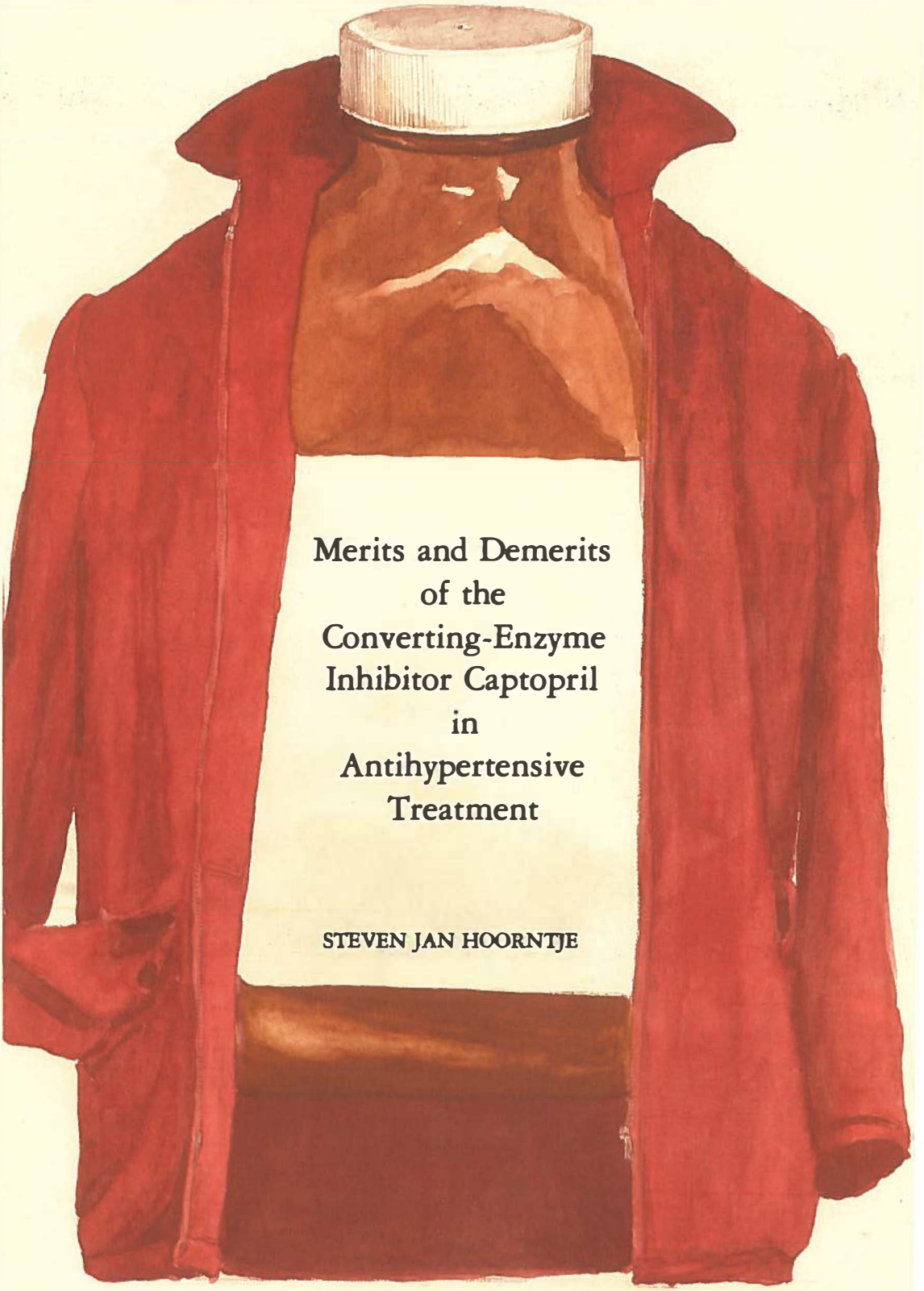

Merits and Demerits of the Converting-Enzyme Inhibitor Captopril in Antihypertensive Treatment

STEVEN JAN HOORNTJE

MERITS AND DEMERITS OF THE CONVERTING-ENZYME INHIBITOR CAPTOPRIL IN ANTIHYPERTENSIVE TREATMENT

Stellingen

I.

Captopril is - ook bij langdurig gebruik - een effectief antihypertensivum.

II.

Dubbelzijdige nefrectomie is een achterhaalde therapie voor de behandeling van zeer ernstige hypertensie.

III.

Voortdurend onderzoek op bijwerkingen is vooral geïndiceerd bij die geneesmiddelen, die langdurig gebruikt moeten worden.

IV.

Onderzoek naar aetiologie en pathogenese van het syndroom van Bartter kan bevruchtend werken op het inzicht in de pathofysiologie van de bloeddrukregulatie bij de zwangere.

V.

Het ontstaan van (orthostatische) hypertensie staat en valt met de nier.

VI.

Het is mogelijk dat bij patiënten met de ziekte van Goodpasture de aanwezigheid van nierinsufficiëntie bijdraagt aan de binding van auto-antilichamen aan de alveolaire basaalmembraan. Ter voorkoming van haemorrhagische pneumonitis lijkt bij deze patiënten vroegtijdige en intensieve nierfunctievervangende behandeling aangewezen te zijn.

VII.

De stormachtige ontwikkelingen op het gebied van de chronische ambulante peritoneale dialyse zullen ook in Nederland moeten leiden tot een herbezinning op de behandelingswijze van de patiënt met terminale nierinsufficiëntie.

VIII.

Het verdient aanbeveling een onderzoek in te stellen naar die factoren, die van doorslaggevend belang zijn bij de keuze van een patiënt met terminale nierinsufficiëntie tussen thuis- en centrumdialyse.

IX.

Een - terechte - bezorgdheid over de vervuiling van het milieu met radioactieve afvalstoffen mag niet leiden tot een individueel suboptimale behandeling van hyperthyreoïdie.

X.

Het dragen van autogordels reduceert niet alleen het aantal slachtoffers bij auto-ongevallen, maar leidt tevens tot een afname van de ernst van het opgelopen letsel.

XI.

De opvatting dat het terugschroeven van de honoraria van medische specialisten zal leiden tot een wezenlijke bezuiniging op de kosten van de gezondheidszorg, is zeer discutabel.

XII.

De opvatting dat alleen lang-benigen kunnen basketballen is kort-zichtig.

Stellingen
behorende bij het proefschrift van
Steven Jan Hoorntje

Merits and Demerits of the Converting-Enzyme Inhibitor Captopril
in Antihypertensive Treatment

Groningen, mei 1981

RIJKSUNIVERSITEIT TE GRONINGEN

Merits and Demerits of the Converting-Enzyme Inhibitor Captopril in Antihypertensive Treatment

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. M. R. van Gils
in het openbaar te verdedigen op woensdag 27 mei 1981
des namiddags te 2.45 uur precies
door

STEVEN JAN HOORNTJE

geboren te Groningen

1981
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

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Printing of this thesis was financially supported by the Dutch Heart Foundation (Nederlandse Hartstichting).

Aan Jan, Wouter, Lotte en Bart

Acknowledgements

The studies described in this thesis were performed in the Department of Nephrology (Prof. Dr. G. K. van der Hem) and in the Outpatient Clinic for Hypertension (Dr. B. S. ten Berge, Dr. A. J. M. Donker, Dr. J. F. May) of the State University Hospital Groningen, Groningen, The Netherlands.

I am much indebted to Prof. Dr. E. Mandema, Prof. Drs J. Scholten and Dr. J. T. Panders, who offered me the opportunity for my education and training in Internal Medicine in the Departments of Medicine of the State University Hospital Groningen and the St. Joseph's Hospital Deventer, respectively.

I am much indebted to Prof. Dr. G. K. van der Hem for my education and training in Nephrology and for being my promotor.

I am grateful to Prof. Dr. H. Doorenbos and Prof. Dr. Ph. J. Hoedemaeker, who gave their skilful advice during the studies and preparations of the manuscript.

I am much obliged to Dr. A. J. M. Donker and Dr. D. de Zeeuw, who were the great stimulators of this study. Their expert knowledge has greatly contributed to the realization and rounding off of the studies performed.

I gratefully acknowledge the cooperation of Drs A. J. Smit and the technical staff of the Hypertension Outpatient Clinic (Miss V. van der Heide and Mrs. W. Kramer-Beerlings).

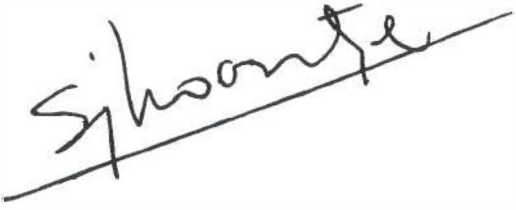
Thanks are also due to Mrs. J. Jager-van den Berg (cover design); Mr J. Brouwer and Mr A. de Lange (drawings); Miss A. Bremer; Miss G. von Dülmen Krumpelmann and Mrs. E. Konneman-van Zalk (renal function studies and blood sampling); Mrs. A. van Zanten and Dr. J. J. Pratt (PRA and PAC determinations, Department of Nuclear Medicine, Prof. Dr. M. G. Woldring); Ir. W. A. Dijk (computer programming), Miss G. Sinot (Laboratory of Clinical Chemistry, Prof. Dr. A. Groen), Mr A. J. Schaalma and Mrs. E. Muskiet-Stuut (library), Drs I. Wierenga (grammatical suggestions) and Mrs. G. E. Hoorntje-Boonstra (secretarial assistance).

I wish to thank the patients and nursing staff for their valuable help.

During these studies invaluable contributions were made by Drs S. van der Laan, Drs C. G. M. Kallenberg, Dr. E. J. L. Prins, Dr. W. Sluiter and Dr. J. J. Weening.

The manufacturers of captopril (E. B. Squibb & Sons, Inc) gave financial support; this study was also supported by grant C_9^V from the Dutch Kidney Foundation ("Nierstichting Nederland").

I am really thankful to my parents and my wife for providing me with the education and opportunity to do the kind of work which I enjoy.



Contents

List of abbreviations

A I	: angiotensin I
A II	: angiotensin II
ANA	: antinuclear antibodies
anti-ds DNA	: anti-double stranded DNA
BK	: bradykinin
BP	: blood pressure
CE	: converting-enzyme
CT ratio	: cardiac-thoracic ratio
EH	: essential hypertension
EM	: electron microscopy
ERPF	: effective renal plasma flow
FF	: filtration fraction
GFR	: glomerular filtration rate
GBM	: glomerular basement membrane
IF	: immunofluorescence
KW	: Keith-Wagener
LVH	: left ventricular hypertrophy
MAP	: mean arterial pressure
MGP	: membranous glomerulopathy
PRA	: plasma renin activity
PAC	: plasma aldosterone concentration
RAS	: renin-angiotensin system
RVH	: renovascular hypertension
RPD	: renal parenchymal disease
SDBP	: supine diastolic blood pressure
SSBP	: supine systolic blood pressure

Chapter 1
Introduction

1.1 The rationale of antihypertensive treatment

This thesis deals with the vascular and renal response of hypertensive patients to the converting-enzyme inhibitor captopril (SQ 14,225). It seems appropriate to open this chapter with some brief remarks on modern concepts of blood pressure regulation before reviewing the role of inhibition of the renin-angiotensin system (RAS) in the treatment of high blood pressure.

Hypertension, defined according to WHO-criteria as a blood pressure (BP) level above 160 mmHg systolic and/or above 95 mmHg diastolic[282], has a morbidity of approximately 15-20 per cent in the adult population of the Netherlands[26]. Facing this impressive percentage, it will be clear that management of hypertension imposes an enormous burden on society. Therefore, possible benefits of antihypertensive treatment should be carefully evaluated.

Life insurance statistics have revealed an important point: the lower a person's BP, the longer he is likely to survive[240]. More generally applicable information has come from prospective community studies, for example the Framingham study[235]. In this particular study, persons with hypertension (i.e. supine diastolic blood pressure [SDBP] of $>$ 95 mmHg) had more than seven times as many strokes, four times as much congestive heart failure, three times as much coronary heart disease and twice as much occlusive peripheral arterial disease as normotensive individuals. The conclusion must be drawn that there is a good theoretical reason for trying to lower BP.

Before advocating mass treatment, the results achieved by lowering BP must be examined. In the past decade , most physicians have come to the conclusion that treatment of moderate to severe hypertension (SDBP $>$ 105 mmHg) will reduce overall morbidity and mortality from cardiovascular disease. The most impressive evidence in support of this view is the Veterans Administration Cooperative Study, a randomized, double blind, placebo controlled trial in 500 middle-aged men [269 270]. Drug treatment lowered the incidence of morbidity of cardiovascular events among those with moderate or severe hypertension. However, a statistically significant fall of morbidity and mortality could not be shown among men with mild hypertension

(SDBP between 90 and 104 mmHg). Therefore, the question "to treat or not to treat" mildly hypertensive persons remained open.

Recently, three important studies (The Hypertension Detection and Follow-up Program[137], The Australian Therapeutic Trial in Mild Hypertension[171] and The Oslo study[113]) have provided evidence that effective BP regulation of hypertensive persons with a SDBP between 90 and 104 mmHg will lower cardiovascular casualties in this group. Though the results of these studies need further confirmation, the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure has already advised that a SDBP of $>$ 90 mmHg on 2 successive examinations should be an indication for antihypertensive treatment[155]. Therefore, it seems that the pendulum is going to swing away from "benign neglect" to rigid BP control in the treatment of mild hypertension[219].

1.2 Current possibilities in antihypertensive treatment

Aetiology and exact pathogenesis are obscure in most cases of hypertension. Therefore, therapy can only be aimed at correction of the abnormal pressure. Treatment of hypertension should start with general measures as relief of stress and regular exercise. Dietary management includes caloric restriction for the patient who is overweight[76]. Restriction of the intake of saturated fats is recommended by some on the basis of identification of hypertension as a risk factor in the development of atherosclerosis[144]. Because of the documented efficacy of sodium restriction in lowering BP, it seems to be a rational, though controversial, approach to prescribe dietary sodium restriction[76].

Many hypertensive patients, however, require additional treatment with drugs to achieve normotension. The aim of drug therapy is to restore arterial pressure to normal levels through antihypertensive agents, alone or in combination. For practical purposes, antihypertensive agents can be divided into five groups: diuretics, beta-adrenoceptor blocking drugs, drugs acting on the central nervous system, adrenergic neuron blocking drugs and vasodilators[64]. Many investigations have now demonstrated that BP can be successfully controlled by these currently available agents. Especially the combined use of beta-blockers, vasodilators, and diuretics has appeared to be an effective approach in the management of moderate to severe hypertension[290]. Though hypertension is controllable in most patients, it is estimated that only a small percentage on drug therapy is close to optimal control[24]. There seem to be two main reasons for the failure of treatment. First, anti-

hypertensive agents produce quite significant adverse effects. This is the more arresting as hypertension *per se* is accompanied by few symptoms. Strikingly, several reports on antihypertensive treatment have included a comment that hypertensive patients "feel better" when given a placebo in comparison with antihypertensive therapy[64]. Secondly, a subset of patients, many of whom have renal or renovascular disease, fail to respond to all currently available drugs[136 176 204 205].

Considering the above, it is clear that there is still a need for potent antihypertensive agents, which would correct hypertension with no or few adverse effects. Now that active participation of the renin system is recognized as an important factor in the maintenance of human hypertensive disease, inhibition of this system might provide an effective tool in treating elevated arterial pressure.

1.3 Inhibition of the renin-angiotensin system

Much evidence has accumulated that the renin-angiotensin system (RAS) plays a pivotal role in the regulation of renal and adrenocortical function, the electrolyte and water balance and the arterial pressure. In the next few sections a brief description of the RAS and its role in the maintenance of BP in normal and hypertensive subjects will be provided. After that, the clinical implications of antirenin drugs - available before the advent of captopril - will be reviewed.

The renin-angiotensin system

The first mention that kidney disease may produce cardiac dysfunction goes back to 200 BC when Choun-Yow-I wrote: "when the pulse upon depressing is very firm and upon superficial palpation tight, then the disease has its seat in the kidney". It was not until 1898 that Tigerstedt and Bergmann established a role of the kidney in BP regulation in their classical experiment in which they produced hypertension in rabbits by injecting a crude extract of kidney, which they called renin[260]. In the following years the cascade of the renin-angiotensin system, as depicted in Figure 1, was gradually discovered.

Renin, a proteolytic enzyme, is mainly synthesized, stored and secreted by the juxtaglomerular apparatus. Its release is effected by a variety of physiologic stimuli, including the pressure in the renal afferent arteriole (baroreceptor mechanism), the concentration of sodium in the macula densa, the stimulation of renal sympathetic nerves and the serum potassium concentration[60 62 99 265]. Renin cleaves - after activation - its substrate angiotensino-

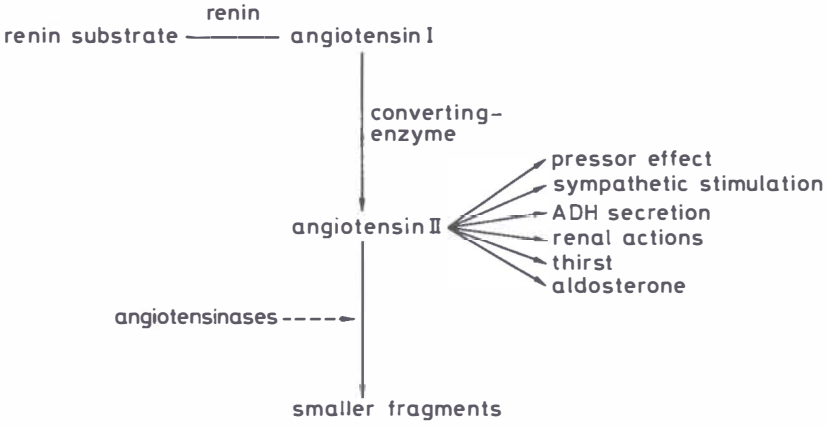


Figure 1. The renin-angiotensin system.

gen, which is an α_2-globulin, to release angiotensin I (A I). This decapeptide may be considered a prohormone as it is in itself an almost inactive substance[115 195 196 197]. Activation occurs by the proteolytic action of converting-enzyme (CE) which was discovered by Skeggs in 1954[238]. This enzyme cleaves the dipeptide His-Leu from A I; the remaining octapeptide is angiotensin II (A II).

A II is the principal circulating hormone of the RAS. Little is known about the cellular mechanism of action of this hormone. For a positive identification of a physiologic receptor a conclusive demonstration is required of the relation between angiotensin binding and a metabolic response, which is not available at the present time[63]. The main actions of A II are its immediate vasopressor effect and its dominating role in aldosterone secretion. Beyond the vascular smooth muscle and the adrenal cortex, A II acts within the central nervous system, at the adrenergic nerve endings and on the adrenal medulla. These actions mainly amplify its vasoconstrictive effect on the peripheral vascular system. In addition, the hormone decreaseas renal outer cortical blood flow, increases tubular sodium reabsorption, stimulates secretion of antidiuretic hormone and induces thirst, all of which augment the action of the hormone on other volume-expanding mechanisms[30 47 80 108 158 227 243].

The role of the renin-angiotensin system in the maintenance of arterial pressure in normotensive and hypertensive subjects

Though A II is a potent arteriolar vasoconstrictor, its mere presence in circulating blood does not necessarily imply a physiological role in the maintenance of arterial pressure. However, studies combining graded infusions of A II with its assay in plasma indicated that both in recumbent normotensive subjects and patients with essential hypertension on a normal intake of sodium, peripheral A II concentrations are within a range having only a small, but distinct effect on arterial pressure[20]. Sodium depletion, while elevating A II, diminishes the pressor effect and increases the aldosterone-stimulating action of a given plasma A II concentration. In spite of this, the arterial pressure is mainly dependent on A II in this situation[30 198].

One may speculate whether average Western sodium intake represents the normal physiologic situation in man. Several "unaccultivated" societies are known to have a low daily sodium intake. Plasma renin activity and urinary aldosterone excretion have been measured in one of these societies where daily urinary sodium excretion averaged only 1 mmol. Both variables were found high according to our standards of normality[201]. Therefore, it is probable that the RAS once played an important role in pressure and volume homoeostasis, whereas it became less important when renin was suppressed by an increase of salt consumption.

A primary pathogenetic involvement of the RAS in the maintenance of arterial pressure in patients with essential hypertension was postulated by Laragh[157]. Inappropriate renin secretion in relation to sodium intake would lead to vasoconstriction in patients with high and normal values of plasma renin activity. However, haemodynamic studies have revealed that total peripheral resistance is higher in patients with low renin values than in those with normal or high renin[80]. It may be concluded that this vasoconstriction-volume hypothesis is too much challenged by these facts.

The role of the RAS in hypertension caused by renal artery stenosis is not quite clear yet. In experimental studies it has been shown that the rise in arterial pressure, following application of the clip, can be explained by the direct pressor action of the immediate consequent rise in A II. In a later phase A II levels are proportionally lower though BP remains high. This phase is probably encountered in most patients with renovascular hypertension. It has been proposed that a slow, progressively developing pressor action of A II is responsible for the elevated BP. It may well be that additional factors other the the RAS have become involved[9 21 22 31 53 165 180].

Finally, high blood pressure in patients with chronic renal disease is mostly secondary to sodium and water retention which occurs as the kidneys lose the capacity to rid the body of even modest amounts of dietary salt and water. The A II levels in these patients are usually normal. "Normal" in this clinical setting may, in fact, be inappropriately high. Antagonism of the RAS will probably have at most a modest effect on BP since correction of hypertension can be achieved by dialysis without difficulty in most cases. However, in a subset of patients high BP is maintained by very high plasma levels of A II; one would expect in these a reduction of arterial pressure as a result of inhibition of the RAS[59 204 205 229].

Inhibitors of the renin-angiotensin system

Drugs that specifically antagonize actions of biologically active endogenous substances or prevent their formation have traditionally provided some of the most powerful tools for analysis of the physiological and pathophysiological functions of these substances. In addition, these drugs may also possess therapeutic properties. Inhibitors of the RAS have therefore been sought; the more so as they could turn out to be effective antihypertensive agents.

Inhibition of the RAS has been studied at different sites of the system (Figure 2). The RAS is biochemically complex and is therefore vulnerable at

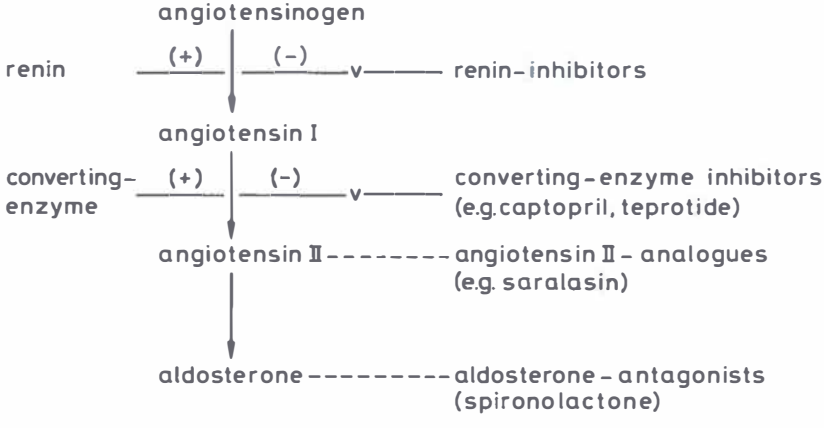


Figure 2. Blockade of the renin-angiotensin system by drugs operative at different sites of the system.

a number of distinct sites. As early as 1953 *antibodies against renin* were shown to be successful in preventing the development of renal hypertension[274]. Recent purification of renin has paved the way to the study of the therapeutical usefulness of a specific antibody in man. *Inhibitors of the renin-renin substrate reaction* form also a field of intensive interest. Both renin antibodies and inhibitors of the renin-renin substrate reaction have already been used in experimental studies; it seems to be only a matter of time before they will be available for clinical use[108 226 244 252].

Numerous *analogues of angiotensin II* have been synthesized. These compounds have all substituted an alternative amino-acid in stead of alanine in position 8 of the molecule of authentic A II. The intrinsic agonist activity of these drugs, which is dependent on the prevailing A II-level, hampers the identification of the exact role of A II in the maintenance of BP. *Saralasin* (1 sar-8 ala-angiotensin II) is the most widely used, both clinically and experimentally, of these A II-antagonists. The use of the drug has been studied by van Hoogdalem in our clinic[124 125]. An extensive review of experience with this compound is given in the symposium edited by Vaughan and Peach[268]. The drug was used to study the involvement of the RAS in normotensive and hypertensive cardiovascular homoeostasis. In addition, the drug has been employed extensively as a prognostic guide to predict a favourable outcome of renal artery surgery in patients with renovascular hypertension[68 245]. After initial enthusiasm the usefulness of the drug for this purpose has been questioned because of a high score of false positive and negative results[257]. In addition, saralasin has two serious drawbacks which limit clinical application. First, the drug has to be given intravenously, which limits its therapeutical value. Secondly, a rebound of the BP may occur after withholding the drug. This is a dangerous complication: fatal accidents have been described[146].

For about a decade it has also become possible to inhibit the RAS by *blockade of converting-enzyme* (CE). In 1970, several inhibitors of converting-enzyme were found in the venom of the South American snake Bothrops jararaca[86 87]. Based on the structure of one of the snake-venom peptides the nonapeptide *teprotide* was synthesized[78 203]. Several investigators observed that teprotide lowered BP acutely in hypertensive patients, generally in proportion to the pretreatment plasma renin level. But again, the need to give the drug intravenously seriously limited its therapeutical value.

The prospects of clinical application of CE inhibition were greatly expanded by the development of the orally-active converting-enzyme inhibitor *captopril*[202 203 225 241 251]. After its introduction in clinical medicine in 1977, extensive studies have been started on its place in antihypertensive treatment. The drug has also been studied in our clinic by Prins. His final, tentative conclusion was that the drug possesses antihypertensive properties, which exceed the results obtained with other currently available therapeutics[217]. A definite conclusion as to its proper place in antihypertensive treatment could then not be provided since long-term results were not available.

1.4 Captopril

In the following sections a general survey of captopril will be given. Because the stream of publications on the drug is progressively swelling, only a selection of clinically relevant data will be reviewed. Experimental animal studies will only be referred to if relevant for practical purposes.

Design and development

Early studies with substrates and inhibitors of converting-enzyme (peptidyl-dipeptidase) had indicated that the enzyme is a zinc-containing metalloprotein, rather similar to pancreatic carboxypeptidase A. Based on this analogy and the fact that the latter enzyme was known to be inhibited by D-benzylsuccinic acid, Ondetti and Cushman argued that inhibitors of CE might be produced by succinyl amino-acids that correspond in length to the dipeptide cleaved by CE[57 58 202]. On the basis of these considerations succinyl-1-proline (SQ 13,745) was synthesized which turned out to be an only moderately potent inhibitor of CE. Subsequent manipulation with this compound ultimately led to the synthesis of series of carboxyl alkanoyl and mercapto alkanoyl derivates that acted as potent competitive inhibitors of the enzyme. Most active was D-3-mercapto-methylpropanoyl-1-proline or captopril (Figure 3).

