activity of nicotinamide-adenine-dinucleotide diaphorase, glucose-6-phosphate and succinic dehydrogenases, adenosine triphosphatase, alkaline and acid phosphatase and nonspecific esterases in normal and pathological human thyroid glands, however, similar to Tremblay and Pearse (1960) they did not establish any important differences between the normal and adenomatose glands.

Niwelinski and Zamorski (1962) found an increased activity of nicotinamide-adenine-dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate diaphorases, succinic dehydrogenase and citochrom oxidase in normal thyroid glands of rats, treated with thiouracil, while the goiterous glands show no increase at all.

Lindsay (1963), found a weaker alkaline phosphatase activity and adenosine triphosphatase in hypoplastic, than in the hyperplastic thyroid gland of rat. Lindsay and Arico (1963), examining 19 different hydrolitic and oxidative enzymes in normal and pathological human thyroid gland, found out that proliferative processes of the epithel are usually followed by an increased enzymatic activity, which is connected with the growth or with the metabolic function of thyroid cells. The nonspecific esterases and acid phosphatase activity was almost identical in the normal and pathological gland, while the alkaline phosphatase activity slightly decreased in the glands with pathological changes.

Zawistowska and Zawistowski (1963) found in the thyroid gland of the rat that enzymes: alkaline and acid phosphatase, adenosine triphosphatase and nonspecific esterases, participate in the production and transport of hormones, while the phospholipides and mucopolysaccharides are considerably constant. Sobel (1964) found out that the alkaline phosphatase activity in the thyroid gland of

the rat is parallel with the secretoric activity of the glands, but he did not establish an increase of the oxidative enzymes in the different phases of the secretoric activity. Similarly, Haley et al. (1955), stated an increase of the alkaline phosphatase activity in human hyperplastic thyroid gland. Gruca and Wegmann (1964) stated in the thyroid gland of the rat, that the activity of succinic-, lactic- and glucose-6-phosphate-dehydrogenases remains unchanged, and that these enzymes do not participate in the synthesis of the hormones in the thyroid gland.

Feustal et al. (1969), found a decrease of the acid phosphatase activity in the thyroid gland of the rat, after reducing the partial oxygen pressure. Mitin et al. (1972) found an increase of the alkaline phosphatase activity in the thyroid gland of cattle hyperplastic and parenchymal goitre. The acid phosphatase activity did not change considerably in relation to the pathological condition of the gland, while the adenosine triphosphatase activity of the thyroid gland in cattle increased in the glands with hyperplastic, colloid and parenchymal goitre in relation to the thyroid gland without notable pathological changes.

MATERIAL AND METHODS

The examinations were carried out on 53 thyroid glands of pigs of both sexes, aged 8-10 months, weight 80-120 kg. Pigs were brought to the slaughter-house to the regular slaughter. Immediately after, the thyroid glands were taken out and either fixed in neutral formolcalcium, after Baker, or fresh frozen and kept in cryostate. One part of the glands served for pathohistological examination, the other one for the histochemical examination. On the ground of pathohistological examination, the glands were divided into three groups: cystic goitre glands, hyperplastic goitre gland and adenomatose goitre glands. From a total of 53 glands, all had pathological changes.*

On the formolcalcium fixed samples of the thyroid glands, cut into 8-10 microns thin sections, the activity of alkaline phosphatase after Gomori (Pearse, 1968), acid phosphatase after Barka and Anderson (1963) and nonspecific esterases after Pearse (1963) with naphthol-AS-acetate as substrate, were investigated.

On the fresh frozen samples of the thyroid glands, cut into 8-10 microns thin sections, the activity of α -glycerophosphate-, glutamic-, lactic-, succinic-, β -hydroxybutyric- and glucose-6-phospha-

* Pathohistological examination was performed by prof. dr. Marijan Hecceg, Department of Pathology, Veterinary Faculty University of Zagreb — and we use this way to express our sincere thanks.

ie- dehydrogenases, as well as nicotinamide-adenine-dinucleo-

tide- and nicotinamide-adenine-dinucleotide-phosphate- diaphorases (NADH₂-and NADPH₂-DH) after Hess et al. (1958) and Scarpelli et al. (1968), were investigated.

