α- and β-D Galactosidase Activity of Rat Thyroid and Spleen. A Quantitative Study.* (29311)

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We could not duplicate histochemically(1) the biochemical observations with fresh homogenates that α-D galactosidase activity of thyroid and spleen was higher in females than males(2). In the survey of rat tissues by a histochemical technic it was observed that the parathyroid glands are very active for α-D galactosidase(2). Similar histochemical observations were made in rat parathyroids for β-D galactosidase. On the other hand, striated muscle showed little activity for the two galactosidases.

Since the parathyroid glands are closely applied to the anterolateral surface of the thyroid lobes, we speculated that the discrepancy between the histochemical and the quantitative observations was due, among other factors, to erratic inclusion of minute bits of parathyroid and/or striated muscle in the homogenized samples of thyroid gland. The histochemical observations on spleen(1) failed to confirm the quantitative observations of a sex-linked difference for the two galactosidases. Our observations on rat spleen indicated that megakaryocytes and heterophils showed the highest reactivity for α- and β galactosidases(1). For these reasons, we decided to repeat the quantitative study and to make homogenates of the thyroid gland after 1) removing from it all muscle and loose areolar tissue, and 2) isolating the parathyroid. It will be shown here that α- and β-D galactosidases of thyroid and spleen were not significantly different in male and female rats when these enzymes were assayed with the colorimetric procedures previously reported(2).

Material and methods. Nine female and 9 male albino rats of a Sprague Dawley strain belonging to 2 different litters were used for this study. They were fed a regular diet and were fasted overnight before sacrifice. The neck region was exposed and the larynx and trachea were resected. Under a dissecting microscope the thyroid gland lobes were dissected. After peeling off the capsule of the gland, the parathyroid glands were separated with an iris scissor. Both lobes of the thyroid gland were placed in a square of gauze slightly wet in saline. The capsule was removed from the spleen and 3 mm slices were placed in a wet square of gauze. Tissues were weighed while wet and homogenized in distilled water at the concentration of 2.5 mg/ml of thyroid and 5 mg/ml of spleen.

Galactosidase activities were determined by a modification of the method proposed by Gillman *et al*(2) using as substrates the following compounds: (a) 2-naphthyl-α-D-galactoside, (b) 2-naphthyl-β-D-galactoside, and (c) 6 bromo-2-naphthyl-α-D-galactopyranoside. Hydrolysis of these substances releases 2-naphthol or 6 bromo-2-naphthol, which are both measured colorimetrically by coupling with tetrazotized diorthoanisidine. The only modification to the above method was the use of 0.15 ml of trisodium phosphate solution (0.2 M) to bring the pH of the reaction up to 7.5 which is the favorable pH for coupling the diazonium salt. The reaction is very simple and easy to use for measuring the activity of α or β galactosidase.

Results. Tables I and II contain the values for the two galactosidases in male and female rats. Inspection of the values and statistical analysis (Table III) of the data indicate a lack of sex difference. It is apparent that no significant differences exist between male and female average values in either organ for any of the 2 enzymes. In fact, the probabilities of a larger difference occurring by chance (assuming no difference) are so

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TABLE I. α- and β-D-Galactosidase Activity (Male Rats).

Rat #	Thyroid			Spleen		
	α-D-Gal	β-D-Gal	6-B-α-Gal	α-D-Gal	β-D-Gal	6-B-α-Gal
1	100 (.036)	275 (.099)	400 (.179)	468 (.168)	502 (.181)	574 (.257)
2	410 (.148)	500 (.180)	645 (.289)	353 (.127)	472 (.170)	394 (.177)
3	470 (.169)	395 (.142)	410 (.184)	447 (.161)	628 (.226)	471 (.211)
4	472 (.170)	370 (.137)	650 (.291)	452 (.163)	408 (.147)	441 (.198)
5	540 (.195)	700 (.252)	600 (.269)	690 (.249)	850 (.306)	680 (.305)
6	960 (.346)	405 (.146)	550 (.247)	695 (.250)	740 (.267)	700 (.314)
7	825 (.297)	900 (.324)	585 (.262)	738 (.266)	837 (.302)	657 (.295)
8	497 (.179)	450 (.162)	348 (.156)	800 (.288)	875 (.315)	658 (.296)
9	685 (.247)	770 (.278)	670 (.300)	255 (.092)	927 (.334)	755 (.338)

Columns to left indicate Klett units; values in parentheses correspond to micromoles of 2-naphthol or 6-bromo-2-naphthol produced per 2 hr at 37°C under the conditions of the methods (2).

TABLE II. α- and β-D-Galactosidase (Female Rats).