Pharmacological properties

Captopril dissolves in water and is best absorbed in the fasting state. About 70 per cent of the drug is excreted by the kidneys within 8 hours after ingestion. The remainder is excreted by the liver in 24-48 hours. The drug has been shown to accumulate in patients with impaired renal function[138 241].

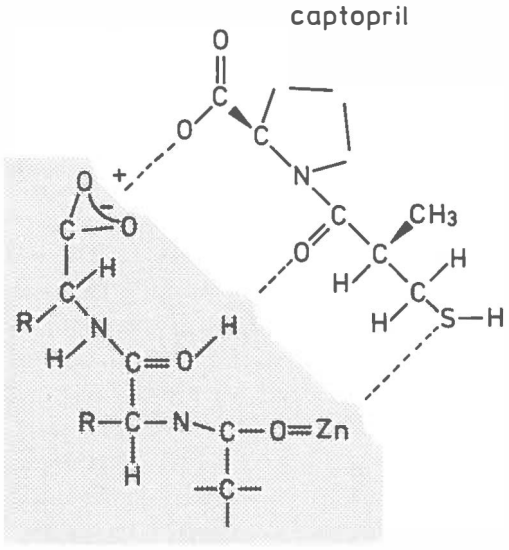


Figure 3. Inhibition of the active site of converting-enzyme by captopril. The shaded area represents the active site of converting-enzyme.

When tested in vitro on smooth muscle preparations, captopril selectively reduced responsiveness to A I without influencing that to A II or to many other vasoactive agents with the notable exception of bradykinin (BK)[57]. The exception is explained by the discovery of Erdös that CE and kininase II, the enzyme that catalyzes BK by cleaving a dipeptide, are identical[79]. It is important to recognize that CE catalyzes both the synthesis of A II, the most potent endogenous pressor substance known, and the destruction of BK, the most potent endogenous vasodilator. Losing sight of this harbours the risk of misinterpretation of the effects of CE inhibitors.

The interpretation of pharmacological studies with captopril are seriously hampered for the following reasons. First, there is no readily available assay to determine plasma levels of the drug at the moment. Secondly, BK has a very short half-life and hence blood sampling and storing is critical in obtaining reliable results[134 135]. Moreover, the immunochemical assay is technically difficult to perform. Thirdly, the assay of A II also presents considerable technical difficulties in the presence of CE inhibitors. Most antibodies raised against this hormone crossreact at least to a small extent with A I.

Since A I accumulates in the circulation during CE inhibition, levels of A II may be falsely found elevated[13 81 189]. Finally, it has been shown that the assay for plasma CE activity is unreliable in the presence of a CE inhibitor unless performed immediately after blood sampling[56 71 224]. The reason for this is that CE activity gradually increases during storage by a hitherto unexplained mechanism. If these analytical difficulties are not accounted for, many publications may lead to serious misinterpretations.

Studies, in which reliable assays have been used, show that captopril lowers A II and aldosterone with a converse rise in PRA and A I[181 189]. The mechanisms by which captopril raises PRA (and A I) are as yet unclear. Possible mechanisms include reflex sympathetic activation, the interruption of the feed-back system which exists between BP and/or A II and PRA release, and stimulation of the synthesis of prostaglandins[6 154 175].

Determinations of changes in plasma BK concentrations during CE inhibition have yielded variable and somewhat conflicting results. Dealing with BK levels during captopril, most authors agree that these remain unaltered[141 181 248].

In the light of the present knowledge on the mode of action of captopril, one would expect its effect on BP to be proportional to the pretreatment level of plasma renin. Such a correlation is described by many authors but at the same time completely denied by others. The correlation, if it was found, was poor in most studies[37 38 43 96 169 170 215 254]. The existence of a correlation does not necessarily imply a cause and effect relationship; factors other than a fall in plasma A II might be involved. A number of alternative possibilities exists. Arterial pressure may be maintained by A II generated not only in the circulation but also within the blood vessel wall[206 247 258]. A second possibility is that captopril potentiates BK in tissues; this local accumulation, which is not reflected in plasma levels, might modify the hypotensive response. Evidence in favour of such a mechanism has been adduced in studies which describe how the hypotensive action of captopril could be diminished by infusions of either aprotin (a potent inhibitor of plasma kallikrein) or kinin antibodies[41 207]. Thirdly, changes in prostaglandins, aldosterone secretion and renal sodium handling induced by captopril might all contribute to the antihypertensive effect[15 187 191 193].

Clinical application

The pharmacological properties of CE inhibitors have led to the hypothesis that these drugs would be useful in assessing the participation of the

RAS in hypertensive disease, in screening for overactivity of this system and, most of all, in treating patients with hypertension[34 46 109 218]. Captopril would be expected to lower BP when associated with high peripheral levels of A II. Therapeutical benefit has actually been reported in patients who suffered from hypertension associated with renin-secreting tumour, renovascular hypertension with high plasma renin, Bartter's syndrome and renal and vascular crises due to accelerated hypertension, scleroderma, necrotizing vasculitis and haemolytic-uraemic syndrome[8 16 129 131 152 166 185 284].

The number of cases in which captopril lowers BP in hypertensive patients with normal or even low plasma renin is surprising[2 9 38 173]. There is an awareness that such a high incidence of depressor responses should not be ascribed exclusively to the involvement of the RAS because of the participation of other systems in these responses[41 174 184 187]. It must be recognized, however, that a depressor response observed in the presence of a "normal" plasma renin level cannot be dismissed as the consequence of some action of the drug beyond the RAS. The persistence of "normal" renin and A II levels in the presence of hypertension (or sodium retention or both) may in fact be inappropriate[14 37 156].

The combination of captopril with diuretics has been proved very effective[12 151 283]. Sodium depletion sets into operation the normal physiologic mechanism of increased renin secretion to maintain arterial pressure; captopril prevents this correction. Apart from this, the vascular wall smooth muscle relaxation induced by captopril is enhanced by a diuretic[49]. Captopril has also been combined with beta-blockers; again, its BP lowering capacity increased by this combination probably through the prevention of reflex sympathetic activation[246].

Captopril decreases vascular resistance without initially changing cardiac output. Pulmonary capillary wedge pressure decreases, which is probably due to an improved unloading of the left ventricle by the decrease in BP or by pooling of the blood through arteriolar and venous dilation[38 50 83 92]. These effects make the drug suitable for treating patients with congestive heart failure. Preliminary studies have shown that captopril improves cardiac function in these patients[61 71 256]. With regard to its renal effects, an increase in renal blood flow has been observed[121 183]. Experimental studies have shown that this effect may lead to application of the drug for prophylaxis or treatment of acute renal failure due to shock, renovascular surgery or surgery that requires extracorporeal circulation[252].

In conclusion, many studies have underlined the efficacy of captopril in

the treatment of hypertension and congestive heart failure. Most of these studies, however, include only a moderate number of patients with a relatively short follow-up.

Adverse effects

Adverse effects include those reactions which are a consequence of an overshoot of the therapeutical action of captopril (untoward reactions) and those which are unexpected (side effects). The adverse effects which have hitherto been described are summarized in Table I.

TABLE I. Adverse effects of captopril.

Untoward reactions	hypotension, shock[7 8 130] rebound hypertension[159] renal function loss[52 84 105 127] myocardial infarction[18] stillbirth (?)[28] hyperkalaemia[275]
Side effects	rash[286] ageusia[177 272] fever[43 217] arthralgia[217] proteinuria[12, 44, 126, 128, 216, 223, 233] angioneurotic oedema[217] pemphigus[210] hepatitis[266] polyneuropathy[11] anaemia[159] agranulocytosis[77 97 242 264] aphthous ulcers[232] eosinophilia[150] serum sickness[132]

Captopril may evoke a steep BP fall leading to shock in patients which are sodium-depleted. Renal function loss and myocardial infarction have been described in patients with too drastic a BP fall, mostly as a consequence of a large volume deficit. The development of renal function loss during captopril may be dangerous because hyperkalaemia will then readily develop as a consequence of the suppression of aldosterone secretion.

Finally, dangerous "rebound hypertension" may occur during the first days of treatment. Figure 4 shows an example of the enormous differences in BP which were observed in one of our patients during the first week of treatment with captopril.

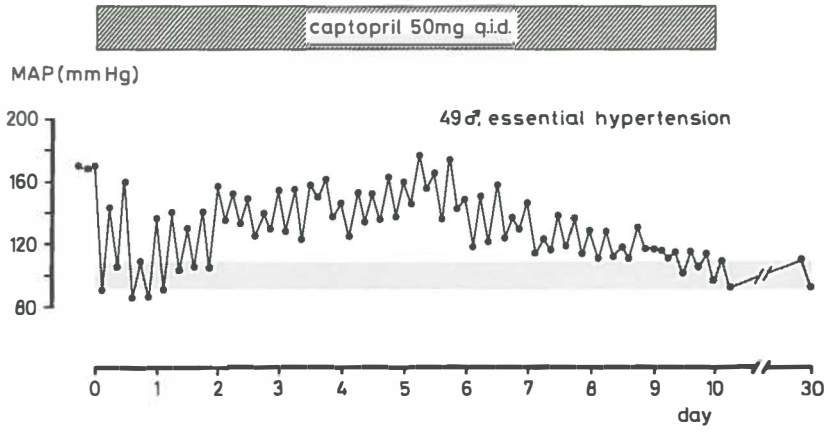


Figure 4. Course of blood pressure in a patient during the initial phase of captopril therapy. Values obtained one hour before and after each captopril dose are shown. Mean arterial pressure (MAP) fell clearly after the first doses but returned toward control levels during the first 6 days before reaching a stable level representative of the BP during prolonged treatment (indicated by the dotted band).

Rash, fever, arthralgia and ageusia are the most commonly described side effects of captopril. In addition, proteinuria due to membranous glomerulopathy, agranulocytosis, bone-marrow aplasia, aphthous ulcers, pemphigus, hepatitis and a serum-sickness-like syndrome have also been published. Most of these were reported incidentally as case reports. The incidence and seriousness of most of these side effects have not yet been established.

1.5 Scope of this thesis

This thesis deals with the antihypertensive effect of captopril in clinical practice. Efficacy and toxicity of the drug have been studied prospectively for a period of up to 18 months in a population of 89 hypertensive patients (chapter 2).

The vascular and renal response to captopril has been investigated in greater detail. As to the former, the relation between the hypotensive response and the blockade of the RAS has been studied in some carefully selected patients. This was done by comparing the time course of the BP response, the changes in renin release and the degree of CE inhibition using the vasopressor effect of exogenous A I (chapter 3). The renal response has been studied by comparing renal function studies before and after 6 and 12 months of treatment. This was done because of the important relationship

that exists between blood pressure, renal sodium handling and renal haemodynamics (chapter 4).

The incidence and the seriousness of immune complex glomerulopathy and agranulocytosis will probably determine the applicability of captopril in clinical practice. The incidence and clinical course of membranous glomerulopathy will be reviewed on the basis of literature and our own experiences. Possibly involved pathogenetic mechanisms and clinical significance will be discussed (chapter 5).

The summary and final conclusions of this thesis are presented in chapter 6. The chapter ends with a look toward the future of RAS inhibition.

Chapter 2
Efficacy and toxicity of captopril
A prolonged study in 89 hypertensive patients

2.1 Introduction

The CE inhibitor captopril was introduced in the treatment of hypertension in 1977. Since then the potency of the drug to lower BP has been established in many studies as summarized in chapter 1. Most of these studies deal with patient groups that are small with a relatively short period of follow-up. At the same time, the number of reports dealing with occasionally observed side effects during captopril treatment is rapidly expanding. So far a prospective study concerning long-term efficacy and safety of captopril treatment in a large group of antihypertensive patients has not been published.

The present study deals with the merits and demerits of CE inhibition by captopril in a group of 89 hypertensive patients. This study was carried out prospectively for a period of up to 18 months; the average duration of treatment was 11 months (range 1-18 months).

2.2 Patients and methods

Patients

The patients were included in the study when supine diastolic blood pressure (SDBP) was $\geqslant$ 95 mmHg on 2 separate occasions and after informed consent. The relevant data are summarized in Table II. All patients had a thorough examination including rapid-sequence intravenous urography. Renal angiography and determination of renal vein renin ratios were performed whenever there was any clinical or roentgenologic suspicion of renovascular disease. Renal biopsy was performed when renal parenchymal disease was suspected. The patients were classified into 3 groups: essential hypertension (EH), renovascular hypertension (RVH) and hypertension associated with renal parenchymal disease (RPD). Additional data on the last of these 3 groups are provided in Table III.

TABLE II: Characteristics of the studied patient population. Mean (± SD) is given numbers in parentheses represent ranges.

Patients (no)	89
Age (years)	42.5 ± 11.6 (18-65)
Sex (no)	
Male	57
Female	32
Diagnosis (no)	
Essential hypertension	35
Renovascular hypertension	38
Renal parenchymal disease	16
Duration of hypertension (years)	5.7 ± 6.0 (0-28)
Weight (kg)	73.9 ± 13.1 (45-116)
Blood pressure (mmHg, with previous therapy)	
Systolic	169 ± 31 (117-260)
Diastolic	110 ± 14 (85-155)
Blood pressure (mmHg, without therapy)	
Systolic	185 ± 35 (136-260)
Diastolic	118 ± 16 (95-160)
Severe hypertension (no)	47
Refractory hypertension (no)	30
Severe retinopathy (no)	21
Cardiomegaly (no)	38
Left ventricular hypertrophy (no)	45

TABLE III. Diagnosis in patients with renal parenchymal disease.

Analgesic nephropathy	2
Arteriolar nephrosclerosis	2
Haemolytic uraemic syndrome	1
Polyarteritis nodosa	1
Polycystic kidney disease	4
Poststreptococcal glomerulonephritis	1
Systemic lupus erythematosus	1
Transplant kidney, chronic rejection*	2
Tuberculosis renis	1
Tubulo-interstitial disease	1

* in 1 patient also diabetic glomerulosclerosis

Hypertension was classified as "mild" when SDBP did not exceed 104 mmHg, "moderate" when SDBP was between 105 and 119 mmHg and "severe" when SDBP exceeded 119 mmHg. BP was considered therapy-resistant when BP control (SDBP < 105 mmHg) could not be achieved by standard triple therapy or by a comparable drug combination therapy, provided adequate doses of the drugs had been used.

Dose titration and follow-up

Patients with mild or moderate hypertension were treated in the hypertension outpatient clinic whereas those with severe or refractory hypertension were hospitalized.

The outpatients were instructed to adhere to a moderately sodium restricted diet (100-150 mmol sodium/day) and received a placebo for a period up to 8 weeks. Captopril was started in a dosage of 25 mg t.i.d. The dosage was increased in one or two weekly intervals until either SDBP decreased below 95 mmHg or the maximally daily dosage (450 mg) was reached. A diuretic (40 mg furosemide o.i.d. or 50 mg hydrochlorothiazide o.i.d.) was added when SDBP still exceeded 95 mmHg.

The hospitalized patients (n=50) were instructed to keep rigid dietary sodium restriction (10-80 mmol sodium/day); they did not receive a placebo. Captopril was given 72 hours after complete withdrawal of previous antihypertensive medication. Captopril had to be started in 2 patients during clonidine treatment because of the occurrence of a withdrawal syndrome after dose reduction of clonidine. Propranolol was continued in low dosages in 5 patients. The captopril doses were increased faster than in the outpatients; the final dosage was mostly reached within 14 days.

All patients visited the outpatient clinic monthly during the first year and two-monthly afterwards. If hypertension recurred, treatment included either an increase of the captopril dosage or addition of a diuretic. Reduction of the captopril dosage or withdrawal of diuretics was applied occasionally. The patients were requested to contact the clinic immediately whenever rash, fever, arthralgia or a sore throat were noticed.

One patient died of myocardial infarction occurring after 7 months of captopril treatment.

Methods

BP recordings were performed by a specially trained nurse. The BP was recorded both after 10 minutes in the supine position and after one minute standing using the London School of Hygiene sphygmomanometer. Diastolic BP was read when the sounds became muffled (Korotkoff IV).

Body weight was adjusted for height according to the Quetelet index ($10^5 \times$ weight (kg)/[height (cm)]^2).

Blood samples and 24-hour urine were collected before the start of captopril and at each outpatient visit. The performed haematological and biochemical investigations are summarized in Table IV. All determinations

TABLE IV. Laboratory investigations.

Blood	Haemoglobin
	Haematocrit
	White blood cell count (WBC)
	Platelets
	Differential count
	Eosinophil count
	Sodium
	Potassium
	Chloride
	Urea
	Creatinine
	SGOT
	SGPT
	Total protein
	Serum albumin
	ANA
	Anti-ds DNA antibodies
	PNF
Urine	Sediment
	Protein (dipstick)
	Glucose
Urine (24 hour)	Volume
	Sodium
	Potassium
	Creatinine
	Protein

were performed according to routine laboratory procedures. Type and titer of antinuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-ds DNA antibodies) and perinuclear antibodies (PNF) were performed according to methods described in detail elsewhere[1 179 200 288]. Fundoscopy, electrocardiograms and chest X-rays were performed immediately prior to institution of captopril and afterwards at half-yearly intervals.

Fundoscopy was performed by an ophthalmologist and classified according to the Keith-Wagener (KW) classification[147]. Electrocardiograms were coded for voltage criteria, left ventricular hypertrophy (LVH) and S-T abnormalities by two independent observers[221].

Chest X-rays were analyzed by two independent observers. The cardiac-thoracic ratio (CT ratio) was determined by using the method described by Bucher[39]. Cardiomegaly was supposed to exist when this

coefficient exceeded 0.49. Though the CT ratio is a readily accessible tool for the clinician to gain an impression of the size of the heart, the method of Jonsell determining cardiac surface has proved to be more accurate[3] [143]. Hence, we additionally determined cardiac surface using the latter method with the aid of a computer (see appendix 1).

Data management and statistical analysis

The data of all patients were stored and processed on a Cyber 74-16 (CDC). Since the patients have been investigated for different follow-up periods, we used paired data to denote differences between the various treatment intervals (1, 3, 6, 9, 12, 15 and 18 months). Statistical analysis consisted of the following tests: Student t-test testing the significance of any difference between parameters supposed to be normally distributed, otherwise the Wilcoxon test. Furthermore, correlation coefficients were calculated. All p values were considered significant when smaller than 0.05. In the next chapters, the term "significant" will only be used in a statistical sense.

2.3 Results

Blood pressure

Figure 5 depicts the course of BP both in the supine and upright position. BP increased significantly after withdrawal of previous antihypertensive medication. Treatment with captopril resulted in an impressive fall of supine systolic blood pressure (SSBP) and SDBP. The mean SSBP decreased from 185 to 140 mmHg, while the mean SDBP diminished from 118 to 88 mmHg on captopril therapy after one month of treatment. The relative change of MAP* (expressed as percentage change) between the various treatment intervals is shown in Figure 6. A significant, though minimal, further decrease in MAP was observed at the end of month 3 compared to the results after one month of therapy. After that no further changes in the course of MAP could be detected. The course of MAP after the start of captopril treatment was similar in the supine and standing position.

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* MAP is mean arterial pressure, estimated from the sum of SDBP and one-third of the pulse pressure.

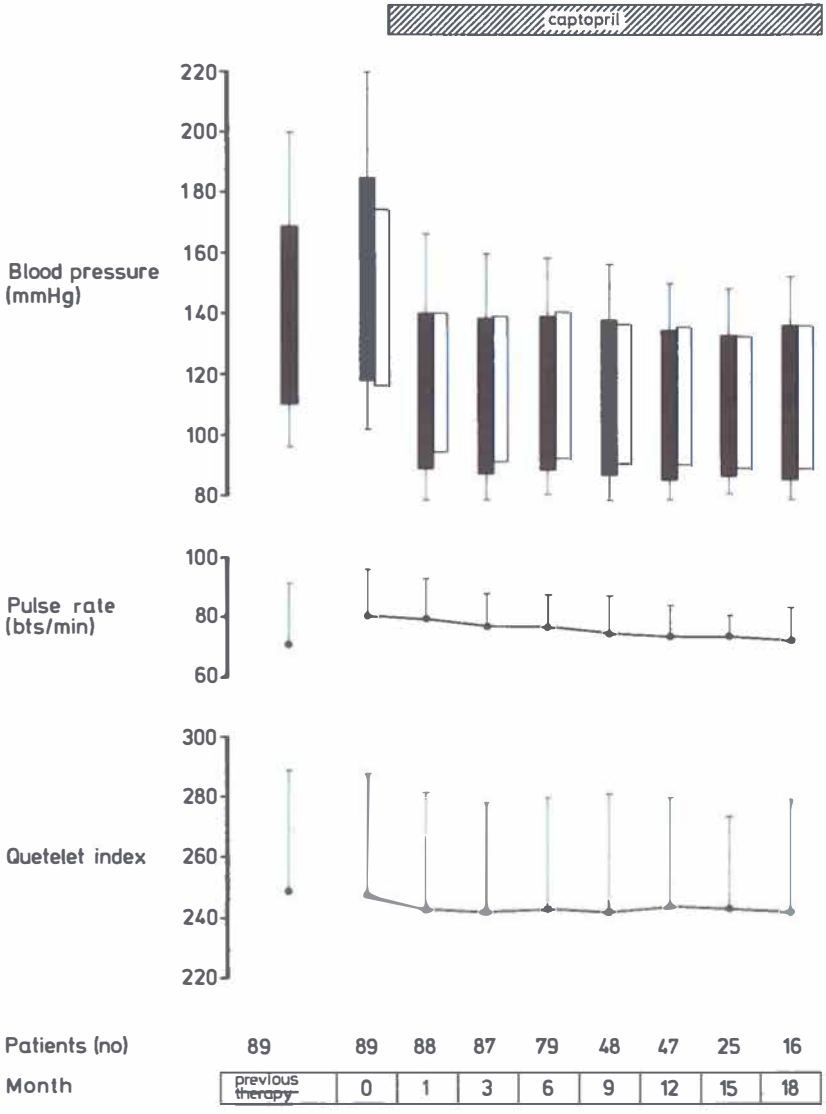


Figure 5. Course of blood pressure (SSBP and SDBP), pulse rate and Quetelet index after withholding previous therapy and during captopril treatment. Mean + SD is shown. The discrepancy in patient numbers is caused by different follow-up periods. Supine BP is indicated by closed bars and BP in the standing position by open bars.

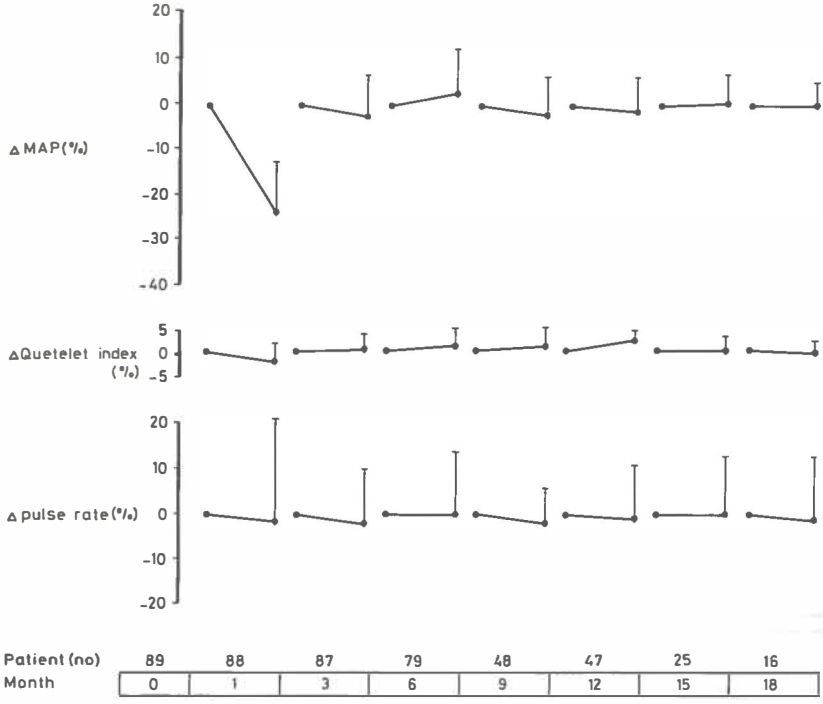


Figure 6. The mean + SD of the individual percentage change in supine MAP, Quetelet index and pulse rate between successive treatment intervals.

Withdrawal of previous therapy resulted in a significant rise in pulse rate from 70 to 80 beats per minute. However, pulse rate gradually decreased during prolonged therapy with captopril. Quetelet index decreased significantly after the start of captopril therapy at the end of month 1. This variable remained unaltered after its initial decrease (Figures 5 and 6).

The relation between initial MAP (without therapy) and the fall of MAP (Δ MAP) is shown in Figure 7. There was a significant relationship at the end of month 1 ($r = 0.79$) and month 3 ($r = 0.84$) of treatment.

The distribution of SSBP and SDBP before captopril and at the different intervals of treatment is given in Figure 8. The proportion of patients with an initial SSBP or SDBP above 150/95 mmHg decreased from 92 to 28 per cent (SSBP) and from 98 to 21 per cent (SDBP) after one month of therapy. The proportion of subjects with SDBP above 105 mmHg decreased from 84 to 6 per cent in that period. These figures tended to improve further

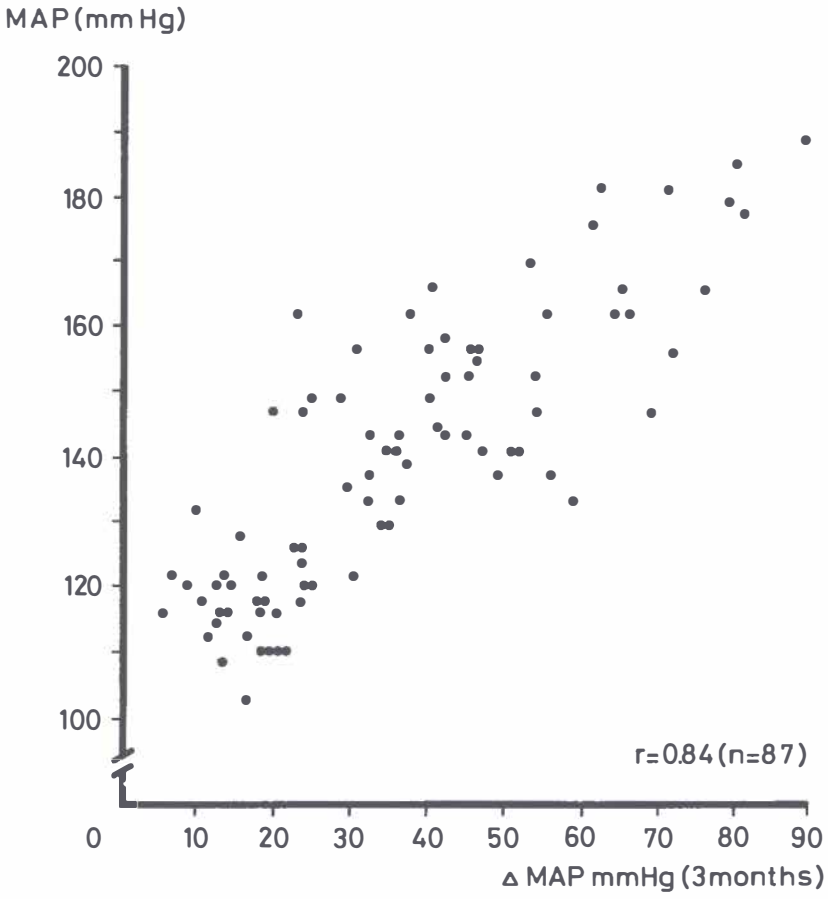


Figure 7. The relation between supine MAP and Δ MAP after 3 months of treatment.

during prolonged treatment. After 12 months of therapy only 10 per cent had a SSBP above 150 mmHg while 7 per cent had a SDBP exceeding 95 mmHg; the corresponding values after 18 months amounted to 12.5 and 0 per cent, respectively.