PERSONAL OBSERVATIONS

The activity of the alkaline phosphatase was shown in the shape of a metalprecipitate on the apical parts of the follicles epithelial cells (Fig. 1). The total intensity of the reaction on the alkaline phosphatase showed that the reaction varies from weak to moderate, no matter to what degree were the glands histopathologically changed. Only the adenomatose goitre showed a stronger reaction on the alkaline phosphatase. This is specially emphasized on the parenchyma, which is permeated with stronger interfollicular

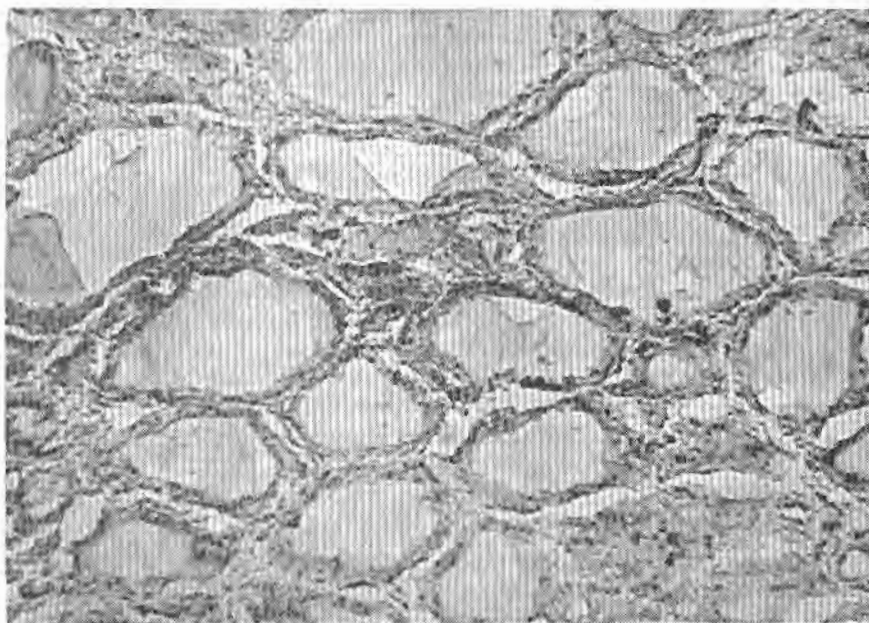


Fig. 1. Thyroid gland of a pig, struma adenomatosa, AP, moderate activity. Enlargement 6×10, overenlarged.

tissue and vascular stroma. Such densely arranged follicles, are as a rule, smaller and show somewhat wider layer of precipitate on the membranes of the epithelial cells. All follicles do not show the same intensity of the reaction, which may bring us to the conclusion that all parts of the gland are not identically active. Partly, the presence of the reactive precipitate can be found on the basal parts of the epithelial cells in the interfollicular tissue as well as the endothelium of the smaller blood vessels. In outstanding big fol-

