zig-zag with hingend apices which deforms by unfolding, and a planar zig-zag with rigid apices which deforms by cantilever bending (the last is derived in ref.[2]). In Figure 2, the data for 2 types of tendon are compared to the predicted curves, and it is seen that the points lie along the curve for the sine wave, or between the sine and cantibending cases. It is also noted that the human diaphragm tendon data lie in the same relation to the predicted curves as do the RTT data, where the waveform can be demonstrated planar by rotation in polarized light. This strongly indicates that the waveform is also planar in the diaphragm tendon.

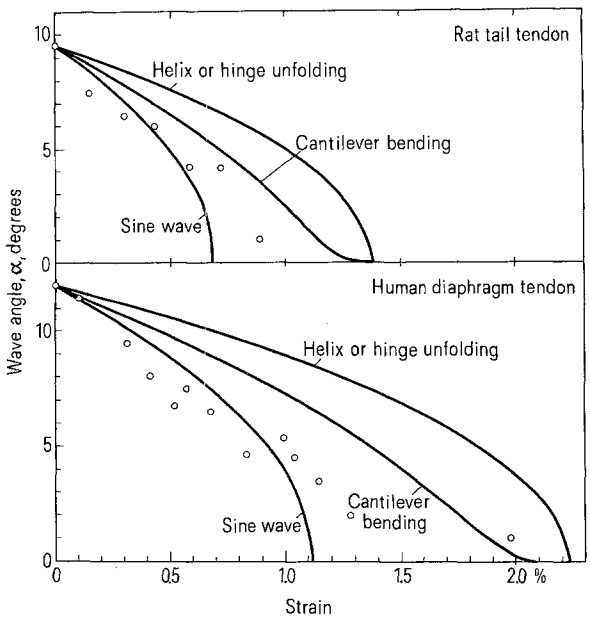

Fig. 2. Decrease of angle of the waveform with strain as tendon is stretched, for rat tail and human diaphragm tendons. (Samples A) and C) in Figure 1). Points are experimental observations and curves are predicted behavior of inextensible fibers whose original wave shape is as marked.

The Table shows that major differences were found in the size parameters of the waveform for tendons from different sources. Also, a range of sizes was found in individual samples of human Achilles and kangaroo tail tendon, including a waveform 100 μm long in the Achilles tendon of an 87-year-old male. Nonetheless, it seems clear that essentially the same planar symmetric waveform as seem in RTT was found in all these tendons from diverse sources. Consequently, its implications for the stress response and function of the tendon in vivo, along the lines of ref.[2], are expected to hold throughout[3].

Zusammenfassung. Es wird gezeigt, dass die zwischen gekreuzten Nicols für die Rattenschwanzsehne nachgewiesenen periodischen Bänder von fibrillären Strukturelementen ebener Wellenform abstammen und deren Ausstreckung das beobachtete Dehnungsverhalten erklärt[2], das auch für andere Säugetiere zutrifft.

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Chemical and Immunological Characterization of Serum Lipoproteins Isolated by Glass-Column Chromatography

The chromatographic separation of lipoproteins by adsorption on glass powder columns, developed for whole serum by Carlson[1], was adopted with some modifications for isolation of serum lipoproteins from human, rabbit and rat subjects. Assay of the column eluates by both chemical and immunoreaction methods demonstrated the reliability of this method for a satisfactory separation.

Experimental. Stock solutions of 1.2 *M* potassium bicarbonate and 0.4 *M* potassium carbonate were prepared and titrated against HCl. Buffer solutions of pH 8.8, pH 9.6 and pH 9.8 were prepared from stock solutions. Glass columns, 15 × 150 mm, were packed with glass-beads of 100–150 mesh and serum samples, diluted with equal volume of buffer pH 8.8, were added to the columns. The walls were washed with 1 ml of pH 8.8 buffer. The samples were allowed to move down the columns by opening the stopcock. The columns were washed with another portion of pH 8.8 buffer in the order: 2 + 2 + 4 + 4 ml, followed by 1 ml of 15 ml of the pH 8.8 eluate, containing mainly albumin, were collected. The subsequent fractions of the buffer eluates were collected in the following order: 5 ml + 5 ml + 3 ml of pH 9.6 and 2 ml of 9.8 were collected together as containing α-lipoprotein. Then 5 ml of 0.4 *M* potassium carbonate buffer were collected as α_2 lipoprotein. Finally 15 ml of potassium carbonate buffer were added to the column for elution of β-lipoprotein.