Damage of "target" organs

Central nervous system, kidney and heart are generally considered to be the organs that suffer most from the deleterious effects of high blood pressure. The effects of captopril treatment on the damage of these "target" organs observed in this study will be given in the next few sections.

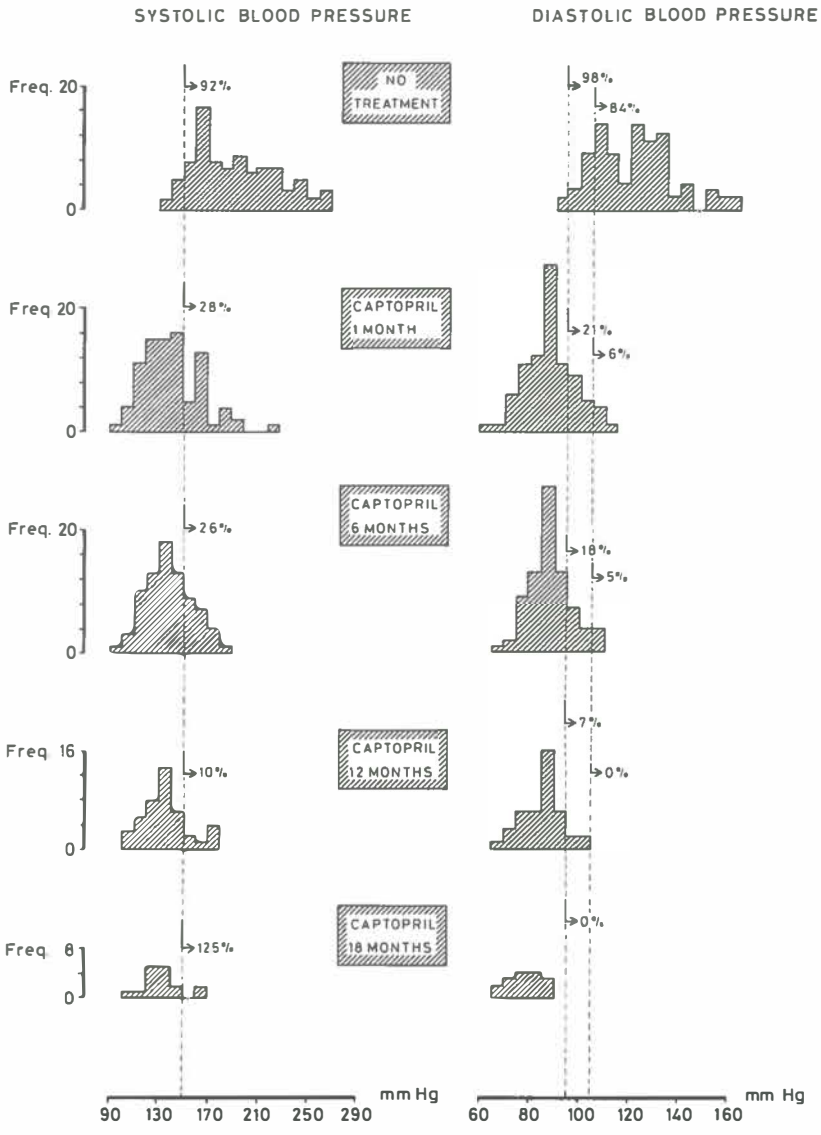


Figure 8. Distribution of supine systolic blood pressure and supine diastolic blood pressure prior to captopril and after the successive treatment intervals. The percentage of patients, which have a systolic BP of > 150 mmHg or a diastolic BP of > 95 and > 105 mmHg, respectively, has been marked by dotted lines.

Retina. Severe retinopathy (KW grades III and IV) was established in 21 patients before captopril treatment (Table V). Fundoscopy had improved in all but one patient after 6 months of therapy. A gradual shift to KW grade 0-I of all patients initially having grade II or more was observed during prolonged therapy.

TABLE V. Results of fundoscopy (Keith-Wagener classification) prior to and during captopril treatment. N = number of investigated patients.

Month	0	6	12	18
KW grade IV	8	0	0	0
KW grade III	13	1	0	0
KW grade II	43	22	10	1
KW grade I	25	46	31	15
	N = 89	N = 69	N = 41	N = 16

Kidney. A slight, significant increase in serum creatinine and a significant decrease of creatinine clearance had occurred after one month of therapy. However, this decrease in renal function disappeared after 3 months of treatment. Renal function did not change afterwards during the remaining period of therapy. Details are given in Table VI.

Heart. Repeated electrocardiography showed an improvement of voltage criteria. Maximum praecordial voltage decreased significantly after 6 months of therapy (Table VII). The number of patients meeting the criteria of LVH decreased from 38 to 13 during this period.

TABLE VI. Serum creatinine and creatinine clearance (mean ± SD) prior to and after 1, 6, 12 and 18 months of captopril therapy. Moreover, the percentage changes (mean ± SD) between the various follow-up periods are shown. N = number of investigated patients.

Month	0	1	6	12	18
Serum creatinine (μmol/l)	121 ± 87	133 ± 95	121 ± 79	113 ± 48	115 ± 74
percentage change		+9 ± 32	−2 ± 14	−2 ± 8	0 ± 8
Creatinine clearance (ml/min)	84 ± 36	80 ± 43	89 ± 37	82 ± 33	90 ± 36
percentage change		−5 ± 40	+10 ± 39	−6 ± 17	+4 ± 12
	N = 89	N = 88	N = 77	N = 47	N = 16

TABLE VII. Electrocardiographic characteristics prior to and during captopril treatment. N = number of investigated patients.

Month	0	6	12	18
Sokolow index (mm)	37 ± 8.2	30 ± 6.6	30 ± 6.1	29 ± 6.1
percentage change		−19 ± 15	−2 ± 19	−1 ± 7
LVH (no)	45	13	7	3
Strain (no)	19	7	4	1
	N = 84	N = 73	N = 30	N = 15

Cardiac size (determined by CT ratio) decreased significantly during the first half year of captopril treatment. A significant further individual decrease was observed after 12 months of therapy (Table VIII). Measurements of cardiac size by the determination of the surface of the frontal projection of the heart on the postero-anterior roentgenogram (see appendix 1) also revealed a significant decrease after 6 months; afterwards no further decrease in heart size could be established.

TABLE VIII. Cardiac-thoracic ratio and cardiac surface (frontal projection on postero-anterior roentgenogram) prior to and after 6, 12 and 18 months of captopril treatment. Mean ± SD is shown. N = number of investigated patients.

Month	0	6	12	18
C-T ratio	0.49 ± 0.6	0.45 ± 0.5	0.45 ± 0.5	0.44 ± 0.6
Cardiac surface, cm^2 ("digitize method")	148 ± 26	134 ± 21	136 ± 21	137 ± 18
Cardiac surface, cm^2 ("4 points-method")	144 ± 32	128 ± 26	130 ± 23	130 ± 18
	N = 74	N = 41	N = 37	N = 12

Pharmacotherapy

The details of antihypertensive therapy are shown in Table IX. The mean daily captopril dosage was 241 ± 125 mg after one month. After that no statistically significant change in this mean dosage occurred. It appeared that 27 patients had been given a diuretic during the first month of therapy; it was successfully withdrawn in 10 of these patients during prolonged treatment. On the other hand, a diuretic had to be newly added in 11 patients.

TABLE IX. Pharmacotherapy. Mean daily captopril dosage and number of patients using a diuretic at the end of the various follow-up periods. N = total number of patients.

Month	0	1	6	12	18
Captopril (mg/24 hours)	0	241 ± 125	246 ± 125	281 ± 119	273 ± 102
percentage change		+5 ± 29	+6 ± 28	−4 ± 10	
Diuretics (no)	4	27	26	22	7
	N = 89	N = 88	N = 79	N = 47	N = 16

Electrolytes

Serum sodium had decreased significantly, though minimally after one month of therapy. This change disappeared after 6 months of therapy (Table X). Urinary sodium excretion increased steadily during prolonged treatment, all these changes between the individual observation periods (except for month 18) being significant.

Hypokalaemia (< 3.6 mmol/l) was never observed despite a frequent use of captopril in combination with a diuretic. On the contrary, serum potassium had increased significantly after one month of therapy and remained at that level during prolonged treatment.

TABLE X. Serum sodium and potassium concentrations and urinary sodium excretion prior to and during captopril treatment. N = number of investigated patients.

Month	0	1	6	12	18
Na (mmol/l)	140 ± 2.4	139 ± 4	140 ± 3	139 ± 3	139 ± 2
percentage change		−1 ± 3	0 ± 2	0 ± 2	0 ± 2
K (mmol/l)	4.1 ± 0.5	4.4 ± 0.5	4.3 ± 0.5	4.3 ± 0.6	4.5 ± 0.5
percentage change		+8 ± 15	0 ± 9	0 ± 6	+4 ± 11
Na (mmol/24 h)	86 ± 58	106 ± 74	119 ± 61	126 ± 66	100 ± 56
percentage change		+24 ± 72	+12 ± 23	+10 ± 18	−3 ± 8
	N = 89	N = 88	N = 79	N = 47	N = 16

Untoward reactions

Untoward reactions are here defined as those reactions that occur as a consequence of an overshoot (i.e. exaggerated response) of the pharmacological action of the drug. The untoward reactions to captopril treatment

observed in this study are summarized in Table XI. A severe drop in BP resulting in shock (SSBP < 90 mmHg and tachycardia) occurred in two patients, both of them being sodium-depleted. Rapid recovery followed after saline infusion in one patient and combined saline and A II-infusion in the other. A rebound of BP after a steep fall in BP together with transient neurological symptoms (hemiparesis) occurred in one patient during the second day of treatment.

Marked loss of renal function (doubling of serum creatinine or a decrease of 25 ml in the creatinine clearance) was observed during the first weeks of treatment in 5 patients. All these patients had renovascular hypertension. Sodium repletion restored renal function in 3 patients whereas one of the remaining 2 showed a marked improvement. Finally, one patient developed anginal complaints during BP reduction with captopril.

TABLE XI. Untoward reactions during captopril treatment.

Hypotension (SSBP < 90 mmHg, tachycardia)	2
Rebounding of BP accompanied by neurological symptoms	1
Renal function loss (loss of > 25 ml in creatinine clearance)	5
Angina pectoris	1
Total number of patients with untoward reactions	9

Side effects

Side effects include all those reactions that happened unexpectedly and that could not be ascribed to the pharmacotherapeutic action of the drug. Table XII lists the side effects that were observed in this study. They were noticed in 27 patients with a total number of 33 occurrences; the drug had to be withdrawn in 8 patients. Because of a possible relationship between

TABLE XII. Side effects. Figures in parentheses represent number of patients in whom drug was withdrawn.

Rash	8 (2)
Rash, arthralgia, fever	5 (2)
Rash, angioneurotic oedema	3 (1)
Ageusia	12
Proteinuria	4 (4)
Anaemia	1
Total number of side effects	33
Total number of patients with side effects	27 (8)

captopril dosage and the occurrence of side effects, the captopril dosages for patients with and without side effects were compared. The daily captopril dosage for these groups did not differ. Since captopril accumulates in patients with an impairment of renal function, the captopril dosages after correction for differences in the creatinine clearance were compared for the two groups. This comparison once more failed to reveal a statistically significant difference.

The occurrence of side effects as observed during the first half year of treatment is shown in Figure 9. The figure provides also data on the development of auto-antibodies and eosinophilia in this treatment period. It

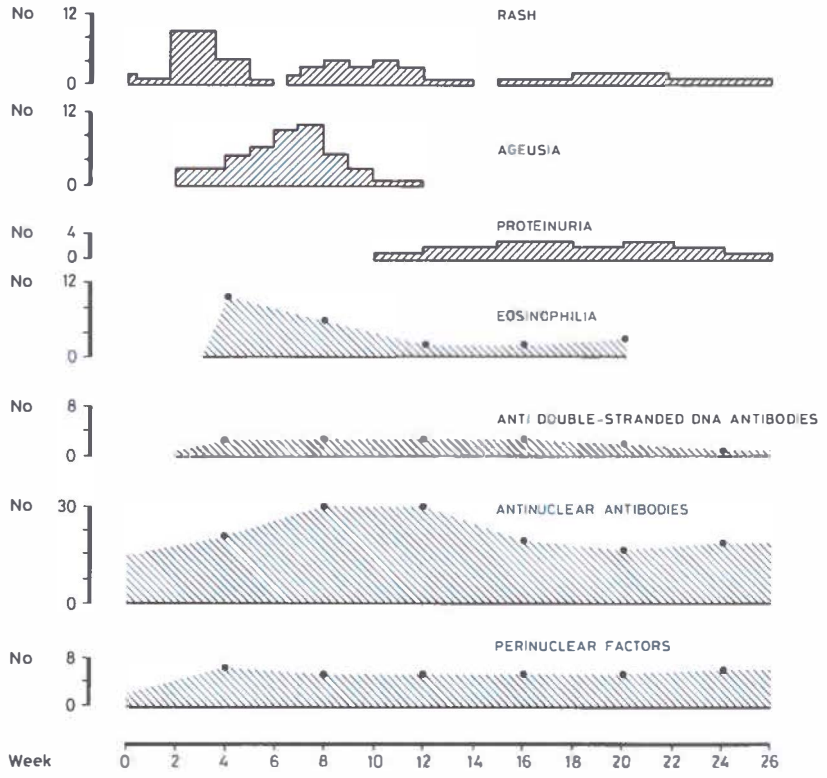


Figure 9. The occurrence of side effects, auto-antibodies and eosinophilia during the first half year of captopril treatment.

can be seen that most of these occurred between weeks 2 and 12 after the start of captopril except for *de novo* proteinuria. A brief description of each type of side effect is given below.

Rash. Rashes developed mostly during the first weeks of therapy. Their character was maculopapular or urticarial while frank epidermolysis developed in one patient. Rash was accompanied by arthralgia and fever in 5 patients, and by angioneurotic oedema in another 3. These 3 patients appeared to have a normal concentration of C_1-esterase inhibitor.

Dermal symptoms disappeared either spontaneously or after dose reduction in most patients. However, symptoms persisted in 5 patients; in these patients the drug had to be withdrawn.

Ageusia. Loss of taste developed in 12 patients after institution of captopril. This side effect was transient and disappeared generally without requiring dose reduction.

Ageusia was accompanied by loss of scalp, pubic and axillary hair in one patient. He developed at the same time rash and crumbly nails. The rash disappeared after dose reduction; the other symptoms, however, gradually worsened. Laboratory investigation disclosed an abnormally low urinary excretion of zinc, the plasma levels being in the lower range of normal. Supplementory zinc therapy resulted in a disappearance of the ageusia within two weeks. Normal scalp, pubic and axillary hair growth gradually reappeared (Figure 10).

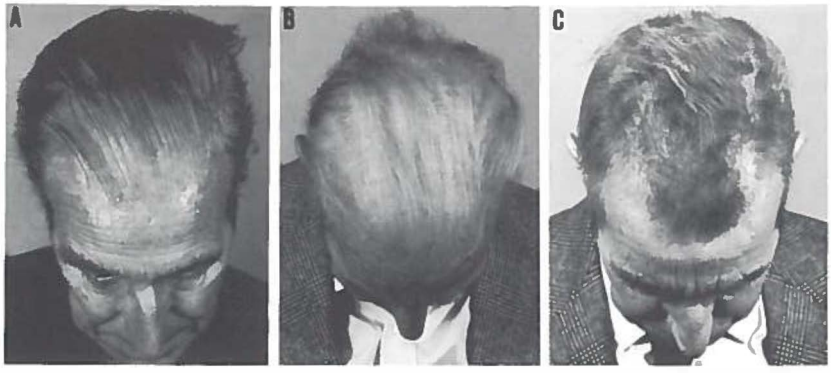


Figure 10. Baldness developing during captopril treatment (A,B). Return of hair growth after oral zinc therapy (oral zincsulphate, C).

Proteinuria. De novo proteinuria developed in 4 patients after 3 to 6 months of treatment. Renal biopsies disclosed stage I membranous glomerulopathy (MGP). The case histories of these patients are described in detail in appendix 3.

Six patients had proteinuria varying between 0.2 and 8 g/24h before the start of captopril treatment. Urinary protein excretion remained either unaltered or decreased during captopril treatment.

Haematopoietic system. Mean haemoglobin, haematocrit, white blood cell count and platelets after 6, 12 and 18 months of captopril treatment did not differ from the pretreatment values. None of the patients developed agranulocytosis or thrombocytopenia. Anaemia developed in one patient with a gradual fall of haemoglobin of 18 to 9.5 g/l. Extensive haematological studies revealed no cause of the anaemia in this particular patient. Eosinophilia (eosinophils exceeding $30 \times 11/mm^3$ while fasting) developed transiently in 15 patients. It was found in 8 patients showing rash.

Serological abnormalities. Auto-antibodies (ANA, anti-ds DNA, PNF) were found often intermittently in 52 patients during captopril treatment. The percentage of patients with one or more of these auto-antibodies in their sera was higher at weeks 8 and 12 after the start of captopril than before treatment. The development of auto-antibodies was not associated with clinically apparent side effects.

Antinuclear antibodies were detected in 15 patients before institution of captopril. These mostly decreased in titer or disappeared during the first month of therapy. Positive ANA titers appeared for the first time, often intermittently, in 36 patients during captopril treatment; they appeared in high titers ($> 1/100$) in 13. These antibodies did never fix complement and were mostly of the homogenous type. They belonged always to the IgM class, whereas in some patients also IgG-ANA and IgA-ANA could be detected.

Antibodies against anti-ds DNA developed in 3 patients. These were detected in the Crithidia-assay. The antibodies all belonged to the IgM class and were of low-avidity which was demonstrated by negative results in the Farr-assay. They did not fix complement. Like ANA, anti-ds DNA antibodies developed within the first few months of treatment and gradually disappeared. Development of these antibodies was not associated with drug-induced systemic lupus erythematosus.

Finally, perinuclear antibodies developed in 5 patients during captopril treatment.

2.4 Discussion

We have documented the sustained efficacy of captopril in antihypertensive treatment. The beneficial effects manifested themselves not only in the BP readings but were also reflected in the regression of hypertensive target organ damage. The other side of the coin was the high incidence of side effects. The antihypertensive effects and the toxicity of captopril will be discussed separately in the following sections.

Efficacy

Effective BP regulation was obtained throughout in a selected population with a predominance of severe and uncontrollable hypertension. Secondary forms of hypertension amounted to 61 per cent in this selected population whereas they occur in 4 - 7 per cent of random populations of hypertensive patients[104 136]. For this reason it is not surprising that many of the patients presented considerable cardiovascular abnormalities.

Several aspects of the hypotensive action of the drug observed in the population concerned are worth noting. Firstly, captopril alone or in combination with a diuretic is highly effective in all forms of hypertension, regardless of severity and aetiology. Acceptable BP, i.e. SDBP $<$ 95 mmHg, was achieved in 93 per cent of all patients treated with the drug for one year whereas the highest observed SDBP was 104 mmHg at that time. These figures are even more impressive when one takes into account that 33 per cent of the population studied was previously therapy-resistant. However, it should be noticed that these results were achieved in patients on a moderately to severely sodium-restricted diet. Activation of the RAS is the most important compensatory mechanism in BP regulation during sodium and volume depletion[30]. Assuming that captopril's main antihypertensive effect is mediated via blockade of the RAS, it is likely that dietary sodium restriction has contributed to the success of the applied therapy. Our results are completely in accord with those reported in recent publications on captopril therapy in severe and therapy-resistant hypertension[12 246 284].

A second important aspect is the absence of tachycardia and of postural hypotension despite the marked lowering of arterial pressure during prolonged captopril treatment. The absence of tachycardia appears to be a feature common to agents that interfere with the RAS at different levels, in-

cluding those that, like captopril, do not seem to cross the blood-brain barrier to any significant degree[92 225]. This may be a consequence of the fact that the drug is a vasodilator which affects veins as well as arterioles[51 140]. Such vasodilators have been reported to produce less tachycardia than those predominantly affecting arterioles. Blunting of the baroreceptor reflex sensitivity via enhanced parasympathetic activity could be an additional mechanism which accounts for the absence of tachycardia[112]. Captopril does not interfere with the homoeostatic cardiovascular response to posture. Cody et al. have demonstrated that heart rate responds adequately to head-up tilt in patients receiving captopril even when combined with diuretics or a low sodium diet[49]. This finding has important clinical implications in view of the frequent need for diuretics in captopril-treated patients. Acute studies with captopril and teprotide in normotensive volunteers had suggested that cardiovascular responses to posture during salt depletion were predominantly dependent on the effectiveness of the RAS[188 198 227]. However, as previously mentioned, orthostatic hypotension has not been a complication of captopril therapy either alone or in combination with sodium depletion during prolonged treatment in this study.

Thirdly, the antihypertensive effect of captopril appears to be sustained. This is the more remarkable as the drug acts like a vasodilator and waning of efficacy has been reported to take place especially during vasodilator therapy[64 290]. Secondary resistance to maintenance treatment developed in only 11 patients and was characterized by a marked responsiveness to diuretics. It has not yet been established whether the lack of secondary resistance in most patients is a consequence of the fact that sodium and fluid retention failed to occur because of a natriuretic effect of the drug via inhibition of the intrarenal renin-angiotensin system[163]. A decrease in synthesis and release of aldosterone may also have prevented fluid retention[15].

Fourthly, it is noteworthy that the antihypertensive effects could already be obtained within 14 days after initiating therapy. Many antihypertensive agents (i.e. diuretics, beta-blockers) take their optimum effect after a longer period[19 33 64 255]. The relatively fast-occurring BP response is clearly an asset when rapid correction of high BP is needed.

In conclusion, the antihypertensive action of captopril, alone or in combination with dietary or diuretic-induced sodium depletion, is highly effective and sustained. However, casual office readings may lead to a misjudgement of antihypertensive therapy[186]. Moreover, normalization of BP is not an end

in itself but is meant to prevent the risks of cardiovascular morbidity and mortality. Therefore, we thought it reasonable to evaluate the value of occasional BP readings by studying the effect of treatment on hypertensive target organs. The favourable results (improvement of hypertensive retinopathy, diminution of hypertensive electrocardiographic abnormalities and roentgenologic decrease of heart size) underline that effective BP control had been achieved. These findings are the more impressive since LVH and retinopathy grade III-IV have been shown to be ominous harbingers of cardiac and cerebral casualties[100 186]. With regard to the kidney as a target organ of elevated BP, it appeared that renal function as measured by serum creatinine and creatinine clearance had not changed. Taking into account the fact that renal function mostly deteriorates in patients with an inadequately controlled BP[111], we consider the stability of renal function a favourable effect.

Our study was not designed to determine the minimum captopril dosage needed for optimum BP control in the individual patient. The mean daily dosage used in this study was about half of the maximally allowed dosage. A common pattern in the evolution of the antihypertensive response to captopril is a triphasic course with a prompt and dramatic drop in MAP after the first doses, a rebound of BP to pre-existent levels which persists over the next 3 - 7 days and finally a definite drop of BP[159]. Captopril dosages were often increased rapidly during the first days of treatment in our hospitalized patients. An unsettled question is whether increases in captopril dosages could have been postponed for 7 days in anticipation of the definite fall of BP. If so, the maintenance therapy of many of these patients may have been too high.

It has been shown that the antihypertensive effect of captopril is enhanced by diuretics and dietary sodium restriction[12 151 283]. The latter was at first fairly well kept by most patients but patient compliance decreased during prolonged treatment which appeared from a gradual increase of the urinary sodium excretion. Nevertheless, approximately 40 per cent of the patients needed a diuretic: a percentage comparable with those in other studies[43 96]. Addition of a diuretic to dietary sodium restriction during captopril treatment lowers BP through a further reduction of the already decreased peripheral vascular resistance without appreciable changes in plasma volume[50 254]. Hence, there is a good case for combining a diuretic with captopril when BP is inadequately controlled by CE inhibition and dietary sodium restriction. This combination is the more attractive since the captopril-

induced fall in aldosterone level limits or, as in this study, abolishes the occurrence of hypokalaemia induced by diuretics.

Though the potency of captopril is a major advance in the treatment of severe hypertensive patients, the drug may initially cause too drastic a BP response. Shock, severe renal function loss and myocardial ischaemia are complications which occurred in this study, especially when captopril was given to patients with a large volume deficit. Thus we advocate caution when starting CE inhibition in such patients. It is prudent to begin captopril treatment in these patients in the hospital with a saline or A II-infusion stand-by. Moreover, BP should be monitored carefully to detect enormous swings of BP which are prone to develop especially after the first doses.

All in all, captopril is a highly effective antihypertensive agent with a sustained action when given alone during dietary sodium restriction or in combination with a diuretic. It should be added that the results have probably been favourably influenced by the organization of our outpatient clinic employing physicians and nurses with a special interest in the treatment of hypertension[5 239]. Nevertheless, the results with captopril in this study group compare favourably with the results of other forms of antihypertensive drug treatment in severe hypertensive patients carried out in an identical setting[5 176].

Toxicity

Most studies on captopril therapy are concerned primarily with efficacy and do not deal prospectively with toxicity. We observed a high incidence of clinically manifest side effects in this study. World-wide experience with captopril now extends to over 5000 patients and manifest side effects occur in about 20 per cent of the patients[72 150]. Side effects occurred in 30 per cent of the patients reported in the present study. Differences emerged in ageusia (6 per cent in the Squibb world-wide survey, 14 per cent in our study) and in the development of proteinuria (see chapter 5). The high incidence of side effects may either reflect the design of this prospective study or point to a special feature of our patient group to develop side effects, or both.

