ANTI-THYROID ACTIVITY OF PURIFIED THYMUS GLAND EXTRACT IN MALE WISTAR RATS

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Summary

The effect of a purified bovine thymus gland extract (Dr. Kurt Mulli, GmbH, Hamburg, West Germany)was studied in 12-week old male Wistar rats on the following: thyroid weights and morphology, T_3-T_4 serum levels, thyroid lactic dehydrogenase, ATP-ase, acid phosphatase, and non-specific esterase activities. Thymus extract was administered intramuscularly daily for 21 days at doses of 0.5, 1.0 and 2.0 ml/kg. Measurements were made on day 3, 7, 14 and 21 of treatment. Thyroid histology and enzyme activity were studied only on 21-day specimens. Thymus extract significantly decreased average thyroid gland weights in a dose-dependent manner irrespective of treatment duration. T_3 serum levels were consistently lower in thymus-treated rats irrespective of treatment dose or duration. Changes from control levels were not statistically significant due to large standard deviations. T_4 serum levels were significantly lower than control levels in rats treated with thymus extract for 14 and 21 days.

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Histology of thyroids from 21-day treated animals revealed a marked reduction in both thyroid follicle size and colloid content with an increase in connective tissue, resorption vacuoles and hyperemia. Histochemical study of thyroid enzyme activity showed lactic dehydrogenase increase in follicle epithelial cells, acid phosphatase increase in follicle epithelial cells and decrease in interstitium, ATP-ase increase in granular storage area and non-specific esterase increase in follicle epithelial cell. The data suggest the presence of an unidentified specific anti-thyroid factor(s) in the thymus gland extract.

Introduction

Comsa (1980), in a recent study concerned with the present high level of thymus research, noted that the number of published reports exceeds 10,000. The initial observation that thymectomy-induced changes in most endocrine organs can be corrected by thymnic extract injections remains firm (Comsa, 1965) despite the use of diverse thymus extract prerparations varying in degree of purity, composition and bilogic effects.

Earlier literature have focused on thymus anatomy, embryology, histology and the physio-pathologic effects of thymectomy and thymus implantation (Gregoire, 1935). Studies during the past two decades have established the endocrine nature of the thymus with its particular essential role in the immune response as reported first by Miller and Osoba (1963) and elucidated by subsequent investigations (White and Goldstein, 1975; Good and Gabrielsen, 1964; Trainin and Small, 1970; Goldstein, et al., 1975; Bach et al., 1975; Bach, 1977).

The following evidence support affirmatively and definitively the endocrine nature and function of the thymus gland: (a) identification and partial characterization of such thymic hormone substances from the blood and thymus as thymopoietin (Goldstein, 1975), thymosin (Goldstein et al.,

1966), thymic humoral factor (Trainin and Small, 1970) and serum thymic factor (Bach et al., 1975); (b) the hormone factors substitute for certain thymic functions deficient in thymectomized rodents (Thurman et al., 1975), attesting to the role of the thymus in maintaining the immune system (Miller, 1961); (c) the recent demonstration of thymosin localization in human thymus (Hirokawa et al., 1982) and presence of thymosin-like factors in human thymic epithelium (Karter et al.,1974)

Endocrine gland secretions commonly influence the effect of other organs, their target organs or the function of other endocrine glands. The thymus also has been associated with the immunopathology of aging (Goldstein et al., 1974) and the interactions between the thymus and the hypophysis, adrenals, gonads and their secretions also have been studied (Deschaux, 1977; Comsa, 1973a). Comsa (1973b) suggested that the homeostatic thymus hormone is a "synergist of the growth hormone" whereas Luft and Hall (1975) proposed that thymic fractions belong to the "somatomedins". Neonatal thymectomy was reported to induce post-natal ovarian follicular developemnt and ovarian dysgenesis in the mouse (Nishizuka and Sakakura, 1971a; Nishizuka and Sakakura, 1971b). Extirpation of the thymus also influenced parathyroid tetany (Nitschke, 1943).

A relationship between the thymus and thyroid gland has been reported as early as 1928 when Nitschke (1928) noted that a calcium- and phosphate-decreasing factor from thymus fractions had anti-thyroid activity. Pohland (1962) studied the effect of thymus extract on metmorphosis and growth of tadpoles. Schliephake (1936) later described the healing of a patient with Basedow's disease by administration of thymus extract. Comsa (1938), Goslar (1958) and Goslar et al., (1961) recognized the relationship between the thymus and thyroid glands. Goslar and Jaeger (1959) reported that injection of thymus extract reduced thyroid growth. Jaeger (unpublished data) also showed that a commercial clinical preparation of

thymus extract decreased protein-bound iodine and thyrotropin levels.