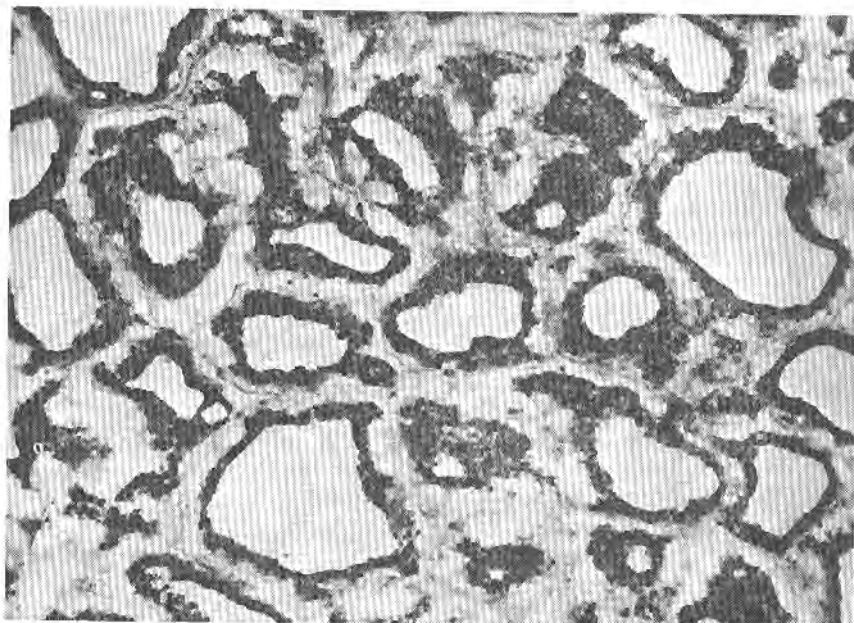


Fig. 2. Thyroid gland of a p.g, struma adenomatosa, SP, strong activity.
Enlargement 6×10 , overenlarged.

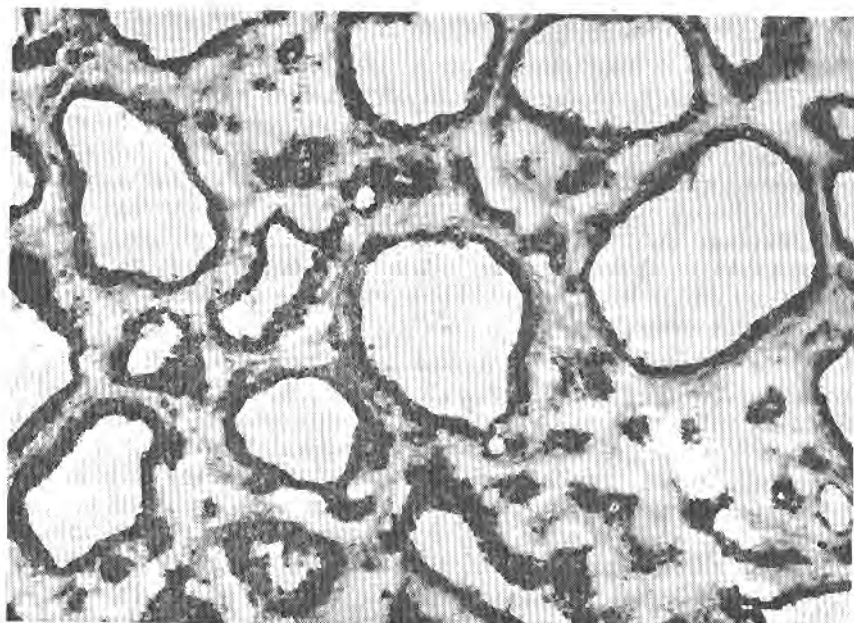


Fig. 3. Thyroid gland of a p.g, struma adenomatosa, SP, strong activity.
Enlargement 6×10 , overenlarged.

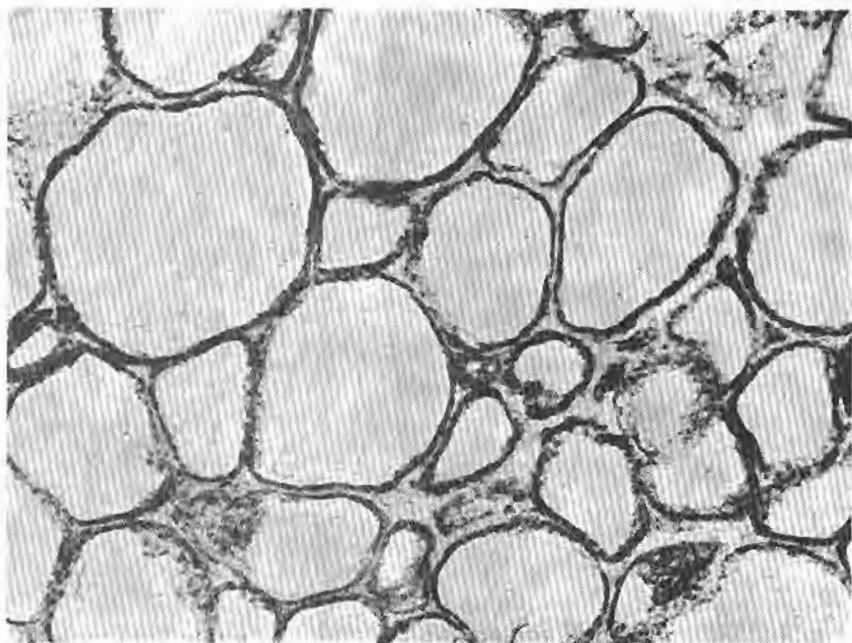


Fig. 4. Thyroid gland of a p.g. struma cystica, SP, strong activity. Enlargement 6×10 , overenlarged.