The lipoprotein fractions obtained after elution from the glass-columns were dialyzed against distilled water for 8 h at 4 °C. The solutions were lyophilized and stored for 6 weeks at −15 °C without any appreciable denaturation. The ultra-centrifugal pattern of the separated α- and β-lipoproteins in artificial boundary cells with Schilieren optics are shown (Figures 1 and 2).

The protein fractions of the lipoproteins and sera were determined according to Lowry et al[2]. Total and free cholesterol were estimated by the method of Sperry and

[1] L. A. Carlson, Clin. chim. Acta *5*, 528 (1960).

[2] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. L. Randall J. biol. Chem. *193*, 265 (1951).

WEBB[3]. The method of VAN HANDEL and ZILVERSMIT[4] was employed for triglyceride estimation, while phospholipids were determined according to CHEN et al[5].

For immunological analyses, the separated lipoproteins were employed as antigens. Antisera were prepared according to SCANU[6]. Immuno-precipitation reactions were performed according to OUCHTERLONY[7] and agar gel immunoelectrophoresis according to SCHEIDEGGER[8]. The antisera were employed to characterize the various antigens, i.e., the isolated lipoproteins, by studying their reactivity by immunodiffusion technique in agar gel. A summary of the findings is presented in Table II.

Results and discussion. By the procedure described, resolution of serum lipoproteins was possible with great reproducibility; they were designated in the study as α- and β-lipoproteins. The intermediate α_2-fraction comprised only a minor fraction and was not investigated. The ultra-centrifugal pattern of the separated α_1- and β-lipoproteins indicated that the α_1-fraction was essentially free of other proteins, while the β-lipoprotein fraction exhibited the presence of a very low amount of another protein, possibly some contaminants of α_2-lipoprotein (Figures 1 and 2).

Chemical analysis of the lipoproteins isolated from the glass-columns (Table I) showed that the lipid content and the ratio cholesterol: phospholipid in the eluates of potassium carbonate-bicarbonate buffer, pH 9.6, and the CO_3 fractions were analogous to those obtained by CARLSON and others[1,9,10] in the α_1- or high-density lipoprotein fraction and the β- or low-density lipoprotein fraction, respectively. The immuno-reaction studies showed that all anti-α_1-lipoprotein and anti-β-lipoprotein sera had similar behaviour and reacted only with their homologous antigens. Agar gel immunodiffusion reaction between α_1-lipoprotein and its antisera showed 2 distinct lines, whereas the reaction between β-lipoproteins and anti β-lipoprotein sera gave a distinct arc of precipitation. The immuno-electrophoretic studies show the same type of reaction. From these findings it can be concluded that the α_1-lipoproteins were composed of 2 immunologically distinct proteins. The ultracentrifugal Figure 2 shows contamination of some other proteins with the β-lipoproteins, but the immunochemical studies showed no evidence of contamination. The immunological experiments demonstrated that the preparation obtained by elution of the glass column with potassium carbonate and bicarbonate buffer of pH 9.6 consisted exclusively of a product with antigenic properties the same as human α_1-lipoprotein. But it has been observed that this product, i.e., the α_1-lipoprotein, is antigenically heterogeneous, albeit the ultracentrifugal pattern showed that this protein was essentially free from other products. The heterogeneity of the high-density of α_1-lipoprotein has been shown by other workers[7,11]. The immunological experiments demonstrated that the preparation obtained

Table I. Lipid distribution of serum and lipoprotein fractions of normal human, rabbit and rat separated on glass-column

	Serum			pH 8.8 fraction				pH 9.6 fraction				CO_3 fraction			
	G	C	P	R	G	C	P	R	G	C	P	R	G	C	P	R
Human	98.2±3.2	200.0±15.6	210.2±14.0	0.95	6.2±0.4	6.2±0.2	21.1±0.6	0.3	18.6±0.02	36.2±0.2	70.2±0.8	0.51	69.3±5.1	156.1±11.2	117.0±6.4	1.38
Rabbit	57.4±4.2	38.1±0.2	59.1± 2.4	0.64	2.4±0.1	0.71±0.01	4.2±0.3	0.16	11.1±0.01	5.5±0.1	14.6±0.5	0.37	39.0±0.8	27.6± 0.3	37.0±0.8	0.74
Rat	28.2±3.0	50.6± 1.2	115.2± 4.1	0.43	1.02±0.001	1.61±0.002	8.9±0.02	0.18	6.2±0.004	15.6±0.6	46.2±0.2	0.34	17.6±0.4	32.3± 1.3	61.0±2.5	0.53

Values are given in mg/100 ml. G, glycerides; C, cholesterol; P, phospholipid; R, ratio of cholesterol to phospholipid.

