THE METABOLISM AND BIOPHARMACEUTICS OF SPIRONOLACTONE IN MAN

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SUMMARY

Spironolactone, a competitive aldosterone antagonist, has been used for almost 30 years in those disorders associated with primary or secondary hyperaldosteronism. This review is confined to its metabolism and biopharmaceutics in man. Spironolactone undergoes extensive metabolism with no unchanged drug appearing in the urine. Its metabolites can be divided into two main categories: those in which the sulfur of the parent molecule is removed and those in which the sulfur is retained. The dethioacetylated metabolite canrenone, belonging to the former category, was long considered to be the major active metabolite of spironolactone. For this reason pharmacokinetic studies have focussed on its kinetic behaviour. However, pharmacodynamic studies indicated that canrenone could only partly explain spironolactone's action. Furthermore, with the advent of modern high-performance liquid chromatographic techniques to measure canrenone concentrations, it was shown that previously employed assay techniques were unspecific and consequently considerably overestimated true canrenone levels. Recently, it was demonstrated that after a single oral dose of spironolactone, 7α-thiomethylspirolactone is the main metabolite and that unchanged spironolactone reaches maximum serum concentrations which are in the same order of magnitude as canrenone. Both spironolactone and 7α-thiomethylspirolactone are known to possess anti-mineralocorticoid activity, and they may be mainly responsible for the activity of spironolactone. It also appears likely that endocrine side effects of spironolactone, such as gynaecomastia, are mediated by these sulfur-containing compounds. The oral absorption of spironolactone is improved by using micronized drug or inclusion complexes of spironolactone with cyclodextrins. Concomitant food intake has also been shown to enhance the bioavailability, by increasing the absorption and decreasing the first-pass effect of spironolactone.

I. INTRODUCTION

The aldosterone antagonist spironolactone has been used clinically for almost three decades in a variety of disorders associated with hyperaldosteronism. Despite its well described therapeutic and pharmacologic effects, the pharmacokinetics of this drug have puzzled many investigators throughout the years. This is reflected in the number and extent of pharmacokinetic studies carried out on spironolactone,

possibly second only to furosemide amongst the diuretic drugs /1/. Unfortunately, most of these studies were carried out in a period during which investigators were hampered by lack of advanced analytical techniques. The results of pharmacokinetic studies, obtained by application of HPLC techniques from the late seventies on, prompted a re-evaluation of prevailing knowledge on spironolactone's fate in the body after its administration.

It is the aim of this review to critically evaluate the contemporary literature on the pharmacokinetics of spironolactone in man.

Since large differences in the disposition and metabolism of spironolactone have been observed between species (including man) /2/, data obtained from animal studies will be presented only if they are relevant to the situation in man.

II. BIOTRANSFORMATION

Spironolactone belongs to a class of drugs which are subjected to very extensive metabolism. The first report on the detection and quantification of a dethioacetylated metabolite (SC 9376) appeared in 1962 /3/. By means of paper chromatography and fluorescence analysis, the major portion of the circulating drug in plasma was found to be in the form of this metabolite, after oral administration of 500 mg spironolactone to a normal subject. Later, SC 9376 received its names, aldadiene and the more commonly used name canrenone. The fluorescence properties of canrenone were used to develop a quantitative fluorimetric method for the determination of its concentration in plasma /3/. This assay became the standard method in studies on the pharmacokinetics of spironolactone, because the activity of spironolactone was mainly attributed to canrenone /4,5/.

The presence of metabolites other than canrenone was reported by other investigators in the sixties, but no attempt was made to identify them rigorously /6-8/. Isolation and identification of most of these metabolites was performed by detailed investigations in the seventies /9-14/. From the results of these studies, the following metabolic pathways for spironolactone were proposed (Figure 1). The first step in the metabolism probably involves a hydrolysis of the thioacetate group of spironolactone to 7α-thiospirolactone /15/. It should be emphasized at this point that 7α-thiospirolactone has, until now, never been identified in biological fluids. Its presence is merely postulated on the chemical structure of identified sulfur containing successor metabolites.

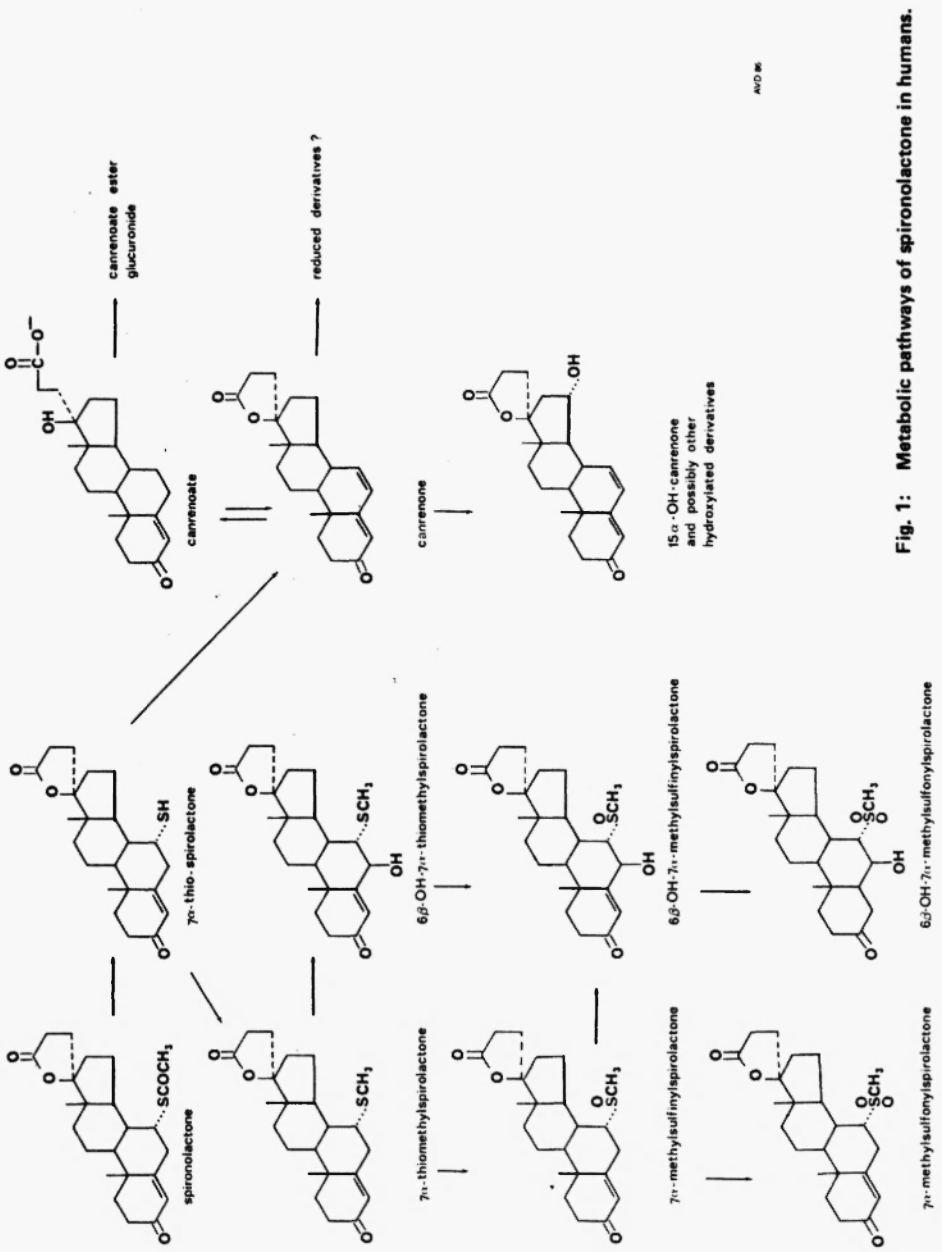

Fig. 1: Metabolic pathways of spironolactone in humans.

It can also be transformed to the non-sulfur containing metabolite canrenone. Canrenone undergoes further transformation by three main pathways. One involves hydrolysis of its γ-lactone ring to form canrenoate, which is excreted in the urine as the glucuronic ester conjugate /11/. An equilibrium exists between canrenone and canrenoate which is probably of an enzymatic nature /16-18/. A second pathway involves hydroxylation. A minor urinary metabolite in man was identified as 15α-OH-canrenone /10/. Other hydroxylated canrenone metabolites were found in animals /14,19-21/, and also in the urine of man after oral administration of potassium canrenoate /22/. In the third pathway, canrenone is reduced to several di-, tetra-, and hexa-hydro derivatives, but so far these metabolites have been identified only in experimental animals /14/.

Although canrenone is also formed after administration of potassium canrenoate, it remains to be established whether the metabolic pathway of canrenone is similar after either spironolactone or potassium canrenoate administration. This is of importance, because a different metabolic pattern may explain why potassium canrenoate has shown tumor inducing effects in animals, whereas no such effects have been observed with spironolactone, as will be discussed later. S-Methylation of 7α-thiospirolactone affords 7α-thiomethylspirolactone (see Figure 1). This metabolite can be hydroxylated on the C6 position to yield 6β-OH-7α-thiomethylspirolactone, but it can also be oxidized to 7α-methylsulfinyl- and 7α-methylsulfonylspirolactone. Sulfoxidation can also occur with 6β-OH-7α-thiomethylspirolactone, leading to 6β-OH-7α-methylsulfinyl- and 6β-OH-7α-methylsulfonylspirolactone. These compounds may also exist as their corresponding γ-lactone open ring forms and their conjugates /13/. Furthermore, other unidentified polar metabolites were found and it has been proposed that these are polyhydroxylated compounds /11,13/.

