pounds was very low, the stock solutions were diluted with 35% ethanol, and 0.02 ml of the solution was injected with a microsyringe directly into the bathing medium (2.0 ml) to get a final dilution. When the medium contained 10^{-5} g/ml of VII, minute crystals were visible. As shown in the Table, this compound seemed to be a little less potent than uzarigenin, but is definitely active, although it lacks 14β-hydroxyl group.

Zusammenfassung. Die kardiotonischen Wirkungen der vier neuen Cardenolid-Derivate auf das isolierte Froschherz wurden untersucht und die Beziehungen zwischen ihren chemischen Strukturen und Wirkungen diskutiert.

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21 December 1967.

On the Metabolism of Prenylamine (Segontin®)

Prenylamine, *N*-[3′-phenylpropyl-(2′)]-1,1-diphenylpropylamine-(3), (see Figure 3) introduced in 1960 for treatment of angina pectoris[1] is able to decrease the catecholamine content of various organs[2–4]. The mechanism underlying its amine releasing action was interpreted as reserpine – like inter alia because prenylamine was also found to release serotonin from brain[2,4,5]; this finding was not confirmed by other authors[6].

Investigations on the pharmacological properties of the drug showed that it has sympathomimetic actions like an indirectly acting amine, e.g. phenylethylamine or tyramine. In addition it has imipramine-like qualities, i.e. it inhibits the uptake of ^{3}H-noradrenaline at the level of the cellular membrane[6].

After i.v. injection of ^{14}C-prenylamine in rats, despite its high lipid solubility, radioactivity declined rapidly in the organs with a half life of about 15 min in heart and brain non-exponentially and in a multiphasic manner[6]. This may be due to a rapid metabolism of prenylamine, which is supported by the fact that after oral administration of the ^{14}C-labelled drug in rats $^{14}CO_2$ appeared very soon in the expiration air[7].

These results prompted us to investigate the metabolism of prenylamine.

Methods and materials. Rats (150 g) were treated orally with 100 mg/kg D,L-^{14}C-prenylamine-lactate[8] (specific activity 30 mC/g; purification of the labelled drug was done by preparative thicklayer chromatography).

Urine was collected in 5 h intervals up to 72 h. During this time only 17% of the radioactivity ingested was excreted into the urine. The urinary radioactive metabolites were characterized by means of highvoltage electrophoresis: paper Schleicher & Schüll 2043 b; pyridine/acetic acid/H_2O = 100/10/890; pH 6.0; 2000 V, 40 mA; 2.5 h; further identification by column chromatography: hydrolyzed urine specimens (2*N* HCl, 2 h, 100°C) after neutralization and addition of amphetamine and derivatives mentioned below were absorbed on Dowex 50 (200–400 mesh), washed with 0.01*N* HCl, and the basic metabolites eluted with 2*N* HCl. The eluted metabolites were separated and identified by thinlayer chromatography (solvent: benzene/pyridine/acetic acid = 62/19/19; Eastman chromagram sheet K 301 R 2, silica gel).

Radioactivity was localized on the chromatograms autoradiographically (Doneo Clear base film Fa. Adox).

Results. Figure 1 shows an autoradiogram of urinary metabolites excreted 5, 10 and 24 h after application of the drug. Qualitatively, the same pattern of metabolites was observed up to 72 h. The main metabolites (I), about 60% of the urinary radioactivity, moved to the anode.