lices, no presence of the reactive precipitate was established for sure. The intensity of the reaction on the acid phosphatase is generally very strong. The reaction of the lysosomal type is specially outstanding on cystic and adenomatose glands (Fig. 2, 3 and 4). An outstanding dropping reaction was located mostly on the apical parts of the follicular cells. In the interfollicular tissue there was no apparent reaction except in some stromal cells, necrophages included. The activity of the acid phosphatase was not noticed in big blood vessels, while in the smaller blood vessels a reaction in the endothelium was established.

The activity of the nonspecific esterases in the follicular epithelial cells, varies from moderate to very strong. The cells of the follicular epithel show the presence of the reactive product mostly in the shape of the cytoplasmatic droplets distributed along the whole cell. In the great number of follicular cells the concentration of the reactive product is stronger on the apical pole of the cytoplasma of the cell, similar to the acid phosphatase. In the interfollicular tissue, the presence of the reactive product can be found only in the stromal elements. In the glands with the adenomatose goitre and gyperplasia the epilhel is a bit higher and the line of the coloring is a bit wider than at the cystic glands (Fig. 5). It is

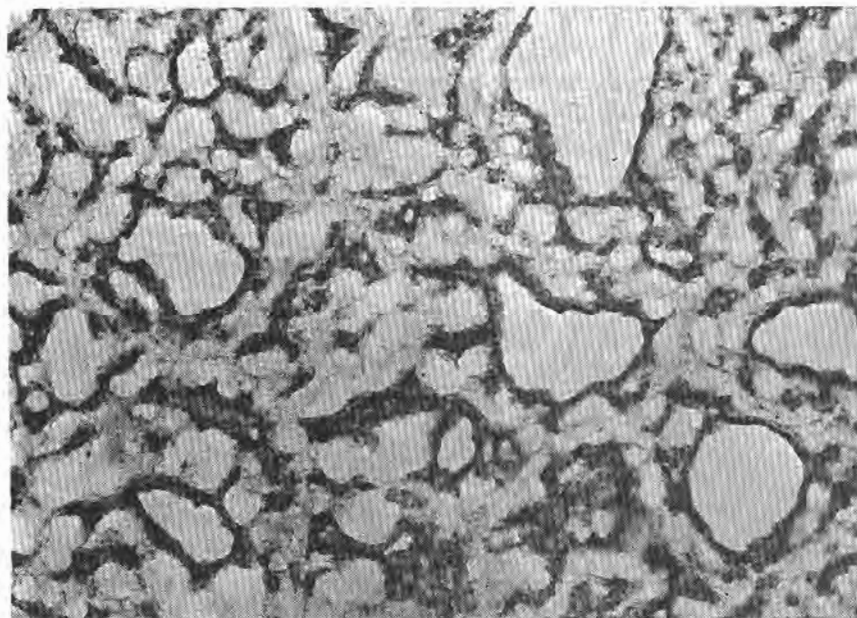


Fig. 5. Thyroid gland of a pig, struma adenomatosa et colloides cystica, NE, strong activity. Enlargement 6×10 , overenlarged.