Rat #	Thyroid			Spleen		
	α-D-Gal	β-D-Gal	6-B-α-Gal	α-D-Gal	β-D-Gal	6-B-α-Gal
1	270 (.098)	348 (.125)		352 (.127)	510 (.184)	
2	232 (.084)	231 (.083)	240 (.108)	442 (.159)	598 (.215)	535 (.240)
3	442 (.159)	270 (.098)	297 (.133)	517 (.186)	658 (.237)	525 (.235)
4	775 (.279)	400 (.143)	625 (.280)	630 (.227)	805 (.290)	600 (.269)
5	495 (.178)	660 (.238)	550 (.247)	545 (.196)	615 (.222)	530 (.238)
6	780 (.281)	865 (.312)	425 (.191)	875 (.315)	963 (.347)	870 (.390)
7	675 (.243)	775 (.280)	290 (.130)	620 (.224)	762 (.275)	650 (.291)
8	610 (.220)	740 (.266)	740 (.332)	250 (.090)	1137 (.410)	900 (.403)
9	670 (.242)	860 (.310)	850 (.381)	265 (.095)	1167 (.421)	940 (.421)

Columns to left indicate Klett units; values in parentheses correspond to micromoles of 2-naphthol or 6-bromo-2-naphthol produced per 2 hr at 37°C under the conditions of the methods (2).

large that it is strongly suggested that no difference exists and even with considerably larger numbers of animals no significant difference would be found. It should be noted that the variation from animal to animal is very large in both males and females.

Discussion. Biochemical literature contains numerous examples of quantitative studies which have led to comparing data, neglecting the fact that the enzyme source was heterogeneous. This heterogeneity may have different causes. It may be due to admixture of normal and diseased tissue, for example, in the study of malignant tumors. In other instances, the anatomic structures are so intimately related that unless special care is taken, the enzyme source will be homogenized with adjacent anatomic structures. In the present study we took special care to prevent inclusion of the parathyroids and striated muscle in the homogenates of the thyroid gland.

Variations of total activity of spleen homogenate may reflect changes in number of megakaryocytes and heterophiles, but our quantitative determinations do not support the view of sex-linked differences in the spleen. This is in agreement with our previous qualitative data which show no sex-linked differences for both α and β galactosidase in the rat(1). That discrepancies in results cannot be explained by the structure of the substrate is shown by the lack of significant differences using 2 naphthol α-D galactoside and 6-bromo-2-naphthol-α-D-galactoside (Tables I and II).

Most references in the literature to α and β galactosidase activity refer to quantitative studies on homogenized tissues(3-7); some to histochemical studies(8,9); and a few to quantitative studies at the microchemical level(10). A combination of quantitative and histochemical methods would seem desirable further to assess tissue enzyme activities.

TABLE III. Statistical Analysis of α- and β-D-Galactosidase Activities in Rat Thyroid and Spleen.

Organ and enzyme	Male	Female	Difference	S.E. of difference	Probability of larger difference (t-test)
Thyroid					
2-naphthyl-α-D-galactoside	551.00 (9)*	549.89 (9)	1.11	107.42	.99
6-bromo-2-naphthyl-α-D-galactosidase	539.78 (9)	502.12 (8)	37.66	86.54	.66
2-naphthyl-β-D-galactosidase	529.44 (9)	572.11 (9)	−42.67	111.08	.69
Spleen					
2-naphthyl-α-D-galactosidase	544 22 (9)	499.56 (9)	44.66	91.91	.62
6-bromo-2-naphthyl-α-D-galactosidase	592.22 (9)	693.75 (8)	−101.53	74.96	.20
2-naphthyl-β-D-galactosidase	693.22 (9)	801.67 (9)	−108.45	102.86	.31

* Numbers in parentheses are numbers of animals.

The need for special sampling of tissue under the dissecting microscope should be emphasized.

Summary. Rat thyroid gland and spleen contain levels of α and β-D galactosidases which show no significant sex-linked differences. We have no satisfactory explanation for the discrepancy in results from those of others, except that the parathyroid glands, very active for the two galactosidases, are closely related to the thyroid. It is suggested that careful dissection may be required to avoid inadvertent dilution or enrichment of enzyme source by adjacent anatomic structures. The quantitative data reported herein confirm previous histochemical observations of intense reactivity of rat thyroid gland for α- and β-D galactosidases. The differences in values obtained for spleen may reflect variations in numbers of heterophiles and megakaryocytes which showed high α-D and β-D galactosidase activity by histochemical technics.

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Distribution of 5,5-Dimethyl-2,4-Oxazolidinedione (DMO) Between Plasma and Erythrocytes. (29312)

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Waddell and Butler(1) described a procedure for evaluating intracellular pH based on distribution of the weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO), between the extracellular and intracellular compartments of skeletal muscle. Among other things, the assumption was made that the muscle cell membranes are freely permeable to the