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# QUANTITATIVE ESTIMATION AND TISSUE DISTRIBUTION OF Kö 592, 1-(3-METHYLPHENOXY)-3-ISOPROPYLAMINO-PROPANOL (2)-HYDROCHLORIDE, A NEW SYMPATHETIC β-RECEPTOR BLOCKING AGENT*

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**Abstract**—A simple, specific and sensitive method for the quantitative estimation in biological material of the newly synthesized sympathetic β-receptor blocking agent Kö 592 (1-(3-methylphenoxy)-3-isopropylaminopropanol) was developed, based on solvent extraction and subsequent fluorimetric determination. As little as 0·06 μg/ml can be measured. The tissue distribution of Kö 592 was studied in rats. The compound was rapidly eliminated from the blood stream and taken up into various tissues. The lungs exceeded all other organs in tissue concentration of Kö 592. Heart, kidneys, brain, and spleen showed approximately equal distribution; only small amounts were found in the liver. Kö 592 was found to be rapidly metabolized by microsomal liver enzymes *in vitro*. Blockage of the microsomal enzymes *in vivo* by SKF 525-A resulted in increased tissue levels of Kö 592, while activation of microsomal enzymes by pre-treatment with phenobarbital resulted in decreased tissue levels of the drug. Only 5% of the administered Kö 592 were excreted unchanged into the urine within 8 hr, at which time only traces of the drug could be found in the tissues.

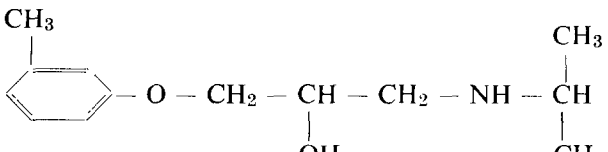
ACCORDING to Ahlquist,[1] the adrenergic actions of epinephrine, norepinephrine and other sympathomimetic agents can be divided into two groups, with each group requiring specific receptors known as α- and β-receptors. This classification is also reflected in the inhibitory patterns displayed by a number of adrenolytic agents. While there is available a variety of α-receptor blocking compounds, only two potent β-receptor blocking substances, dichloroiso-proterenol (DCI)[2] and nethalide (2-iso-propylamino-1-(2-naphthyl-)-ethanol hydrochloride),[3] have been described so far.† In certain tests, however, these substances possess some unwanted β-receptor stimulating action which precedes their β-receptor blocking effect, and which is probably due to structural resemblance of DCI and nethalide to isoproterenol, the most potent β-receptor stimulant.

The present paper deals with a newly developed β-receptor blocking compound, Kö 592, 1-(3-methylphenoxy)-3-iso-propylaminopropanol(2)-hydrochloride which was synthesized recently in the laboratories of C. H. Boehringer Sohn, Ingelheim, Germany and which is practically devoid of stimulatory effects on the β-receptors.‡

* A preliminary report has been given at the 28th Meeting of the Deutsche Pharmakologische Gesellschaft, Bad Nauheim, October 5–8. 1964.

† See addendum.

‡ A. Engelhardt: see Ref. 15.



Kö 592

Preliminary studies had shown that Kö 592 was highly active *in vitro*, whereas in rats relatively high doses were needed *in vivo* to obtain a $\beta$-receptor blockade. Therefore, a simple specific and sensitive method for its quantitative estimation in biological material was developed, based on solvent extraction and subsequent spectrophotofluorimetric determination, and the tissue distribution, metabolism, and excretion of the drug were studied in rats.

## MATERIAL AND METHODS

Male Wistar rats weighing 180–220 g were used throughout and kept on standard pellet diet as obtained from Fa. Altrogge, Lage, Germany. If not stated otherwise, all animals had free access to food and water prior to the experiments.