This study investigates the anti-thyroid effect of a purified thymus extract and the possible correlation between this effect and its effect on immunologic mechanism. Thus, the effect of a thymus gland extract was studied on thyroid weights, T_3-T_4 serum levels and thyroid histology and enzyme activity in 12-week old male Wistar rats.

Materials and Methods

Twelve-week old male Wistar rats, with an average body weight of 250 g, were used (Central Tierversuchsanstalt, University of Dusseldorf). The animals were maintained in a temperature and humidity controlled facility with 12-hour light and dark cycles. Rodent chow and water were available *ad libitum*.

The following four groups of rats, 28 rats per group, were established: Group I - thymus extract, 0.5 ml/kg; Group II - thymus extract, 1.0 ml/kg; Group III - thymus extract, 2.0 ml/kg; Group IV -untreated control. The thymus extract was a purified thymus gland extract (TGE) from the Dr.Kurt Mulli, GmbH, Hamburg, F.R. Germany. The TGE, prepared by a sequential ethanol extraction-gel filtration procedure, had a protein concentration of 11.0 mg/ml and an isoelectric focusing pattern similar to that of Fraction 5 (Schulot et al., 1981) as determined by Bedi and Back (unpublished data). The TGE was injected intramuscularly daily for 21 days.

Seven rats from each group were sacrificed on the 3rd, 7th, 14th, and 21st day of treatment. Five to 6 ml of carotid blood was collected and serum prepared by centrifugation at 2000 rpm for 10 min. The serum was used for the determination of serum T_3 and T_4 by radioimmunoassay (RIA, Diagnostics Products Corp.). For the T_3-RIA, anti-T_3 rabbit serum, anti-rabbit goat γ-globulin and T_3-5125 calibrated with $6T_3$ standards against WHO 68/38 serum was used. The T_4-RIA assay employed anti-T_4 rabbit

TABLE I. MEAN THYROID WEIGHTS (± STANDARD ERROR OF MEAN) FROM CONTROL RATS AND RATS TREATED WITH THYMUS GLAND EXTRACT INTRAMUSCULARLY FOR 3, 7, 14 AND 21 CONSECUTIVE DAYS AT DOSES OF 0.5 ml/kg, 1.0 ml/kg AND 2.0 ml/kg.

Group #	Treatment (ml/kg)	Thyroid Gland Weights (mg) at Varying Durations of Treatment (Days) 3	7	14	21
I	Thymus Extract 0.5 ml/kg	269 ± 25.34	322 ± 5.10*	267 ± 16.16	249 ± 6.59*
II	Thymus Extract 1.0 ml/kg	262.17 ± 33.39	304 ±16.13*	241 ± 20.12	261.67 ± 9.17*
III	Thymus Extract 2.0 ml/kg	236 ± 12.60	229.5 ±19.87**	221.17 ± 8.12**	275 ± 9.65
IV	Control	290 ± 40.77	290 ±36.83	302 ± 20.79	308 ±23.64

*$p < 0.05$ (Group I and II vs. Group III)

**$p < 0.05$ (Group III vs. Group IV)

serum, anti-rabbit goat γ-globulin and T_4-5125 calibrated with T_4 standards against WHO 68/38 serum.

At each time period the thyroid glands were removed and weighed. The right thyroid lobes of the 21-day treated rats were fixed in 4% formalin for subsequent imbedding, sectioning and staining with hemotoxylin-eosin for histologic study. The left thyroid lobes were frozen in liquid nitrogen at -190° and sectioned (5 μm) with a cryostat for histochemical study of lactate dehydrogenase (LDH), adenosine/ triphosphatase (ATP-ase), acid phosphatase and non-specific protease (Pearse, 1972).

Results

Average thyroid weights decreased in the TGE-treated groups of rats (compared to controls) in a dose-dependent manner irrespective of treatment

