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].

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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

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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	

vol.). Initially, the extracted materials were chromatographed two-dimensionally on Whatman No. 52 paper with isopropanol-880 ammonia-water (8:1:1) and benzene-acetic acid-water (125:73:2). Phenolic compounds were detected by spraying with diazotized sulphanilamide and sodium carbonate.

On comparison with urinary chromatograms from 4 untreated control rats, a pink spot corresponding to HMPG was just detectable. Two other new spots were apparent, both orange. These co-chromatographed with and gave the same colour reaction as authentic samples of vanillic acid and vanillyl alcohol (K & K Laboratories, Inc., Plainview, New York), respectively. Vanilloylglycine could not be identified. Measurements by gas chromatography[22-24] (Table) showed that 0.55% of administered DOPS was excreted as HMPG in the first 24 h; corresponding values of vanillic acid, vanillyl alcohol, 3,4-dihydroxybenzyl alcohol (prepared by reduction of protocatechualdehyde with hydrogen and Raney nickel) and protocatechuic acid during this period were 3.9%, 0.8%, 0.75% and 2.1%, respectively. The only other major metabolite noted was 3-*O*-methyl DOPS which was shown by paper chromatography[25,26] to account for approx. 10% of the administered dose. Unchanged DOPS itself was not observed. A trace (approx. 0.3%) of 4-hydroxy-3-methoxymandelic acid (VMA) noted on paper chromatography of urine from DOPS treated rats could not be quantified gas chromatographically because of the presence of interfering substances. In the subsequent 24 h period, vanillic acid accounted for a further 0.5% of the dose; none of the other metabolites was present in increased concentration apart from a trace of vanillyl alcohol. Pretreatment of a further group of 4 rats with neomycin prior to giving DOPS produced substantially the same urinary metabolite excretion values as in the group treated with DOPS alone (Table). Administration of a mixture of *erythro-* and *threo*-DL-4-hydroxy-3-methoxyphenylserine (prepared by condensation of *O*-benzylvanillin with glycine) (4 rats, 100 mg/kg) also yielded urinary vanillic acid and vanillyl alcohol in approximately the same proportion and at about 5 times greater concentration (paper chromatography) as after DOPS treatment.

The residue from the pH 1 extraction step was further purified and subjected to paper chromatography for amines[25]. An increased excretion of noradrenaline or normetanephrine could not be identified in the groups receiving drug treatment.

One surprising feature which emerged was that not more than 20% of administered DOPS could be accounted for. Of this total, a very small proportion only consisted of noradrenaline metabolites. Thus it would appear that administered DOPS is a poor in vivo substrate for DOPA decarboxylase in the rat.

Unexpectedly, the very small production of noradreline from DOPS was quite overshadowed by a conversion of DOPS to vanillic acid and related compounds of the order of 10%. Although vanillic acid is a known but minor metabolite of noradrenaline[27], quantitative considerations would appear to preclude such an origin in the present series of experiments. Vanillic acid and vanillyl alcohol presumably derive from vanillin[28]. This aldehyde might conceivably be formed from an unstable ketoacid analogue of DOPS, generated either from the transamination of L-DOPS or its 3-*O*-methylated derivative, or from the action of D-amino acid oxidase on D-DOPS. The neomycin experiment probably rules out vanillin production as a gut flora phenomenon[29]. It seems much more likely, however, that it results directly from the action of the relatively little studied phenylserine-cleaving enzyme of BRUNS and FIEDLER[30,31] on L-DOPS.

The fate of by far the greater proportion of administered DOPS remains unknown. Because phenolic compounds other than those noted above were absent from chromatograms, the possibility of ring-fission must be seriously invoked[32].

Résumé. Chez le rat, les injections de DL-*thréo*-3,4-dihydroxyphénylsérine, un précurseur pharmacologique synthétique de la noradrénaline, provoquent une conversion de moins de 1% au 4-hydroxy-3-méthoxyphénylglycol, le métabolite urinaire majeur de la noradrénaline dans cette espèce. Un nouveau cheminement qui entraîne une coupure latérale de la chaîne prit environ 10% de la dose et un autre 10% fût excrété comme un amino-acide *O*-méthylé; le sort de quelque 80% est inconnu.

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Endothelial Defects and Blood Flow Disturbance in Atherogenesis[1]

Defects of arterial endothelium have been postulated to play an important role in atherogenesis since they may result in augmented lipoprotein entry into the arterial wall[2,3]. Such defects could arise by cell injury and death which are accelerated in areas of lesion formation. Recently, pulsed injections of ^{3}H-thymidine combined with autoradiography have been used to demonstrate cell turnover rates. These studies indicate that cholesterol feeding leads to increased endothelial cell division in the aortas of rabbits on long-term diets[4] and of miniature swine on short term diets[5]. In the latter investigation electron microscopic evidence of endothelial cell damage was also encountered. Using a similar technique in normally fed guinea-pigs, WRIGHT[6] showed that 50% more mitotic endothelial cells were observed around the mouths of aortic bifurcations than in the vicinity of non-