For the fluorimetric determination of Kö 592 in blood and various tissues rats were decapitated, and the blood of each rat was collected in beakers containing 0·20 ml of 30% sodium citrate. Tissues were rapidly excised, weighed and stored at $-18°$ until further preparation. 1–2 ml of blood or plasma, 5 ml of urine, and 0·2–2·0 g tissue samples resp. were homogenized in 10·0 ml of 0·4 N $HClO_4$ using an "Ultraturrax" homogenizer (Jahnke und Kunkel, Staufen, Germany). After an extraction period of 15 min, the homogenates were centrifuged for 10 min at $1{\cdot}200 \times g$, and an aliquot of 5 ml of the supernatant was transferred to stoppered centrifuge tubes containing 3 g of solid sodium chloride and 20 ml of benzene (reagent grade). Following the addition of 1 ml of 5 N NaOH, the tubes were shaken for 20 min and centrifuged for 10 min at $1{\cdot}200 \times g$. 15 ml of benzene phase were transferred to stoppered centrifuge tubes containing 3 ml of 0·1 N HCl, shaken for 5 min and centrifuged for phase separation. In the case of brain tissue, 6 ml of 0·1 N HCl were used because of incomplete phase separation after centrifuging. The organic phase was removed by aspiration, and 2 ml of the aqueous phase were transferred to quartz cuvettes for the fluorimetric determination in an Aminco-Bowman fluorimeter. The activation monochromator was set at 275 m$\mu$, fluorescence was measured at 312 m$\mu$ (uncalibrated instrument values). Internal standards were always carried through the whole procedure by adding known amounts of Kö 592 to a $HClO_4$ solution. Recovery was practically the same if Kö 592 was added to tissue homogenates, blood or urine, and amounted to $79 \pm 1{\cdot}2\%$. All figures given in the text or tables, expressed as $\mu$g/g wet weight, are corrected for the loss of Kö 592 during the extraction procedure. Likewise, all values are corrected for the fluorescence due to other material normally present in tissues (see also Table 2). Although interference with the fluorimetric assay of Kö 592 may be encountered from some sympathomimetic amines, e.g., norepinephrine, epinephrine and metanephrine when using pure aqueous solutions, none of these compounds will be extracted by the above described method (Table 1), even in tenfold amounts compared to Kö 592.

The *enzymatic degradation* of Kö 592 by microsomal enzymes of the liver was determined by measuring the rate of disappearance of Kö 592 when added to a

microsomal enzyme preparation. The latter was obtained by homogenizing rat liver tissue in two volumes of an ice-cold buffer solution containing 77% 0·129 M KCl, 20% 0·067 M sodium phosphate buffer pH 7·4 and 3% 0·28 M glucose.[4] After centrifuging for 30 min at 9,000 × *g* in the cold, the supernatant was diluted with buffer so that 1 ml corresponded to the amount of liver as indicated in the tables of figures. 2 ml of the supernatant, 25 μmoles of $MgSO_4$, 100 μmoles of nicotinamide, 0·4 μmole of

TABLE 1. SPECIFICITY OF THE EXTRACTION PROCEDURE FOR KÖ 592

| | Fluorescence Units | |
|---|---|---|
| | before extract. | after extract. |
| Kö 592 | 45 | 37 |
| + serotonin | 44 | 36 |
| + histamine | 44 | 37 |
| + norepinephrine | 58 | 37 |
| + epinephrine | 68 | 36 |
| + normetanephrine | 84 | 37 |

Fluorescence of Kö 592 at a concentration of 2 μg/ml alone as well as in the presence of 2 μg/ml of the indicated amines was measured. 3 ml of each solution were extracted as described under 'methods', and fluorescence was measured again.

NADP (previously designated as TPN), 1 μmole of Kö 592 and sufficient buffer solution to bring the volume to a total of 5 ml, were incubated for 60 min at 37° in a Dubnoff shaker. At the end of the incubation period, the remaining substrate was extracted and determined as described above. In some experiments SKF 525-A (diethyl-aminoethyldiphenylpropylacetate, Smith, Kline and French, Philadelphia, Pa., U.S.A.), a known inhibitor of microsomal enzymes,[5] was preincubated for 15 min before the addition of the substrate.

## RESULTS

### *Identification of Kö 592 in biological material*

Figure 1 demonstrates activation (left) and fluorescence (right) spectra of authentic Kö 592 which have the same characteristics as those of Kö 592 extracted from tissue homogenates of rats pretreated with 50 mg/kg s.c. The fluorescence displayed by Kö 592 obeys Beer's Law, and is linear to the concentration up to several μg/ml above which quenching may be observed. Using aqueous solutions, as little as 0·06 μg/ml exceeded the reagent blank by 100%. The practical sensitivity depended largely upon the tissue blank, i.e. unknown material normally present in tissue which yields some fluorescence at the wavelengths characteristic for Kö 592. Table 2 summarizes the tissue blanks of a number of rat organs.