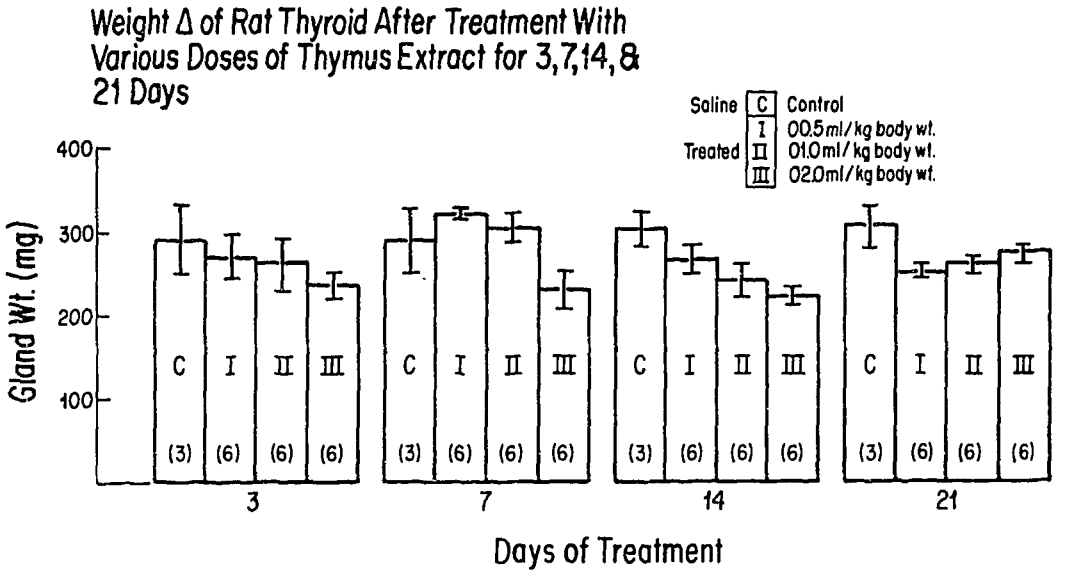

Fig. 1. Mean weights (± standard error of mean) of thyroid glands from rats treated with daily intramuscular injections of thymus gland extract (TGE) at doses of 0.5 ml/kg, 1.0 ml/kg and 2.0 ml/kg compared to weights from control non-treated rats.

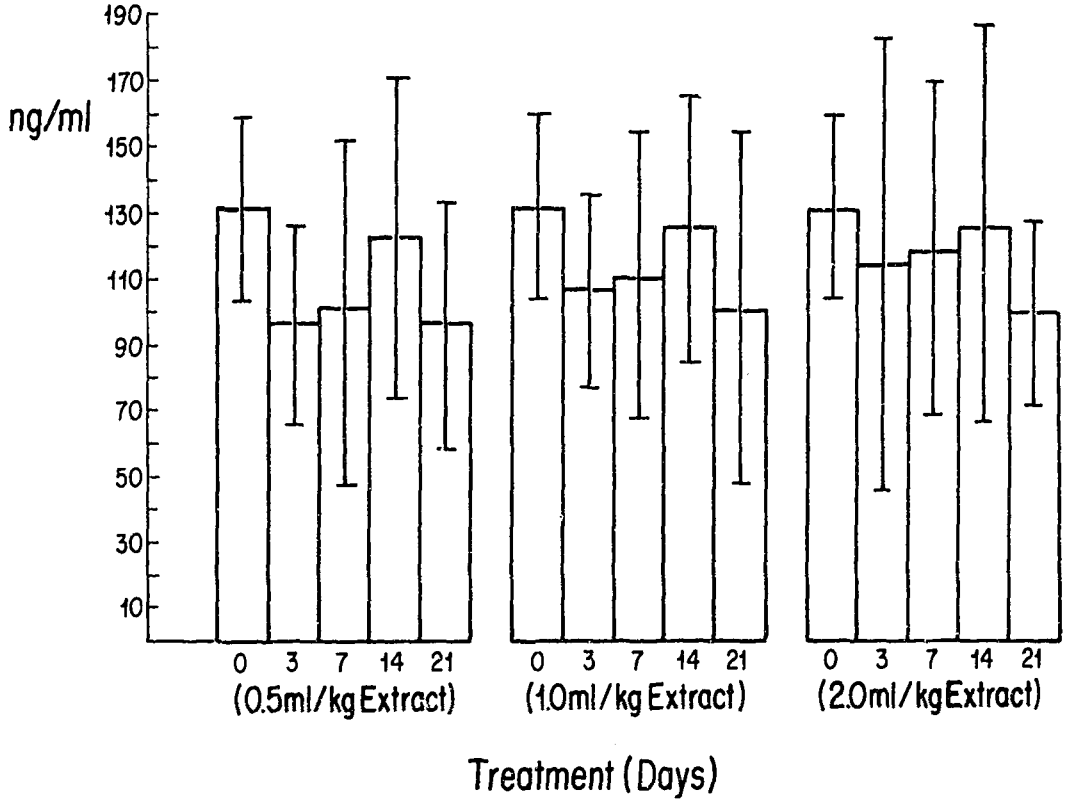

Fig. 2. Serum T_3 levels in rats on day 0,3,7,14 and 21 of the daily intramuscular treatment with thymus gland extract (TGE) at doses of 0.5 ml/kg, 1.0 ml/kg and 2.0 ml/kg.