Although 7α-thiospirolactone has not yet been definitely identified in biological fluids, *in vitro* metabolism studies with guinea pig renal, testicular, adrenal and hepatic microsomes /23,24/, and also with rat liver tissue preparations /25/, have positively identified its existence. From these observations, it may be hypothesized that 7α-thiospirolactone undergoes rapid S-methylation in the blood circulation. This hypothesis is supported by the presence of the enzyme thiol methyltransferase in human red blood cell membranes, which is able to catalyze the methylation of 7α-thiospirolactone /26/.

III. ASSAY METHODS

As already mentioned, the fluorimetric assay method of Gochman and Gantt, developed to measure canrenone concentrations /3/, has been the most widely used method in studies on the pharmacokinetics of spironolactone. Since its introduction, several modifications have been made to extend its applicability to the measurement of canrenoate /27,28/ and the canrenoate ester glucuronide /27/.

However, with the advent of HPLC techniques to measure canrenone levels, the fluorimetric method was shown to be relatively unspecific, as other metabolites were measured concomitantly /29-38/. Consequently, true canrenone concentrations were much lower than had been previously assumed. From this point on, canrenone levels measured with fluorimetry will be referred to as "canrenone levels", or as fluorimetrically measured "canrenone". Gas chromatographic methods, which were developed to measure canrenone concentrations in plasma and urine /39,40/, have been criticized for their lack of specificity, due to thermolytic degradation of some of the spironolactone related products /9,33/.

The only other analytical method which has been employed involves measurement of the radioactive peaks in thin layer chromatograms /11,13/. It will be obvious that this latter method is very time consuming, requires dosing of radioactive drug to subjects, does not allow small biological fluid specimens to be analysed, and is essentially of a qualitative nature.

Summarizing, it will be clear that only HPLC assay methods are suitable for specific and rapid analysis of drug related material in biological fluids. In addition to the many HPLC methods which are now available for measuring canrenone, we have recently developed an HPLC method capable of measuring simultaneously spironolactone, 7α-thiomethylspirolactone, 6β-OH-7α-thiomethylspirolactone, and canrenone in serum /41/.

IV. RECOVERY OF RADIOACTIVITY AFTER 20-[^{3}H]-SPIRONOLACTONE ADMINISTRATION

The elimination of radioactivity in urine and faeces from five healthy subjects after oral administration of 20-[^{3}H]-spironolactone has been described in two important studies /11,13/. In the study of Abshagen *et al* /13/, 47-57% of the radioactivity of the given dose could

be detected in urine within six days, whereas Karim *et al* /11/ only found 23-38% within five days. Complete collection of faeces was possible from only two persons in the study by Abshagen *et al*. The radioactivity excreted by this route in these volunteers was 35 and 38% respectively. Karim *et al* found a recovery ranging from 3 to 41% in their volunteers but they failed to obtain a complete collection in four out of five subjects. The total (urine + faeces) percentage of the dose which was recovered ranged from 40-69% /11/ and 83-95% /13/, respectively. The low recoveries found by Karim *et al* can be attributed to a failure of most subjects to furnish complete faecal specimens or to a possible exchange of the tritium from the drug molecule with water molecules.

V. PHARMACOKINETICS OF SPIRONOLACTONE IN HEALTHY SUBJECTS

Almost all pharmacokinetic studies on spironolactone have focused on the kinetic behaviour of canrenone. It will be clear from earlier arguments that those studies which have used fluorimetry as the means of analysis do not provide information of much value. Therefore, only studies in which a specific assay has been used will be discussed in this section.

5.1 Blood compartment

Single as well as multiple dose studies have been performed to estimate pharmacokinetic parameters for canrenone, after spironolactone administration.

a) **Single dose studies**: Most investigators have used a 100 mg oral dose of spironolactone /29,31,34,38,42/. From these studies the following pharmacokinetic parameters for canrenone were derived: time of maximum serum/plasma concentration (t_{max}) = 2-3.2 h, maximum serum/plasma concentration (C_{max}) = 92-148 ng/ml, elimination half life time (t½) = 17.8-20.1 h, and area under serum/plasma concentration time curve (AUC) = 1403-1541 ng/ml/h (0-24 h) and AUC (0-_{∞}) = 1564-1935 ng/ml/h.

Doses of 50 and 200 mg spironolactone led to proportionally smaller or higher C_{max} and AUC values, whereas no difference in t_{max} and t½ was apparent /35,37,43/, suggesting linear pharmacokinetics in the dose range of 50 to 200 mg spironolactone.

In one study, data have been obtained after a 500 mg and a 100 mg dose of spironolactone /31/. It appeared that, after the 500 mg dose, saturation kinetics must be assumed since the AUC values did not increase in proportion to the 100 mg dose. This may have been due to a reduced absorption capacity for spironolactone at this higher dose /44/, or to saturation of the metabolic pathway leading to canrenone.

However, it should be noted that in this study a group of five women who received the 500 mg dose was compared with a group of ten males and ten females, who ingested 100 mg of spironolactone.

Only one study has described the kinetic parameters for formation of canrenoate, the open lactone ring form of canrenone, after an oral dose of 100 mg spironolactone /38/. Whereas t_{max}, C_{max}, and AUC (0-24 h) for canrenone in this study were found to be 3.2 h, 116 ng/ml, and 1403 ng/ml/h respectively, the corresponding values for canrenoate were 4.2 h, 85 ng/ml, and 1151 ng/ml/h.

Until recently, studies directed to the parent drug and/or metabolites other than canrenone had not been very reproducible and were merely of a qualitative nature. They indicated the presence of the following substances in the blood compartment: spironolactone in two studies only /3,13/, 6β-OH-7α-thiomethylspirolactone, and extremely low levels of 15α-OH-canrenone and 6β-OH-7α-methylsulfinylspirolactone /10,11,13/. Furthermore, an unidentified polar compound was present /13/. However, neither the serum concentration time course, nor pharmacokinetic parameters were obtained for any of these compounds.

In 1985, we were able to gain more insight into the fate of spironolactone in the serum compartment (Figure 2). By using a specific HPLC method we found 7α-thiomethylspirolactone to be the major metabolite in serum after a single oral dose of 200 mg spironolactone to four healthy volunteers /43/. Surprisingly, this metabolite had never been described before. Furthermore, unchanged spironolactone was detected with C_{max} of the same order of magnitude as canrenone and 6β-OH-7α-thiomethylspirolactone. These findings were contrary to the widely accepted belief that spironolactone is metabolized too rapidly to be detected in serum /45-47/, and that canrenone is the principal metabolite of spironolactone /4,11/. Table 1 summarizes the pharmacokinetic data of spironolactone and its metabolites which were obtained from this study.

b) **Multiple dose studies:** Dosage regimens of 50 mg twice a day /37,48/, 100 mg once a day /31/, and 100 mg twice daily /42/ have

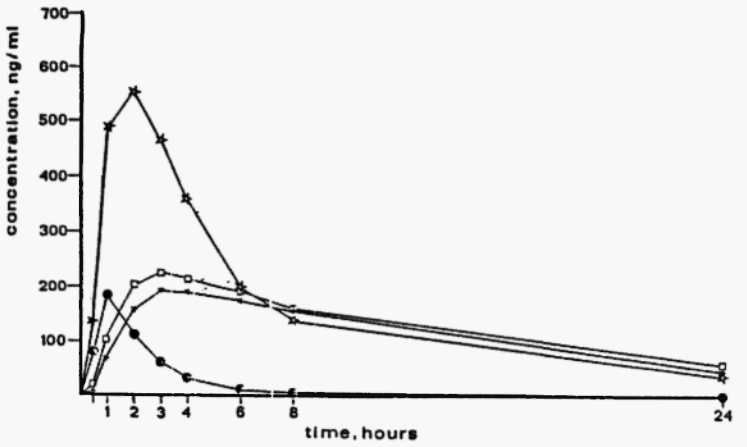

Fig. 2: Mean serum concentrations of spironolactone (●), 7α-thiomethylspirolactone (☆), 6β-hydroxy-7α-thiomethylspirolactone (★) and canrenone (□), after dosing 200 mg orally spironolactone to 4 volunteers. From reference 43.

TABLE 1

Pharmacokinetic parameters of spironolactone and its metabolites. From reference /43/.

	spirono-lactone	7α-thio methyl-spiro-lactone	6β-OH-7α-thio-methyl-spiro-lactone	canrenone
C_{max} (ng/ml)	185 ± 51	571 ± 74	202 ± 54	231 ± 49
t_{max} (h)	1.0 ± 0	1.8 ± 0.5	3.1 ± 0.9	2.9 ± 0.6
t½ (h)	1.3 ± 0.3	2.8 ± 0.4*	10.1 ± 2.3**	11.2 ± 2.3**
		8.9 ± 2.1**		
AUC (0-24)				
($ng.ml^{-1}.h$)	473 + 149	3880 ± 869	2812 ± 785	3107 ± 551

Data are $\bar{X} \pm SD$

* half life time of first phase of elimination.