### *Tissue distribution, excretion and metabolism of Kö 592.*

The tissue distribution of Kö 592 at various time intervals following a single injection of 50 mg/kg *subcutaneously* are summarized in Table 3. At this dose, Kö 592 exhibited a slight sedative action which was more pronounced if higher doses were

given. The lungs were found to contain by far the highest amounts at any time within 8 hr when compared to other organs. The maximal content of 70 $\mu$g/g was found already 15 min after the administration, while the blood levels, for instance, never exceeded 3 $\mu$g/ml. Similarly, only small amounts (2–4 $\mu$g/g) were isolated from the liver. These findings suggest that the lungs seem to be able to store this compound,

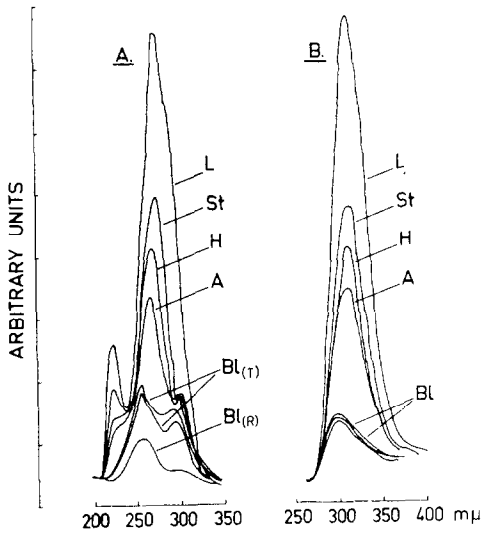



FIG. 1. Activation (A) and fluorescence (B) spectra of authentic Kö 592 and Kö 592 extracted from rat organs. To obtain activation spectra, the fluorescence monochromator was set at 312 m$\mu$ and the activation monochromator was scanned. To obtain fluorescence spectra, the activation monochromator was set at 275 m$\mu$ and the fluorescence monochromator was scanned. $Bl_{(R)}$: reagent blank; $Bl_{(T)}$: tissue blank; St: authentic Kö 592; L: Kö 592 extracted from lung; H: Kö 592 extracted from heart; A: Kö 592 extracted from adipose tissue.

TABLE 2. TISSUE BLANKS OF VARIOUS RAT ORGANS

| Organ | n | Kö 592 Equival $\mu$g/g ± SE. |
|---|---|---|
| Brain | 7 | 0·45 ± 0·07 |
| Lungs | 7 | 0·12 ± 0·05 |
| Heart | 7 | 0·73 ± 0·10 |
| Liver | 8 | 0·17 ± 0·08 |
| Spleen | 7 | 0·67 ± 0·31 |
| Kidneys | 6 | 0·68 ± 0·14 |
| Epidid. Fat | 4 | 0·31 ± 0·17 |
| Blood | 8 | 0·16 ± 0·05 |
| Urine | 5 | 0·29 ± 0·08 |

Activation and fluorescence wavelengths were identical with those characteristic for Kö 592 (see Fig. 1). Fluorescence of the tissue blanks was calculated in terms of Kö 592-equivalents ($\mu$g/g ± SE or $\mu$g/ml ± SE resp.)

while the extraordinary small amounts in the liver could be due to a high metabolic rate of Kö 592 in this organ. The results are also indicative for a rapid disappearance of Kö 592 from the blood stream.

TABLE 3. TIME CURVE OF DISTRIBUTION OF KÖ 592 IN VARIOUS RAT ORGANS FOLLOWING THE INJECTION OF 50 MG/KG S.C.

| Min<br>Organ | 15<br>μg/g ± SE | 30<br>μg/g ± SE | 60<br>μg/g ± SE | 120<br>μg/g ± SE | 240<br>μg/g ± SE | 480<br>μg/g ± SE |
|---|---|---|---|---|---|---|
| Blood | 1·15 ± 0·35 | 1·70 ± 0·33 | 2·81 ± 0·34 | 0·69 ± 0·30 | 0·32 ± 0·20 | 0·0 |
| Lung | 69·40 ± 10·00 | 55·80 ± 3·50 | 54·70 ± 7·40 | 35·00 ± 3·10 | 14·20 ± 2·70 | 4·30 ± 1·00 |
| Heart | 17·30 ± 2·40 | 11·50 ± 2·50 | 10·80 ± 1·40 | 5·30 ± 1·00 | 0·48 ± 0·04 | 0·0 |
| Kidneys | 20·10 ± 2·40 | 19·70 ± 3·40 | 14·70 ± 2·20 | 9·80 ± 1·10 | 0·93 ± 0·60 | 0·0 |
| Spleen | 15·50 ± 1·40 | 14·00 ± 1·10 | 16·90 ± 2·20 | 11·50 ± 2·40 | 3·20 ± 0·60 | 0·0 |
| Epidid. fat | 12·20 ± 3·00 | 18·40 ± 3·50 | 8·20 ± 1·20 | 5·30 ± 0·70 | 2·60 ± 0·80 | 0·46 ± 0·35 |
| Liver | 2·70 ± 0·39 | 4·30 ± 1·10 | 2·80 ± 0·49 | 1·20 ± 0·14 | 0·53 ± 0·08 | 0·48 ± 0·14 |
| Brain | 13·00 ± 1·90 | 15·50 ± 1·90 | 16·70 ± 1·80 | 11·90 ± 1·20 | 3·40 ± 0·50 | 1·30 ± 0·30 |