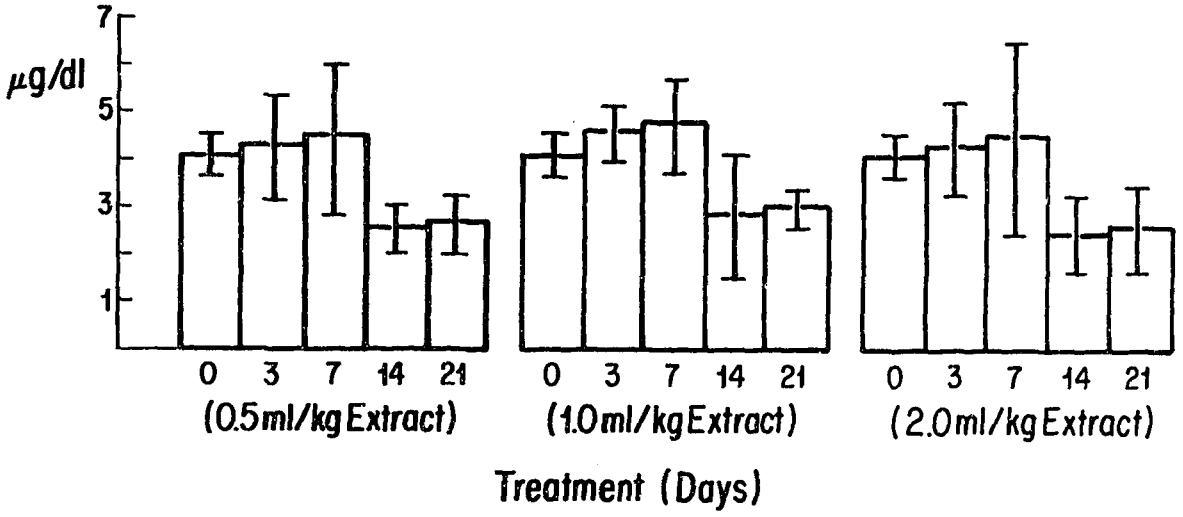

Fig. 3. Serum T_4 levels in rats on day 0,3,7,14 and 21 of daily intramuscular treatment with thymus gland extract (TGE) at doses of 0.5 ml/kg, 1.0 ml/kg and 2.0 ml/kg.

duration, Table I. These decreases were statistically significant at $p<0.05$ in the highest TGE-treated group on the 7th and 14th treatment day and in the two lowest TGE-treatment groups on day 21 ($p<0.05$). The results are seen graphically in Fig. 1.

Serum T_3 levels remained relatively constant in the control animals throughout the experimental period, Fig. 2. TGE decreased T_3 levels irrespective of dose and treatment duration. Initially T_3 levels tended to increase by the 7th to 14th day of treatment, but decreased to their lowest levels in all treatment groups by the 21st treatment day. These changes were not statistically significant.

Serum T_4 levels are represented in the bar graph, Fig. 3. On day 3 and 7 of the TGE treatment T_4 levels increased above those at zero time before treatment. These changes were not statistically significant. However, serum T_4 levels decreased significantly ($p<0.05$) in TGE-treated rats at all TGE concentrations on days 14 and 21 of treatment compared to the zero time before treatment.

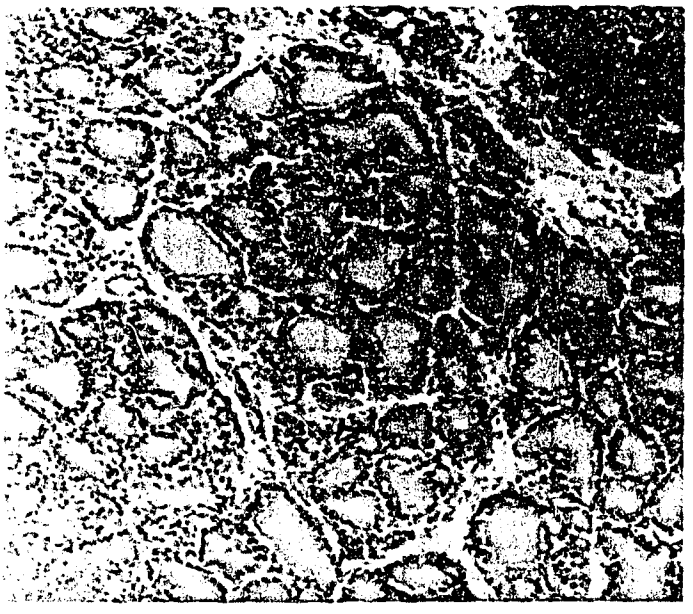

Fig. 4. Thyroid control rat showing normal histology with follicles of normal volume containing homogeneously-staining colloids, H + E.X. 150.

The thyroids from control animals showed normal histology with follicles containing homogeneously-staining colloids and normal follicular volume. The follicle epithelia had a monolayer form with flat to cubical and some highly prismatic cells. The interstitium showed occasional para-follicular groups of cells (Fig. 4).