With regard to the pathogenesis of the captopril-induced side effects either toxic, allergic or idiosyncratic mechanisms may be involved[102 103]. Toxic mechanisms could include the development of rashes as a result of potentiation of kinin-mediated cutaneous reactions or the development of ageusia as a result of binding of the drug to heavy metals[177 286]. However, evidence for such toxic mechanisms is circumstantial.

Much evidence in our study favours an immunologically mediated pathogenesis of the side effects. Arthralgia, fever, serum-sickness, rashes and immune complex glomerulopathy are typical manifestations of drug allergy. The spectrum of captopril-associated side effects is strikingly similar to the one observed during treatment with penicillamine and aurothiomalate[167]. It is generally accepted that the pathogenesis of side effects occurring during treatment with these drugs is mediated by the immune system. However, the way in which captopril induces these allergic manifestations is not clear.

The development of a serum-sickness-like syndrome in one patient indicates that the drug may act as a hapten and induce antibody formation[132]. Alternatively, as shown for procainamide and methyldopa, captopril may induce immunodysregulation by altering lymphocyte function which may result in auto-immune phenomena[103 281]. Such a mechanism is suggested by the increase in auto-antibodies during the first months of therapy. In this latter concept the auto-antibodies may either directly participate in the development of clinically manifest side effects or merely be the result of immunodysregulation.

Whatever mechanisms may be involved, it should be noted that most of the side effects observed did not prejudice the long-term treatment with captopril. What is even more, most patients felt very well and appeared to have a more ideal correction of hypertension than could be obtained with most other antihypertensive agents. However, the drug should only be prescribed in these patients where the benefits of reducing the blood pressure outweigh the potentially dangerous side effects. It may be that these - usually therapy resistant - patients are especially prone to develop side effects mediated by the immune system[73 106].

Careful surveillance of these patients is therefore necessary, especially during the first half year of treatment.

Chapter 3
The vascular response to captopril
A study concerning the role of inhibition of the renin-angiotensin system in lowering blood pressure.

3.1 Introduction

In the previous chapter it was shown that captopril is an effective antihypertensive agent. However, the exact mechanisms by which the drug lowers BP are as yet incompletely understood. To date the pharmacological action of the drug seems entirely attributable to its inhibitory effect on CE[36 69 85]. Most investigators in the field agree that blockade of the RAS by CE inhibition plays an important role in the lowering of BP. However, the evidence for this is mainly circumstantial and often inconclusive (see chapter 1).

The present study was designed to investigate the role of blockade of the RAS in the hypotensive action of captopril in a more direct way. This was done by comparing BP changes and the inhibition of CE to convert its substrate A I. Moreover, changes in renin release were related to the observed changes in BP. Since mechanisms operative in the BP response to captopril during acute studies may differ from those during prolonged treatment[254], both single dose and prolonged dose studies have been performed. The investigations were carried out in 4 patients. They were selected because their respective BP was known to be either renin-dependent (2 patients) or renin-independent (2 patients).

3.2 Patients and methods

Patients

Four patients were selected for the investigations. The case histories will be briefly described in the next few sections.

Patient 1. In this 31-year-old man Bartter's syndrome was diagnosed because of the findings of hypokalaemia, hypochloraemic alkalosis, hyperreninaemia, hyperaldosteronism and a normal BP. A more elaborate case history of this patient has been published elsewhere[67]. Administration of indomethacin had resulted in complete correction of all biochemical abnormalities (Table XIII). This correction was sustained during a 5-year outpatient survey period. Withdrawal of indomethacin before the present investigations resulted in an immediate natriuresis and urinary potassium loss with complete recurrence of all biochemical abnormalities characteristic of Bartter's syndrome within 10 days (Figure 11, Table XIII).

Captopril (50 mg) and saralasin (10 µg/kg/min, during an infusion period of 60 minutes) both lowered BP in this patient to a similar degree (Figure 12). Saralasin infusion, when started one hour after an oral captopril dose, had no additional effect on arterial pressure. We concluded from these results that BP in this patient was A II-dependent. The observation that a CE inhibitor and an A II-antagonist had an identical effect on BP strongly sug-

TABLE XIII. Summary of laboratory data of patient I.

		I	II	III	IV
Blood pressure	(mmHg)	120/80	110/80	110/70	110/70
Body weight	(kg)	57.9	59.1	60.1	58.3
Haemoglobin	(g/l)	15.5	12.1	14.0	14.5
Haematocrit	(%)	43	36	41	43
Sodium	(mmol/l)	138	141	140	139
Chloride	(mmol/l)	92	102	98	89
Potassium	(mmol/l)	2.4	3.9	3.8	2.3
pH		7.46	7.43	7.37	7.49
Bicarbonate	(mmol/l)	35	26	27	37
Calcium	(mmol/l)	2.50	2.45	2.50	2.60
PRA	(nmol A_1/l/h)	12.6	1.2	0.9	8.0
PAC	(nmol/l)	n.d.	n.d.	0.44	1.44
GFR	(ml/min)	108	92	104	n.d.
ERPF	(ml/min)	358	354	394	n.d.
FF		0.30	0.26	0.26	n.d.
Pressor dose angiotensin II (ng/kg/min)		n.d.	n.d.	4	16

I : before indomethacin treatment (1974)
II : after two weeks indomethacin (1974)
III : after five years sustained treatment with indomethacin (1979)
IV : two weeks after withdrawal of indomethacin (1979)
n.d. : not done

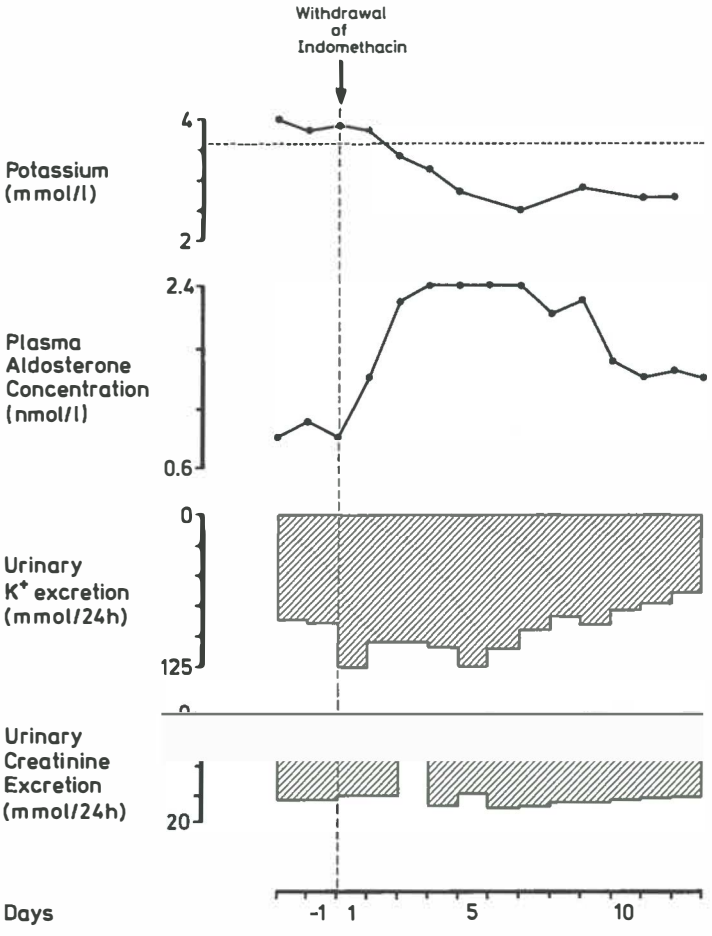


Figure 11. Effects of indomethacin withdrawal on serum potassium, urinary potassium excretion and PAC in patient 1.

gested that only diminished A II-formation was responsible for the decrease in BP.

Patient 2 is a 28-year-old man who had been hospitalized in 1977 for evaluation of fatigue, headache, periods of palpitations and hypertension. Extensive studies, published in detail elsewhere[142], revealed a renin-dependent hypertension in combination with a state of hyperprostaglandinism. The

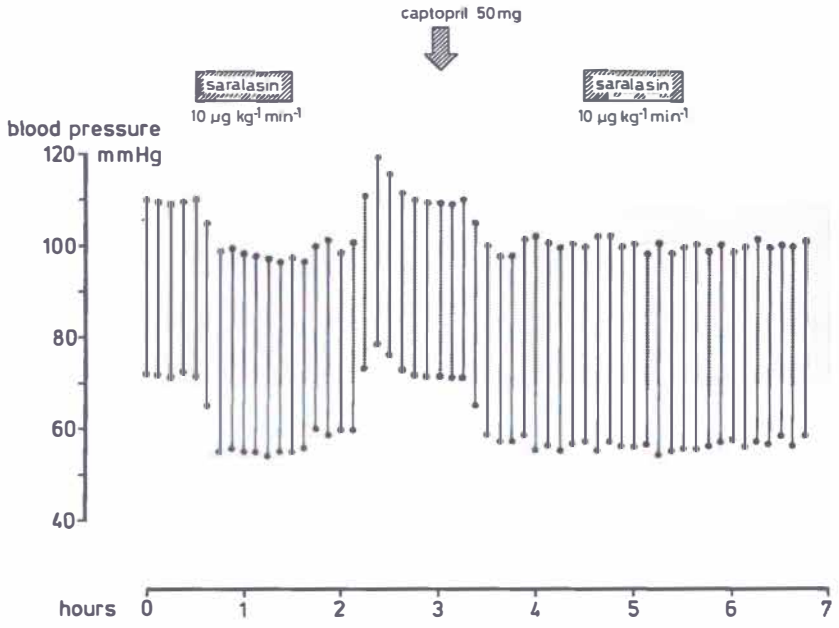


Figure 12. Blood pressure recordings of patient 1 during the administration of saralasin and captopril.

state of hyperprostaglandinism was thought to be secondary to hypertension with increased sympathetic activity.

When the investigations were started BP was 160/100 mmHg and there was slight hypokalaemia (3.5 mmol/l).

Patient 3 is a 54-year-old woman. She was hospitalized because of evaluation of hypertension and hypokalaemia (2.9 mmol/l). A clinical diagnosis of primary hyperaldosteronism was made. After our investigations patient was operated upon and adrenalectomy of the left adrenal disclosed a cortical adenoma (Figure 13). Serum potassium normalized and BP decreased (160/95 mmHg without therapy) after the operation.

Patient 4 is a 56-year-old man in whom hypertension was discovered in 1970 (160/110 mmHg). Secondary forms of hypertension were ruled out during

Figure 13. Adenoma in left adrenal gland (patient 2).

an admission to the hospital in 1967; a diagnosis of essential hypertension was made. Blood pressure showed a pressor response on saralasin infusion both in the sodium repleted and depleted state. These studies have been published in detail elsewhere[125].

Methods

During the observation period the patients were hospitalized. They were kept on a diet constant in sodium (200 mmol/24 h : patients 1 and 2; 100 mmol/24 h : patients 3 and 4), potassium (100 mmol/24 h) and fluid (2500 ml/24 h). All medication had been withdrawn for at least 7 days before the studies. The patients were familiarized with both the various procedures and personnel involved; they gave informed consent.

The following studies were performed:

1. Single dose studies. These studies were performed exclusively in patient 1 and included:

— determination of the effect of single captopril doses on BP, plasma renin activity (PRA) and plasma aldosterone concentration (PAC). These studies were performed for increasing doses (1, 2, 4, 8, 12 and 25 mg) on consecutive days,

— determination of the time-relationship between decrease in BP, inhibition of the pressor effect of a fixed A I dose (16 ng/kg/min during 5 minutes) and changes in the vasodepressor dose of bradykinin (BK), respectively. These 3 variables were determined separately on consecutive days for captopril doses of 12, 25 and 50 mg.

2. *Prolonged dose studies.* These studies were performed in the patients 2, 3 and 4. The patients were treated with captopril in a fixed dosage of 25 mg q.i.d.. Captopril was administered at 3 a.m., 9 a.m., 3 p.m. and 9 p.m. precisely. The studies included:

— determination of the course of BP. Values were obtained daily at 8 a.m., the patients being in recumbent position,
— determination of fasting PRA and PAC at 8 a.m. after overnight recumbency, starting 2 days before captopril administration till the end of the investigations,
— determination of the pressor dose of A I before the first, second, fifth (day 2) and ninth (day 3) doses; subsequently every other day always exactly one hour before the captopril dose of 9.00 a.m.,
— determination of time-relationship between BP response and inhibition of the pressor effect of the A I-pressor dose after the aforementioned captopril doses.

BP recordings during the study were performed with an automatic BP-recorder (type Statham-Godart 1A). PRA and PAC were measured by radioimmunoassay-methods previously described[89 214]. The pressor dose of A I (the amount needed to cause a sustained rise of the SDBP of 20 mmHg) was determined as described by Kaplan[145]. The vasodepressor response to BK was assessed in an identical way and was defined as the dose needed to obtain a sustained 15 per cent increase of the pulse rate or a decrease in the SDBP of 10 mmHg. A I (Ileu^5-angiotensin I, Schwartz-Mann) and BK (Sigma) were of analytical grade and were diluted freshly before each study.

3.3 Results

Single dose studies. The lowest administered captopril dose (1 mg) did not result in a decrease of the SDBP or the MAP. However, blockade of CE manifested itself in a rise of PRA and a decrease of PAC (Table XIV). The maximum decrease of BP was already observed at a captopril dose of 4 mg and amounted to 13 mmHg (MAP). Higher single doses did not affect the degree of the fall in BP, but resulted in a prolongation of its duration (Figure 14, Table XIV). The dose of 4 mg captopril, which was the minimum needed to obtain the maximum hypotensive effect, resulted also in a maximum rise in PRA. The duration of the PRA rise outlasted the hypotensive effect of captopril.

TABLE XIV. The effect of increasing captopril doses on MAP, SDBP, PRA and PAC in patient 1. n.d. = not done.

Captopril dose	MAP (mmHg)			SDBP (mmHg)			PRA (nmol A_1/l/h)			PAC (nmol/l)		
	0	1h	2h	0	1h	2h	0	1h	2h	0	1h	2h
0 mg (day 1)	90	88	88	78	77	77	8.6	8.5	8.2	1.34	1.40	1.25
1 mg (day 2)	86	85	86	76	75	78	8.0	18.4	18.2	1.58	1.11	1.02
2 mg (day 3)	90	82	90	73	66	69	7.9	22.0	18.4	1.29	0.83	0.68
4 mg (day 4)	86	71	78	74	62	70	11.1	42	30	1.17	0.95	0.82
8 mg (day 5)	86	72	75	72	60	67	11.3	40	41	1.27	1.02	0.83
12 mg (day 6)	83	68	75	72	59	65	11.7	42	43	1.16	0.97	0.85
25 mg (day 7)	88	74	73	72	59	59	12.6	37	43	n.d.	n.d.	n.d.

A clear time-relationship was observed between BP-fall and inhibition of the A I-induced pressor response during the 3 captopril doses tested (Figure 14). The pressor effect of A I disappeared completely 30 minutes after administration of captopril. This inhibition gradually decreased and the pressor effect returned to control levels 30 to 60 minutes after normalization of BP.

The vasodepressor effect of exogenous BK was potentiated for a much longer period than the duration of both the decrease in BP and the inhibition of the pressor effect of A I (Figure 14). Initial values of the BK- depressor dose were not reached within 8 hours after administration of each of the 3 captopril doses tested.

Prolonged dose studies. The effect of captopril treatment in a fixed dosage in patient 2 is shown in Figure 15. After the first dose of captopril a transient reduction in arterial pressure was observed which soon returned to pretreatment levels before the second dose was administered (Table XV). In the first 2 days of the fixed captopril dosage regimen this pattern was observed at every captopril dose studied. The BP decrease which immediately occurred after a captopril dose averaged - except for the first one - between 8 and 10 mmHg (SDBP), irrespective of the basal BP (Table XV). The duration of the BP fall was between 2 and 2.5 hours. Inhibition of the A I-induced pressor effect closely parallelled the decrease in BP which occurred after a captopril dose.

Basal SDBP in patient 2 began to decrease gradually 2 days after the start of captopril treatment (Figure 15). As for plasma renin levels, PRA decreased at day 3 after a rise during the first two days of captopril with another fall at day 12. The pressor dose of A I showed a course that was similar to that of PRA (Figure 15).

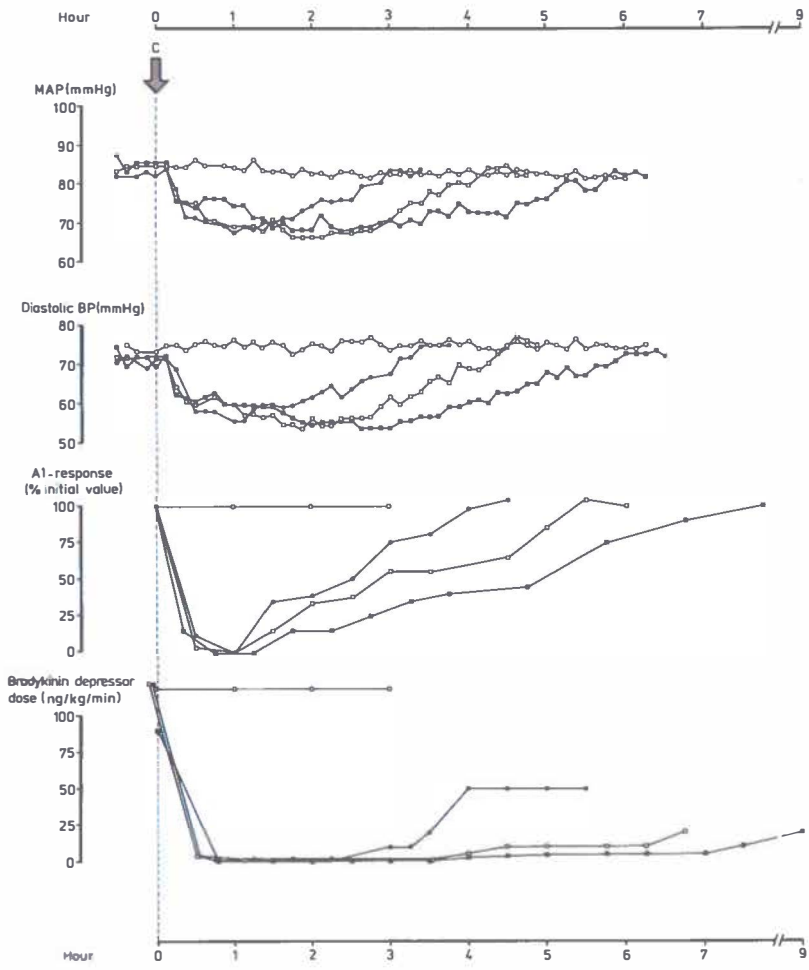


Figure 14. The effects of placebo (o) and single captopril doses (12 mg: ●, 25 mg: □, and 50 mg: ■) on blood pressure (MAP and diastolic BP, respectively), the pressor effect of exogenous angiotensin I and the vasodepressor dose of bradykinin in patient 1.

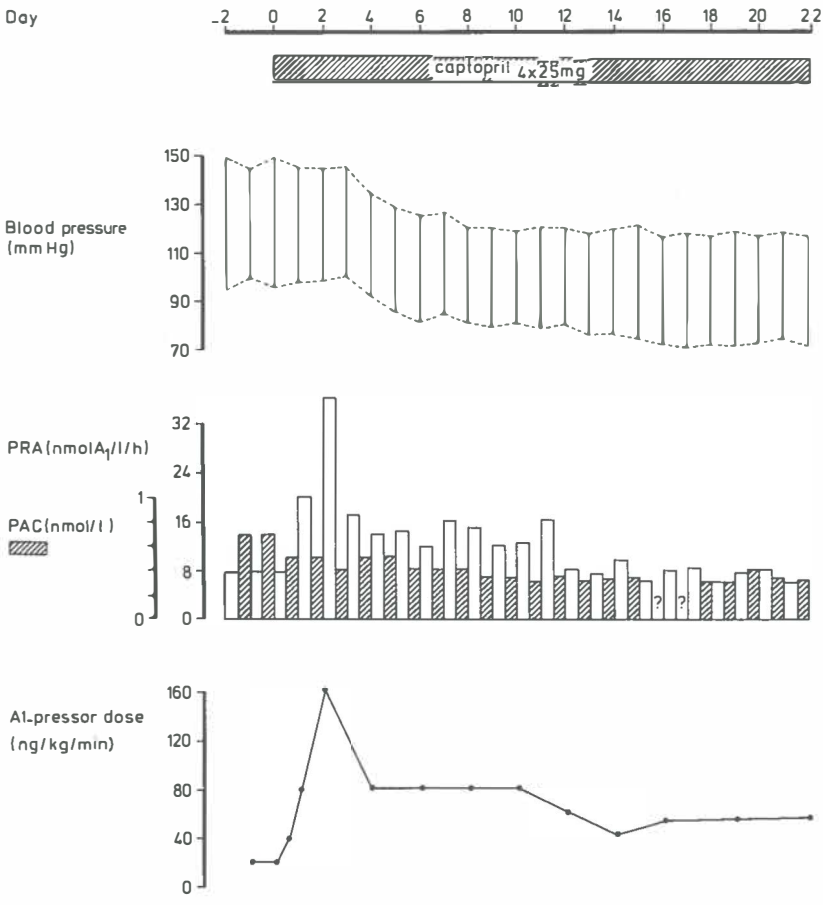


Figure 15. The effect of captopril on BP, PRA, PAC and A I-pressor dose in patient 2.

No effect of captopril on blood pressure was observed in patients 3 and 4. PRA and PAC remained unaltered. However, a slight increase in the amounts of A I, required for a sustained rise in SDBP of 20 mmHg (the pressor dose), was observed during prolonged treatment in both patients (Figures 16 and 17). The effectiveness of CE inhibition was demonstrated by the inhibition of the pressor effects of A I (Table XV).

TABLE XV. The effect of single captopril doses given during a fixed dosage regimen of 25 mg q.i.d. on SDBP and on the pressor effect of the A I-pressor dose in patients 2, 3 and 4.

		SDBP (mmHg)					Pressor dose (ng A I/kg/min)	Pressor effect (expressed as percentage of basal BP rise)				
Time (minutes)		0	60	90	120	180		60	90	120	180	210
Patient 2.	Dose 1	96	91	98	97	97	20	10	55	70	100	-
	Dose 2	98	90	94	96	96	40	10	15	100	100	-
	Dose 5	96	88	86	94	98	80	20	40	90	100	-
	Dose 9	93	84	94	92	96	160	10	40	90	100	-
	Dose 17	94	85	85	89	94	80	25	60	100	100	-
	Dose 33	85	77	74	85	85	80	30	40	75	100	-
	Dose 65	70	62	62	72	71	54	15	35	100	100	-
Patient 3.	Dose 1	120	125	122	120	118	3	20	20	20	40	100
	Dose 2	115	113	114	120	120	3	35	25	40	-	100
	Dose 5	120	124	121	120	118	10	0	5	45	85	100
	Dose 9	123	120	118	117	117	6	10	20	75	75	100
	Dose 17	120	120	116	118	118	6	20	20	-	90	100
	Dose 33	119	118	117	118	116	6	10	10	35	85	100
Patient 4.	Dose 1	102	108	103	103	108	7,5	15	10	15	-	100
	Dose 2	102	102	108	108	104	7,5	15	30	45	40	100
	Dose 5	101	102	100	98	103	10	30	45	40	100	100
	Dose 9	110	105	100	107	104	10	25	20	20	75	100
	Dose 25	104	110	102	96	98	10	8	10	50	90	100

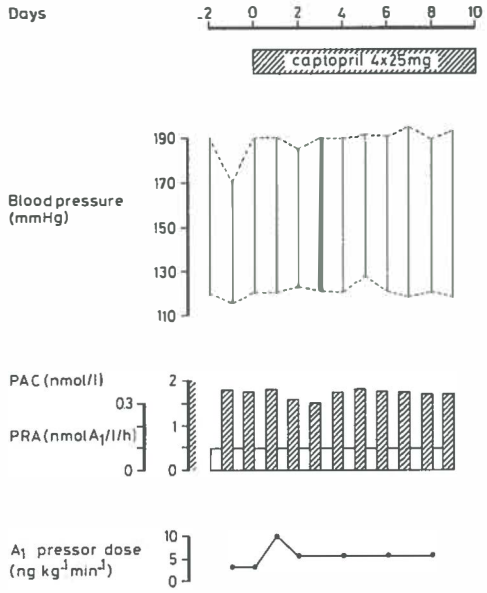


Figure 16. The effect of captopril on BP, PRA, PAC and A I-pressor dose in patient 3.

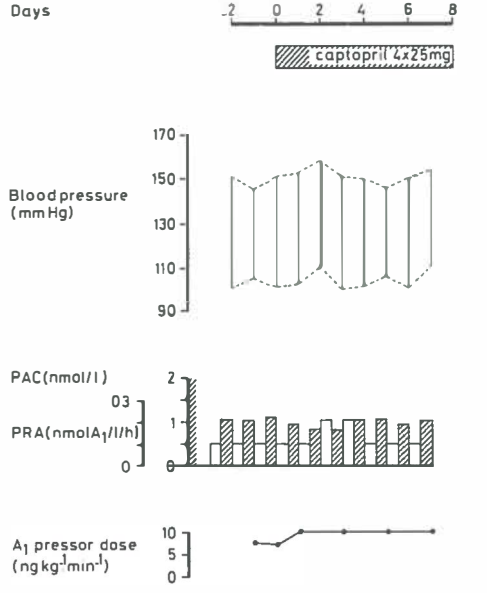


Figure 17. The effect of captopril on BP, PRA, PAC and A I-pressor dose in patient 4.

3.4 Discussion

The mere presence of a significant correlation between antihypertensive action of captopril and control basal renin values has been adduced as "evidence" for causality between renin inhibition and decrease in BP[156 159]. However, such a correlation does not necessarily imply a cause and effect relationship[264]. Moreover, studies by many investigators have yielded widely divergent results as for the relation between basal PRA and BP response during CE inhibition (see chapter 1). Tarazi et al. found that although such a relation did exist initially, it disappeared during prolonged treatment[254]. Most evidence linking interference of captopril with the RAS and BP response is therefore inconclusive.

This study provides more direct evidence that inhibition of the RAS mediates the hypotensive action of captopril. First, it appeared that the time course of inhibition of the pressor effect of exogenous A I ran parallel to changes in BP in those patients in whom captopril lowered arterial pressure (patients 1 and 2). The similarity of the time course of BP response and inhibition of the A I-induced pressor effect strongly suggests that BP reduction and RAS-blockade by CE inhibition are related as cause and effect.

Secondly, captopril did not lower BP in the patients with low renin hypertension and primary hyperaldosteronism, respectively (patients 4 and 3). Since other investigators have studied such patients with teprotide and captopril with similar results[10 36 46 94 159 162], it is fair to conclude that captopril (or other CE inhibitors) show little or no depressor activity in two hypertensive states associated with little or no renin.

