J. Pharm. Pharmacol. 1981, 33: 772–777
Received April 23, 1981

0022-3573/81/120772–06 $02.50/0
© 1981 J. Pharm. Pharmacol.

Pressor effect of L-*threo*-3,4-dihydroxyphenylserine in rats

HIROMASA ARAKI*, CHIKAKO TANAKA†, HIROSHI FUJIWARA, MASAFUMI NAKAMURA AND IPPEI OHMURA**

Department of Pharmacology, Kobe University School of Medicine, Chuo-ku, Kobe 650, Japan

The pressor effect of L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS) in rats and its decarboxylation in vivo have been examined. On i.v. administration, it produces a slow-onset and long-lasting pressor response, but no significant change in heart rate or e.c.g. The pressor effect was markedly reduced by inhibition of peripheral decarboxylase and by blockade of α-adrenoceptors. The slow-onset and long-acting pressor effect was also evident when the drug was given orally, while intracerebroventricular administration produced a long-lasting decrease in blood pressure. Noradrenaline (NA) concentrations in the plasma were significantly increased by both i.v. and oral administration of L-*threo*-DOPS. Elevation of plasma NA concentration by L-*threo*-DOPS given i.v. was suppressed by inhibition of decarboxylase. The plasma concentration of the drug was highest immediately after its i.v. administration. Its pressor effect was enhanced in rats made hypotensive by chemical sympathectomy with 6-hydroxydopamine (6-OHDA), compared with control rats, nevertheless, L-*threo*-DOPS produced the same increase in plasma NA concentrations in sympathectomized rats as in the controls. These results indicate that L-*threo*-DOPS is gradually converted to NA by L-aromatic amino acid decarboxylase in vivo. These findings suggest that L-*threo*-DOPS may be clinically useful as an oral pressor agent for the treatment of certain disorders related to hypotension.

That noradrenaline (NA) is formed from 3,4-dihydroxyphenylserine (DOPS) in various mammalian tissues has long been known (Blaschko et al 1950; Schmiterlöw 1951; Creveling et al 1968; Puig et al 1974; Bartholini et al 1975). Recently, the isomers, L-*threo*-, D-*threo*-, L-*erythro*- and D-*erythro*-DOPS have been separated and purified and their enzymic decarboxylation demonstrated (Bartholini et al 1975; Fujiwara et al 1976; Inagaki et al 1976; Ohmura et al 1978). L-*threo*-DOPS was suggested to be an effective precursor of (−)-NA (Inagaki et al 1976) and the D-isomer an antagonist in the conversion of L-isomer to NA (Inagaki et al 1976; Ohmura et al 1978). We also found L-*threo*-DOPS, but not D-*threo*-DOPS, to be converted to NA by decarboxylase in rat isolated atrium and to increase the atrial rate (Araki et al 1978). Using racemic *threo*-DOPS, Redmond et al (1975) found no significant effect on heart rate, e.c.g. or blood pressure. We have attempted to demonstrate the effect of L-*threo*- DOPS on blood pressure as well as in vivo formation of NA from it in rats.

* Present address: National Institute of Hygienic Sciences, Osaka Branch, Department of Pharmacology.
** Research Laboratories, Nippon Merck-Banyu Co., Ltd.
† Correspondence.

MATERIALS AND METHODS

Male Wistar rats, 280–380 g, were anaesthetized with urethane (800 mg kg^{-1} i.p.) which was also used at 100 mg kg^{-1} every 60 min to maintain anaesthesia. The right femoral artery was cannulated for the direct measurement of blood pressure. For the oral administration of drugs, a polyethylene tube and gavage were used. For intracerebroventricular (i.c.v.) administration of drugs, solutions were stereotaxically injected using a microlitre syringe. Arterial blood pressure was recorded through a pressure-displacement transducer (Nihonkohden Kogyo Co. Ltd., SB-1T) on an ink-writing oscillograph (Nihonkohden Kogyo Co. Ltd., RJG-3018) and calibrated with a mercury manometer. (−)-NA (1.0 μg kg^{-1} i.v. or 1·0 μg/animal i.c.v.) was given about 1 h before L-*threo*-DOPS to verify that the monitoring apparatus was functioning and that a pressor response could be evoked in each rat. With i.v. and oral doses, an e.c.g. was simultaneously recorded and the heart rate calculated from it.