The thyroids from the group treated with the lower concentration of TGE (Group I) showed a marked reduction in both size and content of follicles (Fig. 5). Large follicles appeared only occasionally and singly at the edge of the follicles. Epithelia were commonly flat to cubical. High prismatic cells were not present. Resorption vacuoles were identified. The tissue appeared hyperemic with an increase in connective tissue. Thyroid glands from rats treated with the higher TGE concentration (group II) were dominated by numerous small follicles with little colloid content (Fig. 6). The epithelia were flat and cubical with only few large, highly prismatic follicles on the edge. Many resorption vacuoles were seen and the gland generally was hyperemic with enlarged connective tissue.

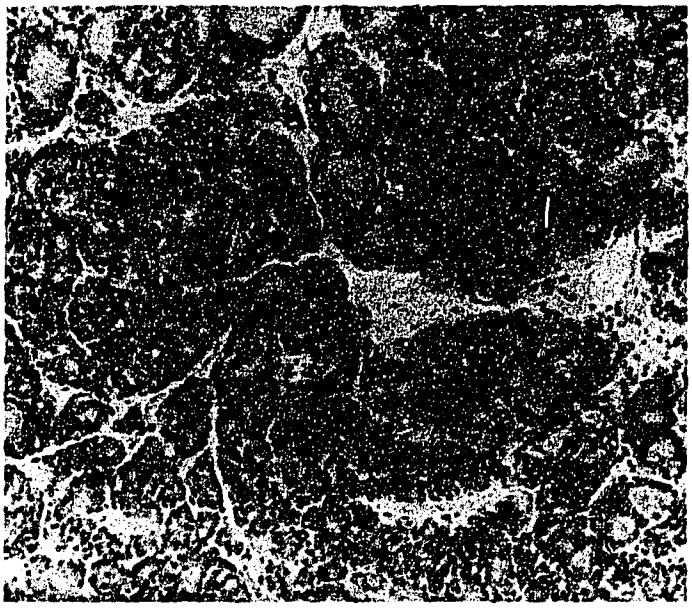

Fig. 5. Thyroid from rat treated with 0.5 ml/kg thymus gland extract for 21 days. Note marked reduction in follicular size and content. H+E X 150.

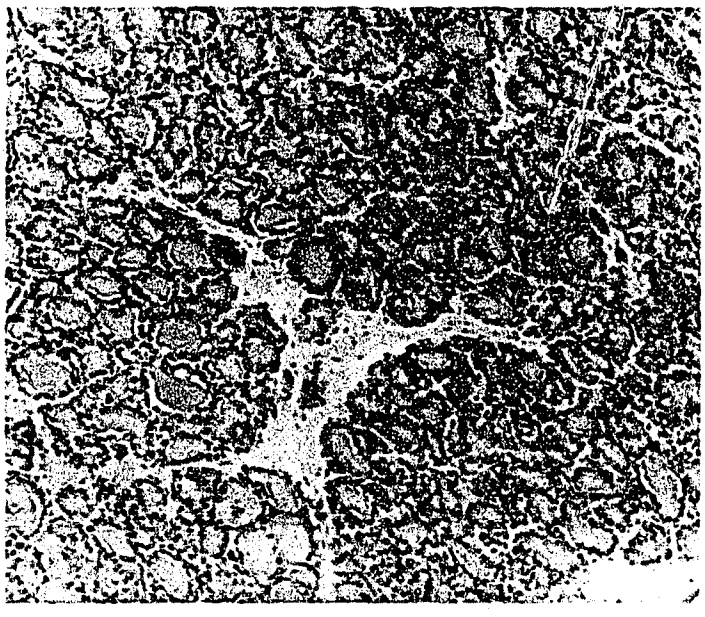

Fig. 6. Thyroid from rat treated with 1.0 ml/kg thymus gland extract for 21 days. Follicles are small with little colloid content H+E X 150.

Histochemical study of frozen sections revealed LDH activity to be increased strongly in thyroid samples from TGE-treated rats (Fig. 7b) compared to controls (Fig.7a) as noted by strongly stained follicle epithelia, particularly in apical areas (Fig. 7b). Connective tissue in control glands showed only sporadic positive-reacting cells. No significant differences in ATP-ase activity were found in glands from TGE-treated rats (Fig. 8b) and those from control rats (Fig. 8a). However, a trend toward a decrease of ATP-ase activity in the glandular storage area and connective tissue was noted in thyroid from TGE-treated rats (Fig. 8). A clear increase in acid phosphatase activity was seen in the follicular epithelial cells and a decrease in activity in the interstitium of thyroid sections from rats treated with high TGE doses (1.0 ml/kg) (Fig. 9b). These changes were not seen in glands from control rats (Fig. 9a) and those treated with lower doses of TGE. Non-specific esterase activity was definitely and distinctly increased in the thyroid sections from rats treated with higher doses of TGE (1.0 mg/kg). Glands from control rats and low dose TGE-treated rats (0.5 mg/kg) showed average and linear activity respectively in the follicle epithelial cells. The interstitium in the thyroids from all groups of rats showed strongly positive but sporadic activity.