All values are means ± standard error (SE) from 5 animals.

This became particularly obvious, if Kö 592 was injected *intravenously* to rats at a dose of 10 mg/kg. A rapid decline of the blood levels was already apparent within 10 sec, and 60 sec after the injection the level was about one tenth of the initial value

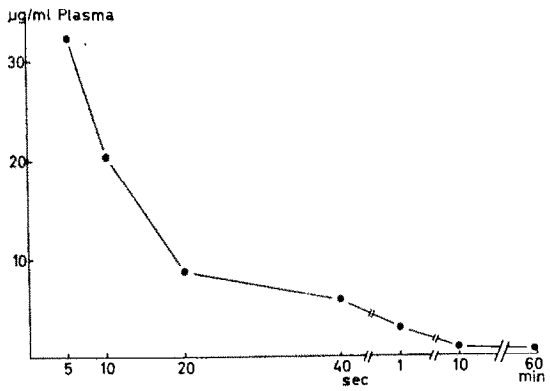



FIG. 2. Rat plasma contents of Kö 592 after intravenous injection of the drug (10 mg/kg). At the time intervals given on the abscissa, two animals were decapitated, the plasma pooled and tested for the contents of Kö 592.

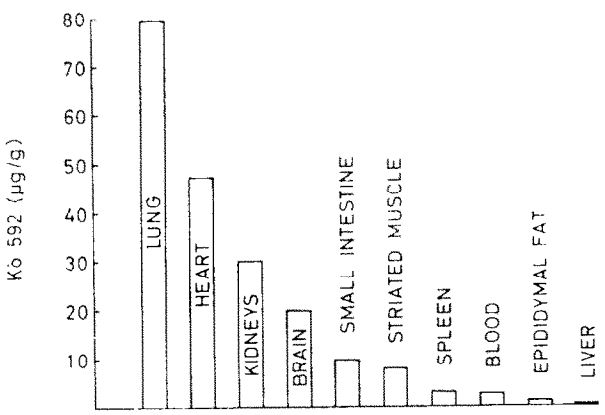



FIG. 3. Contents of Kö 592 in various rat organs 60 sec after intravenous injection. Dosage and method see Fig. 2. The organs were taken from the animals in Fig. 2 which had been decapitated 60 sec after the injection of Kö 592.

(Fig. 2). At this time, tissue levels as shown in Fig. 3 were found. Again, the lungs exceeded all other organs, confirming the results of Table 3.

*Oral administration* of Kö 592 essentially did not change the distribution patterns in tissues. Using this route of administration, the values found in the respective organs were always lower than after subcutaneous injection with the exception of the liver where three times as much was found after oral administration (Fig. 4).

*Urinary excretion* of Kö 592 was followed in fasted rats which had been loaded with 5 ml/100 g water orally before receiving Kö 592 subcutaneously. As can be seen from

Table 4, only about 5% of the injected dose were excreted into the urine within 8 hr. A definite peak was observed between 1–2 hr after the injection. Urinary elimination of Kö 592 apparently occurred in the unbound form, since acid hydrolysis of the urine which should have led to a cleavage of conjugated products, did not alter the values

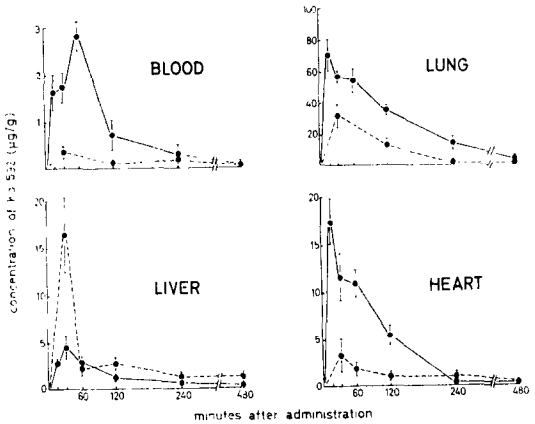



FIG. 4. Comparison of the contents of Kö 592 in some rat organs after subcutaneous (straight line) and oral (dotted line) administration. The values for s.c. injection were taken from Table 1. Groups of 5 rats which had been fasted overnight received 50 mg/kg Kö 592 by stomach tube. At each of the given time intervals after the administration, one group was decapitated and Kö 592 was determined in various organs. All values are means ± standard error.