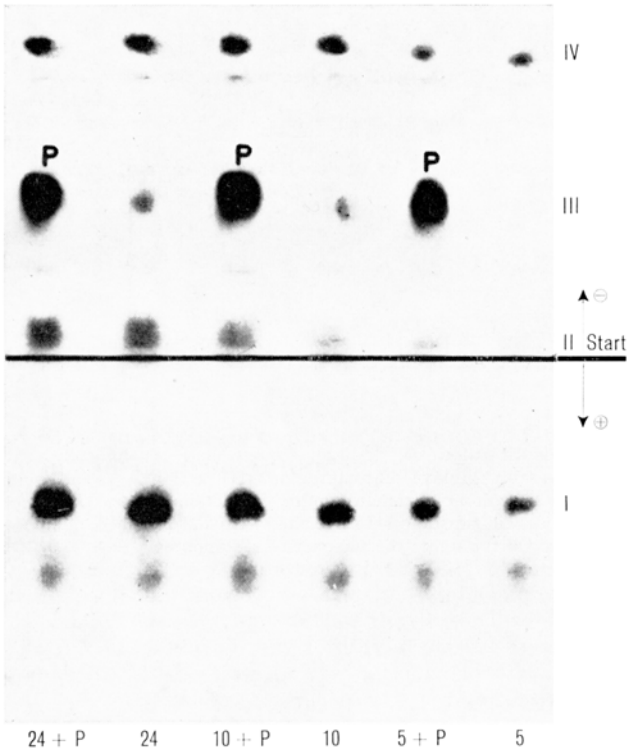

Fig. 1. Autoradiographic demonstration of urinary metabolites of prenylamine excreted 5, 10, and 24 h after administration of 100 mg/kg D,L-^{14}C-prenylamine-lactate orally, separated by highvoltage electrophoresis. + P = ^{14}C-prenylamine added to the respective fraction before separation (for details see text).

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[1] E. LINDNER, Arzneimittel-Forsch. *10*, 569 (1960).

[2] H. H. SCHÖNE and E. LINDNER, Arzneimittel-Forsch. *10*, 583 (1960).

[3] H. GROBECKER, D. PALM and H. J. SCHÜMANN, Arch. exp. Path. Pharmak. *251*, 158 (1965).

[4] H. OBIANWU, Acta pharmac. tox. *23*, 383 (1965).

[5] A. CARLSSON, N. A. HILLARP and B. WALDECK, Acta physiol. scand *59*, Suppl. 215 (1963).

[6] H. GROBECKER, D. PALM and P. HOLTZ, Arch. Pharmak. exp. Path. *251*, 174 (1968).

[7] H. A. E. SCHMIDT, A. HÄUSSLER and W. HOFFMANN, Arzneimittel-Forsch. *13*, 173 (1963).

[8] We are indebted to the Farbwerke Hoechst AG for a generous gift of ^{14}C-prenylamine.

They are presumably glucuronides of metabolite II- not yet identified, because after treatment of the urine with glucuronidase or after hydrolysis with hydrochloric acid, the spots under I disappeared and behaved electrophoretically like metabolite II. Only traces of unchanged prenylamine were excreted. Fraction III consisted mainly of *N*-[3′-*p*-hydroxy-phenylpropyl-(2′)]-1, 1-diphenylpropylamine-(3), i.e. *p*-hydroxy-prenylamine (see Figure 3); it was identified as its *bis*-dinitrophenyl-derivative (reaction with dinitrofluorobenzene) by twodimensional thinlayer chromatography.

From fraction IV (about 20–35% of the urinary radioactivity), moving faster to the cathode and therefore being more basic than prenylamine (see Figure 1), 2 fractions (A and B) could be separated by means of column chromatography. Fraction A contained norephedrine and *p*-hydroxynorephedrine, fraction B amphetamine and *p*-hydroxyamphetamine as identified by thinlayer chromatography (Figure 2). Quantitative measurements revealed, for instance in the urine sample excreted from the 10th to the 24th h after application of the drug, 60 μg amphetamine + *p*-hydroxyamphetamine and 20 μg of their β-hydroxylated metabolites. These metabolites were found in urine up to 72 h after application of prenylamine.

Also in man after a 2 days treatment with 3 × 60 mg prenylamine/day orally and application of ammonium chloride – in order to render the urine acedic[9] – excretion of amphetamine (400μg per 24 hrs) into the urine could be demonstrated.

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[9] A. M. ASATOOR, B. R. GALMAN, J. R. JOHNSON and M. D. MILNE, Br. J. Pharmac. *24*, 293 (1965).

