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-

branching areas. Since regions such as branch orifices, curvatures and bifurcations are known sites of predilection for atherogenesis, predictable disturbances of blood flow in these areas may play a role in producing cell damage. Cholesterol feeding and blood flow instability could then act synergistically to accelerate endothelial cell injury in favored sites, but it appears from WRIGHT's study, that such injury can result from a flow disturbance alone. In order to obtain further information on the likelihood of this event, we examined vascular endothelium by electron microscopy in an arterial region of this type where blood flow disturbances had been previously demonstrated.

Heated wire velocity probes were inserted into ilioaortic junctions (experimental areas) and downstream in the iliac arteries (control areas) of 5 young Yorkshire swine (4 sows and 1 boar, averaging 30–32 kg body weight each, and approximately 4–6 weeks of age). The probe tips were advanced to less than 1 mm from the lateral wall and oriented to face the streamlines orthogonally. When placed in the junction, they bisected the obtuse angle between abdominal aorta and iliac artery. Local blood velocity signals were recorded on an oscilloscope screen after passage through an anemometerlinearizer circuit[7]. Essential agreement in the nature of the records

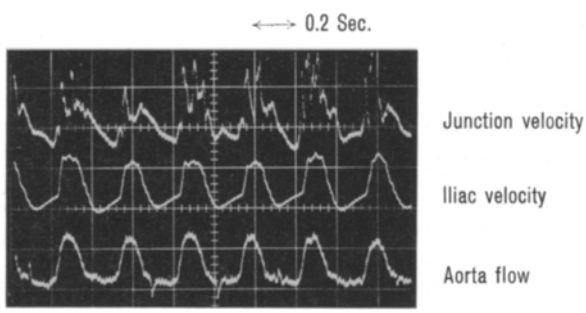

Fig. 1. Oscillograph traces of blood velocities and volumetric flow rate. Lower = aortic flow rate (200 ml/min). Upper = velocity in ilioaortic junction. Disturbed pattern is evidenced by superposition of high frequency components on primary (pulse) waveform. Middle = velocity in iliac artery showing undisturbed 'smooth' pattern.

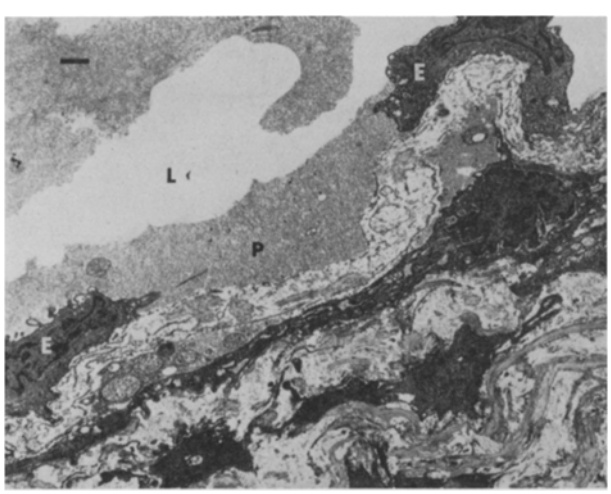

Fig. 2. Electron micrograph of the arterial wall from the ilioaortic junction, from which the data in Figure 1 was obtained. The surface endothelium (E) shows a defect due to the loss of a single cell; L, lumen; P, plasma. Defects of this type were found frequently at the junction, but not in the iliac artery, despite extensive searching. Length of bar = 1 μm.

was observed in all 5 animals. Figure 1a is a typical record showing a markedly disturbed junction velocity characterized by high frequency components superimposed on the usual pulse waveform as compared to 'normal' iliac artery velocity and aortic flow recordings.

Following velocity determinations the animals were allowed to recover and placed on commercial swine rations. At 10 to 11 months of age (allowing time for spontaneous atherogenic changes of the intima to occur[8]) the endothelium of the corresponding probed areas was examined macroscopically as well as by conventional and electron microscopy after immediate fixation in KARNOVSKY's medium[9]. On gross and light microscopic inspection of the vessels on the side contralateral to that of probe entry (in order to avoid instrumentation artifacts), lesions were not detected, emphasizing the early or 'preatherosclerotic' stage of the investigation. On ultrastructural examination, no unusual cellular changes were found in the iliac arteries despite extensive searching. In the ilioaortic junction, however, a variety of nuclear and cytoplasmic degenerative alterations were encountered, including total loss of individual endothelial cells accompanied by inflow of plasma into the arterial wall (Figure 2).

We believe, therefore, that the higher cell turnover rate observed in areas where blood flow is presumed to be disturbed does indeed represent an increased rate of endothelial cell degeneration and loss related to the existence of unstable velocities. Facilitated entry of lipoprotein-rich plasma in these regions could favour atherogenesis as stated by FRENCH[2].

Since endothelial cells are directly exposed to the adjacent stream, a turbulent fluid structure may result in injury to the vessel lining in a number of ways[7,10]. The flow disturbances we have recorded (Figure 1) are of a qualitative nature. Quantitative studies of velocity amplitudes, turbulence intensity, kinetic energy and shear stress levels as well as experimentation concerning the rate of endothelial cell regeneration under different conditions are required for further elaboration of the cell injury mechanism.

Résumé. Les observations faites sur le porc de Yorkshire montrent que certaines perturbations du flot sanguin normal se manifestent dans une zone prédisposée au developpement de lésions athéromateuses et sont accompagnées de pertes de substances endothéliales. Cette destruction des cellules endothéliales permettrait au plasma riche en lipoprotéines de pénétrer librement dans la paroi artérielle, ce qui favorise l'athérogénese.

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