** these data should be interpreted with some reserve as not enough blood samples may have been drawn in the elimination phase to determine these values accurately.

been employed to study the pharmacokinetics of canrenone after several days of spironolactone treatment. In general, steady state concentrations of canrenone were attained after approximately three days of treatment.

Twice daily dosing of 50 mg spironolactone led to maximum and minimum steady-state serum levels of 146-188 ng/ml and 50-70 ng/ml, respectively /37,48/. An oral dose of 200 mg spironolactone, followed by once daily dosing of 100 mg for four days, yielded peak and trough steady-state serum canrenone levels of 201 and 49 ng/ml, respectively /31/. Finally, when spironolactone was administered in a dose of 100 mg twice daily for nine days, steady-state peak and trough levels of canrenone were 250 and 100 ng/ml /42/. One study found an accumulation factor for canrenone of 2.53 by dividing the AUC in a dose interval at steady-state by the corresponding AUC value after the first dose /37/. The t_{max} at steady-state reported in these studies ranged from 2.5 to 3 h and the t½ from 17.8-22.6 h.

No indication of nonlinear kinetics can be derived from these studies, when one compares the AUC values in a dose interval which were obtained with the employed dosage regimens.

5.2 Urinary excretion data

Unaltered spironolactone has not been detected in urine in any study. One group of investigators found that in the 0-24 h urine, 5.93%, 2.73%, 2.36%, 1.04% and 0.87% of the given spironolactone dose (500 mg), was excreted as 6β-OH-7α-methylsulfinylspirolactone, 6β-OH-7α-methylsulfonylspirolactone, 7α-methylsulfonylspirolactone, canrenone and 6β-OH-7α-thiomethylspirolactone, respectively /13/. Furthermore, 1.4% of the dose was excreted as an unidentified metabolite. It was also shown that probably all of these compounds exist in the form of an opened γ-lactone ring, or additionally, as conjugates. Furthermore, very polar material with an unknown identity was present in the extractable fraction of urine. From its extraction and chromatographic behaviour it appeared to be one or more polyhydroxylated steroids.

Another study showed that in the 0-24 h urine 4.5%, 2.9%, 1.8%, 0.8% and 0.5% of the given spironolactone dose (200 mg) was excreted as canrenoate ester glucuronide, canrenone, 6β-OH-7α-methylsulfinyl-spirolactone, 15α-OH-canrenone, and 6β-OH-7α-thiomethylspirolactone, respectively /10/. Very small quantities of probably 7α-methylsulfinyl-spirolactone and 7α-methylsulfonylspirolactone were also identified.

These same investigators reported that in the 0-5 day urine 5.0%, 5.2% and 6.2% of a 200 mg dose of spironolactone was recovered as canrenone, 6β-OH-7α-methylsulfinylspirolactone and canrenoate ester glucuronide, respectively /11/. The fact that these latter investigators did not find traces of 6β-OH-7α-methylsulfonylspirolactone was explained by a different work-up procedure during which this unstable compound was converted to canrenone /13/. This might explain the higher quantities of canrenone in urine by this group /10,11/.

The contrasting qualitative and quantitative results found by these groups stress even more the necessity of employing specific assays in pharmacokinetic studies on spironolactone. Only urinary excretion data obtained with HPLC appear to be valid, but unfortunately, at present not much information has been obtained by using this assay method. One such study indicated that after a 200 mg dose of spironolactone, 0.636 mg (0.32% of dose) of canrenone was excreted in the 0-24 h urine /35/, whereas another study showed that after a 100 mg dose of spironolactone, 0.93 mg (0.93% of dose) of canrenone and 6.24 mg (6.24% of dose) of canrenoate ester glucuronide could be recovered in the 0-24 h urine /38/.

5.3 Faeces

The chemical nature of spironolactone derived substances in the faeces has been investigated in only two studies. One study reports that not more than 1.8% of the given radioactive dose spironolactone was in the form of spironolactone and/or canrenone /11/. Also, the presence of at least 6 metabolites more polar than canrenone was indicated. With chloroform, 65% of the radioactivity was extractable. In the other study, canrenone and 6β-OH-7α-methylsulfinylspirolactone were the only constituents of the lipophilic fraction of the faeces /49/.

In conclusion, significant amounts of unaltered spironolactone could not be found in the faeces. The metabolites originated from the biliary excretion of the absorbed drug, and possibly also from the gut flora metabolism of unabsorbed spironolactone /49/.

5.4 Biliary excretion

Biliary excretion of spironolactone has been studied in eight patients who had undergone choledochotomy /49/. All the bile excreted by

these patients was collected by means of a special tube after oral administration of radioactive spironolactone. Within four days, 5.4-32.7% of the dose was excreted in bile. Of this, 50-70% consisted of polar material, 10-20% of canrenone, 5-15% of 6β-OH-7α-methylsulfinylspirolactone, and 3-10% of 6β-OH-7α-thiomethylspirolactone. Enterohepatic cycling of spironolactone metabolites was demonstrated by comparing the elimination half life time of the radioactivity in the patients with that in control subjects. There were also differences noted in the composition of the urinary metabolites between normal subjects and these patients. Thus, interruption of enterohepatic circulation had resulted in a shift of metabolic pathways. A significant reduction of all sulfoxidation reactions was observed, which indicates that these reactions occur in the liver or in the intestinal mucosa.

5.5 Conclusion

Investigators, using specific assays, have proven that the role of canrenone in the metabolism of spironolactone is less important than had previously been assumed. Recently the unaltered parent drug has been shown to reach a maximum serum concentration of the same order of magnitude as canrenone after a single oral dose, and it could still be detected eight hours after dosing /43/. The major metabolite in serum was identified as 7α-thiomethylspirolactone /43/. However, since the elimination half life time of the parent drug and 7α-thiomethylspirolactone are shorter than that of canrenone, it appears likely that the relative proportion of canrenone after multiple dosing will be higher.

VI. PHARMACOKINETIC STUDIES IN PATIENTS

The present knowledge on the pharmacokinetics of spironolactone in patients is not very extensive. Moreover, most investigators have reported data obtained only by fluorimetric measurements in biological fluids. As has been mentioned before, only specific assays (like HPLC) will yield reliable information on the disposition of spironolactone.

The elimination plasma half life time (t½) of fluorimetrically measured "canrenone" was 59 h (range 32-105 h) in five patients with chronic liver disease /46/. In comparison, the t½ in normal subjects in the same study was found to be 20.5 h. Despite the delayed elimination of "canrenone" in these patients, there was no evidence of greater

accumulation of "canrenone" in the plasma of those patients with a prolonged t½. Other investigators similarly found no relationship between the steady-state "canrenone" concentration and the severity of liver disease, when the bromsulphalein elimination kinetics were employed as a measure of liver function /50/. Possible explanations include decreased absorption of spironolactone, decreased "canrenone" formation, increased volume of distribution, or alteration in protein binding in disease states /46/. Again, it should also be noted that the fluorimetric assay was used in these studies, which overestimates the true canrenone concentrations.

Another study showed that the highly complex metabolism of spironolactone in patients with decompensated liver disease, occurred in a uniform manner and was not different from that observed in normal test subjects /51/. One study compared patients with liver cirrhosis with hospitalized controls and used gas chromatography as the means of analysis for canrenone /52/. No significant difference in the urinary derived half-life of gas chromatographic determined canrenone was observed (10.0 and 12.8 h, respectively). However, in healthy volunteers without hospitalization, a considerably shorter t½ was found (6.0 h). Recently, we studied the multiple dose kinetics of spironolactone in a patient with liver cirrhosis and ascites, who was treated with 100 mg spironolactone by mouth, once daily for 15 days /53/. A specific HPLC method was used to measure pre-dose serum concentrations of spironolactone and its metabolites throughout the therapy (Figure 3). Canrenone and 7α-thiomethylspirolactone accumulated, canrenone reaching a minimum steady-state serum concentration (C_{ss} = 116 ng/ml) only after 14 days of spironolactone therapy, as compared with nine days for 7α-thiomethylspirolactone (C_{ss}= 167 ng/ml). No accumulation was found for the parent drug 14 ng/ml). The t½ for 7α-thiomethylspirolactone and canrenone after termination of the treatment was found to be at least 62 and 111 h, respectively.