Drugs

L-*threo*-DOPS (Kyowa Hakko Kogyo Co. Ltd., $[\alpha]_D^{20} = -42.6$ (c = 1; M HCl), purity 99·5%) and (−)-NA bitartrate (Sigma) were dissolved in sterile 0·9% NaCl (saline) immediately before use, except

for oral administration. In saline, L-*threo*-DOPS was insoluble over 2 mg ml^{-1} at room temperature (23 °C). Drug solutions in a volume of 1·0 ml kg^{-1} (i.v.) and 20·0 μl/animal (i.c.v.) were administered at the rates of 0·1 ml s^{-1} and 2·0 μl min^{-1}, respectively. In the oral administration, L-*threo*-DOPS was suspended at a concentration of 30 mg ml^{-1} in 0·5% carboxymethylcellulose (CMC) solution. The contamination of L-*threo*-DOPS by NA was less than 0.001 μg mg^{-1}. Benserazide hydrochloride (Hoffman-La Roche Co.) 50 mg kg^{-1} dissolved in saline was given i.p. 1 h before administration of L-*threo*-DOPS. Tolazoline hydrochloride (Yamanouchi Co. Ltd.) 20 mg kg^{-1} i.p. was given 10 min before the L-*threo*-DOPS. 6-Hydroxydopamine (6-OHDA) hydrobromide (Sigma) was dissolved in saline containing 0·5% (w/v) ascorbic acid to give a 100 mg ml^{-1} solution (calculated as the free base). The solution was prepared just before use and given i.p. in a volume of 1·0 ml kg^{-1} on the fifth and fourth days before the experiments. The dose of each drug is expressed as a base.

Chemical assays for NA and L-threo-*DOPS*

NA and L-*threo*-DOPS contents in rat plasma were measured at various periods after administration of the drug. Five ml of blood was withdrawn from the abdominal aorta of rats anaesthetized with urethane. One sample was taken from each animal. Heparinized blood was centrifuged, the plasma was made 0·4 M with 0·8 M perchloric acid and 10 mg sodium metabisulphite and 0·5 ml 100 mM EDTA were added. The mixture was shaken and then centrifuged at 8000 *g* for 15 min at 4° C. The supernatant was transferred to a glass beaker containing 10 mg sodium metabisulphite and 1·0 ml of 100 mM EDTA-Tris HCl buffer (pH 4·2) and the pH of the mixture adjusted to 4·0–4·2 with 2 M NaOH. The mixture was passed through the column of Dowex 50 × 4 in the Na^+-form. After the column had been washed with distilled water, NA was eluted with 10 ml of 1·0 M HCl. The solution that had been passed through the column, and the washings, were used for the determination of L-*threo*-DOPS. The resulting eluates were subjected to the alumina absorption procedure of Anton & Sayre (1962). NA and L-*threo*-DOPS in the alumina eluates were measured using a high performance liquid chromatograph (Yanaco, L-2000) equipped with an electrochemical detector (Yanaco, VMD-101). The mean recoveries were 93% for NA and 78% for L-*threo*-DOPS.

Statistical analysis

The measures of blood pressure were converted to an approximation of mean blood pressure using 'diastolic + (systolic-diastolic)/3'. The statistical significance of differences between the means was determined by the unpaired Student's *t*-test.

RESULTS

Pressor effect of intravenous administration of L-threo-*DOPS*

Intravenous administration of L-*threo*-DOPS produced a dose-related pressor effect in rats anaesthetized with urethane. The pressor response to 2·0 mg kg^{-1} L-*threo*-DOPS occurred at approximately 11 s, reached the maximum about 10 min after the injection and remained elevated for 3 h (Table 1). The mean rise in blood pressure within 3 h was 16·3 ± 1·4 mmHg, compared with blood pressure in control rats, given 1·0 ml kg^{-1} saline, which gradually subsided during 2 to 3 h post injection (Fig. 1). The heart rate was not significantly increased and no change in e.c.g. was observed. On the other hand, the pressor effect of (−)-NA 1·0 μg kg^{-1} was fast in onset, fast-rising and short-lasting although the maximum rise was greater than that caused by L-*threo*-DOPS at 2 mg kg^{-1} (Table 1). (−)-NA, 0·2 μg kg^{-1} had no effect on the blood pressure.