Altogether these data provide strong evidence for the view that the major antihypertensive action of captopril is mediated by blockade of the RAS. Our results and conclusions seem at first sight to contradict the studies reported by Man in 't Veld et al.[173]. These investigators observed that captopril lowered BP in fluid-depleted anephric patients with very low serum levels of A II. However, these results can be interpreted as evidence for the importance of angiotensin II-generation in the vascular wall in some conditions; a phenomenon not reflected in serum levels of the hormone. The existence of such a mechanism during CE inhibition has been demonstrated by Oparil et al.[206]. In our opinion, the studies of Man in 't Veld et al. only reinforce the relativity of citing a relation between BP response and basal PRA (or A II) levels as evidence for a cause and effect relationship.

Our studies also show that duration and degree of the decrease in BP is - at least initially - determined by the ability of the RAS to overcome the

competitive inhibition of CE by captopril. This can be concluded from the observations in the single dose studies, since the minimum captopril dose needed to obtain the maximum reduction of BP also triggered the maximally observed renin release. Higher doses only prolonged the duration of the BP reduction but did not result in a further elevation of PRA levels. The lack of enhancement of the amplitude of the antihypertensive effect that occurs beyond a certain captopril dose has been described by Brunner et al.[35]. This study does not provide an answer to the question which mechanisms are involved in the stimulation of renin release by captopril. It has been suggested that only sympathetic stimulation of the baroreceptor cells situated in the afferent arterioles is involved in the activation of renin release by captopril[6]. However, interruption of the A II-PRA axis might also result in PRA release during CE inhibition[154].

It is striking that the opposition to the antihypertensive action of captopril in patient 2 during the first days of treatment was accompanied by an increase in PRA and A I-pressor dose. A gradual lowering of BP occurred after 2 days of captopril treatment with a simultaneous decrease in PRA and A I-pressor dose. These observations suggest that the RAS transiently defended pre-existing BP. Afterwards, a resetting or adaptation of the RAS occurred which was synchronous with a decrease in BP. Though the evolution of the antihypertensive response to captopril observed in patient 2 is a common pattern in many patients treated with the drug[43 284], it is unclear whether resetting of the RAS is a mechanism which is operative during the multiphasic antihypertensive action of captopril. In the future, studies are needed to investigate a possible role of resetting of RAS in the hypotensive effects of captopril.

Continuous BP control was achieved during captopril treatment despite intermittent resumption of normal CE activity in patient 2. Similar results have been reported by Waeber et al., who used a plasma assay of CE activity to denote inhibition of conversion of A I[273]. These observations suggest that specific effects of chronic captopril administration (inhibition of formation of A II) may act together with other mechanisms to control BP throughout the day.

Though our studies provide strong evidence that the main antihypertensive action of captopril is mediated by inhibition of the RAS, the data do not rule out the possibility that captopril-induced changes in vasodepressor activity play an additional role in the lowering of arterial pressure. In this respect it is worth noting that the A I-pressor dose increased during prolonged

treatment in patients 3 and 4, whereas PRA and BP did not change. Though changes in a pressor dose are a crude marker for vascular responsiveness[30 250], its consistent increase in both patients is very well compatible with an increase in vasodepressor activity. Because CE has been shown to be identical with kininase II[79], accumulation of BK might account for the increase of vasodepressor activity during CE inhibition with captopril. However, several studies have revealed that BK plasma levels do not increase during captopril treatment[181 248]. Bradykinin accumulation is most likely prevented either by a decrease in production rate or by breakdown via alternative pathways. The observation in patient 1 that potentiation of BK amply outlasted the fall in BP excludes a direct role for BK potentiation in the vascular response to captopril. This is the more striking since patients with Bartter's syndrome have elevated serum levels of BK[271]. It has been suggested that it is more likely that an increase of prostaglandin synthesis is responsible for an increase of vasodepressor activity during captopril[187 249].

All in all, our studies indicate that captopril lowers BP by inhibition of the RAS. Changes in vasodepressor activity probably reinforce this effect. The decrease of BP is achieved in the face of an intermittently unblocked converting-enzyme.

Chapter 4
The renal response to captopril
The results of renal function studies during converting-enzyme inhibition in hypertensive patients.

4.1 Introduction

Many investigators have underlined the importance of the kidney in sustaining hypertension[27 116 190]. Failure of the kidney to effect appropriate natriuresis when arterial pressure rises is central in their analyses. This inappropriate natriuresis is thought - at least in part - to be a consequence of abnormal renal vasoconstriction, which is present in most hypertensive patients. Repeated observations over the years have shown that angiotensin II plays a key role in the regulation and maintenance of renal blood supply. Evidence has accumulated that abnormal renin secretion could account for a substantial contribution to the increased renal vasoconstriction and the reduction of renal blood flow in hypertensive patients[91 117 122 123 178].

For these reasons it would be worthwhile to study the renal response of hypertensive patients to the converting-enzyme inhibitor captopril. Preliminary studies have shown that the drug decreases renal vasoconstriction and increases renal blood flow[121 183]. The present study was designed to examine the renal response to captopril in a large group of patients treated with captopril over a prolonged period. In addition, factors that might possibly influence the renal response to captopril have been investigated.

4.2 Patients and methods

Patients

The patients enrolled in this study have been described in detail in chapter 2. Renal function (here defined as the glomerular filtration rate and the effective renal plasma flow), was determined just prior to the start of captopril treatment and again after 6 and 12 months of therapy. In addition, these studies were performed at the end of the dose-titration period in most of the hospitalized patients.

Methods

The glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) were determined by using ^{125}I-iothalamate and ^{131}I-hippuran, respectively, as described by Donker et al.[66]. Clearances were proportioned by conversion to 1.73 m^2 body surface area. Filtration fraction (FF) represents the ratio of GFR and ERPF.

4.3 Results

The renal function was studied in 82 patients, treated with captopril alone or in combination with a diuretic. Figure 18 illustrates the effect of captopril on the mean GFR, ERPF, FF and MAP after 6 and 12 months of treatment. It appears that the mean ERPF had increased significantly from 326 ± 124 to 368 ± 145 ml/min after 6 months of therapy. Since the mean GFR remained unaltered, a significant drop in FF occurred. No further changes in these parameters were found after 12 months of therapy.

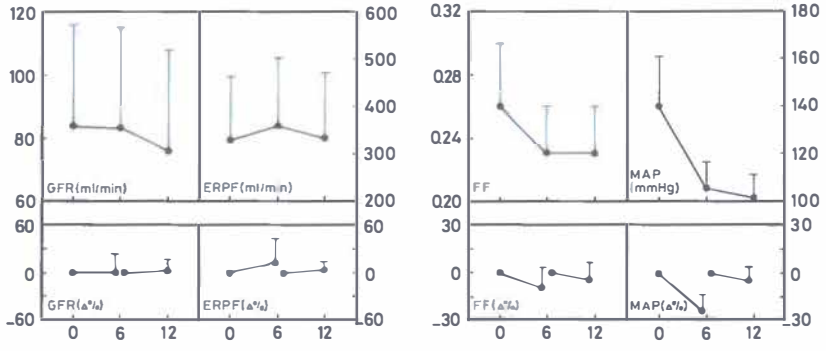


Figure 18. The course of GFR, ERPF, FF and MAP prior to captopril (n=82) and after 6 (n=72) and 12 months (n=32) of therapy, respectively. Mean ± SD of both the absolute values and the percentage change between various treatment intervals is shown.

It is known that GFR is dependent mainly on both pressure and flow. We therefore investigated the relation between the individual changes in the GFR on the one hand and those occurring in the MAP and the ERPF on the other. Figure 19 shows the relation between the percentage change of GFR and of the product of MAP and ERPF after 6 months of therapy compared to their baseline values. A strong correlation (r=0.82) was found.

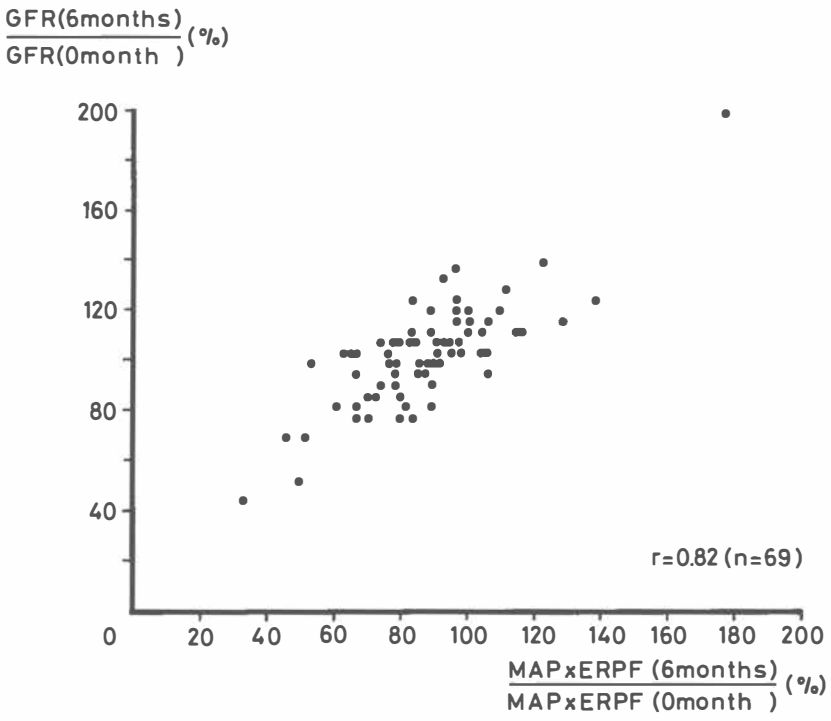


Figure 19. Relation between the percentage change of the GFR and of the product of MAP and ERPF after 6 months of therapy compared to baseline values.

In order to determine a possible role of diuretics in the renal response to captopril, the renal functions of the patients treated with captopril alone during the whole observation period were compared with those of the patients treated with captopril and a diuretic. The results are shown in Table XVI. It appeared that captopril in combination with a diuretic had been applied in patients with an initially higher BP than patients who did not need a diuretic. The mean GFR remained unaltered in both patient groups. A significant increase in the mean ERPF was observed in both the patients treated with captopril only (from 361 ± 124 to 388 ± 130 ml/min) and those treated with the combination (from 297 ± 138 to 326 ± 141 ml/min). Though the mean ERPF at the end of month 12 was lower than that measured after 6 months of therapy in both groups, a further individual percentage increase in ERPF was observed at the end of that treatment period compared with values obtained after half a year of treatment. The filtration fraction dropped in both groups.

TABLE XVI. Renal function and MAP (mean ± SD) in patients on captopril alone (−) versus patients on captopril and a diuretic (+) before and after 6 and 12 months of therapy. Numbers in patentheses indicate percentage change (mean ± SD) compared to the previous period.

Month		0	6	Δ %	12	Δ %
GFR	−	92 ± 29	91 ± 28	(0 ± 16)	79 ± 36	(+3 ± 15)
(ml/min)	+	77 ± 33	75 ± 34	(+2 ± 37)	67 ± 33	(+1 ± 13)
ERPF	−	361 ± 124	388 ± 130	(+7 ± 17)	348 ± 176	(+3 ± 9)
(ml/min)	+	297 ± 138	326 ± 141	(+21 ± 52)	283 ± 128	(+5 ± 12)
FF	−	0.25 ± 0.04	0.24 ± 0.02	(−6 ± 11)	0.23 ± 0.03	(0 ± 11)
	+	0.26 ± 0.04	0.23 ± 0.04	(−14 ± 13)	0.24 ± 0.04	(−4 ± 10)
MAP	−	133 ± 20	101 ± 9	(−23 ± 12)	98 ± 9	(0 ± 9)
(mmHg)	+	145 ± 20	108 ± 8	(−24 ± 10)	105 ± 11	(−3 ± 8)
Number of	−	42	36		16	
patients	+	19	16		12	

TABLE XVII. Renal function and MAP (mean ± SD) in patients with essential hypertension (EH), renovascular hypertension (RVH) and hypertension associated with renal parenchymal disease (RPD).

Month		0	6	12
GFR	EH	100 ± 20	100 ± 21	102 ± 8
(ml/min)	RVH	82 ± 27*	79 ± 30*	70 ± 24*
	RPD	55 ± 42*,**	46 ± 32*,**	50 ± 38*
ERPF	EH	410 ± 90	437 ± 111	433 ± 95
(ml/min)	RVH	301 ± 117*	332 ± 131*	306 ± 130*
	RPD	205 ± 140*,**	193 ± 123*,**	229 ± 179*
FF	EH	0.24 ± 0.05	0.23 ± 0.03	0.23 ± 0.03
	RVH	0.27 ± 0.04*	0.24 ± 0.03*	0.23 ± 0.03*
	RPD	0.27 ± 0.04*	0.24 ± 0.03*	0.22 ± 0.04*
MAP	EH	128 ± 19	105 ± 10	102 ± 8
	RVH	150 ± 19*	107 ± 11	103 ± 11
	RPD	142 ± 18*	101 ± 12	98 ± 7
Number of	EH	36	32	17
patients	RVH	32	30	13
	RPD	15	10	8

* denotes significance as compared to EH; ** denotes significance as compared to RVH.

The renal response to captopril in patients with the various forms of hypertension can be read in Table XVII. The mean GFR and the mean ERPF were significantly lower in patients with renovascular hypertension (RVH) than in patients with essential hypertension (EH) whereas MAP and FF were significantly higher in the former group. Renal function was significantly more impaired in patients with renal parenchymal disease (RPD) than in patients with RVH or EH. Treatment with captopril resulted in an

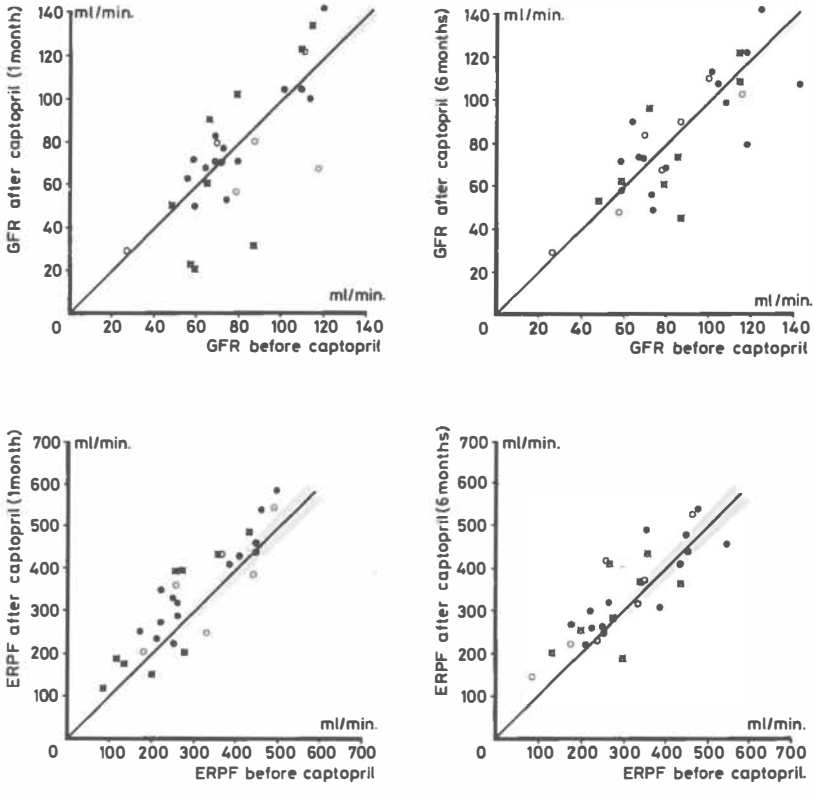


Figure 20. GFR and ERPF prior to captopril versus GFR and ERPF after one month and 6 months of therapy, respectively, in patients with renovascular hypertension (RVH). The lines of identity are drawn with the 2.5% (GFR) and 5% (ERPF) deviation.
o = unilateral RVH, treatment with captopril alone; ● = unilateral RVH, treatment with captopril and a diuretic; □ = bilateral RVH, treatment with captopril alone; ■ = bilateral RVH, treatment with captopril and a diuretic.

ultimate MAP that was the same for the 3 groups. The mean GFR remained stable in all 3 groups; the mean ERPF increased in the patients with EH and RVH whereas it did not change in patients with RPD. FF decreased in all 3 patient groups. There was no difference between renal function after 6 and 12 months of therapy in the 3 patient groups.

The baseline values of GFR and ERPF of the individual patients with RVH have been plotted against the values obtained after 1 and 6 months of treatment in Figure 20. It is interesting to see that after one month of treatment 4 patients had developed severe renal function loss. These 4 patients were severely sodium and volume depleted as a consequence of rigid dietary sodium restriction (20 mmol Na/day) and the use of diuretics. Gradual sodium repletion restored renal function in 2 patients, whereas the remaining 2 patients showed a partial improvement of GFR. Though the mean GFR did not change during captopril treatment, it can be seen in Figure 20 that the glomerular filtration in the individual patient with RVH did either improve or decrease. The patients with an improved GFR did not differ from the patients showing a decrease in GFR during captopril treatment as far as age, initial BP, BP response, captopril dosage, GFR and ERPF are concerned.

Figures 21 and 22 show the individual pretreatment values of GFR and ERPF plotted against the values obtained after 6 months of treatment in the patients with EH and RPD, respectively. It appears that the patients with

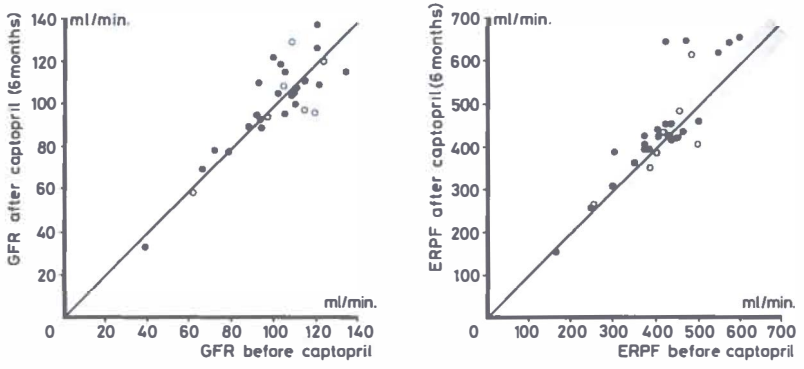


Figure 21. GFR and ERPF prior to captopril versus GFR and ERPF after 6 months of therapy in patients with essential hypertension (EH). The lines of identity are drawn with the 2.5% (GFR) and 5% (ERPF) deviation.
o = treatment with captopril alone; ● = treatment with captopril and a diuretic.

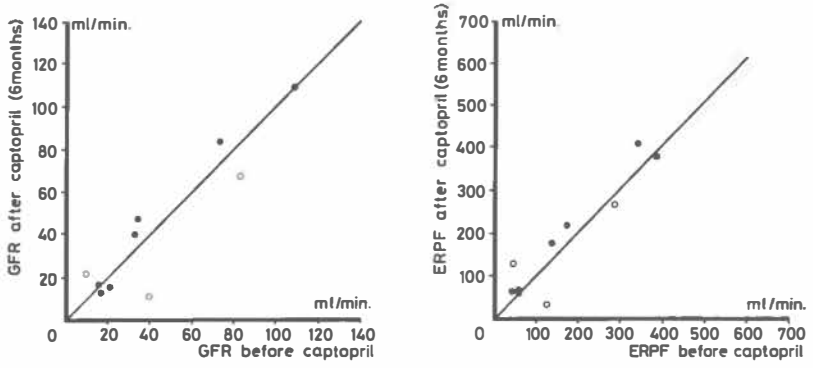


Figure 22. GFR and ERPF prior to captopril versus GFR and ERPF after 6 months of therapy in patients with renal parenchymal disease (RPD). The lines of identity are drawn with the 2.5% (GFR) and 5% (ERPF) deviation.
o = treatment with captopril alone; ● = treatment with captopril and a diuretic.

EH showing an improvement in GFR after 6 months of treatment (n=12) were significantly younger than those showing a decrease in GFR (n=7). No differences were found upon testing for initial MAP, BP response, captopril dosage, GFR and ERPF between the two groups. Data of the patients with RPD were too scattered to allow any conclusions.

4.4 Discussion

This study shows that the long-term effects of captopril on renal function are similar to those observed in acute and short-term studies. The drug diminishes renal vasoconstriction and increases renal blood flow whereas glomerular filtration rate is unaffected despite a considerable drop in arterial pressure. However, there is a restriction that the observations were made during dietary and diuretic-induced sodium depletion.

Changes in renal perfusion pressure should produce corresponding changes in renal blood flow provided renal arteriolar tone remains unaltered. Antihypertensive treatment with most currently used drugs results in a decrease of renal vascular resistance relative to the total peripheral resistance[33 220 277]. Renal blood flow is maintained by the capacity of the kidney to stabilize blood flow over a wide range of perfusion pressures[107]. Captopril is unique in that the drug causes an increase in renal blood flow. This cannot be explained by an increase of the cardiac output since this remains the

same[82]. Therefore, the decrease of renal vascular tone and the increase of renal blood flow must be a consequence of active renal vasodilation. The exact mechanism whereby captopril causes renal vasodilation is as yet unknown. Several studies have attributed a pivotal role for A II in the inappropriate renal vasoconstriction in hypertensive patients. However, before drawing the conclusion that the renal response to captopril reflects only a reduction in the influence of A II, further studies will be needed to determine the possible role of kinins and prostaglandins in this renal vascular response.

The strong correlation between the percentage change in GFR and the product of MAP and ERPF indicates that the GFR was influenced by the opposite change in MAP and ERPF which occurred during captopril treatment. Renal vasodilation must have prevented a decrease in the glomerular filtration rate which is usually encountered during a fall in arterial pressure induced by antihypertensive agents. Indeed, the GFR increased in some patients despite the reduction of BP. This indicates that the GFR in a subset of hypertensive patients is suppressed by abnormalities, which appear reversible during CE inhibition.

It has long been known that restriction of sodium intake reduces renal perfusion and glomerular filtration by activating the RAS[119 148 163]. Hence, one might conclude that the beneficial effects of captopril represent a reversal to the original state of renal function as present before sodium depletion. However, short-term studies have revealed that captopril increases renal blood flow both in hypertensive and normotensive persons while on a liberal salt intake. Hypertensive persons showed an enhanced vascular response in comparison with normotensive controls[178]. These observations suggest that the influence of captopril on renal function extends beyond a reversal of renal vasoconstriction induced by sodium restriction in that the drug corrects functional abnormalities of renal vasculature in hypertensive patients.

The renal response in the patients on captopril alone was similar to that in patients using captopril in combination with a diuretic. It has been shown that captopril and diuretics have a synergistic action on the vascular tone of the systemic circulation[49 254]. It may well be that the same holds for the combined action of these drugs on renal vascular tone. However, renal function decreases when rigid dietary sodium constriction is combined with the administration of captopril and a diuretic. The effect of a developing large volume deficit, resulting in a decrease of renal perfusion and glomerular filtration, cannot be reversed by captopril. The same observations have

been made in experimental studies[91 148 178]. As it generally appears that pharmacologic interruption of the RAS does not entirely reverse the renal response in these settings, it seems likely that under these circumstances an additional effector system - perhaps a direct action of the sympathetic nerves on the renal blood supply - has come into operation.

Contrary to the observations made by Prins, we could not establish a difference between the mean GFR of patients with renovascular hypertension before and after CE inhibition by captopril[217]. Moreover, our studies now revealed an increase in the mean ERPF in these patients. Initial BP and BP-responses to captopril were comparable in the two studies. It is probable that the different outcome is explained by the degree of sodium depletion of the patients since the applied sodium restriction has been more rigid in Prins's study.

Though the mean GFR did not alter during captopril treatment in patients with RVH, most individual patients reacted either with a fall or an increase in GFR. We could not establish discerning features in these categories of patients. One of the possible reasons that might explain different renal responses in individual patients with RVH is that captopril exerts opposite effects on the stenotic and the non-affected kidney. Experimental studies have shown that a stimulated RAS protects a stenotic kidney against ischaemia, thereby suppressing the function of the non-stenotic kidney[174]. Provided the same mechanisms are operative in human pathology, the ultimate renal response to captopril is the sum of the opposite changes.

Our results with regard to the renal response in patients with essential hypertension are in agreement with former observations. Studies by De Bruyn et al. - as yet unpublished - have shown that the extraction of hippuran decreases during captopril treatment in patients with EH. Consequently, our results may well be an underestimation of the real increase in renal blood flow in these patients. It appeared that patients with EH, who showed a decrease in GFR during captopril were older than the patients in whom captopril resulted in an increase of the GFR. This can be explained by the observation that progressive changes in renal vascular morphology and renal haemodynamics occur in ageing patients with EH[153 211]. Intrarenal blood flow is then passively redistributed to the juxtaglomerular region. The decrease in cortical blood flow is an age-dependent phenomenon, which causes renin suppression via the baroreceptor mechanism. It is likely that captopril does not have a major influence on the renal situation in the older patients and that a decrease in GFR as a consequence of a drop in BP will not

be corrected for by renal vasodilation. On the other hand, it has been shown that in younger patients with EH a decrease in renal blood flow is a consequence of functional changes in the renal vessels; abnormal activation of the RAS is thought to play an important part in these functional changes[153]. It is not surprising that the renal response to captopril in this category of patients is characterized by an improvement of renal perfusion and glomerular filtration.

Finally, important relationships exist between renal haemodynamics, arterial pressure and renal sodium handling[110]. A functional RAS is important in allowing sodium balance to occur without large fluctuations in arterial pressure or renal function. The ability of the kidney to excrete sodium is suppressed by inappropriately high levels of A II. Hence, captopril treatment may result in an improved capacity of the kidney to get rid of excessive sodium when A II levels are inappropriately high. This mechanism may - at least partially - explain the absence of sodium retention during captopril therapy in hypertensive patients. On the other hand, the increased capacity of the kidney to excrete sodium may lead to a large sodium and volume deficit, and hence renal function loss, when sodium intake is rigidly restricted. It is quite striking that patients using captopril have much in common with patients having the syndrome of "hyporeninaemic hypoaldosteronism"[213]. Several case reports of patients with this syndrome have described that the GFR drops when salt is withheld[212 280]. In our own experience and that of others, it seems that patients with RVH are especially prone to develop renal function loss during sodium depletion[52 84 105]. A critical drop in BP relative to the severity of the renal artery stenosis as well as volume depletion may both contribute to this condition.

In conclusion, captopril is a unique antihypertensive agent in that the drug increases renal blood flow, thereby maintaining - or sometimes improving - the GFR. Further research is needed to establish to what degree the kidney plays a part in the antihypertensive action of captopril.

