der C-5-Methylengruppe von (*S*)-β-Lysin. Dieser Austausch ist rascher als die unter den angewendeten Bedingungen praktisch irreversible Wanderung der Aminogruppe (Exp. 2–4). d) Austauschreaktion und H-Wanderung sind beide stereospezifisch, und zwar diskriminieren sie auf gleiche Art zwischen den diastereotopen H-Atomen am C-5 des Substrates (Exp. 5).

Über die genaue Stereochemie der Reaktion soll an anderer Stelle berichtet werden.

Summary. Using tritium labelled substrates it is shown that in the rearrangement of (*S*)-β-lysine to 3,5-diaminohexanoic acid catalysed by β-lysine mutase a stereospecific hydrogen migration from C-5 to C-6 of the substrate occurs. When the reaction is carried out in the presence of [5′-^{3}H]-coenzyme B_{12}, the heavy isotope is transferred both to C-6 of 3,5-diaminohexanoate and to C-5 of β-lysine. In the latter the labelled atom occupies the same diastereotopic position as the H atom that is transferred to C-6 of the product.

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A Method for the Fluorimetric Determination of 4-(2-Hydroxy-3-isopropylaminopropoxy)-indole (LB 46), a β-Blocking Agent, in Plasma and Urine

For the determination of plasma levels of LB 46[1], a β-blocking agent, we needed a sensitive method, since only low doses of the drug are employed for clinical treatment. A very sensitive method was obtained by reacting LB 46 with *o*-phtalaldehyde. The condensation product has a 10^4-times higher intensity of fluorescence than the parent molecule in 3 *N* hydrochloric acid.

Ortho-phtalaldehyde has been described as a reagent for the fluorimetric determination of several amines (histamine[2], spermidine[3] and indoles[4]). By condensation of LB 46 with *o*-phtalaldehyde in acidic solution an intensely fluorescent product is obtained. The fluorophor formed is not very stable, especially in the assay of minute amounts of LB 46, but it can be stabilized by the addition of ascorbic acid solution, which also lowers the blank readings.

Extraction of LB 46 from aqueous solution. The optimum conditions for extraction of LB 46 from aqueous solution into a nonpolar solvent have been tested by studying the distribution of the compound between various buffer solutions and ethyl acetate. 100 µg of the drug in 3 ml of buffer were shaken with 10 ml of ethyl acetate for 15 min, then the LB 46 content of the organic phase was determined (Table I). The data in Table I demonstrate that for the transfer of LB 46 from an aqueous into an organic phase a pH larger than 11 has to be applied, and that re-extraction into water should occur at a pH lower than 3.

Extraction from plasma and urine. The extraction of the compound from plasma into several organic solvents was tested. The highest recoveries were obtained with diethyl ether, about 89% of the drug added to plasma samples being recovered. Saturation of the aqueous phase with sodium chloride did not improve this yield.

The recovery of LB 46 from urine by extraction with ether is low and depends on the volume of urine assayed: Only 15% of 1 µg of the drug added to 5 ml of urine was recovered. An improved yield of approximately 40% was obtained by substituting benzene for the ether. Treatment of the urine samples with zinc sulphate improved the results further, and the combination of these modifications resulted in a recovery of almost 80% of the amount added to urine.

Sensitivity and specificity of the method. The method allows the measurement of 20 ng of LB 46 in a plasma sample of 1–4 ml and approximately 50 ng of the substance in 5 ml of urine.

Since the *o*-phtalaldehyde seems to react with the indole nucleus of the LB 46 molecule, it was to be expected that propranolol[5], a naphtalene derivative with the same side chain, would not interfere with the determination of LB 46. Indeed, it was found that the fluorescence intensity of propranolol in a concentration up to 10 µg corresponds to the blank reading of the LB 46-assay.

Stability of LB 46 in plasma. Adding the drug to cattle blood or plasma and keeping the samples for 72 h at room temperature did not cause a significant decrease of fluorescence. Similar results were obtained with plasma from LB 46-treated patients, where again no change of fluorescence intensity was observed when the samples were allowed to stand for 48 h at room temperature.