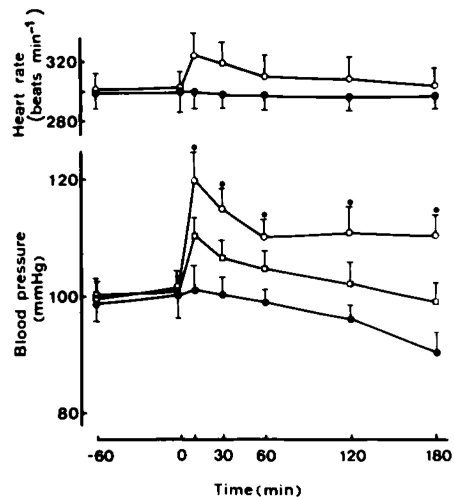

FIG. 1. Effect of i.v. administration of L-*threo*-DOPS on blood pressure and heart rate in rats. Each point represents the mean ± s.e. of the values from 5–8 animals treated with saline (●, control) and L-*threo*-DOPS in doses of 1·0 (□) and 2·0 (○) mg kg^{-1}. Figures on the abscissa represent time after i.v. administration of L-*threo*-DOPS or saline, 0 is just before the injection. * Significantly different from control ($P < 0.05$).

Table 1. Maximum increase, time up to onset and maximum response and duration of pressor effect of L-*threo*-DOPS and l-NA in control, benserazide-, tolazoline- and 6-OHDA-treated rats.

Drug	Route	Maximum increase in blood pressure (mmHg)	Time up to onset of response (s)	Time up to maximum response (min)	Duration of response (min)
Control rats					
L-*threo*-DOPS 2·0 mg kg^{-1}	i.v.	18·4 ± 1.9	11·63 ± 1.02	9.93 ± 0·70	180
L-*threo*-DOPS 50 mg kg^{-1}	oral	15·0 ± 1·4	2406·4 ± 222·7	84·3 ± 7·8	200
(−)-NA 1·0 μg kg^{-1}	i.v.	22·9 ± 2·4	0·38 ± 0·21	0·22 ± 0·02	4·75 ± 0·32
Benserazide-treated rats					
L-*threo*-DOPS 2·0 mg kg^{-1}	i.v.	5·9 ± 0·7***	18·52 ± 2·43	10·55 ± 1·04	12·46 ± 1·78***
Tolazoline-treated rats					
L-*threo*-DOPS 2·0 mg kg^{-1}	i.v.	5·3 ± 0·6***	12·74 ± 1·57	10·09 ± 0·98	9·88 ± 1·14***
(−)-NA 1·0 μg kg^{-1}	i.v.	3·5 ± 0·6***	0·48 ± 0·18	0·28 ± 0·06	1·05 ± 0·26***
6-OHDA-treated rats					
L-*threo*-DOPS 2·0 mg kg^{-1}	i.v.	26·8 ± 2·6*	17·00 ± 2·55*	10·14 ± 0·63	180
(−)-NA 1·0 μg kg^{-1}	i.v.	63·8 ± 5·2***	0·10 ± 0·10	0·20 ± 0·03	6·70 ± 0·54**

Each value represents the mean ± s.e.
4–8 rats were used.
Significantly different from control animals, *: $P < 0·05$, **: $P < 0·01$, *** $P < 0·005$.

Inhibition of peripheral L-aromatic amino acid decarboxylase with benserazide (50 mg kg^{-1} i.p.) abolished the pressor response to L-*threo*-DOPS (2·0 mg kg^{-1} i.v.) (Table 1) and blockade of α-adrenoceptors with tolazoline (20 mg kg^{-1} i.p.) markedly inhibited the response as well as that to (−)-NA (1·0 μg kg^{-1}) (Table 1).