[3] W. M. SPERRY and M. WEBB, J. biol. Chem. *187*, 97 (1950).
[4] E. VAN HANDEL and D. B. ZILVERSMIT, J. Lab. clin. Med. *50*, 152 (1957).
[5] P. S. CHEN JR., T. Y. TORIBARA and H. WARNER, Analyt. Chem. *28*, 1756 (1956).
[6] A. SCANU, J. Lipid Res. *7*, 295 (1966).
[7] O. OUCHTERLONY, Acta path. microbiol. scand. *26*, 507 (1949).
[8] J. J. SCHEIDEGGER, Int. Arch. Allergy appl. Immun. *7*, 103 (1955).
[9] R. J. HAVEL, H. A. EDER and J. H. BRAGDON, J. clin. Invest. *34*, 1345 (1955).
[10] R. T. LEVY and O. S. FREDRICKSON, J. clin. Invest. *44*, 426 (1965).
[11] J. L. ONCLEY, K. W. WALTON and D. G. CORNELL, J. Am. chem. Soc. *79*, 4666 (1957).

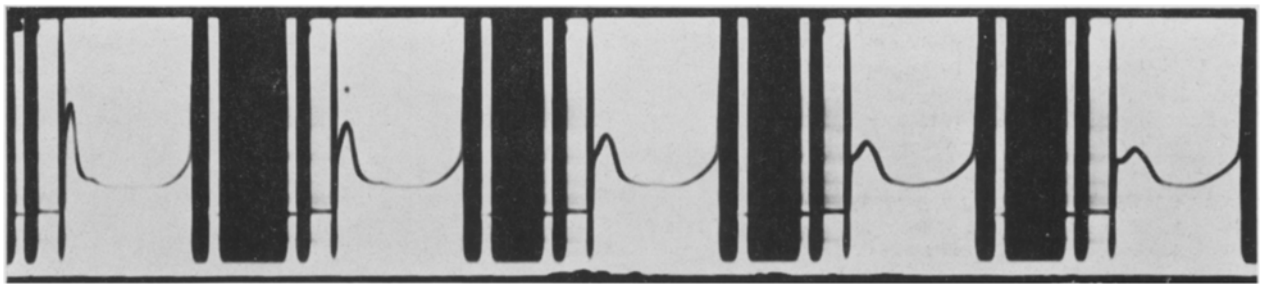

Fig. 1. Ultracentrifugal pattern of isolated α-lipoprotein.

Table II. Characteristics of antisera

Antibody	Antigen	Reactivity		
		α_1-Lipoprotein	β-Lipoprotein	Whole serum
Antihuman rabbit serum	Human α_1-LP (pH 9.6 fraction)	+[a]	−	+[a]
Antihuman rabbit serum	Human β-LP (CO_3 fraction)	−	+[b]	+[b]
Antihuman rabbit serum	Human albumin (pH 8.8 fraction)	−	−	+
Anti-human α_1-LP rabbit serum	Human α_1-LP (pH 9.6 fraction)	+[a]	−	+[a]
Anti-human β-LP rabbit serum	Human β-LP (CO_3 fraction)	−	+[b]	+[b]
Antirat rabbit serum	Rat α_1-LP (pH 9.6 fraction)	+[a]	−	+[a]
Antirat rabbit serum	Rat β-LP CO_3 fraction)	−	+[b]	+[b]
Antirat rabbit serum	Rat albumin (pH 8.8 fraction)	−	−	+
Anti-rat α_1-LP rabbit serum	Rat α_1-LP (pH 9.6 fraction)	+[a]	−	+[a]
Anti-rat β-LP rabbit serum	Rat β-LP (CO_3 fraction)	−	+[b]	+[b]

[a] Double arc of precipitation. [b] Single arc of precipitation.