Chapter 5
Captopril-associated membranous glomerulopathy

5.1 Introduction

Membranous glomerulopathy (MGP) is a well-known entity which may be recognized in renal biopsy specimens when immunoglobulins and complement are present in a regular granular pattern along the capillary walls and spikes are found on the epithelial side of the glomerular basement membrane (GBM) in light microscopy. Electron microscopy (EM) discloses electron dense deposits along the subepithelial side of the GBM[75]. (Figures 23, 24).

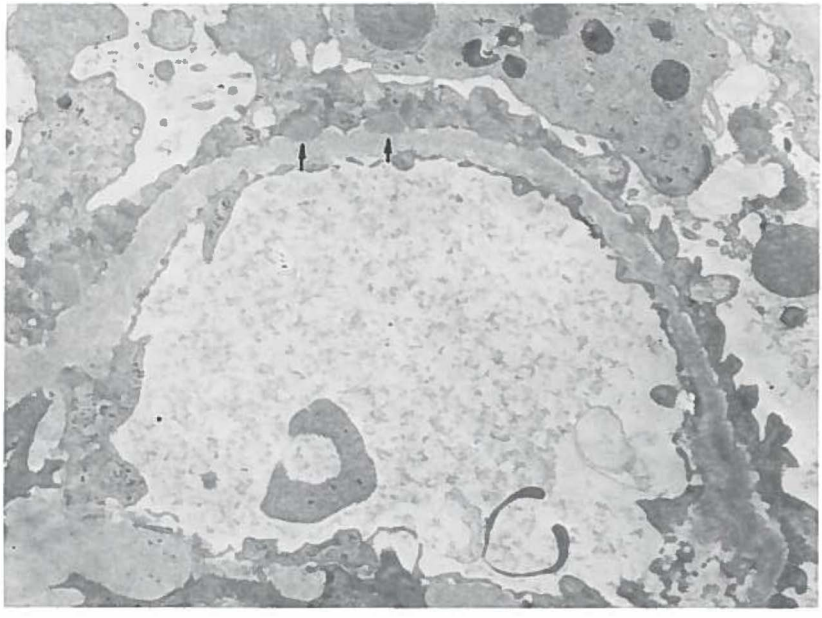


Figure 23. Electron micrograph of a renal biopsy of a patient with an early stage of captopril-associated membranous glomerulopathy. Electron dense deposits are clearly visible at the subepithelial side of the glomerular basement membrane (Uranyl acetate, lead citrate, x 10.000).

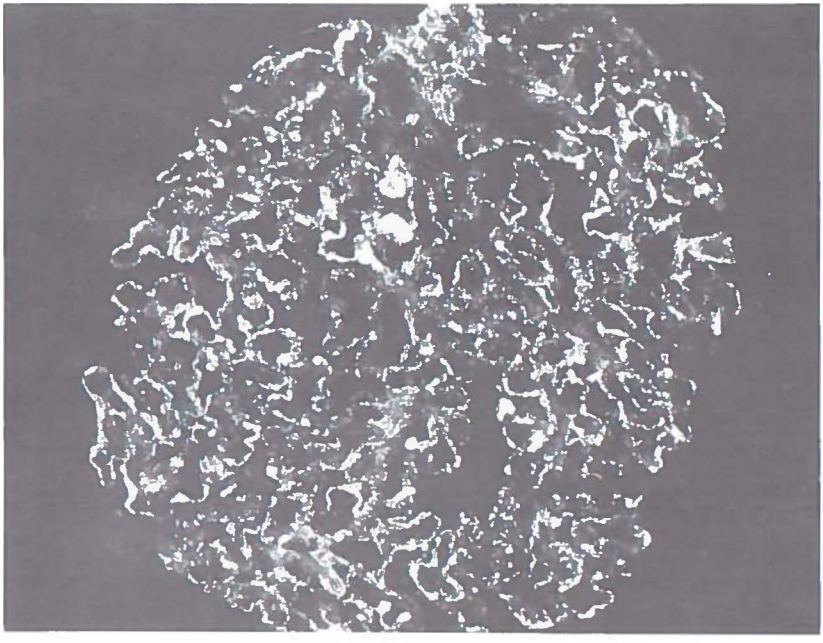


Figure 24. Immunofluorescence (using antibodies to human IgG) of a glomerulus from a patient with captopril-associated MGP showing a brilliant granular fluorescence along the GBM (x 220).

In August, 1979, we reported the development of proteinuria in a patient on captopril[216]. Histology of a renal biopsy disclosed MGP, stage I. Some months later we reported the association of MGP with a serum-sickness-like syndrome, which developed during captopril treatment[132]. Both patients had transiently developed ANA during captopril treatment. These findings - together with the development of anti-ds DNA antibodies in two other patients - prompted us then to investigate whether an early stage of MGP was also present in captopril-treated patients without clinical signs of glomerulopathy[128]. Renal biopsy specimens in 11 such patients disclosed 2 other cases of MGP, stage I. In the remaining 9 patients immunofluorescence revealed a patchy, granular deposition of a variable combination of one or more immunoglobulins (Figure 25). Electron microscopy did not show MGP but atypical, small, very dense deposits (Figure 26). We considered that these findings might represent a very early stage of MGP although hypertension itself or previous antihypertensive treatment could also have caused these atypical particles in the GBM and the patchy immunofluorescence.

Figure 25. Glomerulus showing a patchy immunofluorescence using antibodies against human IgM (x 400).

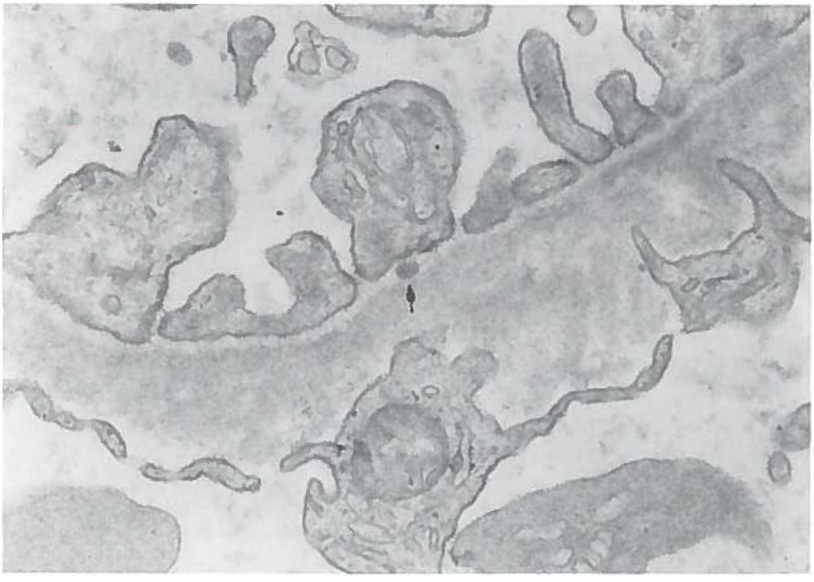


Figure 26. Electron micrograph of a renal biopsy of a patient on captopril. One very dense, small deposit is clearly visible in the GBM (Uranyl acetate, lead citrate, x 33.000).

Because captopril-associated MGP is a newly recognized entity, we have reviewed the clinical data of all published cases of captopril-associated proteinuria[12 44 126 132 223 233]. This review also includes data on 4 unpublished cases (2 from other centers* and 2 from our own study - elaborately described in appendix 3). Moreover, this chapter also contains the results of a prospective study to determine whether the hitherto unknown immunohistological abnormalities, observed in the asymptomatic patients on captopril, could be ascribed to the drug. Therefore, 13 patients underwent a renal biopsy before and after 6 months of treatment.

5.2 Methods

Renal biopsies as part of a drug evaluation study are not commonly practised in clinical research. However, we felt justified in performing these biopsies because the results of this study might profoundly influence clinical applicability of the drug. Moreover, this technique has been proved to be a safe technique in our hands. The studies were approved by an independent committee. All patients were informed and gave informed consent. Renal biopsies were processed for immunohistochemistry as described in detail elsewhere[279]. Light microscopy, electron microscopy (EM) and immunofluorescence (IF) studies were interpreted by two independent observers. With regard to EM, quantification of the atypical, small, very dense deposits was performed by counting these deposits in the GBM of three representative capillary loops. Immunofluorescence staining was graded semiquantitatively. Readings were done without any knowledge of the clinical state of the subject.

5.3 Results

Review of captopril-associated proteinuria

This review contains the clinical data on 15 patients who either developed proteinuria or who showed deterioration of proteinuria during captopril treatment. These data have been summarized in Table XVIII. All patients had moderate to severe hypertension. Nine patients had impaired renal function (serum creatinine $>$ 110 μmol/l). Eleven patients used a captopril dosage of 400 mg or more (including 8 out of 9 patients with impaired renal

* courtesy of dr. J. I. M. Drayer, Nijmegen and Prof. dr. J. Grünfeld, Paris.

TABLE XVIII. Clinical characteristics of the patients who developed proteinuria during captopril treatment.

	case/centre	age/sex		diag-nosis	BP	captopril dosage (24 h)	ANA		serum creatinine (μmol/l)	
							C−	C+	C−	C+
1	Groningen *	48	F	EH	200/120	150	N	P	71	94
2	Groningen *	52	M	RVH	300/125	450,D,P	N	P	113	230
3	Groningen *	51	M	RVH	260/130	225	N	N	96	83
4	Groningen *	60	M	RVH	230/120	150,D,P	N	N	210	486
5	New York	42	M	EH	-	400,D	N	N	115	61
6	New York	32	M	RVH	-	400,D	-	N	107	115
7	New York *	50	F	EH	-	300	-	P	71	107
8	New York *	54	F	RVH	-	400,D	-	P	115	107
9	New York	68	M	RVH	-	450,D	-	P	257	221
10	New York	43	F	EH	-	600,D	-	P	150	124
11	Glasgow *	41	F	EH	208/118	450,D	N	N	180	176
12	Nijmegen *	57	F	EH	180/110	450,D,P	N	P	96	98
13	Natal *	37	F	EH	158/110	600,P	N	P	124	142
14	Paris *	52	F	EH	160/120	600,D,P	-	P	133	-
15	Johannesburg	72	F	EH	214/108	450,D	N	N	88	-

D = diuretic; P = propranolol; F = female; M = male; N = negative; P = positive;
C− = without captopril therapy; C+ = during captopril treatment
* indicates patients who underwent a renal biopsy

function). ANA were (often intermittently and in low titers) found in 9 patients.

Proteinuria developed within a half year of treatment in all but 3 patients (Table XIX). Maximal daily protein excretion amounted to 24 grams. Proteinuria was aselective in all cases tested. Nephrotic syndrome [defined by proteinuria in excess of 3 g/24 h with hypalbuminaemia (<30 g/l), hypercholesterolaemia (> 8.0 mmol/l) and oedema] developed in 10 of the 15 patients. Microscopic haematuria or red cell casts were absent in all cases.

A renal biopsy was performed in 10 of the 15 patients. Light microscopy revealed in all biopsies vascular changes typical of those seen in hypertensive subjects: there was some thickening of arterial and arteriolar walls and partial or total sclerosis of glomeruli. Spike formation had not occurred. Immunofluorescence studies were performed on biopsies of 7 patients. A granular fluorescence for IgG and complement along the capillary walls was observed in all cases while positive staining for IgA and IgM was established in 4. EM revealed the presence of electron dense deposits with a subepithelial localization in all 10 biopsies.

To sum up the findings, in all 10 technically valid biopsies the immunohistological results were compatible with a diagnosis of a stage I membranous glomerulopathy. A second biopsy was performed one year after the first in 2 patients. Histological examination of one biopsy showed no changes while light microscopy revealed "progression" to spike-formation in the other.

The course of proteinuria in the 15 patients is shown in Table XIX. In the 9 patients in whom captopril was withdrawn, proteinuria disappeared immediately (1 patient) or gradually (3 patients); it decreased in 3 patients whereas the clinical course is obscure in 2. The drug was continued in 6 patients; proteinuria either disappeared (2 patients) or decreased (2 patients) whereas urinary protein excretion remained the same in one; the clinical course is obscure in one patient.

TABLE XIX. Characteristics of captopril-associated proteinuria.

case	onset of proteinuria (month)	maximal observed urinary protein loss (g/24 h)	course of proteinuria
1	6	6.5 (A)	C−; immediate disappearance
2	2.5	3.3 (−)	C−; subsiding; 18 months: dipstick +
3	3	8.2 (A)	C−; gradual disappearance (8 months)
4	3	10.1 (A)	C−; gradual disappearance (9 months)
5	4	1 (−)	C+; gradual disappearance (11 months)
6	3	2.4 (−)	C+; subsiding; 0.5 g (12 months)
7	3.5	2.6 (−)	C+; gradual disappearance (12 months)
8	3	7.4 (−)	C+; subsiding; 3 g (12 months)
9	4*	13.8 (−)	C−; course unknown
10	1	1.2 (−)	C+; unchanged proteinuria
11	3*	14 (A)	C−; course unknown
12	9	11.1 (A)	C−; gradual disappearance (11 months)
13	4	24 (A)	C−; subsiding; 15.6 g (15 months)
14	12	5.8 (−)	C+; too short follow-up
15	8	6.6 (A)	C−; subsiding; 0.9 g (12 months)

A = aselective; C+ = captopril continued; C− = captopril withdrawn; − = not tested
* This represents the month of marked worsening of pre-existing proteinuria

Prospective study

The clinical data of 13 patients who underwent a renal biopsy prior to and after half a year of treatment are summarized in Table XX. Ten of the 13 patients had essential hypertension. Captopril medication, alone or in combination with a diuretic, resulted in acceptable BP control in all patients. GFR remained unaltered during captopril treatment.

TABLE XX. Clinical characteristics of patients in the prospective study.

case	age/sex	diagnosis	captopril dosage	MAP month 0	MAP month 6	GFR month 0	GFR month 6
1*	57 M	EH	300	122	108	118	114
2	58 F	EH	400,D	190	96	62	58
3	36 F	EH	225	140	89	126	106
4	21 M	EH	300	127	103	82	85
5	36 F	RVH	150	117	101	110	98
6	32 M	EH	150	126	102	150	170
7*	29 M	EH	75	122	103	120	116
8	46 F	RVH	300	135	102	148	109
9	22 M	EH	75	112	103	112	135
10	46 M	RVH	300	130	105	90	70
11	21 M	EH	300	127	110	99	113
12	51 M	EH	300	155	108	124	127
13	50 M	EH	100,D	119	106	107	116

* denotes patients previously untreated

Light microscopy, EM and IF did not reveal one single case of MGP either before or after captopril treatment. Light microscopy showed changes typical of those seen in hypertension. Immunofluorescence of the pre-captopril biopsies showed mostly a patchly linear or granular fluorescence with IgM and C_3 in the vascular walls and capillary loops. The findings after half a year of treatment were roughly the same in all patients with the notable exception of patient 1, in whom IgG, IgM and IgA had been deposited (Table XXI).

The atypical, small, very dense EM-deposits were spotted in both pre- and post-captopril biopsies. The number of deposits had not changed during captopril treatment (Table XXI). No relation was found between the IF findings and these EM-deposits. Moreover, the small deposits were also observed in 5 out of 5 control biopsies of cadaveric transplant kidneys performed one hour after transplantation.

5.4 Discussion

A diagnosis of an early stage of MGP was made in all patients who underwent a renal biopsy for either *de novo* proteinuria or deterioration of pre-existing proteinuria. Development of proteinuria is the only marker for a clinician to suspect the presence of MGP during captopril treatment. Proteinuria either may be transient (despite continued captopril treatment)

TABLE XXI. Results of immunofluorescence and electron microscopy studies of the patients in the prospective study.

	Immunofluorescence								electron microscopy (no. of very dense, small deposits)	
	IgG		IgM		IgA		C_3			
Case	0	6	0	6	0	6	0	6	0	6
1	N	++ (l)	N	+++ (l)	N	++ (l)	N	N	13	2
2	N	N	+	+++ (g)	+	N	+	+++ (g)	12	8
3	N	N	N	++ (g)	N	N	+++ (g)	N	43	16
4	N	N	++ (g)	++ (g)	N	N	+++ (g)	++ (g)	9	7
5	N	N	+	N	N	N	+	N	7	4
6	N	N	+++ (g)	N	++ (l)	++ (l)	+++ (g)	+	3	3
7	N	N	+	N	+	N	N	N	4	8
8	N	+	++ (g)	++ (g)	++ (g)	N	+++ (g)	++ (g)	4	10
9	++ (l)	N	++ (g)	+	++ (l)	+	+++ (g)	+ (g)	3	11
10	N	N	+	++ (g)	N	+	+++ (g)	+ (g)	19	8
11	N	+	+++(g)	++ (g)	++ (l)	+	++ (g)	++ (g)	6	15
12	ND	ND	ND	ND	ND	ND	++ (g)	N	6	16
13	ND	++ (l)	ND	ND	ND	N	ND	N	5	12

N = negative immunofluorescence; +, ++, +++ denotes weakly, finely and coarsely positive fluorescence, respectively; l = linear, g = granular deposition.

or not develop at all[132]. In spite of this, the incidence of proteinuria does enable us to make an estimate of the minimum incidence of captopril-associated MGP, since all renal biopsies of patients with captopril-associated proteinuria disclosed membranous glomerulopathy. Proteinuria developed in 4.5 and 7.4 per cent of the patients in two captopril single-center studies (Groningen, 89 patients, New York, 81 patients)[44]. All these patients were discovered as part of a meticulous screening program to test the safety of captopril in clinical practice. Twenty-two of 899 captopril-treated patients (2.4 per cent) were reported to develop proteinuria exceeding 3 g/24 h after 8 months of therapy in a recent survey of the Squibb Company[150]. When the criterium of proteinuria exceeding 3 g/24 h is applied to the patients of Groningen and New York together, this percentage is 3.5 per cent.

These observations clearly show that captopril is associated with MGP in a subset of patients receiving the drug. The nephropathy is indistinguishable from secondary MGP produced by other drugs such as mercury, gold (aurothiomalate) and penicillamine[17 114 167 172 278]. Like captopril, these drugs have a heavy metal or a heavy metal binding site (SH-group) in common.

The association of captopril with immune complex glomerulopathy raises questions about disease mechanisms. Captopril or one of its metabolites may act as a hapten and initiate antibody production. This mechanism is suggested by the development of a serum-sickness-like syndrome in one of the patients[132]. The negative test for circulating immune complexes in this patient suggests that in situ formation of immune complexes took place in the GBM. Indeed, it has been shown that subepithelial immune deposits involving exogenous antigens may form locally in the GBM[88]. Alternatively, the immune complex glomerulopathy may be a consequence of an auto-allergic response induced by captopril[103]. Weening has shown that auto-antibodies are involved in mercury chloride-induced MGP in rats[278 279]. He found that immune complexes were deposited in the glomerulus when ANA appeared in the serum. Glomerular eluates contained antibodies directed against a nuclear antigen. This can be seen as evidence for the mediation of glomerular damage by a nuclear auto-antigen-antibody system. Auto-antibodies may therefore initiate a membranous glomerular lesion provided they are present in low titers or have sufficiently low avidity to allow deposit formation to proceed without affecting immune elimination of the antigen[75]. Although there is no conclusive evidence that captopril produces MGP in a similar way, it should be borne in mind that captopril is associated with the development of antibodies against nuclear antigens (chapter 2).

These antibodies develop in low titers and do not fix complement. Some of these auto-antibodies (anti-ds DNA antibodies) are of low avidity since they could be demonstrated in the Crithidia-assay while not being demonstrable in the Farr-assay (chapter 2). All in all, it may well be that captopril induces MGP in patients via auto-antibody formation. The way captopril generates auto-antibodies has not yet been ascertained.

Another point of interest is the mechanism of proteinuria in captopril-associated MGP. Immune complexes interfere with the integrity of the glomerular polyanion[75]. However, other mechanisms must be involved. This can be concluded from the observation that withholding captopril resulted in disappearance of proteinuria in 2 of our patients, whereas a second renal biopsy in these patients one year later showed that the histological abnormalities were still there. Potentiation of kinins and prostaglandins by captopril may possibly influence the occurrence of proteinuria. In addition, captopril might transiently induce lymphokines as a consequence of immunodysregulation which might also promote urinary protein loss.

This study definitely rules out an association between captopril and the presence of the atypical, small, very dense deposits along the GBM on EM. In an earlier publication we suggested that these lesions could represent a very early stage of MGP[128]. However,their presence in biopsies of transplant kidneys and in pre-captopril biopsies of patients with hypertension is conclusive proof that captopril is not associated with their development. With regard to the patchy immunofluorescence found in pre-captopril biopsies, captopril did not change the deposition of immunoglobulins along the vascular wall. Moreover, we observed a dissociation between immunoglobulin deposition and the occurrence of the atypical, small, very dense EM deposits. This suggests that both ultrastructural abnormalities probably represent different histological processes. Aetiology, pathogenesis and clinical significance of the deposition of immunoglobulins in patients with hypertension are not clear yet.

Finally, an important question is to be raised about the prognosis of captopril-associated MGP. An early stage of MGP might develop into two distinct ways (Figure 27). The membranous lesion may develop into a chainlike thickening of the GBM; this development is accompanied by renal function loss[48]. The other pathway is characterized by the same evolution as in the first pathway; however, the membranous lesion never reaches a stage of development which is visible in light microscopy while renal function is maintained (Figure 27)[261]. So far, no cases have been described of capto-

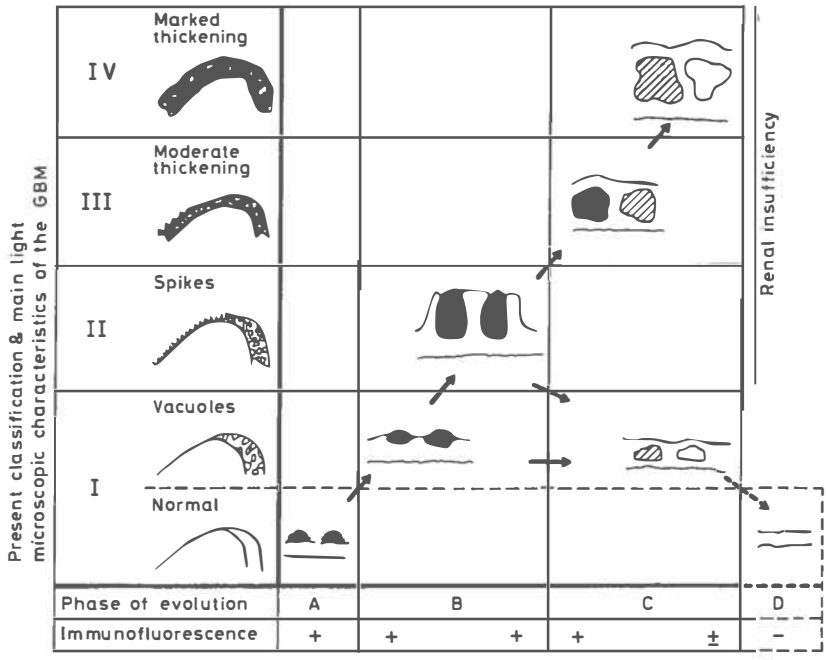


Figure 27. Schematic representation of various morphologic findings related to the evolution in membranous glomerulopathy. The two evolutionary pathways (described in the text) are shown. Dotted arrow represents presumptive evolution.

pril-induced MGP which developed into thickening of the GBM and, consequently, to renal insufficiency. The question as to whether captopril-associated MGP will develop - as has been suggested for penicillamine[17] - as a non-progressive, histologically mild renal disease appearing in all evolutionary phases only at the ultrastructural level, cannot be decided here. It is important from a practical point of view to know whether withdrawal of captopril in patients with MGP might influence the development of the histological lesions.

Chapter 6
Summary, conclusions and look toward the future

6.1 Summary

The Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure has recently advised that diastolic BP readings of 90 mmHg or more on 2 successive examinations should be regarded as confirmation of hypertension and should be an indication for therapy. Most hypertensive patients can be controlled with dietary regimens alone or in combination with currently available antihypertensive agents. However, the number of patients who are moderate or poor responders to antihypertensive therapy will increase with the rigorous requirements for BP control. There will be, therefore, an increasing need for potent antihypertensive agents. One of these might be the converting-enzyme inhibitor captopril. This antirenin drug is the product of intensive research which was started in the sixties when the influence of the renin system in initiating and sustaining hypertension was increasingly recognized. Preliminary studies with captopril have shown that its antihypertensive properties exceed the results with other currently available therapeutics.

The aim of the present investigation was to study the efficacy of captopril in a large group of hypertensive patients. The studies described are in part a continuation of Prins's studies. However, in this thesis the effects of captopril on blood pressure and the cardiovascular system have especially been described during prolonged treatment. In addition, the vascular and renal responses to the drug were studied in order to gain a better insight into captopril's BP lowering effects. Finally, special attention has been paid to the toxicity of captopril since this might limit clinical applicability (chapter 1).

This study established that captopril is a potent antihypertensive agent. A sustained lowering of BP for periods up to 18 months was observed in a group of 89 patients. These results are the more impressive as this group consisted predominantly of severe or previously uncontrollable cases. The combination of captopril and dietary sodium restriction - with an additional diuretic in 30-40 per cent of all cases - resulted in an adequate BP response

(SDBP < 95 mmHg according to WHO-criteria) in 93 per cent of all patients after one year of treatment. Additional advantages were the rapid occurring BP-response and the absence of reflex tachycardia as well as the absence of postural hypotension. Secondary resistance to the drug developed only in 10 per cent of the patients and was characterized by marked responsiveness to diuretics. Finally, fluid retention did not occur to the same extent as occurring with comparably potent vasodilators.

The therapeutic effect of captopril was objectivated by studying changes in those organs that can be considered target organs for elevated arterial pressure. A significant improvement was observed in fundoscopic, electrocardiographic and roentgenologic parameters of hypertensive damage of arterial vessels and heart. These changes were most dramatic in the first few months of therapy. Therefore, the casual BP readings also most probably indicated adequate BP control (chapter 2).

The way in which captopril lowers BP is incompletely understood. Most investigators agree that blockade of the RAS by CE inhibition plays an important part in the decrease in BP. However, the evidence for this is mainly circumstantial. Our studies disclosed that inhibition of the pressor effects of exogenous A I ran parallel to the decrease in BP. Captopril showed no depressor activity in two different hypertensive states associated with little or no renin. Though there is every reason to conclude from these data that decreased formation of A II plays an important part in the captopril-induced decrease in BP, it appeared that continuous BP control was achieved despite intermittent resumption of normal CE activity. This indicates that the specific effect of captopril administration (inhibition of A II generation) acts together with other mechanisms to control BP throughout the day (chapter 3).