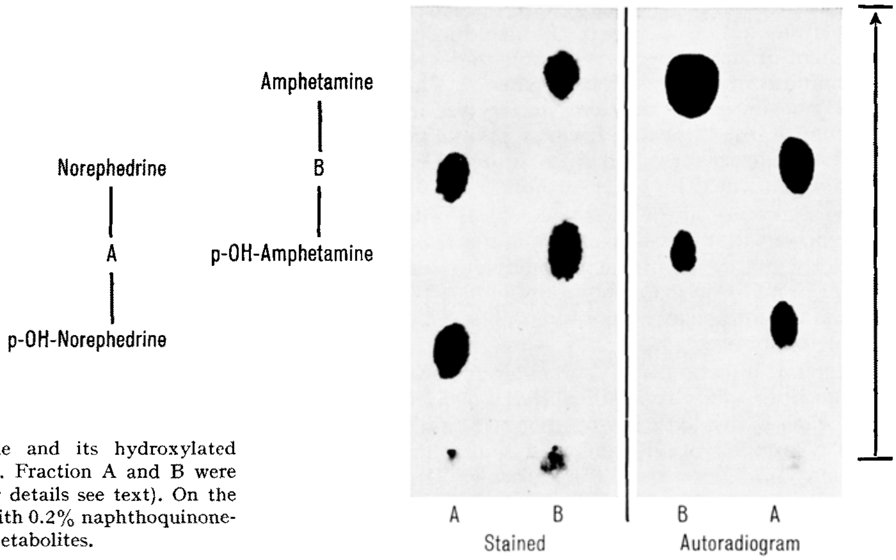

Fig. 2. Identification of ^{14}C-amphetamine and its hydroxylated derivatives by thinlayer chromatography. Fraction A and B were obtained by column chromatography (for details see text). On the left: the marker substances were stained with 0.2% naphthoquinonesulfonate. On the right: the radioactive metabolites.

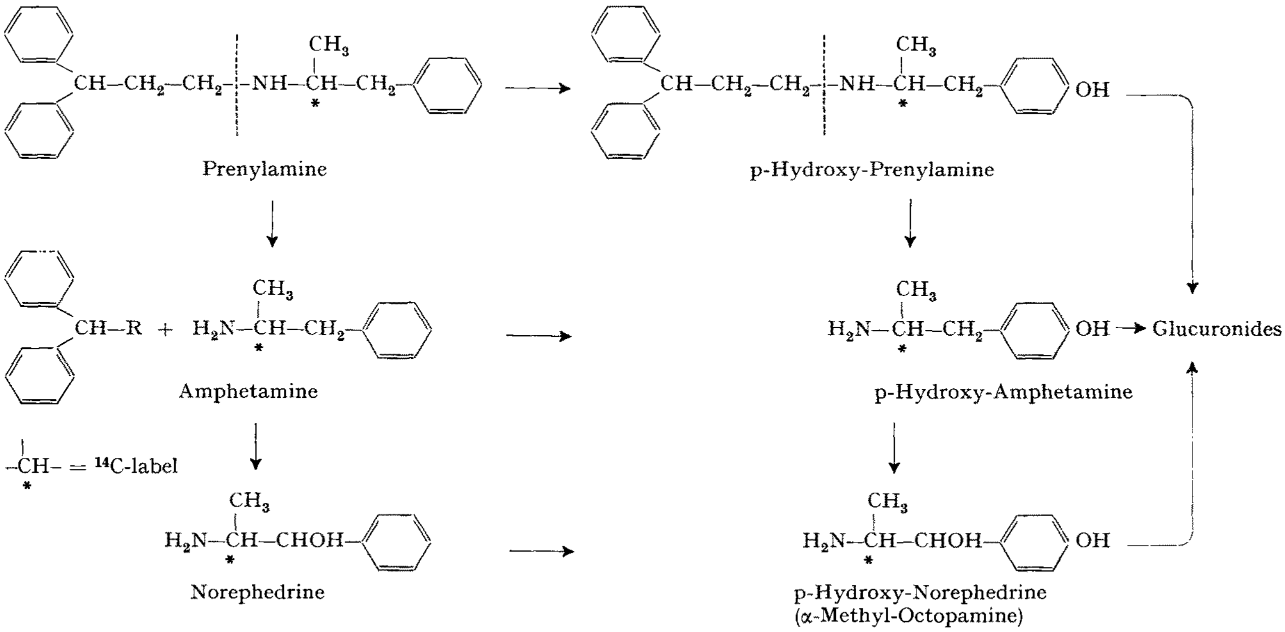

Fig. 3. Metabolism of prenylamine in the rat, as proposed from metabolites identified in the urine.

From the metabolites identified in the urine of rats after oral administration of ^{14}C-prenylamine the following, still incomplete metabolic pathway of prenylamine may be assumed (Figure 3):

(1) Prenylamine is hydroxylated at the amphetamine moiety yielding *p*-hydroxy-prenylamine. This compound is more water soluble than prenylamine itself and therefore can be excreted by the kidneys to a certain extent as such and as a glucuronide.

(2) Presumably by oxidative cleavage of the propyl-chain, amphetamine is formed which is partially hydroxylated to *para*-hydroxy-amphetamine. This metabolite might also be formed from *para*-hydroxy-prenylamine.

(3) The β-hydroxylated metabolites norephedrine and *para*-hydroxy-norephedrine (α-methyloctopamine) are presumably formed in the sympathetic nerves – and may act there or in the central nervous system as false transmitters[10].

