Table III. Oxidation of 2-oxoglutarate

Time (min)	µl of oxygen consumed/mg of protein of mitochondria					
	Normal liver		*A. flavus* affected liver		*P. rubrum* affected liver	
	in absence of DNP	in presence of DNP ($3\times10^{-5}M$)	in absence of DNP	in presence of DNP	in absence of DNP	in presence of DNP
20	1.20 ± 0.12	3.42 ± 0.34	3.22 ± 0.42	2.84 ± 0.40	1.45 ± 0.15	4.33 ± 0.60
40	2.83 ± 0.41	6.25 ± 0.44	6.74 ± 0.38	6.56 ± 0.32	3.16 ± 0.18	8.67 ± 0.32
60	4.61 ± 0.36	8.17 ± 0.78	7.45 ± 0.35	5.82 ± 0.26	4.58 ± 0.53	9.12 ± 0.82
80	5.18 ± 0.43	8.88 ± 0.80	7.62 ± 0.26	5.68 ± 0.32	4.82 ± 0.41	10.23 ± 0.76

2.7 ml of the reaction system contained 30 µmoles of inorganic P; 10 µmoles of $MgCl_2$; 30 µmoles of 2-oxoglutarate and 830 µmoles of sucrose in the main compartment; 0.3 ml of the mitochondrial suspension in the side-arm and 0.2 ml of 20% KOH in the centre-well.

The present studies indicate that mitochondria are not affected even secondarily until the focal necrotic stage of the poisoning (by *A. flavus*) at which these studies have been performed.

Zusammenfassung. Es wurde die funktionelle Integrität der Mitochondrien nach Verunreinigung der Nahrung mit *A. flavus* und *P. rubrum* aus vergifteter Mäuseleber untersucht. Die Mitochondrienfunktion der mit *P. rubrum* vergifteten Leber ist in bezug auf ihre ATPase-Tätigkeit und oxydative Phosphorylierung gestört, während im mit *A. flavus* vergifteten Gewebe alle wichtigen Mitochondrienfunktionen unverändert blieben.

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Distribution of Norepinephrine Uptake Within Rabbit Aorta Between Adventitia and Media[1]

Evidence has been presented that exogenous norepinephrine (NE) is taken up and bound to non-specific extraneuronal storage sites in tissues containing postganglionic adrenergic nerve fibers from which it can be released by tyramine[2-5]. In the present study, we have examined the uptake of tritium-labelled NE (^{3}H-NE) by neuronal and extraneuronal sites in the isolated rabbit aorta taking advantage of the anatomical arrangement in this vessel of 2 distinct circular layers: the smooth muscle containing tunica media and the adrenergic neurone containing tunica adventitia.

Methods. The general method described in detail by Nedergaard et al.[6], was used. Chromatographically pure (±)-7-^{3}H-norepinephrine hydrochloride (^{3}H-NE) was obtained commercially[7]. Rabbit aortic rings were placed in a tissue bath filled with physiological salt solution maintained at 37 °C. After appropriate incubation periods, the rings were removed, partially digested by means of a toluene-soluble quaternary base[8,9], and the radioactivity determined with a liquid scintillation spectrometer[10]. In some experiments following incubation with ^{3}H-NE the adventitia was stripped from the media in a manner similar to that described by Pease and Paule[11]. The completeness of the removal was confirmed histologically[12].

Extracellular space of intact aorta was determined using (carboxyl-^{14}C)inulin[7] (25 γ/ml).

Results. Rabbit aortic rings in vitro accumulated ^{3}H-NE (10^{-8} and $10^{-6}M$) when they were incubated with the labelled amine for varying time periods lasting from 2–60 min (Figure). Part of the uptake is accounted for by extracellular space. The mean uptake of (carboxyl-^{14}C)inulin after 60 min by 6 aortic strips was 0.47 ± 0.02 (S.E.M.) ml/g.

The relationship between extracellular concentration of ^{3}H-NE and the distribution of the uptake of this amine by aorta into adventitia and media was determined (Table). At a low concentration of ^{3}H-NE ($10^{-8}M$), the major part of this amine was taken up by adventitia, while only a small portion was localized in the media. As the bath concentrations of ^{3}H-NE was raised from $10^{-8}M$ to either 10^{-6} or $10^{-4}M$, the percentage of the

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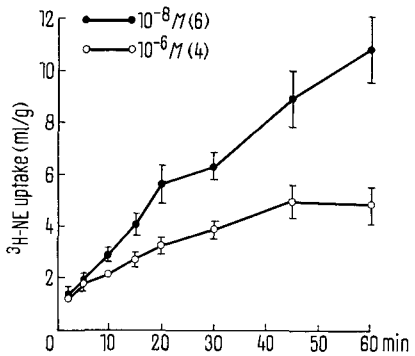
uptake into the adventitial layer decreased correspondingly. At the high concentration ($10^{-4}M$) most of the uptake was localized in the media.