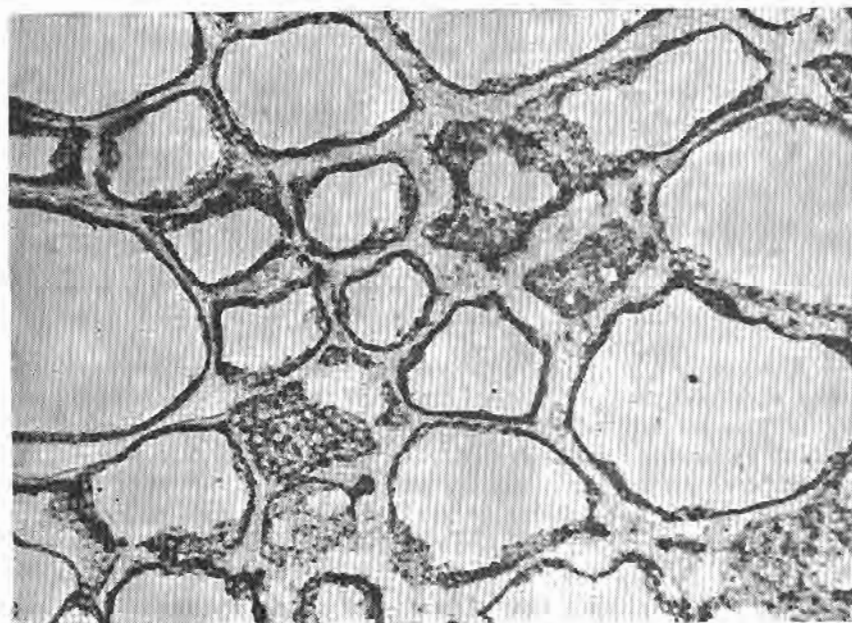


Fig. 6. Thyroid gland of a pig, struma hyperplastica, NE, strong activity. Enlargement 6×10 , overenlarged.

possible to say for the hyperplastic glands, that they show a moderate reaction (Fig. 6). It is a general impression that the intensity of the reaction in smaller and bigger follicles is more uniform than in the reaction of acid phosphatase.

The activity of oxidoreductase is being show in the precipitate of small formazan grains in the epithelial cells of the thyroid gland. The intensity of the reaction between some supstrat specific dehydrogenases is different. The activity of the glutamic dehydrogenase and succinic dehydrogenase is generally the weakest.

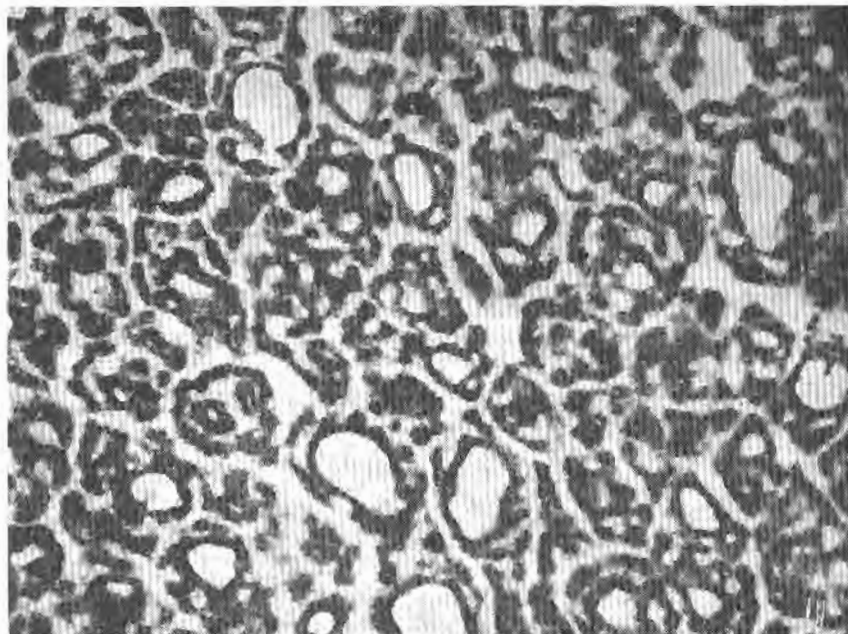


Fig. 7. Thyroid gland of a pig, struma hyperplastica, α -GPDH, moderate to strong activity. Enlargement 6×10 , overenlarged.

A moderate reaction was obtained by showing the activities of the α -glycerophosphate dehydrogenase and lactic dehydrogenase (Fig. 7). The activity of the β -hydroxybutyric dehydrogenase in the material examined is generally gentle to moderately strong, but the samples where the activity was moderately strong, belonged mostly to the hyperplastic glands (Fig. 8). Glucose-6-phosphate dehydrogenase, as well as the NADH₂- and NADPH₂-diaphorases show mostly a very strong reaction (Fig. 9, 10 and 11), but the preparations which show a moderate reaction belong to the adenomatose goitre type. A weaker reaction can be observed on some cystic goitre preparations.