PJD-positive reaction in the cytoplasm of follicle epithelial cells of thyroids from control animals was not consistant whereas strong positive reaction occured in the follicle epithelial cell cytoplasmic granules of thyroids from TGE-treated rats.

Discussions

The thyroid weight, biochemical and thyroid histochemical data all suggest the presence of a specific anti-thyroid factor(s) in the thymus gland extract confirming earlier studies (Nitsche, 1928; Pohland, 1962; Schliephake, 1936; Comsa, 1938; Goslar, 1958a; Goslar et al., 1961; Goslar

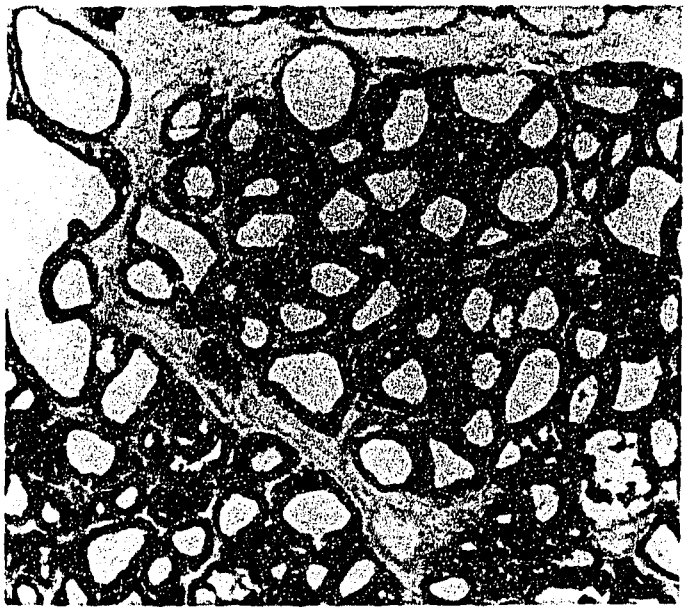

Fig. 7a. Histochemical study of lactate dehydrogenase (LDH) activity in thyroid from control rat. (X150)

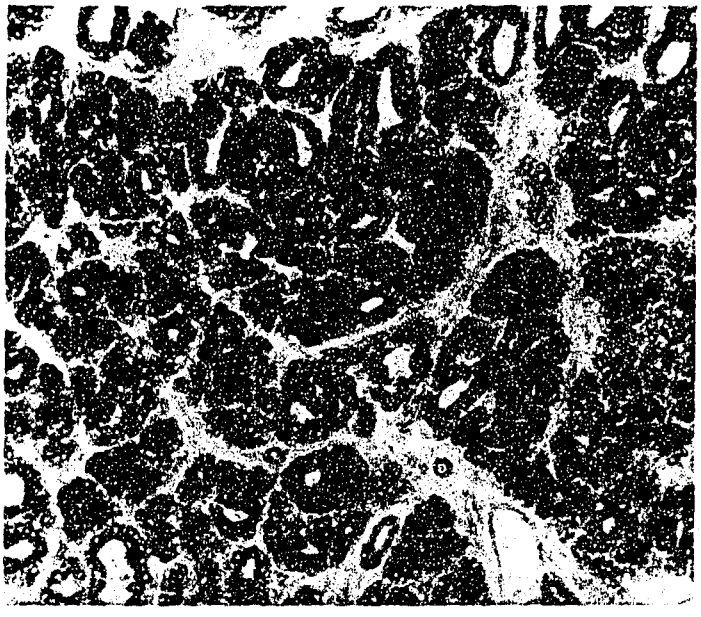

Fig. 7b. Histochemical study of lactate dehydrogenase (LDH) activity in thyroid from rat treated with 0.5 ml/kg thymus gland extract daily for 21 days. (X 150)

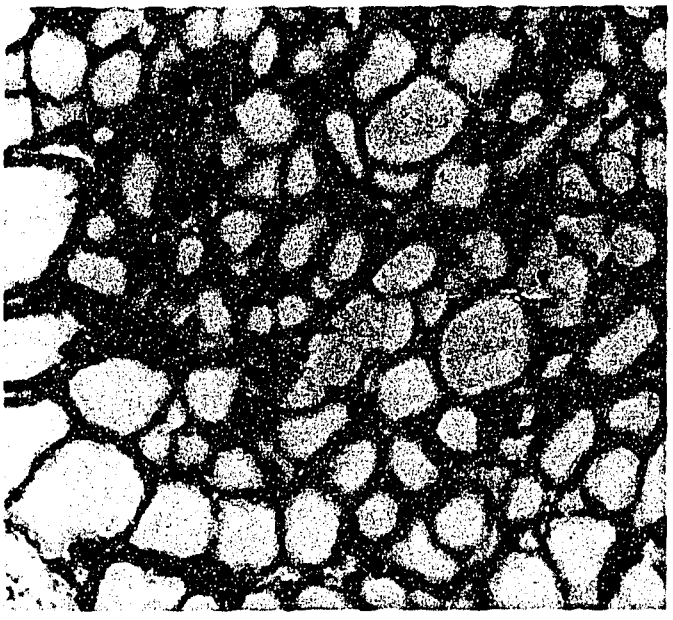

Fig. 8a. Histochemical study of adenosine triphosphatase (ATP-ase) activity in thyroid from control rat (X150)

