Table 9.5: Cell membrane ATPase activity in the combined black and white groups.

	Normotensive group (n = 78)	Hypertensive group (n = 76)
Erythrocyte membrane		
Na^+-K^+-ATPase	10,7 ± 2,1	7,6 ± 1,58**
Ca^{2+}-ATPase	11,2 ± 1,98	10,2 ± 1,81*
Mg^{2+}-ATPase	60,7 ± 5,06	57,6 ± 5,39*
Platelet membrane		
Na^+-K^+-ATPase	14,0 ± 3,06	10,9 ± 2,41**
Ca^{2+}-ATPase	14,5 ± 2,79	11,6 ± 3,04*
Mg^{2+}-ATPase	65,3 ± 7,20	59,7 ± 8,05**

Students-t-test compares the normotensive and hypertensive groups
* $p < 0,05$; ** $p < 0,01$

ATPase activity is expressed as:-

nmol/mg membrane protein/minute at 37°C

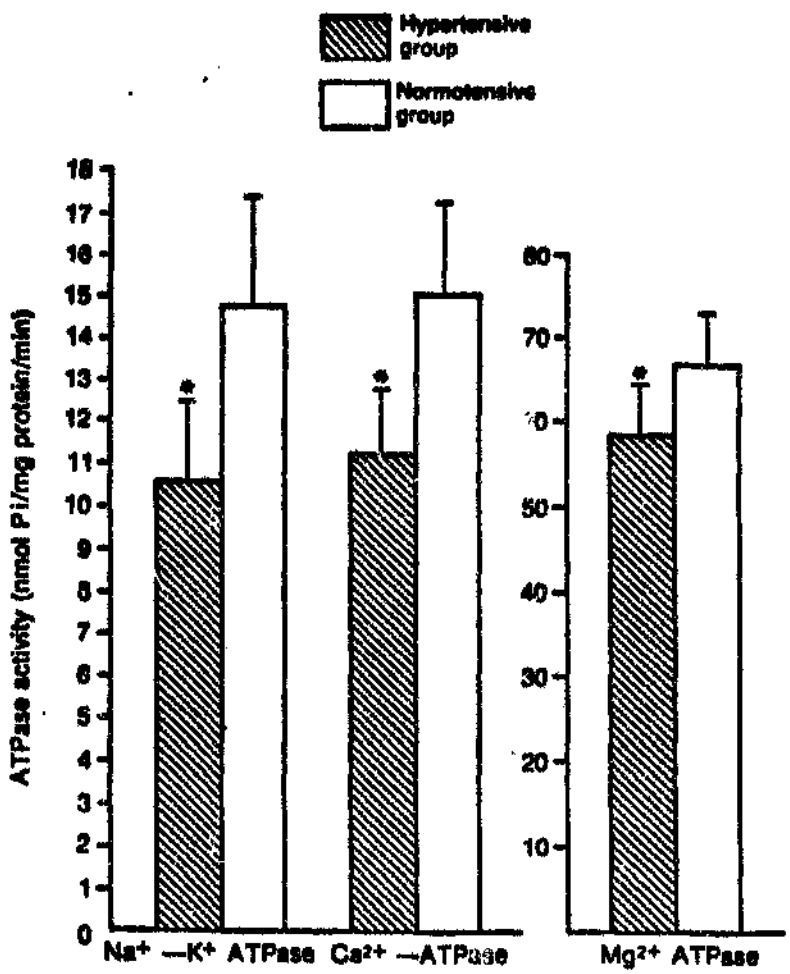


Figure 9.3:

Histogram of platelet membrane Na^+ —K^+ —ATPase, Ca^{2+} —ATPase and Mg^{2+} —ATPase activity in the hypertensive and normotensive groups

Pi = Inorganic phosphate

* $p < 0,05$ Normotensive group vs hypertensive group

Table 9.6 Pairwise-t-test comparisons for platelet membrane ATPase activity between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	0,0001			
BNT	0,0001	-		
	0,0001			
	0,0001	NS		
WHT	NS	0,0001	-	
	0,02	0,05		
	0,0001	0,02	NS	
WNT	0,01	0,01	NS	-
	NS	NS	NS	

Top value represents platelet membrane Na^+-K^+-ATPase activity, middle value Ca^{2+}-ATPase activity, bottom value platelet Mg^{2+}-ATPase activity.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