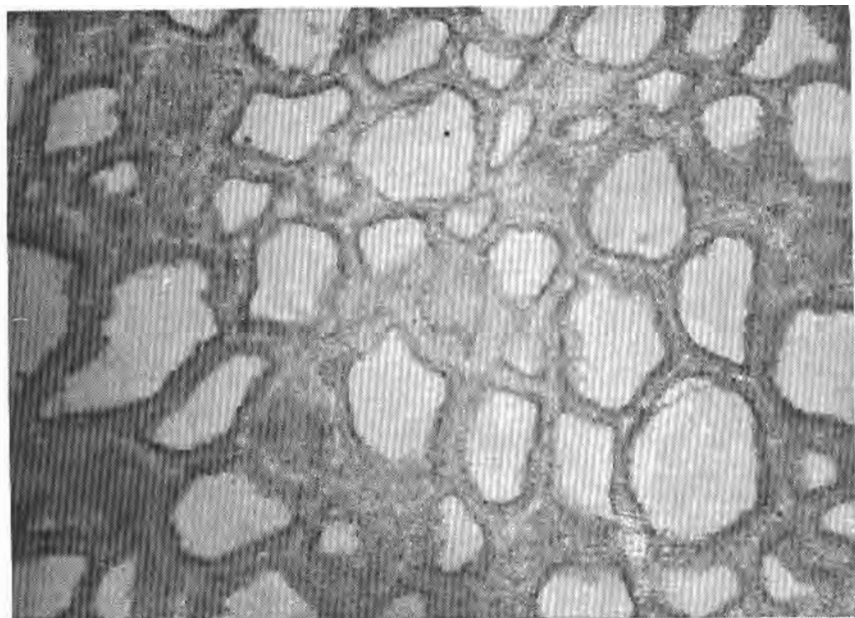


Fig. 8. Thyroid gland of a pig, struma adenomatosa, β -OH-BDH, moderate activity. Enlargement 6×10 , overenlarged.

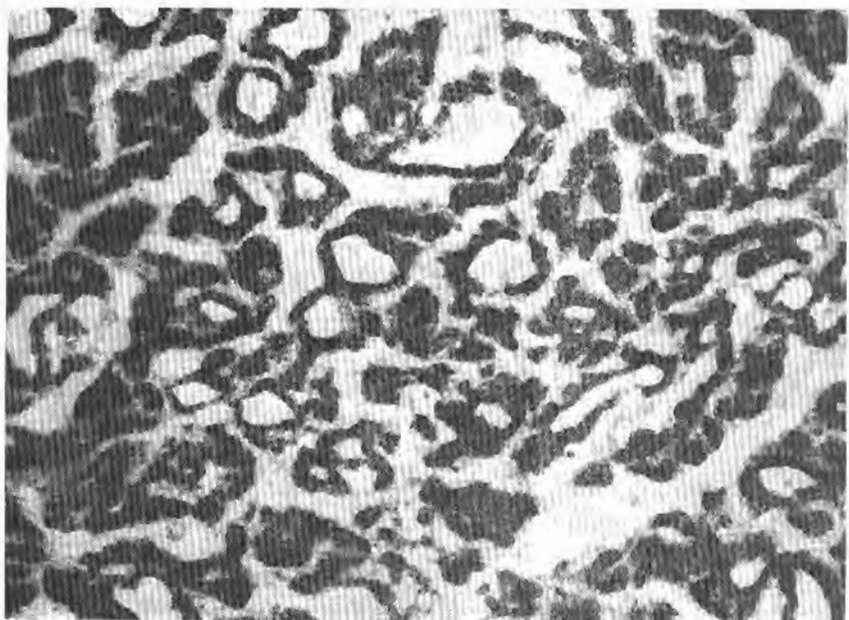


Fig. 9. Thyroid gland of a pig, struma adenomatosa, G-6-PDH, strong activity. Enlargement 6×10 , overenlarged.

