Influence of D-Thyroxine on Vascular Connective Tissue in Rabbits.* (26927)

IB LORENZEN (Introduced by G. Asboe-Hansen)
Connective Tissue Research Lab., University Inst. of Medical Anatomy, Copenhagen, Denmark

It has been demonstrated that l-thyroxine can induce alterations in the mucopolysaccharides of the aortic wall in rabbits(1) and intensify epinephrine-induced alterations(2). The aim of the present experiments was to investigate whether dextro-thyroxine, the optical isomer of levo-thyroxine, has a similar effect on the aortic wall. By injecting doses of d-thyroxine with an inhibitory effect on the release of thyrotrophic hormone equal to the l-thyroxine doses previously used(1), it was desired to demonstrate whether the thyroid hormone exerts its influence on the aortic wall by a direct peripheral action or *via* thyrotrophin inhibition.

Material and methods. Two separate experiments were carried out (with an interval of 10 months). Male albino rabbits weighing about 2 kg were kept on a uniform, adequate laboratory diet under regular control of body weight. In the *first experiment* one group of 22 rabbits received intravenous injections of epinephrine† daily for 11 days in doses increasing from .025 mg/kg to .050 mg/kg during the last 7 days. Simultaneously the animals were injected with 2 ml physiological saline intraperitoneally once a day. Another group of 31 rabbits received epinephrine as the previous group, and d-thyroxine,‡ suspended in physiological saline, .25 mg/kg once a day for 11 days injected intraperitoneally. In both groups one-fourth of the animals died before the 6th day and were excluded. The *second experiment* comprised 3 groups of rabbits: Twelve animals were injected with d-thyroxine .25 mg/kg intraperitoneally and physiological saline 1 ml intravenously daily for 2 weeks (corresponding to the epinephrine-injections in the first experiment). Eleven animals were injected with physiological saline 1 ml intravenously and 1 ml intraperitoneally daily for 2 weeks, while a third group of 10 animals served as untreated controls. The following technic was common for both experiments. Forty-eight hours before being sacrificed the animals received 1.5 mc of ^{35}S sulfate§ intraperitoneally without regard to body weight. A correction for weight was made after the final measurements. The rabbits were sacrificed by air embolism, the entire aorta and the heart removed and the hearts weighed. The gross alterations in aortae were estimated by grading from 0-4 as in previous experiments (1,2). Specimens for microscopic examinations removed from the arch, the thoracic and the abdominal part of aorta were fixed in a 4% aqueous solution of lead subacetate. Sections were stained with hematoxylin-eosin (Harris), orcein (Unna), the van Gieson-Hansen connective tissue stain and with a .5% aqueous solution of toluidine blue for metachromasia. In the first experiment each aorta was divided into 3 parts for biochemical investigation: I from the aortic valve to the first intercostal arteries, II from the first intercostal arteries to the diaphragm and III the abdominal aorta. In the second experiment the whole aorta was analysed as one. The adventitia was stripped off. Water content was determined after drying in a vacuum desiccator for 48 hours at room temperature. Thereupon the tissue was homogenized as described previously(2). Total content of mucopolysaccharides was estimated by determination of the content of hexosamine by the method of Elson and Morgan(3) as modified by Boas(4) and later by Kirk and Dyrbye(5) and Dyrbye(6). The content of hydroxyproline, representing the amount of col-

* Supported by grants from Danish State Research Foundation and Danish Foundation for Advancement of Medical Science.

† A .01% solution of epinephrine hydrochloride.

‡ Generously supplied by Travenol Laboratories Inc., Morton Grove, Ill., as d-thyroxine-sodium.

§ Carrier-free ^{35}S sulfate obtained from the Radiochemical Centre, Amersham, England. Diluted to a suitable volume with a 10 mg per 100 ml solution of sodium sulfate in physiological saline.

TABLE I. Content of Hexosamine and Hydroxyproline in Rabbit Aortae, μg/mg Dried Tissue and Hexosamine/Hydroxyproline Ratio Following Injection of Epinephrine plus d-thyroxine and Epinephrine plus Physiological Saline.

Group	I§	II	III	IV	P values‡ I-II	I-III	II-III
			Hexosamine				
Epinephrine + d-thyroxine	8.9 ± .35* (20)†	7.9 ± .23 (20)	8.2 ± .40 (17)	8.4 ± .26 (17)	<.05	n.s.	n.s.
Epinephrine + physiol. sal.	8.0 ± .45 (15)	6.9 ± .29 (14)	7.6 ± .29 (13)	7.4 ± .32 (13)	<.05	n.s.	n.s.
P values	n.s.	<.02	n.s.	<.05			
			Hydroxyproline				
Epinephrine + d-thyroxine	21.3 ± .53 (20)	20.9 ± .76 (23)	23.5 ± 1.6 (20)	21.9 ± .62 (18)	n.s.	n.s.	n.s.
Epinephrine + physiol. sal.	21.3 ± .66 (15)	21.6 ± .56 (16)	24.9 ± .87 (15)	21.8 ± .55 (15)	n.s.	<.01	<.01
P values	n.s.	n.s.	n.s.	n.s.			
			Hexosamine/hydroxyproline ratio				
Epinephrine + d-thyroxine	.42 ± .024 (20)	.37 ± .017 (20)	.36 ± .021 (16)	.38 ± .019 (16)	n.s.	<.05	n.s.
Epinephrine + physiol. sal.	.38 ± .026 (15)	.32 ± .019 (14)	.30 ± .016 (13)	.34 ± .017 (13)	n.s.	<.05	n.s.
P values	n.s.	n.s.	n.s.	n.s.			