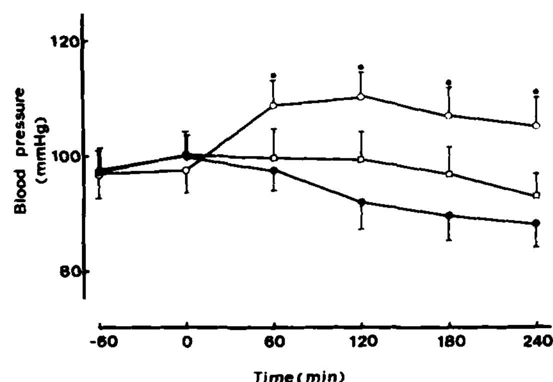

FIG. 2. Effect of oral administration of L-*threo*-DOPS on blood pressure in rats. Each point represents the mean ± s.e. of the values from 4–6 animals treated with the vehicle (●, control) and L-*threo*-DOPS in doses of 20 (□) and 50 (○) mg kg^{-1}. Figures on the abscissa represent time after oral administration of L-*threo*-DOPS or the vehicle, and 0 is just before the administration. *: Significantly different from control ($P < 0·05$).

Pressor effect of oral administration of L-threo-*DOPS*

Oral administration of L-*threo*-DOPS 20 mg kg^{-1} prevented the slight fall in blood pressure in rats given 0·5% CMC solution 1·7 ml kg^{-1} orally. At 50 mg kg^{-1} the drug produced a significant elevation in blood pressure that developed gradually 40 min after dosing and continued for up to about 200 min (Table 1). The mean rise in blood pressure from 1 to 4 h after drug administration was 15·9 ± 1·0 mmHg, compared with that of rats given CMC solution 1·7 ml kg^{-1}. There was no significant change in the heart rate or e.c.g. of rats given 50 mg kg^{-1}.

Depressor effect of intracerebroventricular administration of L-threo-*DOPS and NA*

Intracerebroventricular (i.c.v.) administration of L-*threo*-DOPS 40 μg/animal produced a significant depressor effect in anaesthetized rats. At 20 μg/animal there was no significant change, compared with the controls given 20 μl saline similarly (Fig. 3). The depressor effect to the drug had a slow onset and was prolonged for over 2 h, while that to (−)-NA 1·0 μg/animal occurred immediately and lasted for 7–8 min.

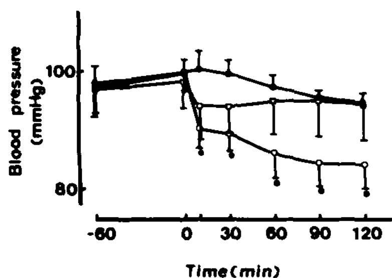

FIG. 3. Effect of i.c.v. administration of L-*threo*-DOPS on blood pressure in rats. Each point represents the mean ± s.e. of the values from 5–8 animals treated with saline (●, control) and L-*threo*-DOPS in doses of 20 (□) and 40 (○) μg/animal. Figures on abscissa represent time after i.c.v. administration of L-*threo*-DOPS or saline, and 0 is just before the injection. *: Significantly different from the control ($P < 0.05$).

Effect of chemical sympathectomy with 6-hydroxydopamine on the pressor effect of L-threo-*DOPS and (−)-NA*

The loss of adrenergic function induced by chemical sympathectomy was confirmed by testing the pressor response to tyramine. Blood pressure in rats treated with 6-OHDA (100 mg kg^{-1}, i.p., twice) was lower, compared with the controls and the pressor response to tyramine was markedly reduced. However, peripheral adrenergic denervation with 6-OHDA significantly augmented the pressor response to L-threo-DOPS (Fig. 4). The mean rise in blood pressure within 3 h following the i.v. administration of 2·0 mg kg^{-1} was 24·3 ± 1·6 mmHg compared with that of

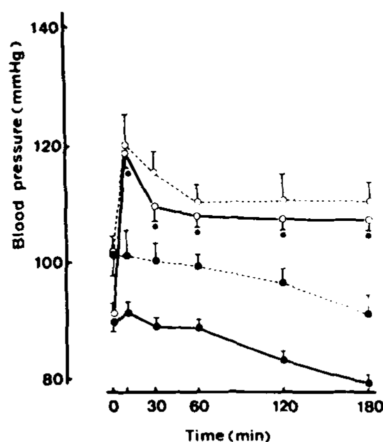

FIG. 4. Effect of chemical sympathectomy with 6-OHDA on pressor effect of L-*threo*-DOPS in rats. Each point represents the mean ± s.e. of the values from 4–8 animals injected with saline (●) and L-*threo*-DOPS in dose of 2·0 mg kg^{-1} (○). Broken and solid lines represent the change of blood pressure in normal and 6-OHDA-treated rats respectively. Figures on the abscissa represent time after i.v. administration of L-*threo*-DOPS or saline, and 0 is just before the injection. *: Rise in blood pressure after L-*threo*-DOPS as compared with blood pressure in rats given saline differed significantly from that in control animals ($P < 0·05$).