Since only the D-isomers of amphetamine or *para*-hydroxyamphetamine can be hydroxylated in the β-position[11] it is D-amphetamine, which – at least in part – originates from prenylamine.

Quantitative measurements of amphetamine and its derivatives in sympathetically innervated organs and in the brain after administration of prenylamine will further elucidate the mechanism underlying its pharmacological actions[12].

Zusammenfassung. Nach oraler Verabfolgung von ^{14}C-Prenylamin (Segontin®) an Ratten werden nur Spuren des unveränderten Pharmakons im Harn ausgeschieden. Folgende Metabolite (ca. 40% der ausgeschiedenen Radioaktivität) wurden identifiziert: *p*-Hydroxyprenylamin, Amphetamin, *p*-Hydroxy-amphetamin, Norephedrin und *p*-Hydroxy-norephedrin. Auch beim Menschen konnte nach zweitägiger Behandlung mit Segontin® in therapeutischer Dosierung das Auftreten geringer Mengen von Amphetamin im Harn nachgewiesen werden.

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6000 Frankfurt/Main (Germany), 13 December 1967.

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[10] J. E. Fischer, W. D. Horst and I. J. Kopin, Br. J. Pharmac. *24*, 477 (1965).

[11] M. Goldstein and B. Anagnoste, Biochim. biophys. Acta *107*, 166 (1965).

[12] Acknowledgement: We are indebted to Miss S. Leonhardt for skilful technical assistance.

Serotonin Metabolism and Kinin-Forming Activity of Plasma During Anaphylactic Shock in Guinea-Pigs and Their Modification After E-Aminocaproic Acid and Trasylol Administration

The role of serotonin (5-HT) and kinins as the mediators of anaphylaxis, responsible for some symptoms of anaphylactic shock, is not explained in a synonymous way.

Probably, in the release of these substances during anaphylaxis, an important role is played by the activation of some enzymatic systems (mainly the proteolytic enzymes).

In the present experiments, the behaviour of 5-HT metabolite-5-hydroxyindolacetic acid (5-HIAA) and blood amino-oxidase connected with metabolism of this amine, as well as kinin formation in plasma, were determined after induction of the anaphylactic shock. Simultaneously the behaviour of the fibrinolytic system was observed and the influence of fibrinolysis inhibitors (ε-aminocaproic acid, trasylol) on the 5-HT and kinin release during the shock was investigated.

Material and methods. Guinea-pigs of 280–300 g body weight and of both sexes were used. Acute or chronic anaphylactic shock was induced by intracardial or i.p. injection of horse serum to sensitized animals. After induction of shock, the following points were considered: (1) The metabolism of serotonin (excretion of 5-HIAA in urine[1], amino-oxidase activity in serum[2]). (2) Kinin formation of plasma (kinin-forming, esterase activity of plasma)[3]. (3) The activity of fibrinolytic system (time of fibrinolysis, fibrinogen level)[4].

Kinin-forming activity as the expression of kininogen supply in plasma was determined pharmacologically after activation of plasma by contact with glass and dilution with water. The level of released kinins was measured on the oestrus rat uterus in comparison with pure bradykinin[5].

In some groups of guinea-pigs production of shock was preceded by i.p. injection either of ε-aminocaproic acid (EACA) or trasylol and during the shock excretion of 5-HIAA and kinin-forming activity of plasma were determined. EACA and trasylol were administrated 30 min before induction of shock, in doses of 0.5 g and 5000 U/kg body weight respectively.

The determination of 5-HIAA excretion was made during chronic shock, all the rest was performed during acute shock. The blood samples were drawn from the heart of animals at the peak of shock symptoms: about 3 min from injection of serum.

Results. The results are presented in the Table. The significant rise in 5-HIAA excretion and decrease of plasma kinin-forming activity is accompanied by an increase of plasma enzymatic activity. The rise in amino-oxidase activity connected with 5-HT metabolism and esterase activity known as a factor which releases the kinins[6] was observed simultaneously with the fibrinolytic

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[1] R. Lecoq, in *Manuel d'analyses médicales et biologie clinique* (G. Doin, Paris 1962), p. 994.

[2] C. M. McEven and J. D. Cohen, J. Lab. clin. Med. *62*, 766 (1963).

[3] P. S. Roberts, J. biol. Chem. *232*, 285 (1958).

[4] S. Niewiarowski, *Krzepniecie krwi* (PZWL, Warszawa 1960).

[5] Bradykinin, BRS 640, Sandoz.

[6] K. O. Hanstein and F. Markwardt, Acta biol. med. germ. *16*, 658 (1966).