* Mean ± stand. dev. of mean.
† No. of samples.
‡ Statistical significance was determined by comparing mean values in Students' t test.
§ I-III—different parts of aorta, IV—aorta as a whole, calculated on basis of total wt of I-III.

lagen, was determined by the method of Neuman and Logan(7) modified by Martin and Axelrod(8). The exchange of ester-bound sulfate in sulfated mucopolysaccharides was estimated by determination of the *in vivo* uptake of ^{35}S sulfate after ashing of the tissue with fumic nitric acid and precipitation of the sulfur as barium sulfate as devised by Moltke

TABLE II. Uptake of ^{35}S Sulfate in Counts/Min./10 mg Dried Tissue in Aortae and Skin, and Content of Water, % of Wet Weight in Aortae of Rabbits Injected with Epinephrine plus d-thyroxine and Epinephrine plus Physiological Saline.

	^{35}S sulfate uptake							
	Aorta				Skin			
Group	I‡	II	III	IV				
Epinephrine + d-thyroxine	79 ± 7* (16)†	69 ± 8 (16)	80 ±11 (15)	74 ± 7 (15)	31 ± 4 (15)			
Epinephrine + physiol. sal.	56 ± 6 (13)	44 ± 3 (13)	61 ± 7 (13)	51 ± 5 (11)	39 ± 5 (13)			
P values	<.05	<.02	n.s.	<.05	n.s.			
	Content of water					P values		
						I-II	I-III	II-III
Epinephrine + d-thyroxine	75.4 ± .40 (23)	72.6 ± .65 (23)	72.2 ± .89 (22)	73.7 ± .46 (22)		<.001	<.01	n.s.
Epinephrine + physiol. sal.	74.9 ± .84 (16)	72.2 ± .85 (15)	70.3 ±1.6 (16)	72.8 ± .84 (15)		<.05	<.02	n.s.
P values	n.s.							

* Mean ± stand. dev. of mean.
† No. of samples.
‡ I-III—different parts of aorta, IV—aorta as a whole.

(9). Activity was measured at infinite layer-thickness with a Geiger-Müller tube, counting to a statistical error of about 3%. The *in vivo* uptake of ^{35}S sulfate in the skin was also determined. The dose of d-thyroxine was 5 times as large as the dose of l-thyroxine used in the previous experiment(1) to obtain a similar suppression of the thyroid stimulating hormone (TSH) production. The inhibitory effect of d-thyroxine on endogenous production of TSH is estimated to about one-third of that of l-thyroxine(10). In a preliminary experiment the inhibitory effect of l- and d-thyroxine on release of thyroid hormone from the thyroid gland was compared in 4 rabbits injected intraperitoneally with .05 mg/kg l-thyroxine and 4 with .25 mg/kg d-thyroxine daily, using ^{131}I and daily counting over the thyroid gland with a scintillation counter, recording a "release curve" as described by Brown-Grant(11).

Results. In the *first experiment* the rabbits injected with epinephrine plus d-thyroxine averaged 8.0% loss in weight in contrast to a weight gain of 4.5% in the epinephrine-saline group. The weight of the hearts expressed in per cent of body weight was highest in the epinephrine-d-thyroxine group (.36 ± .009 and .30 ± .010 g respectively), whereas absolute weights did not differ. Two-thirds of the epinephrine-d-thyroxine injected rabbits showed rather pronounced gross alterations (grade 2-3) in aorta I and II in contrast to slighter alterations (grade 1-2) in half of the epinephrine-saline injected animals. The microscopic lesions consisted of calcified media lesions, stretching and splitting of the elastic membranes, accumulation of amorphous metachromatic substance between the elastic membranes and intimal thickening, most pronounced in the epinephrine-d-thyroxine group and similar to the alterations previously described(1,2,12). The content of hexosamine in part II of aorta (Table I) and the uptake of ^{35}S sulfate (Table II) in part I and II was highest in the epinephrine-d-thyroxine group. In the *second experiment* all 3 groups gained weight, but the d-thyroxine group less than the 2 others (Table III). No gross alterations were seen in the aortae except for a few small

TABLE III. Alterations in Body Weight and Weight of Hearts, Content of Hexosamine and Hydroxyproline, μg/mg Dried Tissue, Hexosamine/Hydroxyproline Ratio, Content of Water in % of Wet Weight in Rabbit Aortae and Uptake of ^{35}S Sulfate in Counts/100 Sec./10 mg Dried Tissue in Aortae and Skin Following Injections of d-thyroxine plus Physiological Saline, Physiological Saline and in Untreated Controls.