With regard to the renal response to captopril, we observed a decrease in renal vasoconstriction and an increase in renal blood flow. The strong correlation between the percentage change of GFR and of the product of MAP and ERPF during captopril treatment compared with baseline values justifies the conclusion that GFR is maintained by the increase in renal blood flow despite a considerable decrease in BP. Though the mean GFR did not change, the individual patient mostly reacted with either an increase or a decrease in GFR. No differences could be detected in the RVH patients having these different renal responses. However, patients with EH who showed an increase in GFR appeared to be younger than the patients who reacted with a decrease in GFR. The probable explanation for this is that younger patients

have functional, i.e. reversible, changes of the renal vessels, often in combination with an activated RAS. Ageing patients have anatomical changes of the renal vasculature, often in combination with a suppressed RAS. It is therefore no surprise that these categories of patients with EH showed a different renal response to captopril. No conclusive answer can be supplied as to whether changes in renal function contribute to the hypotensive action of the drug (chapter 4).

Before a final conclusion can be drawn as to the place of captopril in antihypertensive treatment, the toxicity of the drug has to be considered. We observed one or more side effects in 30 per cent of our patients. These included rash, sometimes in association with arthralgia and fever, ageusia, proteinuria and anaemia. The drug had to be withdrawn in 8 patients. However, most events were transient and did not prejudice the continued treatment with captopril. The character of side effects, in combination with a parallel increase in auto-antibodies, favours an immunologically mediated pathogenesis. Their high incidence may be a consequence of a special feature of the patients enrolled in this study to develop side effects, the design of the study, or both. With regard to the patients, recent studies have shown an increase of immune reactivity in patients with malignant hypertension. Since many patients with severe, therapy-resistant or malignant hypertension were included in this study, it may well be that the high incidence of side effects is a consequence of our patient selection (chapter 2).

Undoubtedly, the most serious complications were renal (nephrotic syndrome) and haematological (agranulocytosis). With regard to the former, we reviewed all available data on development of proteinuria during captopril therapy. It appeared that proteinuria occurred in a frequency of 7.4 % in a single center study in New York whereas we found in our own study 4.5 %. Membranous glomerulopathy at an early stage was unvariably established in all patients who underwent a renal biopsy having captopril-associated proteinuria. However, proteinuria seems to be an unreliable marker for the presence of MGP since urinary protein loss may be transient even without discontinuing the drug. The way in which captopril induces MGP is unknown. It is possible that auto-antibodies, which result from immunodysregulation, play a part in the pathogenesis. As for the clinical impact of the histological lesions, it is as yet unknown whether MGP will progress to renal function loss by the development of a thickened GBM or, alternatively, may heal completely.

Our studies indicated that patchy immunoglobulin deposition and atypical, small, very dense deposits in EM are found in most patients with hypertension before captopril treatment. The atypical particles were also seen in biopsies of a control group (transplant biopsies). No differences could be established between pre- and post-captopril biopsies. These observations are conclusive evidence that captopril is not associated with the development of these ultrastructural abnormalities (chapter 5).

6.2 Conclusion

Captopril is highly effective in lowering blood pressure, especially in combination with sodium restriction. The main mechanism of captopril's BP lowering-action is inhibition of the RAS. The drug increases renal blood flow and decreases renal vasoconstriction while GFR is maintained despite a considerable fall of BP. Though captopril is an antihypertensive agent with undoubted potency, its associated side effects, some of them serious, limit unlimited clinical applicability.

Our present knowledge suggests that captopril should be reserved for the treatment of hypertension which is untreatable by other currently available drugs.

6.3 Look toward the future

So far, no studies have come to hand that deal with optimum dosaging of captopril. Future studies should focus on this important issue and should pay attention to the relative significance of dietary sodium restriction and diuretics in obtaining the maximum effect of captopril in the lowest dosages. The question as to whether lower dosages than currently used will diminish the frequency of side effects, may be answered in these studies.

The remarkable efficacy of captopril (i.e. the principle of converting-enzyme inhibition) has stimulated many pharmaceutical industries to develop orally active CE inhibitors. Since it was thought that the SH group might be responsible for the side effects of captopril, the design of this research has been focussed on the development of a CE inhibitor without a mercapto-group[230]. One must be aware, however, that no conclusive evidence is available at the present time that it is only this mercapto-group which is involved in the range of captopril-associated side effects. It should not be forgotten that the principle of CE inhibition per se is by no means beyond suspicion of playing a part in the pathogenesis of side effects.

In our experience - and in that of others - long term blockade of CE has not been proved detrimental to hypertensive patients. Quite on the contrary, high BP decreases towards normal while the haemodynamic status improves. Only in case of severe sodium depletion orthostatic and general hypotension as well as renal function loss may develop, all of which are reversible after sodium repletion. Moreover, one might wonder whether maintenance of BP by angiotensin II-mediated vasoconstriction without an adequate blood volume (as induced by volume depletion) is really beneficial for the organism. Such a vasoconstriction might tend to decrease the already compromised tissue perfusion in some critical areas further[262]. Adequate blood flow to tissues is undoubtedly more important than what we have arbitrarily accepted to be a normal BP. Conversely, if enough sodium and water are available, the renin system does not seem to be needed. It, therefore, appears attractive to assume that inactivation of the RAS may have only beneficial consequences. All things considered, permanent inactivation of the renin system might be considered a proposition for future treatment of hypertension. Before implementing such a drastic therapy, much knowledge has to be gathered about the indispensability of the RAS.

Samenvatting

Captopril is een remmer van het renine-angiotensine systeem (RAS). Het geneesmiddel remt het converting-enzyme en blokkeert daardoor de vorming van angiotensine II, het belangrijkste effector hormoon van het RAS. Uit voorlopig klinisch onderzoek was gebleken dat captopril een effectief geneesmiddel is. In dit proefschrift worden de ervaringen beschreven die door ons met captopril als antihypertensivum zijn verkregen.

Hoofdstuk 1 bevat een algemene inleiding. Met name worden de fysiologie van het RAS en de pathofysiologie van het systeem bij een gestoorde bloeddrukregulatie beschreven. Het hoofdstuk eindigt met een literatuuroverzicht van captopril.

Hoofdstuk 2 bevat het verslag van een studie naar de effectiviteit en toxiciteit van captopril. Het onderzoek werd verricht bij een groep van 89 patienten met een gemiddelde behandelingsduur van bijna één jaar. Bij het merendeel van deze patienten bestond ernstige en vaak onbehandelbare hypertensie. Behandeling met captopril - in combinatie met zoutbeperking en (bij 40% van de patienten) een diureticum - resulteerde bij vrijwel elke patient in een blijvende adequate bloeddrukregulatie. Naast het verkrijgen van normale bloeddrukwaarden bleek het goede effect van de therapie ook uit een vermindering van de afwijkingen gevonden bij fundoscopie, electrocardiografie en röntgenologisch onderzoek (hartgrootte op thoraxfoto). Opvallend was evenwel het grote aantal bijwerkingen: bij 28 van de 89 patienten werden één of meer bijwerkingen geconstateerd, zoals huidafwijkingen, smaakverlies en proteinurie. Bij 8 patienten moest captopril toediening wegens één of meer bijwerkingen worden gestaakt. Daarnaast bleek dat bij meer dan de helft van de patienten auto-antilichaam-vorming optrad, met name gedurende de eerste maanden van behandeling. Het karakter van de bijwerkingen doet vermoeden dat captopril een - vaak tijdelijke - dysregulatie van het immuunsysteem veroorzaakt.

Hoofdstuk 3 bevat een verslag van de studies die verricht werden bij een aantal geselecteerde patienten om de relatie tussen inhibitie van het RAS en bloeddrukdaling te bestuderen. Er werd gevonden dat verminderde vorming van angiotensine II inderdaad van primair belang is voor de bloeddrukdaling bij het captopril gebruik. Het werd verder waarschijnlijk dat - secundair aan de verminderde vorming van angiotensine II - andere, nog niet

nader gedefinieerde mechanismen een rol moeten spelen bij het bloeddruk-verlagend effect van captopril.

Hoofdstuk 4 bevat het verslag van de nierfunctie studies, die verricht werden vóór en na respectievelijk 6 en 12 maanden captopril gebruik in een groep van 82 patienten met een te hoge bloeddruk. Er werd gevonden dat de nierdoorbloeding toenam, tenzij er sprake was van ernstige volumedepletie. Verder kon uit de verkregen gegevens worden afgeleid dat de glomerulaire filtratiesnelheid - ondanks de vaak aanzienlijke dalingen van de arteriële druk - gehandhaafd kon worden door een dilatatie van het renale vaatsysteem.

Hoofdstuk 5 bevat een verslag van de studies over door captopril geinduceerde proteinurie. Op grond van literatuuronderzoek en eigen waarnemingen bleek dat bij patienten, die proteinurie tijdens captoprilgebruik ontwikkelen, bij nierbiopsie altijd een membraneuze glomerulopathie wordt gevonden. Tenslotte wordt in dit hoofdstuk verslag gedaan over een studie van nierbiopsieën die bij 13 patienten werden verricht vóór en na 6 maanden captopril gebruik. Dit onderzoek werd gedaan om na te gaan of captopril - overigens alleen op ultrastructureel niveau zichtbare - afwijkingen in de glomerulaire basaalmembraan veroorzaakt. Hiervoor werden geen aanwijzingen gevonden.

Hoofdstuk 6 bevat een samenvatting van de gevonden resultaten. De slotconclusie is dat captopril het middel van keuze lijkt te zijn bij die vormen van hypertensie, die niet adequaat behandeld kunnen worden met de thans gangbare antihypertensiva.

Appendix 1

The determination of the cardiac surface on the postero-anterior roentgenogram

This appendix deals with the methods used in chapter 2 to estimate the heart size from chest X-rays. Apart from the cardiac-thoracic ratio, the surface of the projection of the heart on the postero-anterior roentgenogram was calculated according to Jonsell's method in order to evaluate the effect of captopril on hypertensive cardiomegaly[143]. However, it was established by Amundsen that the results of Jonsell's method can be influenced profoundly by the observer bias[3]. To exclude this error, a modification of Jonsell's method was developed. In the next few sections this method will be described.

Method

The area of the frontal projection of the heart on the postero-anterior roentgenogram can be considered an ellipse. Jonsell has shown that the error will not be of significance if the distance from the junction of the aortic arch and the right lower heart contour (A) to the apex (B) is submitted for the true long diameter (l) of the ellipse (Figure 28). The true broad diameter of

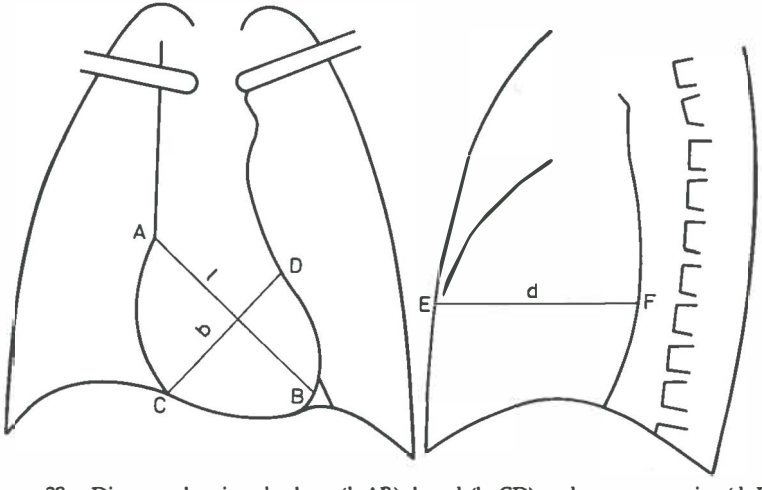


Figure 28. Diagram showing the long (l, AB), broad (b, CD) and anteroposterior (d, EF) diameters. For details, see text.

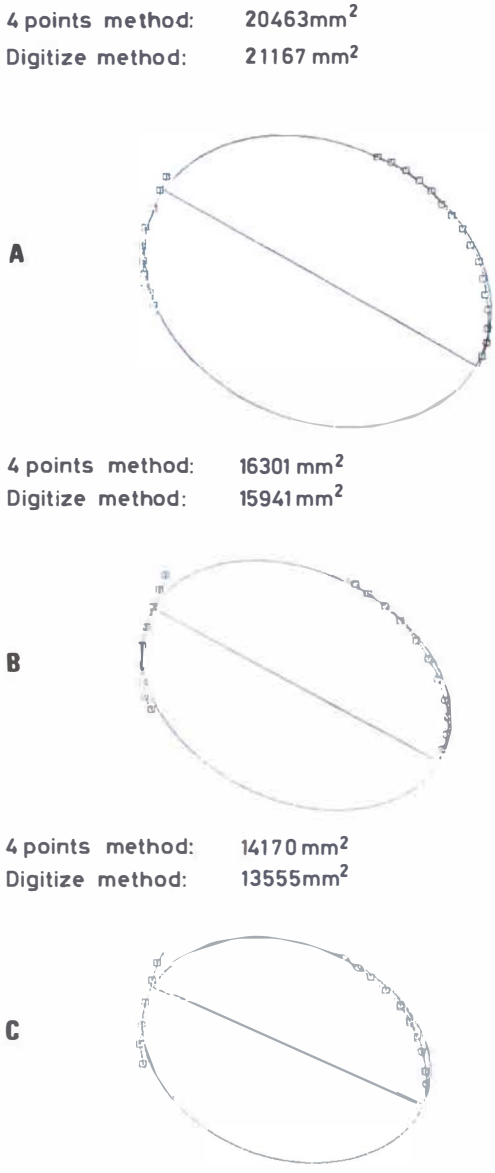


Figure 29. Plotter output of computer-constructed ellipses of 3 roentgenograms of one patient. Each fifth digitized point of the cardiac segments has been marked by a square. A: cardiac surface before captopril; B, C: after 12 and 18 months of therapy, respectively.

the ellipse (b) is found by the intersection of the right heart contour and the diaphragm (C) to a point on the left contour in the region of the left auricular appendix (D). The surface of the ellipse, determined by the diameters l (AB) and b (CD) can be calculated. Moreover, cardiac volume can be calculated by using the antero-posterior diameter d (EF) since the shape of the heart approximates to that of transversely situated ellipsoid.

The observer bias of this method is readily introduced because the crucial points A, B, C and D are not always clearly recognizable. This error can be diminished by means of using more available information than the above mentioned 4 points. Since the segments AC and BD are - by definition - situated on the ellipse, digitizing of these segments provides optimum information.

Therefore, these segments were marked on the roentgenogram and, subsequently, digitized with the aid of a X-Y tablet linked to a computer. The amount of coordinates on a segment was then reduced such that the distance between successive points was equal. This was done to achieve an equal weight of each individual point in the algorythm constructed to estimate the optimum fitting ellipse. A visible check of the computer-constructed ellipse

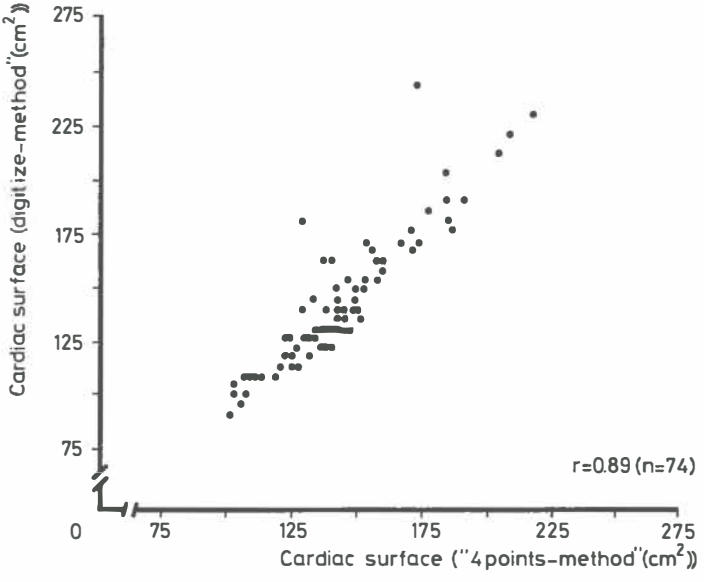


Figure 30. "Digitize method" compared with the "4 points-method".

was possible through a plotter output. An example of such an output is given in Figure 29. It represents the digitized contours of a patient's heart (marked by the squares) and the optimal fitting ellipse from roentgenograms made before the start of captopril treatment and after the 12th and 18th month of treatment.

The results of the measurements of the frontally projected cardiac surface with Jonsell's "4 points-method" compared to the "digitize-method" are displayed in Figure 30. A significant correlation was found ($r = 0.89$). Though it is clear from the figure that the methods are related, it is evident that the results in the individual patient may differ. It is because of this individual variation that we prefer the "digitize-method" which uses the maximum of the available information on the roentgenogram.

Appendix 2
Sodium balance, renal function and the renin-angiotensin system

Two case reports will be presented in this appendix. They have been selected to demonstrate the importance of a functional RAS in allowing sodium balance to occur without large fluctuations in arterial pressure and renal function. The effects of an inappropriately stimulated RAS on BP, renal function and renal sodium handling - including the beneficial effects of captopril - are described in case history 1. The reverse situation is demonstrated in case history 2. Inhibition of the RAS - in combination with dietary and diuretic sodium depletion - may lead to a large sodium deficit and renal function loss.

Case history 1

A 17-year-old female was hospitalized in 1977 because of headache and oliguria. She had been using the oral contraceptive Microgynon 50® (0.05 mg ethinyloestradiol and 0.125 mg d-norgestrel) for 14 months. Laboratory investigation revealed micro-angiopathic haemolytic anaemia, consumption coagulopathy and renal failure (creatinine clearance 7 ml/min). A diagnosis of haemolytic uraemic syndrome was made. She became anuric and intermittent haemodialysis was started. Malignant hypertension (270/150 mmHg) with papilloedema at fundoscopy developed within a week, which was treated with metoprolol, hydralazine and clonidine. Persistant hypertension (180/120 mmHg before dialysis) and recurrent overhydration necessitated continuance of haemodialysis twice weekly, though GFR recovered to 9 ml/min.

After trying various antihypertensive regimens (Table XXII) satisfactory blood pressure control could ultimately be obtained by furosemide, propranolol and minoxidil. However, recurrent overhydration persisted and progressive hirsutism due to minoxidil grew intolerable.

The patient was readmitted in 1979 and it was tried to regulate blood pressure with captopril. High renin secretion was revealed by a fall in BP from 175/115 mmHg to 150/90 mmHg during infusion of the angiotensin II-antigonist saralasin just prior to dialysis. Captopril was begun and caused a marked fall in BP to 130/70 mmHg. Renal function studies revealed an immediate increase in ERPF. Haemodialysis was stopped and overhydration did not recur in spite of an increased fluid intake. Patient was discharged while using captopril 50 mg t.i.d., furosemide 40 mg o.i.d. and a sodium restricted diet. GFR had increased to 24 ml/min. Hirsutism had disappeared.

Five months later the patient presented severe headache and nocturnal dyspnea. Until then, it had gone unnoticed to us that she had used again Microgynon 50® for 3 weeks. Blood pressure was 160/98 mmHg; over the lungs basal rales were heard and slight peripheral oedema was found. Oliguria was noticed (300 ml/24h). Serum creatinine was 400 μmol/l. Because of the presence of mild microangiopathic haemolytic anaemia and consumption coagulopathy a

TABLE XXII. Clinical values at various stages of treatment of patient 1.

Date	Body weight (kg)	Blood pressure (mmHg)	Creatinine (μmol/l)	GFR (ml/min)	ERPF (ml/min)	Plasma renin activity (ng/ml/h)	Plasma aldosterone concentration (ng/dl)	Dialysis	Antihypertensive medication, total daily dose (mg)
10/24/1977†,††	58.7	270/150	1239	-	-	32.4	-	-	none
5/29/1978†	45.2	180/120	506	7	55	8.4	-	+	hydralazine, 160 metoprolol, 400 clonidine, 0.225
9/25/1978†	46.9	135/85	346	7	40	-	-	+	propranolol, 240 clonidine, 0.150 minoxidil, 7.5
12/20/1978†	47.2	145/90	451	7	41	19.2	170	+	propranolol, 240 minoxidil, 12.5 furosemide, 40
1/15/1979†	45	230/130	274	9.4	41	6.5	213	+	none
1/19/1979	45.8	135/70	318	12	82	17.1	45	-	captopril, 150
2/ 5/1979	47.1	140/85	283	14	98	19.6	20	-	captopril, 150 furosemide, 40
5/21/1979	54	140/80	212	24	127	17.9	30	-	captopril, 150 furosemide, 40
7/ 5/1979††	61	160/98	400	-	-	84.6	163	+	captopril, 150 furosemide, 40
7/27/1979	55	155/70	318	15.5	102	13.5	61	-	captopril, 150 furosemide, 40
7/ 3/1980	58	130/85	194	25	156	12.6	54	-	captopril, 150 furosemide, 40
10/ 3/1981	59	130/80	182	28	163	-	-		captopril, 150 furosemide, 40

† Values obtained before dialysis.
†† Admission for (relapse of) hemolytic uremic syndrome.

relapse of the haemolytic uraemic syndrome was diagnosed. PRA and PAC were extremely high (Table XXII). Haemodialysis and fluid restriction were reinstituted; all medication except Microgynon 50® was continued. Within 2 weeks urinary production increased and haemodialysis could be stopped. Since then the patient has remained well with satisfactory BP control (130/80 mmHg) and a gradually improving renal function (Table XXII).

Comments

Our patient had severe hypertension, renal failure and an inappropriately high renin secretion. Inhibition of A II-generation, in combination with haemodialysis if required, has proved to be an efficacious mode of management in similar patients[37 267]. Indeed, captopril restored BP to normal values in our patient.

Previous antihypertensive treatment had resulted in effective BP control prior to captopril. A major difference emerged between both therapies with regard to renal function. Captopril unmasked a functional component in the presumed end-stage renal failure. This may be explained by removal of an excess of A II, though accumulation of vasodilator kinins or prostaglandins may have been operative just as well.

One of the peculiar features of the dialysis period of this patient was the recurrent overhydration in spite of the presence of a GFR of 9 ml/min. In fact, this recurrent overhydration was the main reason for dialysis treatment. It is tempting to speculate that the inappropriately high renin secretion caused both the hypertension and the inability of the kidney to get rid of excessive sodium and water[110]. Captopril reversed both.

As discussed before, captopril's humoral effects include a decrease in A II and aldosterone levels, whereas renin levels increase. However, the renin levels observed during the second period of illness markedly exceeded those repeatedly observed before during captopril treatment. This high renin level with a concomitant rise of aldosterone may indicate that captopril's competitive inhibition of CE was overridden.

Injected renin and angiotensin II have vasculotoxic effects in many species[93]. Therefore, hyperreninaemia resulting in high A II levels may have been an important factor in the serious course of the first crisis. One might speculate about the possibility of a protective role of captopril in the mitigated course of the second attack.

A relation between oral contraceptives and the occurrence of the haemolytic uraemic syndrome has been proposed[29]. The case history of our patient is unique, insofar as the syndrome relapsed after re-exposure to the birth control pill.

Case history 2

The patient, a 32-year-old male, had a history of recurrent urinary tract infections in combination with a hydronephrosis and a hypoplastic left kidney. Because of recurrent infections and a deteriorating renal function, right nephrectomy was performed in March, 1974, and chronic intermittent haemodialysis was started. The patient was normotensive during this period. In July, 1976, a renal transplantation was performed with a graft from a 9-year-old male donor. Two renal arteries on a patch were anastomosed end-to-end to the left hypogastrical artery. The patient had moderate hypertension at discharge (150/100 mmHg) and a vascular bruit was heard over the grafted kidney. Renal function at that time: GFR 35 ml/min, ERPF 103 ml/min and FF 0.34.

A steadily rising BP (240/150 mmHg) was noted at visits to the outpatient clinic. The hypertension prompted admission to the hospital in September, 1976. He developed hypertensive encephalopathy with repeated seizures, ultimately controlled by intravenously administered diazepam and diazoxide. The creatinine clearance at that time was 54 ml/min. Angiography showed a stenosis with post-stenotic dilatation of the main renal artery. At operation a small, hardly patent and tortuated artery of the underpole was ligated; surgical reconstruction of the main artery or a bypass procedure was technically impossible. During this operation the left hypoplastic kidney was removed. After that, hypertension could initially be controlled with alpha-methyldopa 500 mg q.i.d., hydralazine 50 mg q.i.d., clonidine 0.150 mg q.i.d., furosemide 40 mg o.i.d., diazoxide 100 mg t.i.d. and metoprolol 200 mg b.i.d.. However, hypertension gradually returned and the patient was readmitted in February, 1979, in order to be treated with captopril.

On admission BP amounted to 190/130 mmHg. Fundoscopy showed a pattern consistent with severe hypertension (KW grade III). No obvious progression of the artery stenosis was noted at angiography. The patient was placed on a diet including 20 mmol sodium and the antihypertensive medication was gradually tapered down. BP gradually increased to 240/140 mmHg.

Administration of captopril did not result in a clear fall of BP in spite of the severely sodium restricted diet. However, ERPF increased from 290 to 355 ml/min. Plasma renin activity (PRA) rose from 1.8 to 59 nmol A_1/l/h. Hydrochlorothiazide (50 mg o.i.d.) was added; this resulted in a fall in BP to 170/115 mmHg. The patient became normotensive (120/80 mmHg) after addition of the loop diuretic furosemide (40 mg o.i.d.).

Hyponatraemia (118 mmol/l) developed after addition of the two diuretics which was further accompanied by hypochloraemia (79 mmol/l), uraemia (30 mmol/l), an elevated serum creatinine (297 µmol/l) and isosthenuria. Body weight increased slightly (1-2 kg) in this period. Plasma aldosterone concentration rose steadily after the addition of the diuretics (highest value 3.9 nmol), whereas serum potassium did not change (4.0-4.2 mmol/l). GFR and ERPF decreased to 34 and 247 ml/min, respectively. After withdrawal of the diuretics and sodium repletion the biochemical abnormalities disappeared or improved markedly (Na 138 mmol/l, Cl 98 mmol/l, ureum 8.2 mmol/l, creatinine 146 µmol/l). Blood pressure gradually increased during sodium repletion (150/95 mmHg). The patient was discharged with a moderately salt restricted diet (100 mmol sodium daily) and captopril (100 mg t.i.d.). Hydrochlorothiazide 50 mg o.i.d. was added 4 weeks after discharge because of recurrence of hypertension (160/105 mmHg). Since then, the patient has remained normotensive. In March, 1981, BP was 130/80 mmHg, GFR 60 ml/min and ERPF 302 ml/min.

Comments

After the second nephrectomy we were faced with a one-kidney, one clip model of renovascular hypertension. Clipping one renal artery and contralateral nephrectomy creates the experimental model for hypertension due to volume-overload. Blood pressure did not change after administration of captopril, which indicates that the hypertension at that time was not A II-dependent. The combination of dietary sodium restriction, diuretics and CE inhibition resulted in normotension. The recurrence of mild hypertension after sodium repletion could easily be controlled with a diuretic.