Procedure. (A) Reagents. LB 46 standard solution: Prepared by dissolving 10 mg of the drug in 100 ml of 0.1 *N* hydrochloric acid. From this stock solution standards are obtained by dilution with the same acid. 0.1 *N* hydrochloric acid; 1 *N* sodium hydroxide. Zinc sulphate: 10% solution in distilled water. Diethyl ether: Ether, free of peroxides, is redistilled. Impurities can cause complete failure of the determination. Benzene: A reagent grade benzene is redistilled and 1.5% (v/v) isoamyl

Table I. Influence of pH on the extraction of LB 46

pH	3	5	7	11
% extracted[a]	1	6	33	96

[a] Ratio of the LB 46 content in ethyl acetate to total amount × 100.

[1] 4-(2-Hydroxy-3-isopropylaminopropoxy)-indole.
[2] P. A. Shore, A. Burkhalter and V. A. Cohn, J. Pharmac. exp. Ther. *127*, 182 (1959).
[3] B. Elliot and I. A. Michaelson, Analyt. Biochem. *19*, 184 (1967).
[4] R. P. Maickel and F. P. Miller, Analyt. Chem. *38*, 1937 (1966).
[5] 1-(Isopropylamino)-3-(1-naphtyloxy)-2-propanol.
[6] The blood samples were kindly provided by the courtesy of Dr. B. Strässle of the Medizinische Universitäts-Poliklinik, Basel.

alcohol, free of furaldehyde, added. *O*-Phtalaldehyde: 0.5% solution in methanol. Ascorbic acid: 1% solution in distilled water. Distilled water: De-ionized water is twice glass-distilled to avoid high blank readings.

(B) Determination in plasma. The method developed for the determination of LB 46 in human plasma is as follows: 1–4 ml of plasma, from blood containing sodium citrate or heparin as an anticoagulant, are extracted by shaking with 1 ml of 1 *N* sodium hydroxide and 10 ml of diethyl ether for 15 min on an automatic shaker. After centrifuging, an aliquot of the organic layer is removed and the drug returned into 2 ml of 0.1 *N* hydrochloric acid by shaking for 15 min.

Table II. Plasma levels of LB 46 in rats[a]

	Intravenously 5 mg/kg I	II	Perorally 5 mg/kg III	10 mg/kg IV
5 min	–	1662.5 ± 496.0	–	-
10	482.2 ± 210.4	1100.0 ± 123.6	–	-
15	–	975.0 ± 126.1	14.5 ± 5.1	110.1 ± 9.3
30	111.5 ± 18.5	477.5 ± 290.9	25.6 ± 5.7	61.0 ± 9.7
1 h	22.0 ± 2.8	87.5 ± 55.0	17.3 ± 5.5	25.3 ± 6.8
2	2.3 ± 0.2	--	3.3 ± 2.3	19.8 ± 2.6
4	–	--	–	3.1 ± 0.4
8	–	–	–	3.1 ± 2.3

[a] Nanograms per milliliter of plasma, mean ± S.D. of 3 (experiment I, III, IV) and 4 (experiment II) animals respectively.

Table III. Human plasma levels of LB 46[a]

Patient	0.25 (h)	0.50 (h)	1.0 (h)	2.0 (h)	4.0 (h)	8.0 (h)	12.0 (h)	$T_{1/2}$[b] (h)
1	0	0	11.0	15.1	16.9	2.8	0.8	2.1
2	0	0.4	4.1	31.2	18.3	11.5	4.5	3.7
3	0	1.2	6.0	13.3	11.4	6.0	3.6	5.1
4	0	2.4	2.9	6.6	16.0	3.4	3.2	2.7
5	0.9	9.0	9.4	10.6	9.4	4.0	0.9	2.6
Mean ± S.D.								3.2 ± 1.2

[a] Nanograms per milliliter of plasma. [b] Biological half-life.