Group		Alterations in body wt, % of initial wt	Wt of hearts		He	Hp	He/Hp	Water content	^{35}S sulfate uptake	
			g	% of body wt					Aortae	Skin
1. d-thyroxine + physiol. sal.		6.8 ± .63* (12) †	4.54 ± .24 (12)	.22 ± .010 (12)	7.1 ± .14 (12)	21.4 ± .56 (12)	.34 ± .014 (12)	69.9 ± .26 (12)	274 ± 21 (12)	139 ± 15 (11)
2. Physiol. saline		12.7 ± 2.0 (11)	5.19 ± .23 (11)	.21 ± .005 (11)	7.5 ± .18 (11)	21.0 ± .76 (11)	.36 ± .009 (11)	70.8 ± .30 (11)	203 ± 10 (11)	157 ± 22 (10)
3. Untreated controls		12.8 ± 1.1 (10)	4.73 ± .20 (10)	.20 ± .005 (10)	7.1 ± .22 (10)	21.7 ± .52 (10)	.33 ± .014 (10)	71.0 ± .50 (10)	193 ± 15 (10)	143 ± 19 (9)
P values	1-2	<.01						<.05	<.01	
	1-3	<.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<.01	n.s.
	2-3	n.s.						n.s.	n.s.	

† No. of samples.
* Mean ± stand. dev. of mean.

white nodules just above the aortic valve in a few rabbits in all 3 groups. Except for these lesions, microscopically visible as subintimal, fibrous calcified lesions, none of the animals showed severe medial alterations, but half of the d-thyroxine-injected and 2 of the saline-injected animals had scattered accumulations of metachromatic substance in the media between the elastic membranes. Considering the content of water, hexosamine, hydroxyproline and the hexosamine/hydroxyproline ratio no difference was seen between the 3 groups, whereas the uptake of ^{35}S sulfate in aorta showed a significant increase in the d-thyroxine group. The injected doses of d- and l-thyroxine were found to have a complete inhibitory effect on release of ^{131}I-labelled hormone from the thyroid gland. The effect was noted 24 hours after onset of injections.

Discussion. D-thyroxine showed an influence on the aortic wall similar to that of l-thyroxine(1,2). It enhanced the damaging effect of epinephrine and induced alterations in the mucopolysaccharides of the arterial wall. The alterations observed might accordingly be interpreted as a repair process elicited by injury to the arterial wall(2). However, even if injected in 5-fold higher doses, d-thyroxine alone in contrast to l-thyroxine (1), was unable to induce quantitative alterations in the hexosamine content. As the doses of d- and l-thyroxine exerted an equally inhibitory effect on release of thyrotrophic hormone, it seems reasonable to assume, that d-thyroxine as well as l-thyroxine exerts its influence on the arterial wall by a direct peripheral action and not *via* TSH.

Summary. D-thyroxine enhanced epinephrine-induced alterations in the aortic wall in rabbits, causing an increase in hexosamine content and uptake of ^{35}S sulfate. D-thyroxine alone increased uptake of ^{35}S sulfate. Compared to l-thyroxine used in previous experiments, d-thyroxine acted similarly but to a lesser extent. As the applied amounts of d- and l-thyroxine exerted an equally inhibitory effect on TSH, d-thyroxine as well as l-thyroxine probably exerts its damaging effect on the arterial wall by direct peripheral action.

1. Lorenzen, I., *Acta Endocrin.*, 1961, v37, 183.
2. ———, *ibid.*, 1961, v36, 197.
3. Elson, L. A., Morgan, W. T. J., *Biochem. J.*, 1933, v27, 1824.
4. Boas, N. F., *J. Biol. Chem.*, 1953, v204, 553.
5. Kirk, J. E., Dyrbye, M. O., *J. Geront.*, 1956, v11, 273.
6. Dyrbye, M. O., *ibid.*, 1959, v14, 32.
7. Neuman, R. E., Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.
8. Martin, C. J., Axelrod, A. E., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 461.
9. Moltke, E., *Acta Endocrin.*, 1957, v25, 179.
10. ———, *ibid.*, 1957, v24, 226.
11. Brown-Grant, K., v. Euler, C., Harris, G. W., Reichlin, S., *J. Physiol.*, 1954, v126, 1.
12. Lorenzen, I., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 440.

Received May 8, 1961. P.S.E.B.M., 1961, v108.

Cytotoxicity of Adrenal Cortex Hormones on Normal and Malignant Lymphocytes of Man and Rat.* (26928)

ROBERT SCHREK
Tumor Research Laboratory, Research and Surgical Services, Veterans Administration Hospital, Hines, Ill.

I have shown by the method of unstained cell counts(1) that cortisol and related hormones have an *in vitro* cytotoxic effect on cells derived from the rabbit thymus. The newly developed slide-chamber method(2) provided a means for extending this study to human lymphocytes. This method was used in this study to measure the toxic effect of cortisol on normal human and rat lymphocytes and also on human leukemic lymphocytes.

* Aided by grants from Damon Runyon Memorial Fund and Nat. Cancer Inst.