In a study where 13 patients with congestive heart failure or hepatic cirrhosis were treated with 200 mg spironolactone daily for at least three weeks, 15 fold inter-individual differences in the minimum fluorimetrically measured plasma levels of "canrenone" were found /50/. Undoubtedly, part of this variation can be accounted for by the use of an unspecific assay, which measures a mixture of several substances, each substance contributing its own specific fluorescence. The same study showed a cumulation t½ of 1-4 days in nine patients who had recovered from myocardial infarction, without congestive

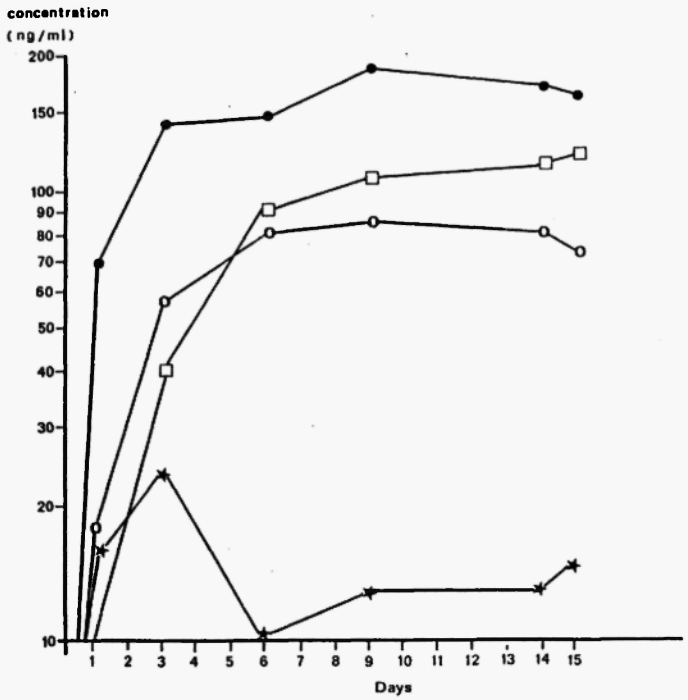

Fig. 3: Pre-dose serum concentrations of spironolactone (★), 7α-thiomethylspirolactone (●), 6β-hydroxy-7α-thiomethylspirolactone (○) and canrenone (□) in a 51 year old male patient with liver cirrhosis and ascites, who received 100 mg spironolactone orally once daily. From reference 53.

heart failure or cirrhosis, and were receiving spironolactone 2 x 100 mg/day.

In patients with congestive heart failure, a longer half life for fluorimetric measured "canrenone" was found (19-48 h) than in normal subjects (18-23 h) /46/. From all of the forementioned information, it can be concluded that at present, no clear picture of the effect of several disease states on the pharmacokinetics of spironolactone has been obtained. Since specific assays are now available, it should be possible to clarify these inconsistencies.

VII. PHARMACOKINETIC STUDIES IN THE ELDERLY

The steady-state pharmacokinetics of canrenone in ten female elderly subjects (mean 77.2 years) have been compared with ten young

females (mean 20.1 years) after multiple oral dosing of once daily 100 mg spironolactone /54,55/. Maximum as well as mean concentrations of canrenone in serum of the elderly subjects were approximately twice as high as those in the young. The half life of canrenone in the elderly was estimated to be 35 h, compared to 17.3 h in the young. This was explained by a reduced capacity of the liver for the breakdown of spironolactone and canrenone with increasing age.

In another study, a group of eight elderly subjects (mean 78 years) was compared with eight young subjects (mean 21 years) following single and multiple doses of twice daily 50 mg spironolactone /56/. After the initial dose on the first day, plasma levels and AUC of canrenone were higher in the young than in the elderly. However, these differences diminished after multiple dosing up to day eight, and the steady-state pre-dose plasma levels of canrenone were significantly higher in the elderly subjects. This was reflected by the finding of a significantly higher accumulation ratio of canrenone in the elderly, as compared to the young subjects (4.99 vs 2.50). In contrast to the former study /54,55/, no significant difference in C_{max} and AUC for canrenone was found between the two age groups at steady-state, although the AUC values were approximately 20% higher in the elderly.

The lower plasma levels of canrenone in the elderly after the first dose may have been due to an impaired formation of canrenone, a decreased oral absorption of spironolactone, or an altered volume of distribution of the drug. The accumulation after long term treatment may have resulted from a delayed elimination in the elderly.

VIII. BIOAVAILABILITY

Bioavailability problems with spironolactone were encountered soon after its introduction. The oral administration of the drug together with a detergent, polysorbate 80, increased the plasma level and urinary excretion of "canrenone" /57/. Further studies showed that this enhanced bioavailability could not be directly attributed to polysorbate 80. A newly formulated tablet, containing no polysorbate 80 but only 22.5 mg of the drug in a finely powdered form, was found to be bioequivalent to the original 100 mg commercial tablet (Aldactone) then available /58/. The latter tablets were replaced by newly formulated bioequivalent and equipotent tablets, containing only 25 mg of spironolactone (Aldactone-A), thoroughly dispersed in a water-soluble matrix /59,60/. Oral absorption of spironolactone could be

increased also by using micronized drug in the tablets /61-64/. It was proposed that differences in absorption were due to differences in the dissolution rates of the drug /65/. Evidence for this was obtained in an *in vitro* study which showed that the dissolution rate of spironolactone from the newly formulated 25 mg tablets (Aldactone-A) was much more rapid than that of the original compression coated tablets /65/. The use of the drug in a slow dissolving form (as in the original Aldactone tablets) probably caused part of the dose to be lost in the faeces. Correlations between the *in vitro* dissolution rate and bioavailability have been found /65,66/, but the relationship can be confounded by major changes in the excipients /66,67/, and also depends on the parameters chosen for bioavailability and dissolution rate /68,69/.

The absolute bioavailability of spironolactone has not been determined in man, owing to the low aqueous solubility of the drug (2.8 mg/100 ml at 25°C /70/), precluding the development of a suitable intravenous formulation. Nevertheless, some investigators have reported on the absolute bioavailability by comparing plasma canrenone concentrations attained after oral spironolactone dosing and after intravenous potassium canrenoate, respectively /18,44/. In evaluating these parameters, it was assumed that potassium canrenoate is an injectable form of spironolactone. From the foregoing biotransformation data, it will be obvious that this assumption is not valid. The extent of absorption must be at least approximately 73%, since 53% and 20% of an orally administered radioactive dose of spironolactone in an alcoholic solution were excreted in urine and bile respectively /13/.

Many comparative studies on the relative bioavailability of several spironolactone formulations have been carried out /42,62-64,66-75/, especially after the U.S. Food and Drug Administration regarded it as a drug with problems related to bioavailability /73/. However, all these studies focussed on the measurement of canrenone, either by fluorimetry or by HPLC. Since in bioavailability testing, measurement of the concentration of all pharmacologically active ingredients is required, it may appear on first sight that the fluorimetric method is the most appropriate assay for such studies, because it most probably also covers substances other than canrenone with pharmacological activity /31/. However, if high concentrations of very active spironolactone derived substances yield only very low fluorescence values, an assay which measures these compounds separately is preferable.

8.1 Effect of food on bioavailability

An enhancement by food of "canrenone" bioavailability from spironolactone was observed, when fluorimetry was used as the means of analysis /76/. With a specific HPLC assay, we investigated the effect of food in more detail by measuring serum concentrations of the unaltered parent drug and its metabolites /77/ in the nonfasting and fasting state, after a single oral dose of 200 mg spironolactone (Figure 4). Statistically significant higher AUC (0-24 h) and C_{max} values were seen for all compounds in the nonfasting state as compared to the fasting state, which indicates an increased absorption by food of spironolactone. Moreover, the mean increase in AUC (0-24 h) of spironolactone was more pronounced than the mean increase in AUC (0-24 h) of its metabolites: 95.4% vs 34.4%, respectively. This finding

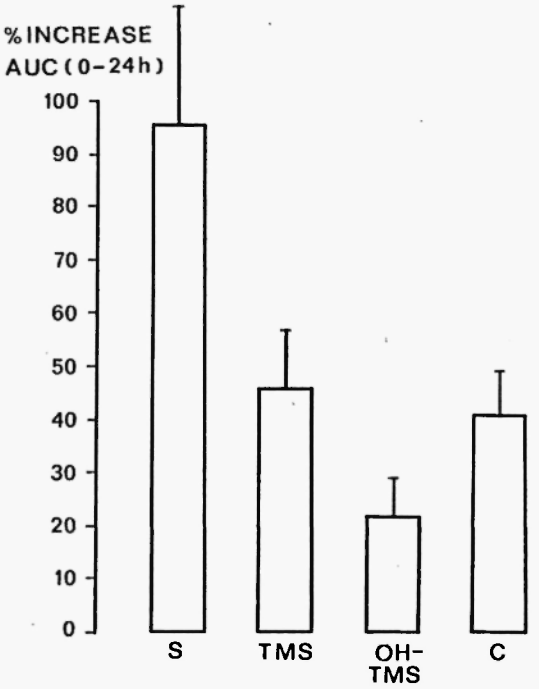

Fig. 4: Mean (± S.E.) percent increase in AUC (0-24h) in nine volunteers after oral intake of 200 mg spironolactone in the fed state compared to the fasting state. S = spironolactone, TMS = 7α-thiomethylspirolactone, OH-TMS = 6β-hydroxy-7α-thiomethylspirolactone, C = canrenone. From reference 77.

may be explained in terms of a food-induced decreased first-pass metabolism of spironolactone /77/.

8.2 Influence of inclusion complexation of spironolactone with cyclodextrins on bioavailability

Recently, the formation of inclusion complexes of spironolactone with cyclodextrins has received attention as an approach to improve oral bioavailability. Inclusion complexation of the drug with β-cyclodextrin (β-CyD) /78-81/ and γ-cyclodextrin (γ-CyD) /80/, resulted in a markedly improved dissolution rate and solubility of spironolactone. In dogs, an enhanced bioavailability of spironolactone (measured as canrenone) was noted, when complexes of spironolactone with β- and γ-CyD were administered, in comparison to the administration of spironolactone alone /80/. The administration of an inclusion complex of spironolactone with β-CyD to rats led to an increase in urinary volume, whereas no increase was observed when either spironolactone alone, or β-CyD alone was given /79/. Until now, only one bioavailability study in humans has been performed with a spironolactone-cyclodextrin complex /82/. Significantly higher quantities of fluorimetrically measured "canrenone" were excreted in urine after oral administration of a spironolactone-β-CyD complex than after oral dosing of spironolactone alone /82/.