Table IV. Excretion of LB 46 in the urine of rats

Hours	Volume ml ± S.D.	Urine levels µg ± S.D.	No.[a]
0–4	2.7 ± 1.6	0.24 ± 0.12	7
4–8	6.3 ± 1.4	0.35 ± 0.11	3
8–24	10.7 ± 5.1	0.70 ± 0.32	7
24–48	16.0 ± 4.2	0.38 ± 0.23	7
48–72	20.9 ± 7.6	0.35 ± 0.10	2
0–48		1.45[b] ± 0.52	5
0–72		1.87[b] ± 0.83	2

[a] Number of determinations obtained with groups of 2 rats. [b] Mean value calculated from the figures for the individual pairs.

An aliquot of the hydrochloric acid solution is used for the fluorimetric assay. To 1.5 ml of the acidic extract 0.1 ml of *o*-phtalaldehyd reagent is added and the mixture heated for 30 min on a water bath at 50 °C. After cooling the tubes to room temperature the fluorophor is stabilized by addition of 0.5 ml of ascorbic acid solution.

The fluorescence of the solution is determined in a spectrofluorimeter at 390 nm excitation, and 440 nm fluorescence. Internal standards, obtained by adding known amounts of LB 46 to plasma samples, and blanks which do not contain the drug are treated in the same way.

(C) Determination in urine. For the assay of LB 46 in urine the modifications mentioned earlier were applied: 5 ml of urine are diluted with an equal volume of distilled water and then are added 2 ml of a zinc sulphate solution and 1 ml of 1 *N* sodium hydroxide. The precipitate formed is separated by centrifugation at 3000 rpm for 10 min. An aliquot of the supernatant is transferred into another tube, and after adding 1 ml of 1 *N* sodium hydroxide the drug is extracted into 10 ml of benzene-isoamyl alcohol by shaking the mixture for 15 min. After centrifuging the samples, the drug is re-extracted from a 9 ml aliquot of the benzene layer into 2 ml of 0.1 *N* hydrochloric acid. The assay is performed as described for the determination in plasma.

Applications of the method. (a) Determination of LB 46 levels in the plasma of rat and man. The data obtained with male rats (average weight 190 g) after i.v. injection of 5 mg/kg and peroral application of 5 and 10 mg/kg of LB 46 are displayed in Table II. The biological half-life for the disappearance of LB 46 from plasma of the rat has been obtained by calculating the regression of the exponential part of a semilogarithmic plot of the data by the 'least squares' method. The half-life was found to be about 30 min.

For studying human plasma levels patients were treated with a single oral dose of 5 mg of LB 46. Blood samples of 10 ml were collected. Before administering the drug one sample was taken to be used for the determination of the blank reading. These blanks did not differ significantly from readings of extracted aqueous samples without added drug. The data obtained from 5 patients are presented in Table III[6]. The data for the disappearance of LB 46 from human plasma were calculated in the same way as described for the rat. The drug was found to be eliminated from human plasma with a half-life of approximately 3 h.

(b) Determination of LB 46 in the urine of rats. The method has also been applied to the determination of LB 46-levels in the urine of male rats (average weight 175 g) treated i.v. with 5 mg/kg of the drug (Table IV). The urine of pairs of animals placed together in a metabolic cage was collected jointly. The data demonstrates that only small amounts of LB 46 are excreted in the rat urine.

Zusammenfassung. Eine empfindliche fluorimetrische Methode zur Bestimmung von 4-(2-Hydroxy-3-isopropylaminopropoxy)-indol, dem β-Blocker LB 46, in Plasma (Erfassungsgrenze 0,02 µg) und in Urin (Erfassungsgrenze 0,05 µg) wird beschrieben. Die mit dieser Methode gemessene biologische Halbwertszeit im Plasma beträgt nach peroraler Verabreichung beim Menschen 3 h und bei der Ratte etwa 30 min.

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