TABLE 4. URINARY EXCRETION OF KÖ 592 AFTER S.C. INJECTION OF 50 MG/KG

| Time after dosage/(hr) | mg/kg of Kö 592 excreted Group A | Group B |
|---|---|---|
| 0–1 | 0·622 | 0·494 |
| 1–2 | 0·873 | 1·270 |
| 2–4 | 0·523 | 0·467 |
| 4–8 | 0·377 | 0·253 |
| Total | 2·395 | 2·464 |
| % of injected dose | 4·8 | 4·9 |

Two groups (A, B) of 6 rats each which had been loaded with 5 ml/100 g water orally were kept in metabolic cages. At the given time intervals following the s.c. injection of 50 mg/kg Kö 592 the pooled and acidified urine was collected and the content of Kö 592 determined in an aliquot of 5 ml. All values were corrected for blank values obtained from a group of control animals.

measured. It should be noted that Kö 592 had a transient antidiuretic effect which was apparent between 0–60 min after the injection.

The possibility of a metabolic breakdown of Kö 592 by microsomal enzymes of the liver was studied *in vitro* and *in vivo*. These enzyme systems are known to metabolize a variety of drugs,[6] requiring the presence of both NADPH and oxygen.[7] Table 5 demonstrates that rat liver microsomes were also able to metabolize Kö 592 when incubated *in vitro*, although metabolic products have not yet been identified. They,

at least, do not seem to be fluorescent, and to interfere with the assay of Kö 592. In the presence of $3 \times 10^{-4}$ M SKF 525-A, the metabolism of Kö 592 was inhibited by 80%, whereas at $3 \times 10^{-5}$ M SKF 525-A, the inhibition still amounted to nearly

TABLE 5. METABOLISM OF KÖ 592 BY RAT LIVER MICROSOMES *IN VITRO*, AND ITS ACTIVATION BY PRETREATMENT WITH PHENOBARBITAL; COMPARISON WITH METABOLISM OF HEXOBARBITAL

| | n | μg/g/hr ± SE metabolized |
|---|---|---|
| Kö 592 | 7 | 166 ± 17 |
| Kö 592 (phenobarb. pretreated) | 5 | 337 ± 25 |
| Hexobarbital | 7 | 475 ± 58 |

Microsomal enzyme preparation corresponding to 250 mg liver were incubated with 1 μmole of Kö 592 (223 μg) or 1 μmole of hexobarbital (236 μg) as described under 'methods'. Rats were pretreated with 100 mg/kg s.c. of phenobarbital 48 and 72 hr prior to the experiment. All values are means ± standard error (SE).

50% (Fig. 5). On the other hand pretreatment of the rats with 100 mg/kg phenobarbital for two days, which activates the metabolism of foreign compounds,[4] increased the degradation of Kö 592 by 100%, thus approximating the metabolic rate of hexobarbital (Table 5), a drug most rapidly metabolized by liver microsomes.[8, 9]

Similar results were obtained *in vivo*. As can be seen from Table 6, the metabolism

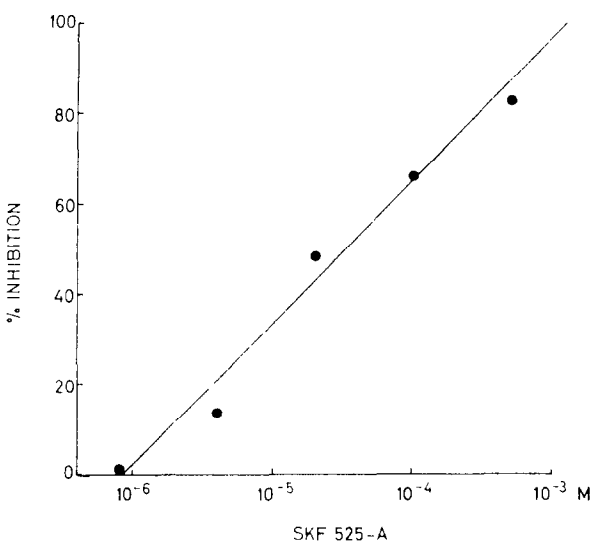



FIG. 5. Effect of SKF 525-A on the metabolism of Kö 592 by microsomal enzymes in vitro. Microsomal enzyme preparations obtained from 5 rats were pooled, and the amount equivalent to 660 mg liver was incubated with 65 μg of Kö 592 for 60 min. SKF 525-A at the indicated concentrations had been added 15 min prior to the addition of Kö 592.