The results of these bioavailability studies with spironolactone-cyclodextrin complexes suggest that it may be possible to use a lower dose of spironolactone in clinical practice. However, bioavailability studies comparing commercially available spironolactone tablets and spironolactone-cyclodextrin complexes have not yet been performed. Furthermore, it has been reported that measuring urinary excretion of fluorimetric "canrenone" alone is not a reliable method of determining the bioavailability of spironolactone /70/. A very interesting feature of the γ-CyD complex is that it may also be applicable in practice to injection preparations, because of its high solubility in water /80/.

IX. PLASMA PROTEIN BINDING

It is generally accepted that only unbound drug is freely diffusable and available for distribution to sites of pharmacological activity /83/.

The percentage plasma protein binding of spironolactone has been reported to be 91.1% and 98.2%, using equilibrium dialysis and

ultrafiltration respectively, at a plasma spironolactone concentration of 550 ng/ml /11/. In another study where only equilibrium dialysis was used, the spironolactone fractions bound to 4% (w/v) albumin and to 1.16% (w/v) γ-globulin were 66% and 18%, respectively /84/. The binding of spironolactone to albumin increased with increasing albumin concentration, whereas this binding did not change significantly when the concentration of spironolactone was varied from 50 to 1300 ng/ml. The percentage plasma protein binding of the metabolite canrenone, at a plasma concentration of 710 ng/ml, was found to be 89.9% and 98.3% using equilibrium dialysis and ultrafiltration, respectively /11/.

Plasma protein binding of canrenone and canrenoate were compared in young and elderly persons by the ultrafiltration technique, at a plasma concentration of 1000 ng/ml for each compound /56/. No significant differences were found between the two age groups. The protein binding of canrenone in the elderly and young group were reported to be 94.0% and 95.2% respectively, and the binding percentages of canrenoate were found to be 91.6% and 93.9% respectively /56/.

To date, no serum or plasma protein binding data of other spironolactone metabolites have been obtained.

X. PHARMACOLOGICAL ACTIVE PRINCIPLE

For a long time, the anti-mineralocorticoid activity of spironolactone was mainly attributed to canrenone /4,5/. Several pharmacodynamic studies, however, indicated that substances other than canrenone must be mainly responsible for the renal anti-mineralocorticoid activity of spironolactone /29,45,85/. For example, after oral administration of single equimolar doses of spironolactone and potassium canrenoate to healthy volunteers, spironolactone was significantly more potent than potassium canrenoate in the bioassay employed, despite the fact that almost equivalent fluorimetric plasma levels of "canrenone" were found /45/. A relative potency (potassium canrenoate: spironolactone) of approximately 0.30 was calculated. This meant that 70% of the renal anti-mineralocorticoid activity of a single dose of spironolactone could not be explained in terms of plasma canrenone. After multiple doses of spironolactone, the contribution of canrenone increased to approximately 70% of the anti-mineralocorticoid activity of spironolactone in this bioassay /5/.

However, these calculations were based upon the assumption that

the fluorimetric assay specifically determined canrenone. This would apply only to the canrenone concentrations after administration of potassium canrenoate /29/, but not to the corresponding levels after administration of spironolactone, as has been previously discussed. These studies have indicated that the real contribution of canrenone to fluorimetrically determined canrenone in human serum amounts to no more than about 20-40% /29-38/. Combining these analytical and pharmacological data led to the conclusion that canrenone exerts only 1/10 and 1/4 of the anti-mineralocorticoid activity of spironolactone, after single and multiple doses, respectively /31,34,86/.

Since canrenoate plasma levels after administration of potassium canrenoate were much higher than after an equivalent dose of spironolactone /38,45/, it was concluded that canrenoate contributed little or no anti-mineralocorticoid activity /45/. The unavoidable conclusion is that other metabolites, or perhaps the unchanged drug itself, must be mainly responsible for the renal anti-mineralocorticoid activity of spironolactone /29,31,34,45,85-90/. Among the metabolites, the sulfur-containing metabolites are proposed as the principal pharmacologically active moieties /45,86,89,90/. This is of particular interest since the recent discovery that 7α-thiomethylspirolactone is the major metabolite in serum after a single dose of spironolactone to man /43/. All the sulfur-containing metabolites which are involved in the metabolic pathway of spironolactone, and also canrenone, have been investigated for their mineralocorticoid blocking activity after subcutaneous injection in rats /91/. In decreasing order of magnitude, 7α-thiospirolactone, 7α-thiomethylspirolactone, and canrenone exhibited significant activity. The other metabolites possessed minor, if any, blocking activity. The oral activity of 7α-thiomethylspirolactone in rats and 7α-thiospirolactone in dogs was estimated to be 1.06 and 0.70 respectively, relative to orally administered spironolactone /91/.

In a bioassay model, developed to determine the renal anti-mineralocorticoid activity of orally administered spirolactones in man, 7α-thiospirolactone and 7α-thiomethylspirolactone were found to have an activity of only 0.26 and 0.33 respectively, relative to spironolactone /89/. However, serum concentrations of these substances were not determined, and an incomplete absorption or an extensive first-pass effect may have complicated the picture. It is possible that 7α-thiospirolactone and 7α-thiomethylspirolactone have high activity, but do not reach the target organ in sufficient quantities. *In vitro* studies have indicated that among the sulfur-containing metabolites, 7α-thiospiro-

lactone and 7α-thiomethylspirolactone possessed a higher affinity than canrenone for aldosterone receptors in rat kidney slices /92/. The affinity of canrenoate to these receptors was much lower than that of canrenone /92,93/. It was found that the presence of a closed γ-lactone ring is of critical importance for binding to the aldosterone receptor /92,93/. However, these receptor binding studies do have their disadvantages. They do not take into account drug absorption, disposition, metabolism and they do not differentiate between agonist and antagonist activity /92,94,95/.

Also, spironolactone itself is mentioned as the possible pharmacologically active principle after its administration /86,89,90/. In comparison to canrenone, it has a higher affinity for rat renal aldosterone receptors /92,93,96/, and it showed anti-mineralocorticoid action in an isolated toad bladder system /94,97/.

In conclusion, the active principles following the administration of spironolactone have yet to be established. Recently described HPLC assays, developed to measure several spironolactone derived compounds separately /41,98/, will make investigations possible which can combine pharmacodynamic and pharmacokinetic aspects of these substances.

XI. METABOLISM AND ENDOCRINE SIDE EFFECTS

A group of endocrine side effects, such as gynaecomastia in men /99-102/ and menstrual irregularities in women /101,103/, have been observed during spironolactone treatment. The most probable mechanism of these side effects is an inhibition of binding of 5α-dihydrotestosterone /101,104,105/ and progesterone /106/ to their respective receptors by spironolactone derived compounds. Several substances have been tested for their affinity to the 5α-dihydrotestosterone receptor of the rat prostate. Spironolactone /105,107/, 7α-thiospirolactone /105/, 7α-thiomethylspirolactone /105/, and canrenone /107/ were able to inhibit 5α-dihydrotestosterone binding to its receptor. The binding of progesterone to isolated human uterine cytosolic progesterone receptors was decreased in the presence of canrenone in a concentration dependent manner /106/.

Interestingly, it has been reported that in patients with hepatic cirrhosis and ascites, the incidence of gynaecomastia is lower when treated with potassium canrenoate, than with spironolactone /108/. Also, spironolactone induced gynaecomastia disappeared when the drug was replaced by potassium canrenoate in a patient with indeterminate

aldosteronism /109/. Furthermore, no gynaecomastia was seen in 12 hypertensive patients treated with canrenone for six months, as compared with four cases of gynaecomastia in 13 hypertensive patients treated with spironolactone for six months /110/. When a mouse kidney cytosol-androgen receptor assay was used, it was found that plasma from mice, chronically administered spironolactone, contained approximately ten times higher levels of androgen receptor active material than plasma from mice administered potassium canrenoate or canrenone /111/. Since canrenone is a common metabolite of potassium canrenoate and spironolactone, it appears that substances other than canrenone and canrenoate are mainly responsible for the antiandrogenic action of spironolactone, resulting in gynaecomastia /53/.

Recently, the German health authorities announced that they planned to restrict the therapeutic indications for spironolactone and potassium canrenoate containing pharmaceuticals /112/. They argued that potassium canrenoate is potentially carcinogenic in man, because it had shown tumor inducing effects in experimental animals. Their decision was also extended to spironolactone because "contemporary data on the metabolism of spironolactone and potassium canrenoate give no reason for a separate risk evaluation of these drugs" /112/. It appeared that it was still assumed that the therapeutic action and side effects of both drugs were mediated by the common metabolite canrenone. The pharmaceutical companies which were involved were allowed a period of four weeks during which they could argue against this proposal. After hearing their comments, the health authorities decided to restrict only the indications for use of potassium canrenoate /113/. This decision was based on toxicological studies carried out until then, which had indicated no enhanced carcinogenic risk for spironolactone. Differences in the metabolism of both drugs were mentioned by the health authorities as a possible explanation /113/.