Hyponatraemia developed after addition of diuretics to the regimen of rigid dietary sodium restriction and CE inhibition. This cannot be explained solely by a decrease in the free water clearance but it was likely to be due to a concomitant sodium deficit. Normally, sodium depletion is a rare consequence of diuretic treatment since its occurrence is prevented by homoeostatic mechanisms. These include a decrease in glomerular filtration, an increase in proximal tubular sodium reabsorption and a stimulation of aldosterone release. Since GFR decreased and PAC increased, it is most likely that proximal tubular sodium reabsorption had failed to increase appropriately during the development of the sodium deficit in our patient. This may have been due to diminished angiotensin II formation by captopril, since angiotensin II in physiological amount increases tubular sodium reabsorption both by its influence on renal haemodynamics and possibly by a direct action on the tubular cell level[90, 163].

GFR and, to a smaller extent ERPF, had decreased substantially in the salt-depleted state at normotension. Salt repletion resulted in an improvement of GFR and ERPF, but the original values were not reached. Apparently, renal vasodilation could not compensate for the decrease in GFR due to the considerable fall in blood pressure. However, systemic normotension with loss of detrimental effects on central nervous and cardiovascular system at the cost of a small diminution of GFR seems to be an acceptable price.

In conclusion, this case history illustrates that treatment with captopril may facilitate severe sodium depletion and renal function loss in some patients who use diuretics and who are on a rigid dietary sodium restriction. Patients using captopril have, in this respect, much in common with patients with the syndrome of the hyporeninaemic hypoaldosteronism[214]. One should be aware of the risks of diuretics in these patients, especially when combined with rigid dietary sodium restriction.

Appendix 3
Captopril-associated proteinuria
A report of four cases

This appendix contains the case histories of 4 patients, who developed captopril-associated proteinuria and/or a nephrotic syndrome.

Case history 3

The patient was a 48-year-old woman in whom hypertension (155/100 mmHg) was found in 1965. Her medical history included an appendectomy. Two pregnancies had been uneventful. Her mother had also suffered from hypertension.

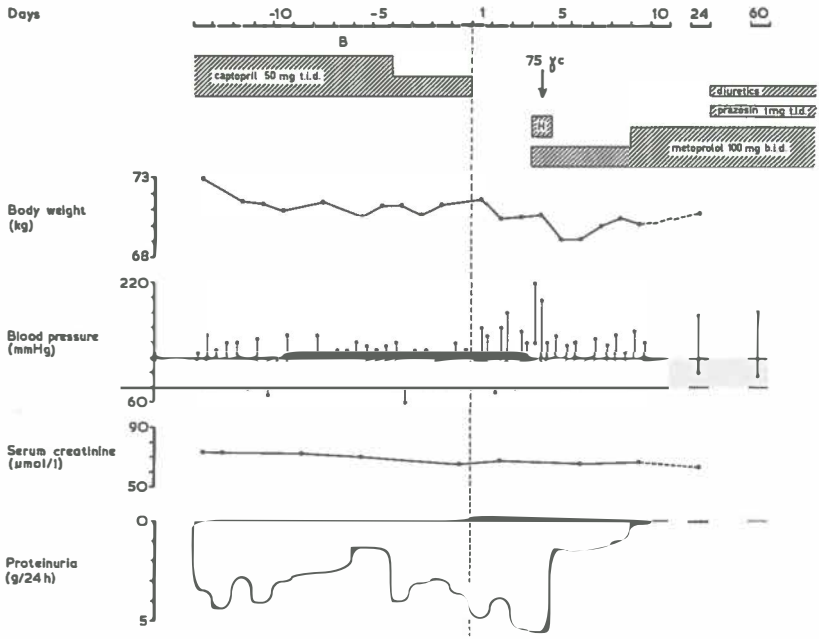
In 1977 the patient was referred to our outpatient clinic because her BP was 260/140 mmHg supine and 270/150 mmHg upright despite treament with sodium restriction, chlorthalidone and α-methyldopa. Chest X-ray and ECG showed no abnormalities. Fundoscopy revealed hypertensive changes KW grade II. Rapid sequence intravenous urography, renography and renal angiography were normal. Without therapy, serum sodium was 139 mmol/l, serum potassium 4.3 mmol/l and serum creatinine 77 μmol/l. Vanilylmandelic acid excretion was normal. The urine contained neither protein nor glucose and the urinary sediment was normal. A diagnosis of essential hypertension was made and she was treated with sodium restriction, hydrochlorothiazide, potassium suppletion and clonidine.

In October, 1978, the antihypertensive therapy was discontinued because her BP control was unsatisfactory (200/120 mmHg supine). The BP rose to 220/140 mmHg. At the time serum sodium was 139 mmol/l, serum potassium 4.2 mmol/l and serum creatinine 71 μmol/l. GFR and ERPF were 94 and 380 ml/min, respectively; FF was 0.25. The urine contained no protein and the urinary sediment was normal again. ANA were not found. An adequate BP control (130/80 mmHg) was obtained with 50 mg captopril t.i.d. and the patient remaind normotensive during the monthly visits to the outpatient clinic. GFR rose to 103 and ERPF to 426 ml/min.

In February and March, 1979, slightly positive (titer 1:10) ANA (homogenous pattern) were obtained. Antibodies against native DNA were absent. In April, 1979, the patient noticed oedema of the legs. Proteinuria was found (5 g/24h). On admission in May, 1979, her BP was normal (120/80 mmHg) as was the urinary sediment. Hypoalbuminaemia (2.6 g/100ml) and hypercholesterolaemia (10.4 mmol/l) existed. Serum creatinine was 74 μmol/l, GFR 96 ml/min, ERPF ml/min and FF 0.22. ANA were positive (titer 1:10); antibodies against native DNA were not detectable. Complement (C_{3c}) amounted to 124% of standard serum. There was no cryoglobulinaemia. HLA-typing revealed the phenotype A2, A3, Bw16, Bw38, B40, Bw60, Bw4, Bw6, DRw6. A renal biopsy was performed and no proliferative or exsudative changes were found on light microscopy. Electron dense deposits along the subepithelial side of the glomerular basement membrane (GBM) were revealed on electron microscopy. Immunofluorescence showed IgA, IgM and C_3 distributed in a granular pattern along the capillary walls.

Captopril was discontinued and BP rose subsequently to pre-existing values (Figure 31). PRA decreased and PAC increased. Hypertension was now treated with hydrochlorothiazide, triamterene, metoprolol and prazosin. A satisfying BP control was achieved. Interestingly enough, proteinuria disappeared completely within 10 days after withdrawal of captopril.

During later visits at our outpatient clinic BP regulation was suboptimal (160/105 mmHg). Proteinuria was not found any more. ANA remained negative. A renal biopsy in May, 1980, disclosed the development of spikes on light microscopy (Figure 32). Electron microscopy and immunofluorescence had not changed.



GFR (ml/min)	103	96	95	88	99
ERPF(ml/min)	426	430	445	460	369
FF	0.24	0.22	0.21	0.19	0.26

Figure 31. The effect of withdrawal of captopril on blood pressure, serum creatinine, proteinuria and renal function in patient 3.

Comments.

A 48-year-old woman with essential hypertension developed a nephrotic syndrome 5½ months after starting captopril treatment. Proteinuria disappeared within 10 days after withdrawal of captopril. Renal biopsy initially disclosed an early stage of MGP (stage I), whereas a second biopsy one year after withdrawal of captopril revealed "progression" to spike-formation.

A remarkable thing was that proteinuria in our patient disappeared completely within 10 days after withholding the drug. In other drug-induced immune complex glomerulopathies, for instance penicillamine-induced,

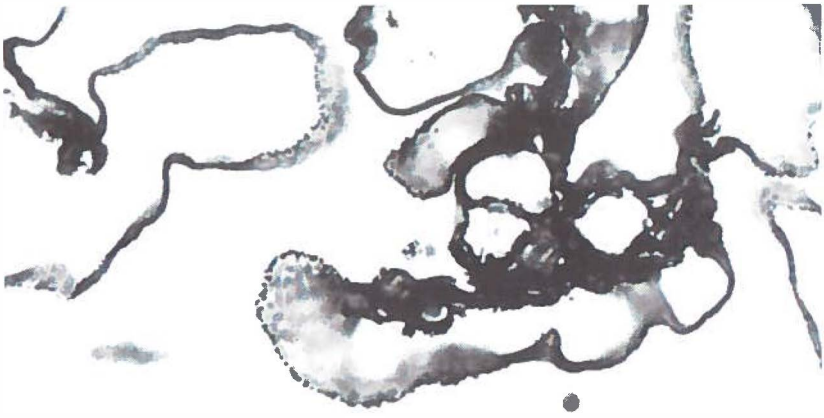


Figure 32. Patient 3. Light microscopy of the renal biopsies of May, 1980 (x 1400). Spike formation is clearly visible in this biopsy.

proteinuria disappears after a much longer time and more parallel with the disappearance of the morphological changes[17]. We wonder therefore if captopril, by decreasing the generation of A II and by inhibiting the degradation of BK, might facilitate glomerular protein loss when immune complex deposition has taken place. In this respect it is noteworthy that the protease inhibitor trasylol reduces aminonucleoside-induced proteinuria in the rat[101]. Trasylol inhibits generation of kallikrein and thereby also the generation of BK. It is even possible for the pharmacological action of captopril to have played a permissive role in the deposition or in situ formation of immune complexes. The antigen in these immune complexes remains, however, to be elucidated.

Case history 4

The patient was a 52-year-old man in whom hypertension was discovered in January, 1978. Blood pressure amounted to 180/105 mmHg. The intravenous urography was described as normal. Chest X-ray and ECG showed slight cardiomegaly and LVH, respectively. Without therapy serum creatinine was 105 μmol/l. A diagnosis of essential hypertension was made. Treatment with sodium restriction, furosemide, propranolol, clonidine and guanethidine was inadequate to lower BP.

The patient was admitted to our hospital in May, 1979. Rapid sequence urography showed a delayed nephrography. The length of the right kidney was 13.5 cm, whereas the left measured 15 cm. Renal angiography disclosed a bilateral renal artery stenosis, with a post-stenotic dilatation of the right renal artery.

The antihypertensive therapy was gradually discontinued, whereupon BP rose to 260/120 mm Hg. Captopril was initiated and increased step by step to a daily dosage of 450 mg; hypertension persisted (220/110 mmHg). After addition of a diuretic, BP gradually decreased to 160/85 mmHg. When seen at weekly intervals at our outpatient clinic, a gradual decrease of renal function was observed (serum creatinine 356 μmol/l) whereas hyponatraemia had developed (126 mmol/l). Rash and arthralgia were noticed. The captopril dosage was decreased to 50 mg t.i.d. whereupon the side-effects disappeared within 4 days. A further decrease in renal function (serum creatinine 762 μmol/l) with hyponatraemia (121 mmol/l) was observed one week later. Sodium repletion restored renal function to pretreatment levels. However, captopril had to be increased to 50 mg q.i.d. in September, 1979, because of a recurrence of hypertension (160/105 mmHg).

The patient was readmitted 3 days later because of a generalized rash with epidermolysis, fever, arthralgia and lymphadenopathy. Laboratory findings included leukocytosis (21300/mm^3) with marked eosinophilia in the peripheral blood smear (40-50%), microscopic haematuria and slight proteinuria (0.8 g/24h). ANA with a homogenous pattern, previously negative, became positive at a titer of 1:1000. Anti-ds-DNA antibodies were not found. Circulating immune complexes were not detectable. Complement levels (C_{3c} and C_4) were normal. HLA typing revealed the phenotype A2, A9, B12, Bw4, DR4, DRw6. Chest X-ray demonstrated reticulonodular changes. A renal biopsy showed no abnormalities on light microscopy. On immunofluorescence granular deposition of IgG, IgA, IgM and C_3 was found along the GBM. Electron dense deposits were demonstrated along the subepithelial side of the GBM on electron microscopy.

Captopril was withdrawn with complete reversal of all clinical and biochemical abnormalities within 2 weeks. A lymphocyte transformation test 6 weeks later, with captopril as an antigen, was positive in the patient whereas there was no stimulation in controls. Moreover, patch tests also revealed the development of delayed hypersensitivity to the drug.

With regard to BP control, the patient was treated with metoprolol, furosemide and minoxidil. The patient was discharged; afterwards dipstick control on proteinuria was occasionally positive, while protein excretion was mostly below 500 mg per day. A second biopsy in September, 1980, disclosed the same abnormalities as were found in the first renal biopsy.

In summary, a 52-year-old male with uncontrollable hypertension due to bilateral renal artery stenosis, developed a serum-sickness-like syndrome 2½ months after starting captopril treatment. Renal biopsy disclosed an early stage of MGP. Withdrawal of captopril resulted in a complete subsidence of all symptoms within 2 weeks. A repeat biopsy one year later disclosed unaltered histological abnormalities.

Comments.

The case history of this patient was characterized by the development of a serum-sickness-like syndrome on captopril therapy. Moreover, renal biopsy disclosed MGP, stage I. This combination is the more interesting as it is tempting to speculate on disease mechanisms. (see chapter 5).

This case history is similar to case history 2 in that both patients developed a severe sodium deficit and renal function loss. Again, this condition was provoked by addition of a diuretic to captopril treatment, the patient being on a severely sodium-restricted diet.

Case history 5

A 60-year-old man was admitted for evaluation and treatment of malignant hypertension in March, 1980. His medical history included angina pectoris since 1978. Hypertension (160/110 mm Hg) developed at the beginning of 1979; treatment was started with propranolol, hydralazine and furosemide with good result (150/90 mm Hg) at first. However, high blood pressure gradually recurred (170/120 mm Hg). The patient complained of headache and blurred vision. Fundoscopy revealed KW-grade IV hypertensive changes. On admission BP amounted to 200/120 mm Hg. Electrocardiography showed LVH and strain. Creatinine clearance was 30 ml/min; urinary sediment was normal while no proteinuria was found. Renal function studies revealed a GFR of 33 ml/min and ERPF of 140 ml/min. Angiography disclosed severe atherosclerotic changes of the abdominal aorta. Vessel wall irregularities were seen in both renal arteries; a stenosis with poststenotic dilation was seen on the left side.

Captopril was instituted and satisfactory BP control was obtained (150/90 mm Hg); the patient was discharged. During outpatient clinic visits a gradual deterioration of the renal function was observed. Subsequently progressive proteinuria and a nephrotic syndrome developed and the patient was readmitted in August, 1980 (Figure 33).

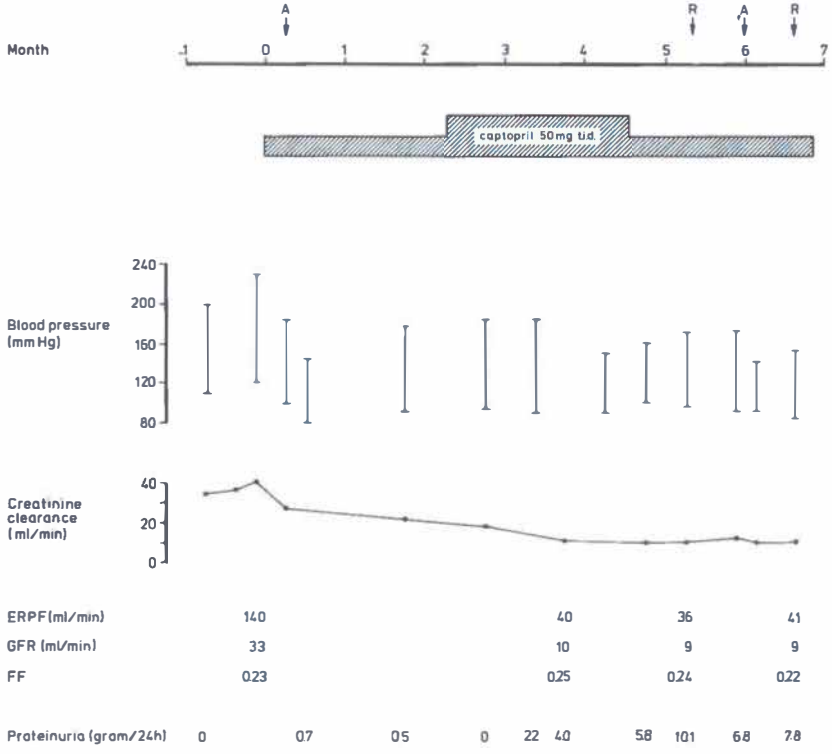


Figure 33. Patient 5. The clinical course on captopril treatment. A = angiography; R = renal biopsy.

At physical examination, blood pressure amounted to 150/90 mmHg; slight peripheral oedema was in evidence. Urinary protein excretion varied between 6.5 and 10.1 grams per day. Urinary sediment was normal. Serum albumin was low (27 g/l); hypercholesterolaemia was found (8.7 mmol/l). Glomerular filtration rate had decreased to 9 ml/min. HLA typing revealed the phenotype A1, A3, B8, B5, Bw6, Cw3, DR3, DR7.

A percutaneous renal biopsy was taken from the lower pole of the right kidney. Light microscopy showed 20 glomeruli; 4 were completely hyalinized. Ischaemic changes of the other glomeruli were observed. The vascular walls were thickened. Immunofluorescence studies revealed a non-specific, patchy fluorescence of C_3. At electron microscopy some sparsely distributed electron dense deposits at the epithelial side of the glomerular basement membrane were observed (Figure 34). The clinical picture could not be accounted for. Therefore renal angiography was repeated. The right renal artery, previously patent, now proved to be completely occluded. Some newly developed collaterals supplied the lower pole of the right kidney, which had shrunk from 13.5 to 10.4 cm. Size and shape of the left renal artery and kidney were unchanged. By now a cause had been found for the deterioration of renal function but the aetiology of the proteinuria was still obscure. It was decided to perform a biopsy of the left kidney.

At light microscopy the histological findings were analogous to the changes observed in the right kidney. Immunofluorescence studies, however, revealed extensive regular granular deposits of IgG, IgM and C_3 along the capillary walls. Electron microscopy showed abundant electron dense deposits along the subepithelial side of the GBM (Figure 34). Based on these findings, a diagnosis of MGP, stage I was made. Captopril was withdrawn and replaced by minoxidil. Four months later blood pressure was 160/95 mmHg. Gradually, proteinuria had completely disappeared.

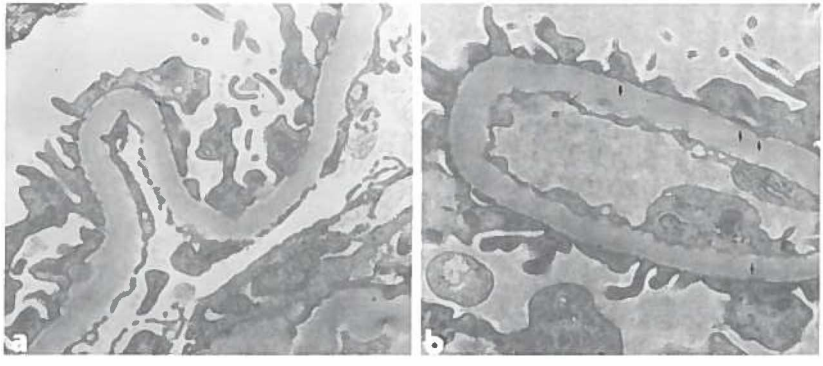


Figure 34. Patient 5. Electron micrographs of the renal biopsies of the right (A) and left (B) kidney. For details see text. (Uranyl acetate, lead citrate, x 10.000).

Comments.

The case history of this patient is remarkable in that both proteinuria and irreversible renal function loss developed in one patient. Though the combination of renal function loss and proteinuria occurs frequently in later stages of idiopathic membranous glomerulopathy, this combination is rarely en-

countered in case of drug-induced MGP. However, further investigation revealed two different processes that were responsible for this particular disorder. Renal failure was – at least partly – due to occlusion of the right renal artery, while unilateral MGP was present in the left kidney.

Unilateral glomerulopathy is a rare human disorder. It has been reported mainly in patients with unilateral hydronephrosis or renal artery constriction. Experimental studies have shown that renal artery constriction protects the kidney against various forms of experimentally induced glomerulopathy. The occlusion of the right renal artery in our patient was documented to have occurred during captopril treatment. Therefore, the marked difference in the distribution of the glomerular lesions between the two kidneys provides strong evidence for a causal role of captopril in the development of MGP in this patient.

Case history 6

A 51-year-old male was admitted for evaluation and treatment of malignant hypertension (270/150 mmHg). His medical history was unremarkable. BP was normal (140/80 mmHg) 6 months before admission. Except for blurred vision the patient had no complaints. Pretreatment physical examination was unremarkable except for KW grade IV fundi and a systolic murmur at the left para-umbilical site. Electrocardiography disclosed LVH and strain. GFR amounted to 65 ml/min, ERPF to 207 ml/min and FF was 0.31. Proteinuria was not detected. Rapid sequence intravenous urography showed delayed nephrography of the left kidney. Angiography revealed a severe renal artery stenosis with post-stenotic dilatation of the left kidney. Renal vein catheterization disclosed lateralization. Captopril was started and normotension was achieved on a dosage of 75 mg t.i.d. in combination with a moderately sodium restricted diet. GFR was 72 and ERPF 293 ml/min at his discharge.

The patient remained normotensive during the next months. He was admitted in March, 1980, for treatment of the renal artery stenosis by percutaneous transluminal angioplasty. BP was 130/80 mmHg; dipstick examination of the urine disclosed a trace of proteinuria. Angiography revealed a complete obstruction of the previously stenotic renal artery. The patient was discharged but the next few months proteinuria gradually increased and he was readmitted in May, 1980.

BP was 150/95 mmHg; except for slight ankle oedema physical examination produced no abnormalities. Hypalbuminaemia (29 g/l) and hypercholesterolaemia (8.4 mmol/l) were found. Tests for ANA (which had been positive in low titers of 1/10 in March, 1980), anti-ds DNA antibodies, cryoglobulinaemia and circulating immune complexes were negative. HLA-typing showed the presence of the phenotype A1, A9, B17, B15, Bw62, Bw4, Cw3, DR7.

Renal biopsy (right kidney) was performed. Light microscopy showed no gross abnormalities. Immunofluorescence studies of the specimen showed IgG, IgA, IgM and C_3 granular deposits. On EM there were electron dense subepithelial deposits. A diagnosis of MGP stage I was made.

Studies were performed on the possible influence of captopril-induced hormonal changes in prostaglandins and bradykinin on urinary protein excretion during this admission. The results are shown in Figure 35. Indomethacin – when given while the patient was on a 200 mmol sodium containing diet – decreased proteinuria from 6.1 to 1.8 g/24h. Indomethacin decreased GFR (82.5 to 75 ml/min) and ERPF (311 to 299 ml/min). Prolonged aprotin-infusion (50.000 kI-U/h

during 3 days) had no demonstrable effect on urinary protein excretion or renal function.

Captopril was subsequently withdrawn and replaced by metoprolol. Proteinuria gradually diminished and had completely disappeared in January, 1981.

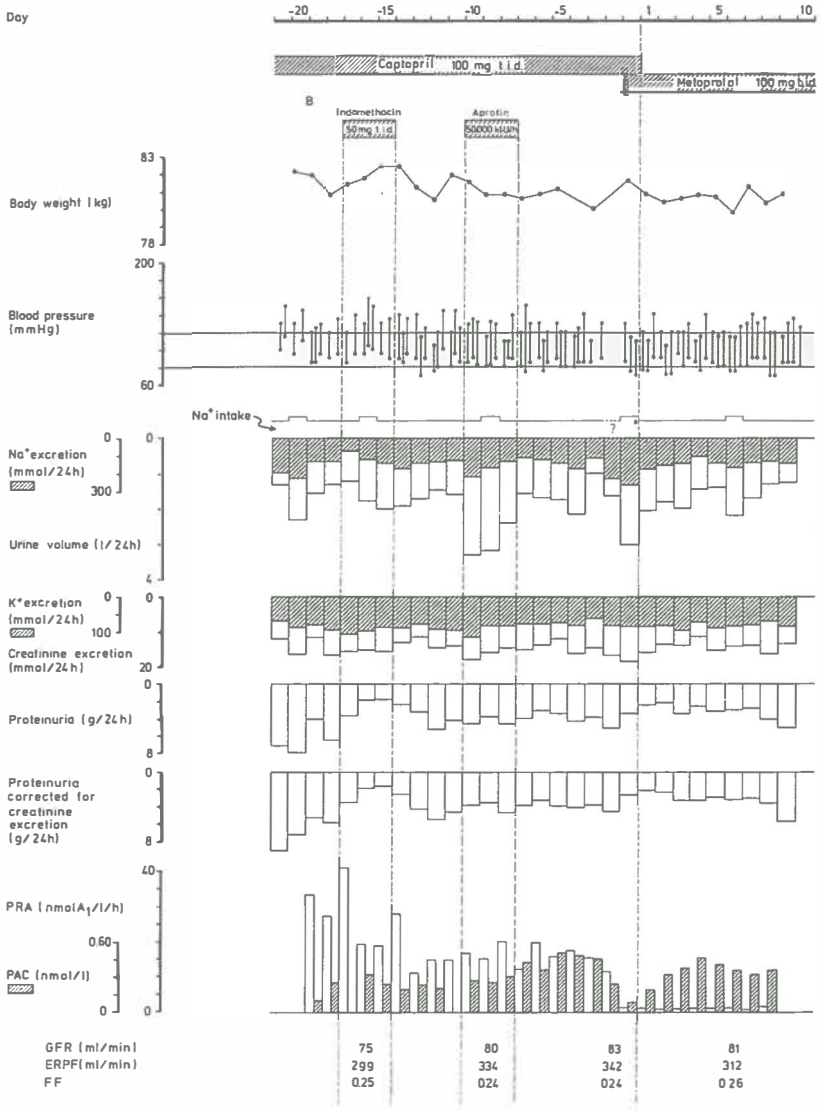


Figure 35. Patient 6. The effects of indomethacin and aprotin on urinary protein excretion and renal function during captopril treatment.

Comments.

A 51-year-old man with renovascular hypertension developed a complete obstruction of the stenotic renal artery and a nephrotic syndrome due to MGP on captopril therapy. Proteinuria gradually disappeared after stopping captopril.

It has been proposed that indomethacin decreases proteinuria by inhibition of renal prostaglandin synthesis. Moreover, it has been shown that the decrease in urinary protein loss is more pronounced by sodium depletion, while sodium depletion itself does not influence the renal capillary protein leak[65]. Therefore, the decrease in proteinuria in this patient, who was sodium repleted, is remarkable. It appeared that aprotin, a potent inhibitor of human plasma kallikrein and other proteases, did not interfere with urinary protein loss. This observation makes an important role for BK as mediator of the captopril-associated proteinuria less probable.

This case history demonstrates that captopril may influence renal function in two opposite ways in patients with renovascular hypertension. Despite the complete obstruction of the left renal artery and an impressive drop in BP, GFR and ERPF improved during CE inhibition. This indicates that the function of the non-stenotic kidney was suppressed by a stimulated RAS before captopril. There is no certainty as to the question whether captopril enhanced the progression to complete obstruction of the stenotic artery by ablating the renin-mediated protection of the stenotic kidney against ischaemia.

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