Table 2. L-*threo*-DOPS and NA concentrations in plasma after administration of L-*threo*-DOPS in non-, benserazide- and 6-OHDA-treated rats.

	Time after drug (min)	Intravenous L-*threo*-DOPS (2·0 mg kg^{-1}) or saline (1·0 ml kg^{-1}) — Plasma concn of L-*threo*-DOPS (μg ml^{-1})	Intravenous — Plasma concn of NA (μg $litre^{-1}$)	Oral L-*threo*-DOPS (50 mg kg^{-1}) or CMC solution (1·7 ml kg^{-1}) — Plasma concn of NA (μg $litre^{-1}$)
Non-treated rats				
Vehicle (control)	30	0	0·62 ± 0·01	0·64 +0·03
L-*threo*-DOPS	2	12·53 ± 1·09	0·70 ± 0·05	—
	30	3·39 ± 0·15	1·11 ± 0·05***	—
	60	1·20 ± 0·13	0·98 ± 0·02***	0·88 + 0·04***
	120	0·20 ± 0·02	0·73 ± 0·03***	0·80 ± 0·03**
	180	0·16 ± 0·04	0·73 ± 0·04**	0·69 + 0·02
	240	—	—	0·65 + 0·01
Benserazide-treated rats				
Saline	30	—	0·63 ± 0·02	—
L-*threo*-DOPS	30	—	0·66 ± 0·03	—
6-OHDA-treated rats				
Saline	30	—	0·57 ± 0·02*	—
L-*threo*-DOPS	30	—	1·06 ± 0·06†	—

Each value represents the mean ± s.e. of the values from 5–7 animals.
—: Not measured.
Significantly different from control (*: $P < 0·05$, **: $P < 0·02$, ***: $P < 0·005$).
Significantly different from 6-OHDA + saline (†: $P < 0·005$).

rats given saline. The maximum rise in blood pressure after i.v. administration of (−)-NA (1·0 µg kg⁻¹) was also markedly augmented by denervation with 6-OHDA (Table 1).

Plasma L-threo-*DOPS and NA concentrations after* L-threo-*DOPS*

L-*threo*-DOPS concentration in the plasma was highest 2 min after i.v. administration of 2·0 mg kg^{-1} and fell to below 0·2 µg ml^{-1} 2 h later (Table 2). On the other hand, plasma NA concentrations rapidly increased with i.v. administration of L-*threo*-DOPS, 2·0 mg kg^{-1}, reaching a maximum of about 1·8 times the value before administration of the drug, thereafter gradually declining but remaining slightly elevated some 40–60 min later.

Oral administration of the drug also produced an elevation in plasma NA concentration which reached a maximum of about 1·5 times the value before administration. About 3 h later, a reversion to the original concentrations had occurred (Table 2). Inhibition of peripheral decarboxylase with benserazide 50 mg kg^{-1} i.p. all but completely suppressed the increase in the plasma NA concentration induced by L-*threo*-DOPS, 2·0 mg kg^{-1} i.v., compared with the plasma NA values in rats treated with benserazide plus saline 1·0 ml kg^{-1} (Table 2).

Chemical sympathectomy with 6-OHDA (100 mg kg^{-1} i.p., twice) significantly decreased plasma NA concentrations in rats given saline. However, elevation in plasma NA concentrations was apparent after i.v. administration of L-threo-DOPS (2·0 mg kg^{-1}), both in sympathectomized and control rats.

DISCUSSION

In anaesthetized rats, the i.v. and oral administrations of L-*threo*-DOPS produced pressor responses which were of a slow-onset and long-acting, but without significant change in heart rate and e.c.g. The pressor effect was markedly reduced by inhibition of peripheral L-aromatic amino acid decarboxylase and by blockade of α-adrenoceptors. Contamination of L-*threo*-DOPS by NA was less than 0·001 µg mg^{-1} so its pressor effect was considered to be negligible, because even at 0·2 µg kg^{-1}, (−)-NA produced no detectable change in the blood pressure. These findings suggest that the pressor effect of L-*threo*-DOPS is the result of its gradual conversion to NA in vivo.