NOTE ADDED AT PROOFS

In the United Kingdom, the Department of Health has recently asked companies to remove the following indications from products containing spironolactone: treatment of essential hypertension and idiopathic oedema. The drug will remain available for other uses /114/. This regulatory action was taken for similar reasons which had led to a restriction of the therapeutic indications for potassium canrenoate in Germany /113/.

It has now been clearly shown that, in contrast to potassium canrenoate, the major pathway of spironolactone's metabolism is not via canrenone or canrenoate, but through pathways that retain the sulfur moiety. Nevertheless, canrenone is a common metabolite of both drugs. The company Searle has done *in vitro* mutagenicity studies which indicated that a metabolite of canrenone (or canrenoate), but not canrenone itself, may be a mammalian cell mutagen, since metabolic activation of potassium canrenoate was necessary for a positive response.

Spironolactone and its sulfur-containing metabolites have recently been shown to inhibit *in vitro* metabolism of ^{14}C-potassium canrenoate by rat liver microsomes /115/. Therefore, the lack of carcinogenity after spironolactone administration may be explained by the absence of mutagenic canrenone metabolites, due to the inhibition of canrenone metabolism by the presence of unchanged spironolactone and its sulfur-containing metabolites.

The policy of the Department of Health in the UK regarding spironolactone is apparently based only on data obtained with potassium canrenoate and is therefore, from a scientific point of view, questionable.

XII. REFERENCES

1. Ings, R.M.J., and Stevens, L.A. Pharmacokinetics and metabolism of diuretics. In: Bridges, J.W. and Chasseaud, L.F., eds, Progress in Drug Metabolism, Volume 7. Chichester: John Wiley and Sons, 1983; 129-45.
2. Karim, A., Kook, C., Zitzewitz, D.J., Zagarella, J., Doherty, M. and Campion, J. Species differences in the metabolism and disposition of spironolactone. *Drug Metab. Dispos.* 1976; : 547-555.
3. Gochman, N. and Gantt, C.L. A fluorimetric method for the determination of a major spironolactone (Aldactone) metabolite in human plasma. *J. Pharmacol. Exp. Ther.* 1962; 135: 312-316.
4. Sadée, W., Dagcioglu, M. and Schröder, R. Pharmacokinetics of Aldactone in humans. In: Brendel, W., Coraboeuf, E., Ebling, F.J.G. et al, eds, Extrarenal activity of Aldosterone and its antagonists. Amsterdam: Excerpta Medica 1972; 40-42.
5. Ramsay, L., Asbury, M., Shelton, J. and Harrison I. Spironolactone and canrenoate-K: Relative potency at steady-state. *Clin. Pharmacol. Ther.* 1977; 21: 602-609.
6. Gerhards, E. and Engelhardt, R. Zum Stoffwechsel von 3-(3-Oxo-7α acetyl - thio - 7β- hydroxy -4 - androsten -17α-yl)-propionsäure-γ-lacton. *Arzneim. Forsch.* 1963; 13: 972-977.

7. Zicha, L., Weist, F., Scheiffarth, F., and Schmid, E. Dünnschicht-chromatographische Untersuchungen des 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)-propionsäure-γ-lactons und seiner Metaboliten beim Menschen. *Arzneim. Forsch.* 1964; 14: 699-705.
8. Wayner, H., Weist, F. and Zicha, L. Aldadien und andere Metaboliten im Blut und Harn nach rectaler Applikation von Spironolacton und intravenöser Injektion von Aldadien-Kalium. *Arzneim Forsch.* 1967; 17: 415-419.
9. Karim, A. and Brown, E.A. Isolation and identification of novel sulfur-containing metabolites of spironolactone (Aldactone). *Steroids* 1972; 20: 41-62.
10. Karim, A., Hribar, J., Aksamit, W., Doherty, M., and Chinn, L.J. Spironolactone metabolism in man studied by gas chromatography-mass spectrometry. *Drug Metab. Dispos.* 1975; 3: 467-478.
11. Karim, A., Zagarella, J., Hribary, J. and Dooley, M. Spironolactone . I. Disposition and metabolism. *Clin. Pharmacol. Ther.* 1975; 19: 158-169.
12. Abshagen, U. and Rennekamp, H. Isolation and identification of a sulfur-containing metabolite of spironolactone from human urine. *Steroids* 1976; 28: 467-480.
13. Abshagen, U., Rennekamp, H. and Luszpinski, G. Pharmacokinetics of spironolactone in man. *Naunyn-Schmied. Arch. Pharmacol.* 1976; 296: 37-45.
14. Karim, H., Hribar, J., Doherty, M., Aksamit, W., Chappelow, D., Brown, E., Markos, C., Chinn, L.J., Liang, D. and Zagarella, J. Spironolactone: diversity in metabolic pathways. *Xenobiotica* 1977; 7: 585-600.
15. Sadée, W., Abshagen, U., Finn, C. and Rietbrock, N. Conversion of spironolactone to canrenone and disposition kinetics of spironolactone and canrenoate-potassium in rats. *Naunyn-Schmied. Arch. Pharmacol.* 1974; 283: 303-318.
16. Karim, A., Ranney, R.E. and Maibach, H.I. Pharmacokinetic and metabolic fate of potassium canrenoate (SC-14266) in man. *J. Pharm. Sci.* 1971; 60: 708-715.
17. Garrett, E.R. and Won, C.M. Prediction of stability in pharmaceutical preparations XVI: Kinetics of hydrolysis of canrenone and lactonization of canrenoic acid. *J. Pharm. Sci.* 1971; 60: 1801-1809.
18. Sadée, W., Dagcioglu, M. and Schröder, R. Pharmacokinetics of spironolactone, canrenone and canrenoate-K in humans. *J. Pharmacol. Exp. Ther.* 1973; 185: 686-695.
19. Sadée, W., Finn, A.M., Schmiedek, P. and Baethmann, A. Aldosterone plasma radioimmunoassay interference by a spirolactone metabolite. *Steroids* 1975; 25: 301-311.
20. Weissenborn, U. and Erbler, H.C. Metabolism of spirolactones by the rat testis *in vitro*. *Steroids* 1983; 42: 365-388.
21. Koch, R.L., Hauswald, C.L. and Oppermann, J.A. Pharmacokinetics and metabolism of canrenone and canrenoic acid after an oral dose of potassium canrenoate or spironolactone in the rat. *Pharmacologist* 1984; 26: 167.
22. Vose, C.W., Boreham, D.R., Ford, G.C., Haskins, N.J. and Palmer, R.F.

Identification of some human urinary metabolites of orally administered potassium canrenoate by stable-isotope labelling techniques. *Drug Metab. Dispos.* 1979; 7: 226-32.

23. Sherry, J.H., O'Donnell, J.P. and Colby, H.D. Conversion of spironolactone to an active metabolite in target tissues: Formation of 7α-thiospironolactone by microsomal preparations from guinea pig liver, adrenals, kidneys, and testes. *Life Sci.* 1981; 29: 2727-2736.
24. Sherry, J.H., O'Donnell, J.P., Flowers, L., Lacagnin, L.B. and Colby, H.D. Metabolism of spironolactone by adrenocortical and hepatic microsomes: Relationship to cytochrome P-450 destruction. *J. Pharmacol. Exp. Ther.* 1986; 236: 675-680.
25. Erbler, H.C. Effect of spironolactone and its main metabolite canrenone on the renin-angiotensin-aldosterone-system during long-term treatment in rats. *Acta Endocrinol.* 1979; 90: 147-156.
26. Keith, R.A., Jardine, I., Kerremans, A. and Weinshilboum, R.M. Human erythrocyte membrane thiol methyltransferase. S-Methylation of captopril, N-acetyl-cysteine, and 7α-thio-spirolactone. *Drug Metab. Dispos.* 1984; 12: 717-724.
27. Sadee, W., Dagcioglu, M. and Riegelman, S. Fluorimetric microassay for spironolactone and its metabolites in biological fluids. *J. Pharm. Sci.* 1972; 61: 1126-1128.
28. Neubert, P. and Koch, K. Simultaneous automated determination of spironolactone metabolites in serum. *J. Pharm. Sci.* 1977; 66: 1131-1134.
29. Dahlöf, C.G., Lundborg, P., Persson, B.A. and Regardh, C.G. Re-evaluation of the anti-mineralocorticoid effect of the spironolactone metabolite, canrenone, from plasma concentrations determined by a new high-pressure liquid-chromatographic method. *Drug Metab. Dispos.* 1979; 7: 103-107.
30. Neurath, G.B. and Ambrosius, D. High-performance liquid chromatographic determination of canrenone, a major metabolite of spironolactone, in body fluids. *J. Chromatogr.* 1979; 163;230-235.
31. Abshagen, U., Besenfelder, E., Endele, R., Koch, K. and Neubert, B. Kinetics of canrenone after single and multiple doses of spironolactone. *Eur. J. Clin. Pharmacol.* 1979; 16: 255-262.
32. Besenfelder, E. and Endele, R. High performance liquid chromatographic method for determination of canrenone in serum. *J. High Resolut. Chromatogr. Commun.* 1981; 4: 419-421.
33. Stuber, W., Mutschler, E. and Steinbach, D. Zur Spezifität der Bestimmung von Canrenon in biologischen Flüssigkeiten. *Arch. Pharm.* 1981; 314: 148-156.
34. Merkus, F.W.H.M., Overdiek, J.W.P.M., Cilissen, J. and Zuidema, J. Pharmacokinetics of spironolactone after a single dose: Evaluation of the true canrenone serum concentrations during 24 hours. *Clin. Exp. Hypertens.* 1983; A5: 239-248.
35. Krause, W., Karras, J. and Seifert, W. Pharmacokinetics of canrenone after oral administration of spironolactone and intravenous injection of canrenoate-K in healthy man. *Eur. J. Clin. Pharmacol.* 1983; 25: 449-453.
36. Overdiek, J.W.P.M., Cilissen, J., Hermens, W.A.J.J. and Merkus, F.W.H.M.