Discussion. The results demonstrate that in vitro ^{3}H-NE is taken up by rabbit aortic rings into both adventitia and media. It is often implied that rabbit aorta is innervated by sympathetic adrenergic motor nerves[13,14], the adventitia probably containing most of the terminal nerve plexus[11,14–16]. At low, but not high concentrations of ^{3}H-NE most of this amine was localized in the adventitia (Table). This is consistent with the report[16] that acute removal of adventitia from rabbit aortic strips before ^{3}H-NE incubation drastically reduced the capacity of this tissue to bind ^{3}H-NE. The increasingly larger proportion of the uptake localized in the media seen with concentrations higher than $10^{-8}M$, suggests that when the capacity of the adrenergic neurones to accumulate the labelled amine is exceeded, extraneuronal uptake becomes predominant.

Cocaine, an inhibitor of the catecholamine uptake mechanism in the axonal membrane of sympathetic nerve fibers[17,18], markedly inhibited the uptake, when the aorta was incubated with a low concentration ($10^{-8}M$) of ^{3}H-NE[19]. Thus, the adventitial accumulation probably is accounted for by uptake into (1) adrenergic neurones (cocaine-sensitive uptake), (2) connective tissue (cocaine-insensitive uptake), and (3) extracellular space.



Effect of concentration on the mean uptake of ^{3}H-NE by aortic rings in vitro. Ordinate: The uptake of ^{3}H-NE (●—●, $10^{-8}M$; ○—○, $10^{-6}M$), expressed as millilitre of bath fluid cleared per gram tissue (ml/g). Abscissa: Length (min) of incubation period with ^{3}H-NE. Numbers in parentheses refer to the number of double determinations made on tissues from different rabbits. The vertical bars represent the ± S.E.M.

Effect of concentration on distribution of ^{3}H-NE uptake into adventitia and media of rabbit aortic rings

^{3}H-NE concentration M	^{3}H-NE uptake, ml/g [a,b] Intact aorta	Adventitia	Media	No. [c]
10^{-8}	10.26 ± 0.77	19.49 ± 2.20	2.21 ± 0.00	11
10^{-6}	5.54 ± 0.33	6.97 ± 0.52	2.30 ± 0.17	7
10^{-4}	2.95 ± 0.10	2.11 ± 0.00	2.59 ± 0.00	5

[a] Uptake of ^{3}H-NE after 60 min incubation. [b] 1 ml/g uptake for 10^{-8}, 10^{-6} and $10^{-4}M$ concentrations corresponds to 0.01, 1, and 100 nmoles/g, respectively. [c] No. of paired observations.

Some of the ^{3}H-NE probably enters into medial tissue by a transport process, since phenoxybenzamine, an inhibitor of NE uptake by sympathetic nerves[18], and cocaine inhibited the uptake, when the aorta was incubated with $10^{-6}M$ ^{3}H-NE[20]. Although the present data do not prove it, this uptake mechanism could possibly be an 'amine-pump' localized in the membrane of smooth muscle which mediates the entry of ^{3}H-NE into these cells. The demonstration (using the FALCK fluorescence technique) of an intracellular accumulation of NE by arterial smooth muscle[21–23] supports this view.

The extraneuronal uptake by rabbit aorta – that into the extracellular space, the smooth muscle and the connective tissue in both adventitia and media – most likely corresponds to the surprisingly rapid uptake ('uptake$_2$') of NE, which IVERSEN[24] observed when hearts were perfused with high concentrations of this amine. On the basis of fluorescence measurements using the FALCK technique, it has been suggested recently that uptake by smooth muscle in isolated arteries of rabbit ear and perfused cat spleen may be responsible for 'uptake$_2$'[21,23,25].

The present findings emphasize that the kinetic analysis of drug uptake into neurones by vascular tissue must take into account not only extracellular space, as is commonly done, but also extraneuronal tissue uptake[26].

Résumé. Dans l'aorte du lapin, la noradrénaline est absorbée par la tunique moyenne qui possède des cellules musculaires lisses de même que par la tunique adventice qui contient des neurones adrénergiques. Il est probable que ces neurones et ces cellules musculaires lisse contiennent une «amine-pump». Le tissu connectif de ces 2 couches adsorbe aussi la noradrénaline. La distribution relative de la noradrénaline entre ces 3 situations est une fonction de la concentration externe de la noradrénaline.

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