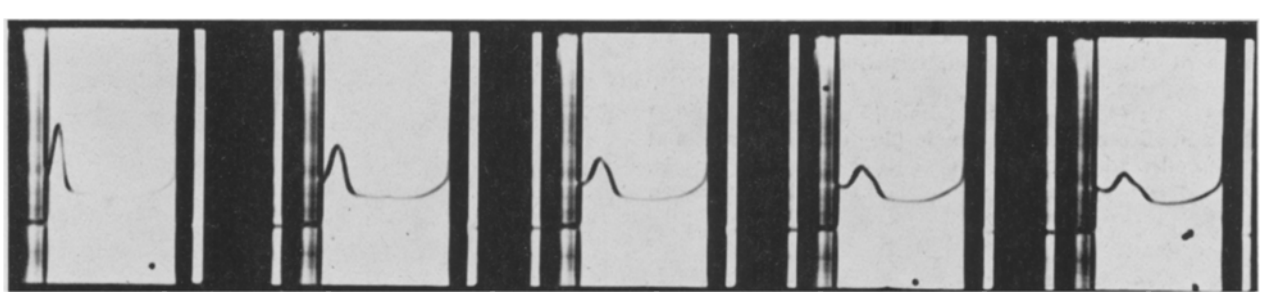

Fig. 2. Ultracentrifugal pattern of isolated β-lipoprotein of normal rat serum in artificial boundary cell with Schilieren optics in 0.02 *M* bicarbonate buffer, pH 7.0, at 259,697 × *g* first plate taken 17 min after attainment of full speed and subsequently at intervals of 10, 20 and 30 min.

by elution of the glass-column with 0.4 *M* potassium carbonate buffer consisted almost exclusively of a product with the same antigenic properties as low-density human β-lipoprotein. It is also shown that this product, i.e., the β-lipoproteins, are antigenically homogeneous. The chemical analysis of this product identified it as similar to the β-lipoproteins. The ultracentrifugal preparation of these eluates had some protein contaminant, different from β-lipoprotein protein. This may be the α_2-fraction of the lipoproteins. This fraction must be antigenically identical with β-lipoprotein protein. Thus the reaction between this β-lipoprotein fraction and anti-β-lipoprotein sera gave

no additional precipitation line. Other workers have sought to refer to the Sf 200–100 subclass separately as very low-density lipoprotein or α_2-lipoprotein; but it has been shown that the protein moiety is antigenically identical throughout the low-density lipoprotein[12].

[12] K. W. WALTON, Immun. Chem. *1*, 267 (1964).
[13] Present address: Department of Biological Chemistry, Harvard Medical School, Boston (Massachussetts 02115, USA).

Zusammenfassung. Eine neue Methode zur Trennung von Lipoproteinen mittels Säulenchromatographie wird beschrieben.

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Threo-3,4-Dihydroxyphenylserine, a Poor Source of Noradrenaline in the Rat

Threo-3,4-Dihydroxyphenylserine (DOPS) was first recognized as a possible intermediate in catecholamine biosynthesis more than 50 years ago[1]. However, despite careful search[2,3], it has never been identified in the organism and any major role as a precursor of noradrenaline must probably be discounted. Nevertheless, as a known substrate of dopa decarboxylase, both in vitro[4–9] and in vivo[10–12], DOPS has been used as a pharmalogical tool to effect a selective increase of noradrenaline in animal brain[13–17]. It is, in fact, an indifferent substrate for decarboxylase in vivo and relatively vast dosage schedules have been necessary to achieve the desired effect.

After DOPA injection in man, dopamine is excreted directly into the urine[18], probably deriving largely but not exclusively from direct renal decarboxylation. DOPS seems to undergo a similar fate, but from the few relevant data on record, it seems to possess no more than $^1/_{10}$ of the ability of DOPA to act as substrate for renal decarboxylase[12]. Observations of this type[10–12] provide an index of decarboxylation in a single organ, the kidney. Whether they can be extrapolated to the whole organism, where more than one form of the enzyme might be present, is open to some doubt[19]. It therefore seemed desirable to obtain further information concerning the degree of in vivo conversion of DOPS to noradrenaline. The present experiment was therefore devised in order to measure 4-hydroxy-3-methoxyphenylglycol (HMPG), the major urinary metabolite of noradrenaline in the rat[20], after DOPS administration. Excretion of this compound appears to reflect noradrenaline production more faithfully than that of other metabolites[21]. During the course of this study, evidence of a wholly unexpected metabolic route of DOPS degradation, resulting from side-chain cleavage, was obtained.