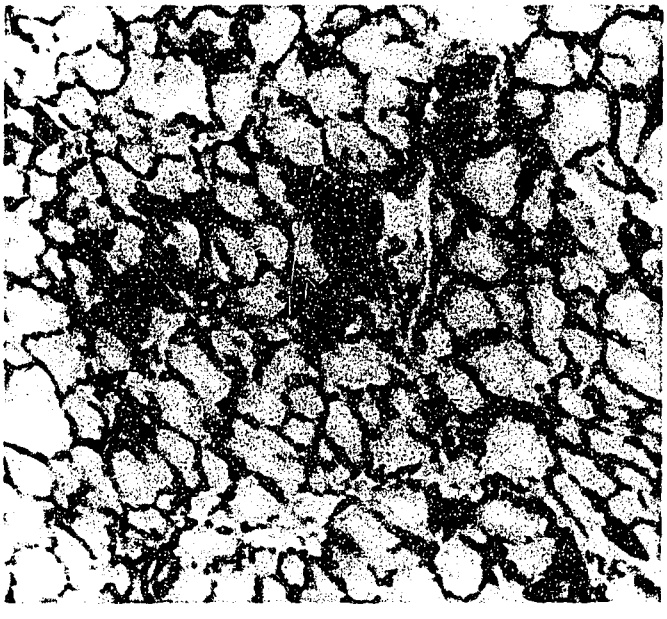

Fig. 8b. Histochemical study of adenosine triphosphatase (ATP-ase) activity in thyroid from rat treated with 0.5 ml/kg thymus gland extract daily for 21 days (X150)

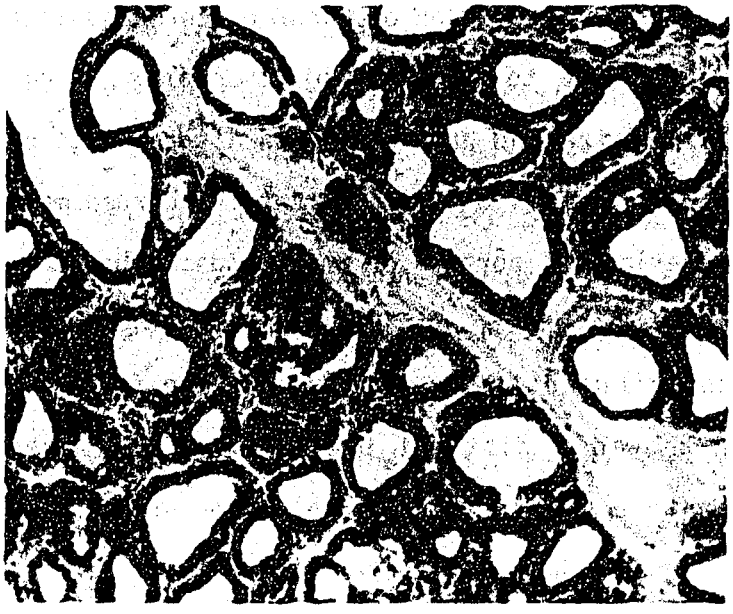

Fig. 9a. Histochemical study of acid phosphatase activity in thyroid from control rat (X150)