The importance of canrenone in the metabolism of spironolactone. *Pharm. Weekbl. Sci. Ed.* 1984; 6: 270.

37. Ho, P.C., Bourne, D.W.A., Triggs, E.J. and Heazlewood, V. Pharmacokinetics of canrenone and metabolites after base hydrolysis following single and multiple dose oral administration of spironolactone. *Eur. J. Clin. Pharmacol.* 1984; 27: 441-446.
38. Kojima, K., Yamamoto, K., Fujioka, H. and Kaneko, H. Pharmacokinetics of spironolactone and potassium canrenoate in humans. *J. Pharmacobio-Dyn.* 1985; 8: 161-166.
39. Chamberlain, J. Gas chromatographic determination of levels of aldadiene in human plasma and urine following therapeutic doses of spironolactone. *J. Chromatogr.* 1971; 55: 249-253.
40. Feher, T., Bodrogi, L. and Váradi, A. Simple gas chromatographic method with flame ionization detection for the determination of aldadiene in human urine. *J. Chromatogr.* 1976; 123: 460-462.
41. Overdiek, J.W.P.M., Hermens, W.A.J.J. and Merkus, F.W.H.M. Determination of the serum concentration of spironolactone and its metabolites by high-performance liquid chromatography. *J. Chromatogr.* 1985; 341: 279-285.
42. Von Vergin, H., Nuss, U., Schwarzländer, F., Strobel, K., Weigand, W. and Hitzenberger, G. Bioverfügbarkeitsuntersuchung zweier Spironolacton-Präparate. *Arzneim. Forsch.* 1981; 31: 1498-1503.
43. Overdiek, J.W.P.M., Hermens, W.A.J.J. and Merkus, F.W.H.M. New insights into the pharmacokinetics of spironolactone. *Clin. Pharmacol. Ther.* 1985; 38: 469-474.
44. Sponer, G., Kaufmann, B. and Kuhr, M. Pharmakokinetik von Aldosteron-Antagonisten. *Krankenhausarzt* 1976; 49: 569-575.
45. Ramsay, L., Shelton, J., Harrison, I., Tidd, M. and Asbury, M. Spironolactone and potassium canrenoate in normal man. *Clin. Pharmacol. Ther.* 1976; 20: 167-177.
46. Jackson, L., Branch, R., Levine, D. and Ramsay, L. Elimination of canrenone in congestive heart failure and chronic liver disease. *Eur. J. Clin. Pharmacol.* 1977; 11: 177-179.
47. Karim, A. Spironolactone: disposition, metabolism, pharmacodynamics, and bioavailability. *Drug Metab. Rev.* 1978; 8: 151-188.
48. Von Vergin, H., Nuss, U. and Strobel, K. Untersuchungen zur Pharmakokinetik und Bioverfügbarkeit von Bendroflumethiazid in Kombination mit Spironolacton. *Arzneim. Forsch.* 1986; 36: 490-495.
49. Abshagen, U., Von Grodzicki, U., Hirschberger, U. and Rennekamp, H. Effect of enterohepatic circulation on the pharmacokinetics of spironolactone in man. *Naunyn-Schmied. Arch. Pharmacol.* 1977; 300: 281-287.
50. Sadée, W., Schröder, R., Von Leitner, E. and Dagcioglu, M. Multiple dose kinetics of spironolactone and canrenoate-potassium in cardiac and hepatic failure. *Eur. J. Clin. Pharmacol.* 1974; 7: 195-200.
51. Abshagen, U., Rennekamp, H. and Luszpinski, G. Disposition kinetics of spironolactone in hepatic failure after single doses and prolonged treatment. *Eur. J. Clin. Pharmacol.* 1977; 11: 169-176.

52. Váradi, A., Feher, T., Bodrogi, L. and Koref, O. Spironolactone metabolism in normal subjects and in patients with liver cirrhosis. *Arzneim. Forsch.* 1977; 27: 1617-1620.
53. Overdiek, J.W.P.M. and Merkus, F.W.H.M. Spironolactone metabolism and gynaecomastia. *Lancet* 1986; 1: 1103.
54. Abshagen, U., Platt, D. and Horn, H.J. Zur Pharmakokinetik von Spironolacton im Alter. *Klin. Wochenschr.* 1981; 59: 909-910.
55. Platt, D., Abshagen, U., Mühlberg, W., Horn, H.J., Schmitt-Rüth, R. and Vollmar, J. The influence of age and multimorbidity on the pharmacokinetics and metabolism of spironolactone. *Arch. Gerontol. Geriatr.* 1984; 3: 147-159.
56. Ho, P.C., Bourne, D.W.A., Triggs, E.J. and Smithurst, B.A. Comparison of plasma levels of canrenone and metabolites after base hydrolysis in young and elderly subjects following single and multiple doses of spironolactone. *Eur. J. Clin. Pharmacol.* 1984; 27: 435-439.
57. Gantt, C.L., Gochman, N. and Dyniewicz, J.M. Effect of a detergent on gastrointestinal absorption of a steroid. *Lancet* 1986; 1: 486-487.
58. Gantt, C.L., Gochman, N. and Dyniewicz, J.M. Gastrointestinal absorption of spironolactone. *Lancet* 1962; 1: 1130-1131.
59. Noel, P.R. and Leahy, J.S. The estimation of the activity of aldosterone antagonist in man: spironolactone (Aldactone) activity. *Clin. Sci.* 1962; 23: 477-483.
60. Kagawa, C.M., Bouska, D.J. and Anderson, M.L. Oral absorption with various preparations of spironolactone in dogs. *J. Pharm. Sci.* 1964; 53: 450-451.
61. Bauer, G., Rieckmann, P. and Schaumann, W. Einfluss von Teilchengrösse und Lösungsvermittlern auf die Resorption von Spironolacton aus dem Magen-Darmtrakt. *Arzneim. Forsch.* 1962; 12: 487-489.
62. D'Athis, P., Richard, M.O., De Lauture, D., Rey, E., Bouvier d'Yvoire, M., Clement, E. and Olive, G. Etude comparative des disponibilites de deux formes de spironolactone. *Therapie* 1981; 36: 443-449.
63. Portal, A., Gaillot, J., Bieder, A., Rodary, C. and Bertharion, J.J. Des aspects de la biodisponibilite physiologique de la spironolactone et de la canrenone. *Sem. Hôp.* 1976; 5: 465-470.
64. McInnes, G.T., Asbury, M.J., Ramsay, L.E., Shelton, J.R. and Harrison, I.R. Effect of micronization on the bioavailability and pharmacologic activity of spironolactone. *J. Clin. Pharmacol.* 1982; 22: 410-417.
65. Levy, G. Availability of spironolactone given by mouth. *Lancet* 1962; 2: 723-724.
66. Clarke, J.M., Ramsay, L.E., Shelton, J.R., Tidd, M.J., Murray, S. and Palmer, R.F. Factors influencing comparative bioavailability of spironolactone tablets. *J. Pharm. Sci.* 1977; 66: 1429-1432.
67. Tidd, K.J., Collins, W.I. and Chamberlain, J. A comparison of the dissolution and bioavailability characteristics of three spironolactone tablet formulations, including preliminary data on two spironolactone metabolites. *J. Int. Med. Res.* 1976; 4: 86-95.
68. Raptis, G., Wendt, H., Meister, R. and Fuchs, P. In vitro and in vivo

availability of spironolactone from various oral preparations. *Drug. Dev. Ind. Pharm.* 1978; 4: 389-411.