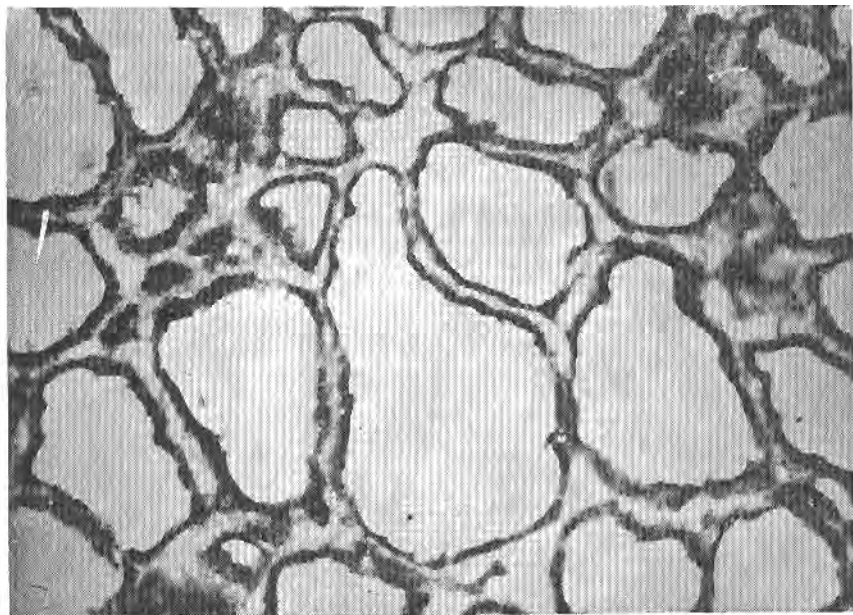


Fig. 10. Thyroid gland of a pig, struma cystica, NADH_2 , strong activity. Enlargement 6×10 , overenlarged.

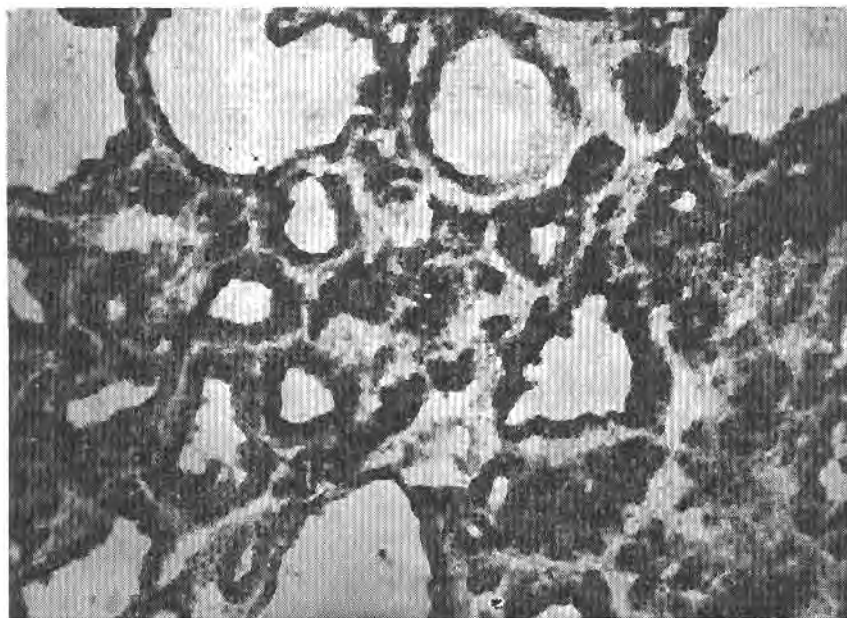


Fig. 11. Thyroid gland of a pig, struma adenomatosa, NADPH_2 , strong activity. Enlargement 6×10 , overenlarged.

DISCUSSION

From the total of 53 examined pigs, not one thyroid gland was without histopathological changes. Animals were grouped in relation to the dimensions of the pathological changes for every kind of the established goitre.

Although some goitre shapes were evaluated pathohistologically as rather strong, not in one group any considerable differences in the acid phosphatase and nonspecific esterases in relation to damage degree of the gland, were established; neither were the differences, in relation to the kind of the goitre, clearly expressed. The activity of both enzymes varied individually from moderate to very strong reaction, as well in the goitre of a weak and in the goitre of a stronger degree, so it can be considered that both examined enzymes play a role in the cell metabolism of the thyrocytes. This observation coincides with the observations of Tremblay and Pearse (1960), Pakdaman et al. (1961), Lindsay and Arico (1963) on the human thyroid gland, as well as with the observations of Niwelinski and Zamorski (1962) on the thyroid gland of the rat. No essential difference was established between the enzymatic activities in relation to the kind and degree of the pathological change in the thyroid gland. So did Mitin et al. (1972) find in the thyroid gland of normal cattle and cattle with various kinds of goitre a prevalently strong activity of the acid phosphatase, so these findings are in the harmony with the observations of the authors mentioned.