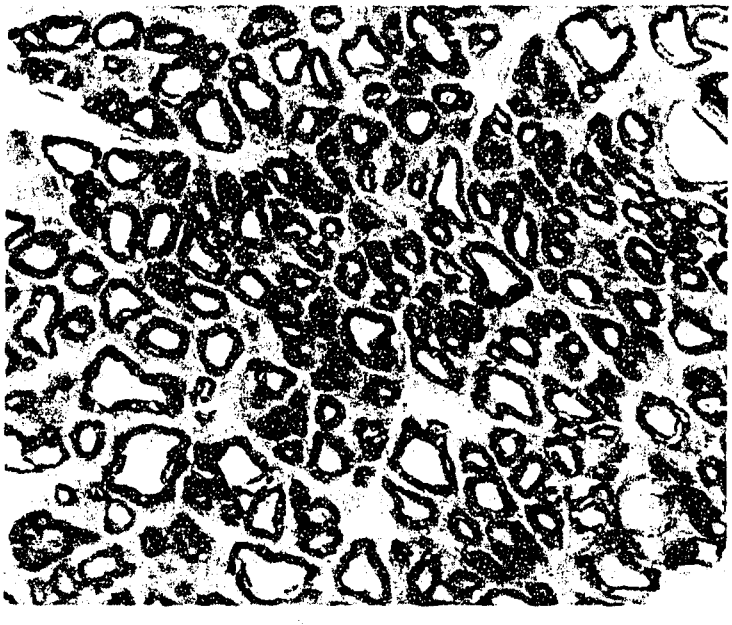

Fig. 9b. Histochemical study of phosphatase activity in thyroid from rat treated with 1.0 ml/kg thymus gland extract daily for 21 days (X150)

and Jaeger, 1959). With the exception of minor differences in the thyroid gland size decreases relative to body weight increases, a reduction in thyroid gland follicle size and an increase in its connective tissue was demonstrated. These histological differences were concerned more with the central rather than peripheral portion of the glands. The numerous single follicle cells in the lumen which appeared with decreased total cell-follicle volumes contributed to the reduction of the follicle size. The histochemical data showed a distinct but discontinuous reduction of adenosine triphosphatase and non-specific esterase while lactic dehydrogenase and acid phosphatase showed an increase of activity in the follicle cells.

The morphologic-histochemical picture correlated with the levels of circulating thyroid gland hormones. T_3 serum levels were consistently lower in the TGE-treated rats irrespective of dose or duration of treatment. These changes, while not statistically significant from the levels in control animals, did indicate a definite trend toward lower levels. T_4 serum levels were significantly lower in the TGE-treated animals compared to levels in the control animals, particularly by the 14th and 21st day of treatment.

A functional link betweeen the thymus and thyroid glands has been proposed recently by Petkova and Cocev(1977) who demonstrated an increase in incorporation of I^{131} in the thyroid gland of rats two days after thymectomy. Pierpaoli and Sorkin (1972) reported a relationship between the thymus, the adrenal cortex and the thyroid glands in genetically hairless "nude" mice which have no thymus. While concentrations of thyroxine always were decreased in the blood of the athymic "nude" mice compared to their normal haired littermates, neonatal implantations of normal thymus into these"nude" mice did not influence the thyroxine levels nor prevent the adrenal and thyroid alterations. The authors suggest that

the thymus in early postnatal life secretes a factor, possibly one or several hormones, which promotes differentiation of the hypophysis-independent zone of the adrenal cortex with alteration of the function of the thyroid gland. Similar relationships were proposed in the extensive review by Comsa (1973b) on the hormonal interactions of the thymus. A long-lasting hyperthymization, it was noted, resulted in degeneration lesions of the thyroid.

The thymus and the thyroid gland derive from the foregut. Thus, it was demonstrated that a total incorporation of I^{125} in the thymus of new-born was equal to or surpassed that of the thyroid (Csaba et al., 1973). Jackson and Graham (1979) also found that normal thymus cells accumulated radioactive iodine and both Csaba, et al. (1975) and Torok, et al. (1975) demonstrated that PAS-positive cells of rat thymus grown in tissue culture take up radioactive iodine. The presence of thymus tissue, furthermore, found in the thyroid by Carpenter and Emery (1976) and Vladutiu and Rose (1972) as described originally by deWiniwartes (1929) reflects the very close embryologic evolution of these two glands.

Investigators studying the biological effects of thymus gland extract did not consider the possible anti-thyroid activity of the extracts (Pohland, 1962; Comsa, 1938; Goslar et al., 1961; Comsa, 1956; Szent-Gyorgyi et al., 1962; Goslar, 1938b). In a recent symposium on the thymus, no references were made to a possible relationship between the immunologic and anti-thyroid principles of thymus extracts (Ainti and Wigzell, 1980). Comsa (1980) discounted the probability that the activity of the thymus on the thyroid gland correlates with the immunologic effects of the thymus. However, Barnes and Irvine (1973) do consider the relationship between thyroid autoimmune disease and thymic disorders and Kojima, et al. (1976) prevented autoimmune thyroiditis by injection of cells of adult thymus into neonatally thymectomized mice.

The thymus gland extract preparation employed in this study has been in clinical use since 1936 (Schliephake, 1936). The extract includes thymosin fraction 5 as characterized recently by isoelectric focusing technique (Bedi and Back, unpublished data). The data suggest that the thymus contains several families of distinct and independent biologically active substances including immuno-modulating peptide hormones and thyroid-depressing substance. Whereas the immuno-modulating components have a relatively short half-life, those substances that act on the thyroid do so only after the 10th day of daily treatment. These two families of substances are being purified for further chemical and endocrine study.

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