DL-DOPS (AB Biotec, Stockholm, Sweden) (100 mg/kg) was administered i.p. to 4 male (200 g) Wistar rats and urine collected over acid[21] for 2 successive 24 h periods. Conjugates were hydrolyzed at pH 5.5–6.0 by incubation at 37°C overnight with suc d'*Helix pomatia* (Industrie Biologique Française, 92-Gennevilliers, France) and acids and alcohols extracted at pH 1 into ethyl acetate (3 × 3

[1] K. W. ROSENMUND and H. DORNSAFT, Ber. dt. chem. Ges. *52*, 1734 (1919).
[2] N. KIRSHNER, J. biol. Chem. *226*, 821 (1957).
[3] R. GELINAS, J. PELLERIN, A. D'IORIO, Rev. Can. Biol. *16*, 445 (1957).
[4] K. H. BEYER, H. BLASCHKO, J. H. BURN, H. LANGEMANN, Nature, Lond. *165*, 926 (1950).
[5] H. BLASCHKO, J. H. BURN, H. LANGEMANN, Br. J. Pharmac. *5*, 431 (1950).
[6] T. SOURKES, P. HENEAGE and Y. TRANO, Arch. Biochem. Biophys. *40*, 185 (1952).
[7] E. WERLE and J. SELL, Biochem. Z. *326*, 110 (1955).
[8] E. WERLE and J. JÜNTGEN-SELL, Biochem. Z. *327*, 259 (1955).
[9] W. J. HARTMAN, R. S. POGRUND, W. DRELL and W. G. CLARK, J. Am. chem. Soc. *77*, 816 (1955).
[10] C. G. SCHMITERLÖW, Br. J. Pharmac. *6*, 127 (1951).
[11] W. DRELL, M. ESHLEMAN and W. G. CLARK, Abstr. IV Int. Congr. Biochem., Wien (1958), p. 107.
[12] L.-M. GUNNE and H.-F. LIDVALL, Scand. J. clin. Lab. Invest. *18*, 425 (1966).
[13] H. BLASCHKO and T. L. CHRUSCIEL, J. Physiol., Lond. *151*, 272 (1960).
[14] A. CARLSSON, Progr. Brain Res. *8*, 9 (1964).
[15] V. HAVLÍČEK, Int. J. Neuropharmac. *6*, 83 (1967).
[16] C. R. CREVELING, J. DALY, T. TOKUYAMA and B. WITKOP, Biochem. Pharmac. *17*, 65 (1968).
[17] O. HORNYKIEWICZ, L. DAVIDSON and F. KRASTER, Abstr. 4th Int. Congr. Pharmac., Basel (1969), p. 71.
[18] P. HOLTZ, K. CREDNER and W. KOEPP, Arch. exp. Path. Pharmak. *200*, 356 (1942).
[19] M. SANDLER, in *Handbook of Experimental Pharmacology: Catecholamines* (Eds. H. BLASCHKO and E. MUSCHOLL; Springer, Berlin 1972), vol. 33, p. 845.
[20] I. J. KOPIN, J. AXELROD and E. GORDON, J. biol. Chem. *236*, 2109 (1961).
[21] P. M. CEASAR, C. R. J. RUTHVEN and M. SANDLER, Br. J. Pharmac. *36*, 70 (1969).

Table. Urinary metabolites of DL-*threo*-3,4-dihydroxyphenylserine (DOPS) in the rat

Regime	Period of urine collection (h)	HMPG (μg/day)		Protocatechuic acid (μg/day)		Vanillic acid (μg/day)		Vanillyl alcohol (μg/day)		3, 4-Dihydroxybenzyl alcohol (μg/day)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
DOPS	0–24	130	109–152	302	274–336	611	474–772	122	108–135	98	67–138
DOPS	24–48	35	29– 40	8	6– 10	134	97–164	12	10– 15	Less than 2	
DOPS + Neomycin	0–24	116	98–137	282	270–294	444	352–501	108	85–138	84	51–106
Control	0–24	38	32– 42	6	5– 8	49	36– 64	Less than 1		Less than 2	