Significant elevations in plasma NA concentrations were evident after 2·0 mg kg^{-1} (i.v.) and 50 mg kg^{-1} (oral) L-*threo*-DOPS and ran parallel with the pressor response it induced. The increased NA concentrations in the plasma after L-*threo*-DOPS 2·0 mg kg^{-1} i.v. were almost completely suppressed by the inhibition of peripheral decarboxylase. In addition, the concentration of L-*threo*-DOPS in the plasma decreased with time immediately post injection. Such evidence supports the concept that the pressor effect of L-*threo*-DOPS is the result of the formation of NA from it by L-aromatic amino acid decarboxylase in vivo and is not due to L-*threo*-DOPS itself.

We also found that the i.c.v. administration of L-*threo*-DOPS as well as of (−)-NA produced a prolonged depressor response in rats. A small amount of L-*threo*-DOPS penetrates the brain through the blood brain barrier and is decarboxylated to NA (Bartholini et al 1975). However, as L-*threo*-DOPS administered peripherally produced only the pressor response, the central depressor effect of this drug appears to be masked by its peripheral pressor effect.

Intravenous administration of L-*threo*-DOPS produced an increase in plasma NA in chemically sympathectomized rats in which the drug appears to be converted to NA, mainly by extraneuronal decarboxylase, as the peripheral adrenergic nerve terminals had been destroyed by 6-OHDA. Nevertheless, L-*threo*-DOPS produced the same increase in plasma NA concentrations in 6-OHDA-treated rats as in the controls and its pressor effect was enhanced after 6-OHDA. This so-called 'supersensitivity' is likely to be due to loss of neuronal reuptake sites.

Although the pressor potency of L-*threo*-DOPS was less than reported for earlier drugs (metaraminol, Siegmund et al 1948, Livesay et al 1954; noradrenaline, Kaczmarek & Puppe 1953; etilefrine, Unna 1951), the response lasted much longer and it was effective by both parenteral and oral routes. Our data suggest that L-*threo*-DOPS may be clinically effective as an exogenous precursor amino acid of NA for the treatment of disorders related to hypotension.

Acknowledgements

We thank M. Ohara, Kyushu University, for critical reading of the manuscript. This work was supported in part by research grants from the Scientific Research Fund of the Ministry of Education, Science and Culture, Japan.

REFERENCES

Anton, A. H., Sayre, D. F. (1962) J. Pharmacol. Exp. Ther. 138: 360–375
Araki, H., Cheng, J.-T., Ohmura, I., Tanaka, C. (1978) J. Pharm. Pharmacol. 30: 456–458
Bartholini, G., Constantinidis, J., Puig, M., Tissot, R., Pletscher, A. (1975) J. Pharmacol. Exp. Ther. 193: 523–532
Blaschko, H., Burn, J. H., Langemann, H. (1950) Br. J. Pharmacol. 5: 431–437
Creveling, C. R., Daly, J., Tokuyama, T., Witkop, B. (1968) Biochem. Pharmacol. 17: 65–70
Fujiwara, H., Inagaki, C., Ikeda, Y., Tanaka, C. (1976) Folia Pharmacol. Jpn. 72: 891–898 (English Abst.)
Inagaki, C., Fujiwara, H., Tanaka, C. (1976) Jpn. J. Pharmacol. 26: 380–382
Kaczmarek, E., Puppe, H. (1953) Arzneimittel-Forsch. 3: 208–210
Livesay, W. R., Moyer, J. H., Chapman, D. W. (1954) Am. Heart J. 47: 745–756
Ohmura, I., Inagaki, C., Araki, H., Tanaka, C. (1978) Jpn. J. Pharmacol. 28: 747–753
Puig, M., Bartholini, G., Pletscher, A. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 281: 443–446
Redmond, Jr. D. E., Olander, R., Maas, J. W. (1975) Toxicol. Appl. Pharmacol. 34: 301–308
Schmiterlöw, C. G. (1951) Br. J. Pharmacol. 6: 127–134
Siegmund, O. H., Nash, V. L., Granger, H. R. (1948) J. Pharmacol. Exp. Ther. 92: 207–213
Unna, K. (1951) Arch. Exp. Pathol. Pharmakol. 213: 207–234