69. Chao, A.Y., Sanvordeker, D.R., Zagarella, J., Mattes, K., Nicholova, B. and Karim, A. In vitro and in vivo availability of spironolactone from oral dosage forms. *J. Pharm. Sci.* 1976; 65: 1630-1634.
70. Karim, A., Zagarella, J., Hutsell, T.C., Chao, A.Y. and Baltes, B.J. Spironolactone, II. Bioavailability. *Clin. Pharmacol. Ther.* 1976; 19: 170-176.
71. Hofmann, L.M., Dutt, J.E., Deysach, L.G., Loncin, H. and Tao, L. Comparison of spironolactone tablet dosage forms in healthy humans. *J. Pharm. Sci.* 1974; 63: 1248-1253.
72. Von Erking, W., Lücker, P.W., Stöcker, K.P. and Wetzelsberger, K. Relative bioverfügbarkeit einer neuen Spironolacton-Zubereitung. *Arzneim. Forsch.* 1979; 29: 1184-1189.
73. Von Rosenthal, J., Jaeger, H. and Specker, M. Vergleichende Untersuchung der relativen Bioverfügbarkeit mehrerer Spironolacton-Zubereitungen im Steady-state-Versuch. *Arzneim. Forsch.* 1979; 29: 1428-1432.
74. Rameis, H., Hitzenberger, G. and Horwatitsch, H. The bioavailability of a spironolactone hydrochlorothiazide combination preparation. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 1982; 20: 327-333.
75. Nijkerk, A.J., Vermeer, J.M., Imanse, M. and De Vos, D. The bioavailability of two new preparations of spironolactone tablets. *Pharm. Weekbl. Sci. Ed.* 1983; 5: 210-212.
76. Melander, A., Danielson, K. Schersten, B., Thulin, T. and Wåhlin, E. Enhancement by food of canrenone bioavailability from spironolactone. *Clin. Pharmacol. Ther.* 1977; 22: 100-103.
77. Overdiek, J.W.P.M. and Merkus, F.W.H.M. Influence of food on the bioavailability of spironolactone. *Clin. Pharmacol. Ther.* 1986; 40: 531-536.
78. Mulgaard Anderson, F. and Bundgaard, H. Inclusion complexation of spironolactone with cyclodextrins. *Arch. Pharm. Chem. Sci. Ed.* 1983; 11: 7-14.
79. Debruères, B., Bretillon, A. and Duchêne, D. Improvement of solubility and bioavailability of spironolactone by inclusion in β-cyclodextrin. *Proc. Int. Symp. Control Rel. Bioact. Mater.* 1985; 12: 118-119.
80. Seo, H., Sunuoka, M., Hashimoto, T. Fujinaga, T., Otagiri, M. and Uekama, K. Enhancement of oral bioavailability of spironolactone by β- and γ-cyclodextrin complexations. *Chem. Pharm. Bull.* 1983; 31: 286-291.
81. Uekama, K. Pharmaceutical applications of methylated cyclodextrins. *Pharm. Int.* 1985; 6: 61-65.
82. Vila-Jato, J.L., Blanco, J. and Vilar, A. Spironolactone/β-cyclodextrin complex: oral bioavailability in humans. *Acta Pharm. Technol.* 1986; 32: 82-85.
83. Koch-Weser, J. and Sellers, E.M. Binding of drugs to serum albumin. *New Engl. J. Med.* 1976; 294; 311-316.
84. Ng, A.S., Kluza, R.B. and Newton, D.W. Distribution coefficients and in

vitro human serum protein binding of spironolactone and its 7α-carboxymethyl analog. *J. Pharm. Sci.* 1980; 69: 30-32.

85. Huston, G.J. and Turner, P. Antagonism of fluorocortisone by spironolactone and canrenone. *Br. J. Clin. Pharmacol.* 1976; 3: 201-204.
86. Merkus, F.W.H.M. Is canrenone the major metabolite of spironolactone? *Clin. Pharm.* 1983; 2: 209-210.
87. Ramsay, L.E., Shelton, J.R., Wilkinson, D. and Tidd, M.J. Canrenone — the principal active metabolite of spironolactone? *Br. J. Clin. Pharmacol.* 1976; 3: 607-612.
88. Casals-Stenzel, J., Schmalbach, J. and Losert, W. Acute effects of aldosterone antagonists in volunteers. In: Addison, G.M., Wirenfeldt Asumussen, N., Corvol, P., et al, eds, Aldosterone antagonists in clinical medicine. Amsterdam-Oxford: Excerpta Medica, 1978; 207-216.
89. McInnes, G.T., Asbury, M.J., Shelton, J.R., Harrison, I.R., Ramsay, L.E., Venning, G.R. and Clarke, J.M. Activity of sulfur-containing intermediate metabolites of spironolactone. *Clin. Pharmacol. Ther.* 1980; 27: 363-369.
90. Ramsay, L.E. Clinical pharmacology and therapeutic use of aldosterone antagonists. In: Agarwal, M.D., ed, Hormone antagonists. Berlin-New York: Walter de Gruyter and Co., 1982; 335-363.
91. Hofmann, L.M. Aldosterone antagonists in laboratory animals. In: Wesson, L.G. and Fanelli, G.M., eds, Recent advances in renal physiology and pharmacology. Baltimore: University Press Park, 1974; 305-316.
92. Funder, J.W., Feldman, D., Highland, E. and Edelman, I.S. Molecular modifications of anti-aldosterone compounds: effects on affinity of spirolactones for renal aldosterone receptors. *Biochem. Pharmacol.* 1974; 23: 1493-1501.
93. Peterfalvi, M., Torelli, V., Foumex, R. and Rousseau, G. Importance of the lactonic ring in the activity of steroidal antialdosterones. *Biochem. Pharmacol.* 1980; 29: 353-357.
94. Sakauye, C. and Feldman, D. Agonist and anti-mineralocorticoid activities of spirolactones. *Am. J. Physiol.* 1976; 231: 93-97.
95. McInnes, G.I., Shelton, J.R., Ramsay, L.E., Harrison, I.R., Asbury, M.U., Clarke, J.M., Perkins, R.M. and Venning, G.R. Relative potency and structure activity relationships of aldosterone antagonists in healthy man: correlation with animal experience. *Br. J. Clin. Pharmacol.* 1982; 13: 331-339.
96. Funder, J.W., Mercer, J. and Hood, J. SC 23992: radioreceptor assays for therapeutic and side effects. *Clin. Sci. Mol. Med.* 1976; 51: 333S-334S.
97. Rossier, B.C., Claire, M., Rafestin-Oblin, M.E., Geering, K., Gäggeler, H.P. and Corvol P. Binding and anti-mineralocorticoid activities of spirolactones in toad bladder. *Am. J. Physiol.* 1983; 244: C24-C31.
98. Sherry, J.H., O'Donnell, J.P. and Colby, H.D. Separation of spironolactone and its biologically active sulfur-containing metabolites by high-performance liquid chromatography. *J. Chromatogr.* 1986; 374: 183-190.
99. Ludbrook, A., Dynon, M., Mendelsohn, F.A.O. and Louis, W.J. Comparison of a single-dose and twice-a-day spironolactone therapy in mild hypertension. *Med. J. Aust.* 1980; 1: 124-125.

100. Caminos-Torres, R., Ma, L. and Snijder, P.J. Gynecomastia and semen abnormalities induced by spironolactone in normal men. *J. Clin. Endocrinol. Metab.* 1977; 45: 255-260.
101. Loriaux, D.L. Spironolactone and endocrine dysfunction. *Ann. Intern. Med.* 1976; 85: 630-636.
102. Huffman, D.H., Kampmann, J.P., Hignite, C.E. and Azarnoff, D.L. Gynaecomastia induced in normal males by spironolactone. *Clin. Pharmacol. Ther.* 1978; 24: 465-473.
103. Levitt, J.I. Spironolactone therapy and amenorrhea. *J.A.M.A.* 1970; 211: 2014-2015.
104. Lobo, R.A., Shoupe, D., Serafini, P., Brinton, D. and Horton, R. The effects of two doses of spironolactone serum androgen and anagen hair in hirsute women. *Fertil. Steril.* 1985; 43: 200-205.
105. Cutler, G.B., Pita, J.C., Rifka, S.M., Menard, R.H., Sauer, M.A. and Loriaux, D.L. SC 25152: a potent mineralaocorticoid antagonist with reduced affinity for the 5α-dihydrotestosterone receptor of human and rat prostate. *J. Clin. Endocrinol. Metab.* 1978; 47: 171-175.
106. Fernandez, M.D., Carter, G.D. and Palmer, T.N. The interaction of canrenone with oestrogen and progesterone receptors in human uterine cytosol. *Br. J. Clin. Pharmacol.* 1983; 15: 95-101.
107. Pita, J.C., Lippman, M.E., Thompson, E.B. and Loriaux, D.L. Interaction of spironolactone and digitalis with the 5α–dihydrotestosterone (DHT) receptor of rat ventral prostate. *Endocrinology* 1975; 97: 1521-1527.
108. Bellati, G. and Ideo, G. Gynaecomastia after spironolactone and potassium canrenoate. *Lancet* 1986; 1: 626.
109. Dupont, A. Disappearance of spironolactone-induced gynaecomastia during treatment with potassium canrenoate. *Lancet* 1985; 2: 731.
110. Fromantin, M. Surveillance clinique et hormonale des traitements prolonges de l'hypertension arterielle par les anti-aldosterones. *Mises Jour. Cardiol.* 1980; 19: 95-98.
111. Armanini, D., Kartowiak, I., Goi, A., Mantero, F. and Funder, J.W. In-vivo metabolites of spironolactone and potassium canrenoate: determination of potential anti-androgenic activity by a mouse kidney cytosol receptor assay. *Clin. Endocrinol.* 1985; 23: 341-347.
112. Anonymus. Kalium-Canrenoat- und Spironolacton-haltige Arzneimittel. *Deutsche Apotheker Zeitung* 1986; 126(36): VII-VIII.
113. Anonymus. Kalium-Canrenoat- und Spironolacton-haltige Arzneimittel. *Deutsche Apotheker Zeitung* 1986; 126(42): VIII-IX and 2312.
114. Anonymous. Spironolactone no longer to be used for treatment of hypertension. *The Pharmaceutical Journal* 1988; January 9: 57.
115. Hauswald, C.L., Cook, C.S., Koch, R.L. and Oppermann, J.A. In vitro metabolism of ^{14}C-potassium canrenoate, ^{14}C-spironolactone and ^{35}S-spironolactone by rat liver microsomes. *Pharmacologist* 1986; 28: 212.