The oxidoreductases show a mostly weak to moderate reaction. An outstanding strong reaction show glucose-6-phosphate dehydrogenase as well as NADH₂- and NADPH₂-diaphorases.

The activity of glucose-6-phosphate dehydrogenase could have here a double meaning: in the production of the reduced coenzyme NADPH₂ and the production of the pentose sugars which are important for the synthesis of the nuclear acids.

Biochemical analyses show that an increased concentration of thyreotropin (TSH) cause an increased concentration of NADP in the follicular cells of thyroid gland. The hormone probably enables the reoxydation of the reduced coenzyme as well as producing NADH from NAD. This output by means of NAD-kinase could be a main control mechanism in the metabolism of the thyroid gland. When thyreotropin acts on the marginal cells of the follicles of the thyroid gland, it stimulates the oxidative activity of the pentose-cycle. This stimulation acts probably in two different ways: the first one is by stimulation of the NADP:I — diaphorase, which NADPH reoxydates into NADP. The reduced coenzyme is inhibitive for glucose-6-phosphate dehydrogenase, which is one of the leading oxidative

enzymes of this way. The second way is the stimulation of the NAD-kinase which phosphorylates NAD in NADP. This could be the critical factor of the control of the pentosephosphate way in these cells, as the glucose-6-phosphate claims NADP as a coenzym and it is inactive in the presence of NAD only.

CONCLUSION

Hystological examination of 53 thyroid glands of pigs of both sexes, aged 8-10 months, showed that all thyroid glands had changes in the sense of hyperplastic, cystic and adenomatose goitre of various degree.

The adenomatose thyroid glands showed an increased activity of the alkaline and acid phosphatase, as well as nonspecific esterases. Nonspecific esterases show sometimes increased activity in the hyperplastic goitre glands, while acid phosphatase shows an increased activity in the cystic goitre glands.

In contrary, adenomatose goitre glands, as well as same of the cystic goitre glands, show a weaker activity of the oxidoreductase, but all the investigated glands show a distinctly strong activity of glucose-6-phosphate dehydrogenase, as well as NADH₂- and NADPH₂-diaphorase.

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S a ž e t a k

PRILOG POZNAVANJU ENZIMATSKE AKTIVNOSTI U ŠTITNJAČI SVINJA U VEZI S PATOLOŠKIM PROMJENAMA ŽLIJEZDE

Istražene su 53 štitnjače svinja oba spola, starih 8-10 mjeseci, težine 80-120 kg. Histološkom pretragom štitnjača, utvrđeno je, da na svim žlijezdama postoje promjene u smislu hiperplazije, cistične i adenomatozne strume različitog stupnja. Praćen je intenzitet aktivnosti alkalne i kisele fosfataze (AP i SP), nespecifičnih esteraza (NE), zatim dehidrogenaza φ -fosfoglicerinske kiseline (φ GPDH), L-glutaminske kiseline (GIDH), mliječne kiseline (LDH), jantarne kiseline (SDH), β -hidroksi-maslačne kiseline (β OH-BDH) i glukoza-6-fosfata (G-6-PDH), kao i NADH₂-i NADPH₂-tetrazolijevе reduktaze.

Na adenomatoznim štitnjačama je primjećena povećana aktivnost alkalne i kisele fosfataze i nespecifičnih esteraza. Nespecifične esteraze pokazuju ponegdje povećanu aktivnost i na hiperplastičnim žlijezdama, dok kiselа fosfataza na cističnim.

Nasuprot tome adenomatozne žlijezde, kao i neke cistične, pokazuju slabiju aktivnost oksidoreduktoza. Izrazito jaku reakciju pokazuju samo glukoza-6-fosfat dehidrogenaza i NADH₂-i NADPH₂-dijaforaze.