of Kö 592 was inhibited by pretreatment with SKF 525-A. Consequently, the contents of Kö 592 in organs of SKF treated animals was significantly higher than in the respective control animals. This was particularly obvious in the case of the liver where the increase in the tissue concentration amounted to 900%. On the other hand, pretreatment with phenobarbital apparently stimulated the metabolism of Kö 592, because in all organs examined the level of Kö 592 was significantly lower than in the controls

TABLE 6. INFLUENCE OF PHENOBARBITAL AND SKF 525-A ON THE DISTRIBUTION OF KÖ 592 IN VARIOUS RAT ORGANS

| Organ | Controls $\mu g/g \pm SE$ | SKF 525-A $\mu g/g \pm SE$ | Phenobarbital $\mu g/g \pm SE$ |
|---|---|---|---|
| Blood | 2·02 ± 0·12 | 4·34 ± 0·19 | 1·29 ± 0·11 |
| Lung | 64·60 ± 2·30 | 90·10 ± 5·20 | 37·40 ± 7·60 |
| Heart | 12·58 ± 1·50 | 26·81 ± 2·40 | 5·64 ± 1·10 |
| Kidneys | 23·03 ± 2·20 | 57·04 ± 7·50 | 13·55 ± 3·00 |
| Spleen | 24·30 ± 3·10 | 35·70 ± 4·00 | 11·57 ± 3·70 |
| Epidid.Fat | 5·76 ± 1·40 | 13·18 ± 2·90 | 6·59 ± 0·90 |
| Liver | 3·94 ± 0·43 | 38·14 ± 3·50 | 1·27 ± 0·15 |
| Brain | 23·70 ± 1·60 | 40·20 ± 2·90 | 11·90 ± 2·50 |

Groups of 5 rats were injected s.c. with 100 mg/kg Phenobarbital 72 and 48 hr, or i.p. with 50 mg/kg SKF 525-A 30 min prior to the s.c. injection of 50 mg/kg Kö 592. 1 hr later the animals were sacrificed to determine the distribution of Kö 592 in various organs. All values are means ± standard error (SE).

except epididymal fat. Essentially the same results were obtained after oral administration of Kö 592.

## DISCUSSION

In the present paper, the tissue distribution, metabolism and the urinary excretion of Kö 592 have been studied. Following the parenteral or oral administration, the compound disappeared rapidly from circulation and was readily taken up by the tissues. It is of interest that the lungs were extremely active in taking up Kö 592, the significance of which is the subject of further studies. After oral administration, Kö 592 seemed to be absorbed readily from the intestinal tract, because maximal values in the tissues were observed at about the same time as was the case with parenteral application. The lower tissue levels after oral administration compared to those following parenteral injection suggest that a single liver passage of the drug is sufficient to inactivate a considerable part of the applicated dose, which has been shown by Westermann[10] to be also true for a variety of other drugs which are metabolized rapidly in the liver. This assumption is consistent with the finding that—although metabolic products have not yet been identified—Kö 592 is metabolized by microsomal enzymes of the liver *in vitro* as well as *in vivo*. The latter fact was best illustrated by a nearly tenfold increase of the tissue concentration of Kö 592 in this organ after these enzymes had been blocked by SKF 525-A. The importance of the metabolism of Kö 592 by the liver was further emphasized by the finding that 8 hr after its administration only traces of the drug—if any at all—could be found in the tissues, while during the same time only very little was excreted unchanged into the urine.

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*Addendum*—After the manuscript had been prepared, the following compounds have also been described to have β-receptor blocking properties: MJ 1999, (4-(2-isopropylamino-1-hydroxyethyl)-methansulfonanilide); its hydroxypropyl-derivative MJ 1998[11]; Inderal[12] (Imperial Chem. Industries Ltd., Alderley Park, Cheshire, England); Segontin[13] (Farbwerke Höchst A.G., Frankfurt/M. Germany), and Isoptin[14] (Knoll A. G., Ludwigshafen, Germany), the latter two being potent coronary vasodilators.

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