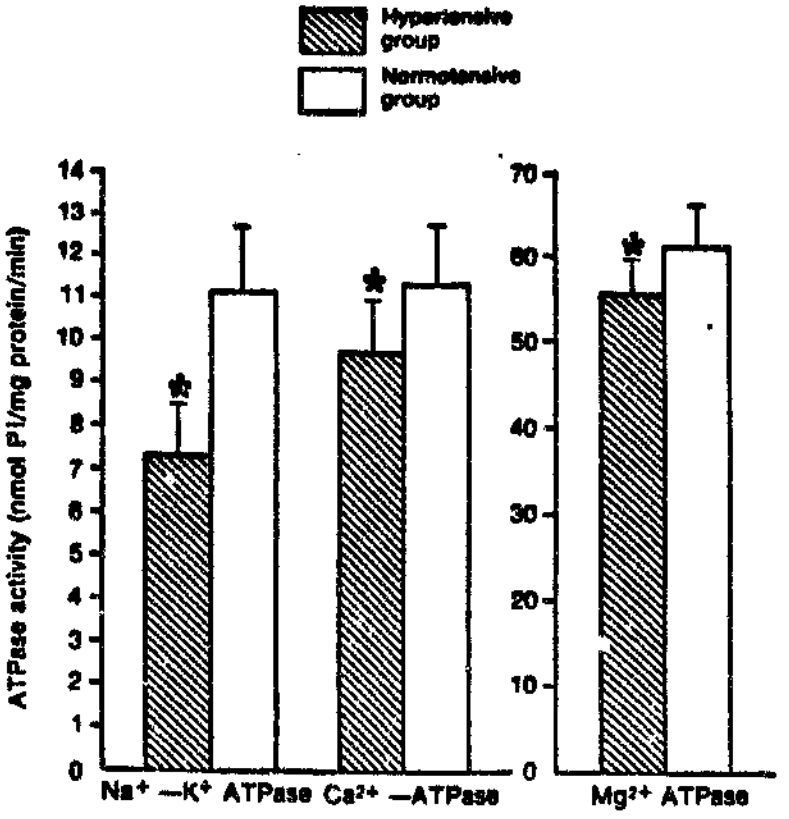


Figure 9.4:
Histogram of erythrocyte membrane Na^+ —K^+ ATPase, Ca^{2+} —ATPase and Mg^{2+} —ATPase activity in the hypertensive and normotensive groups

Pi = inorganic phosphate

* $p < 0,05$ Normotensive group vs hypertensive group

Table 9.7: Pairwise-t-test comparisons of platelet membrane ATPase activity between the male and female, black and white, normotensive and hypertensive groups.
Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS NS	-						
3	0,0001 0,0001 0,0001	0,0004 0,0001 0,0008	-					
4	0,0001 0,0001 0,002	0,0001 0,0001 0,01	0,001 NS NS	-				
5	NS NS NS	NS NS NS	0,01 0,003 0,01	0,0001 0,001 NS	-			
6	NS NS NS	NS NS 0,02	0,02 0,01 NS	0,0001 0,006 NS	NS NS NS	-		
7	0,002 0,01 0,008	0,01 0,003 NS	NS NS NS	0,0006 NS NS	NS NS NS	NS NS NS	-	
8	NS NS NS	NS 0,07 NS	0,01 0,02 NS	0,0001 0,01 NS	NS NS NS	NS NS NS	NS NS NS	-

Top value represents Na^{2+}-K^{+}-ATPase activity, middle value Ca^{2+}-ATPase activity and bottom value Mg^{2+}-ATPase.

Group			Group		
1	=	BHT female	5	=	WHT female
2	=	BHT male	6	=	WHT male
3	=	BNT female	7	=	WNT female
4	=	BNT male	8	=	WNT male
			NS	=	not significant

Table 9.8 Pairwise-t-test comparisons for erythrocyte membrane ATPase activity between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	0,0001			
BNT	0,001	-		
	0,0008			
	0,05	0,0001		
WHT	NS	NS	-	
	NS	NS		
	0,0001	0,0001	NS	
WNT	NS	NS	NS	-
	0,007	NS	NS	

Top value represents erythrocyte membrane Na^+-K^+-ATPase activity, middle value Ca^{2+}-ATPase activity and bottom value Mg^{2+}-ATPase activity.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 9.9: Pairwise-t-test comparisons of erythrocyte membrane ATPase activity between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS 0,08	-						
3	0,0001 0,002 0,01	0,0001 0,001 NS	-					
4	0,0001 0,0001 0,0001	0,0001 0,001 0,01	0,005 0,05 NS	-				
5	0,03 NS 0,01	0,04 NS NS	0,0001 NS NS	0,0001 NS NS	-			
6	NS NS NS	NS NS NS	0,0001 NS NS	0,0001 NS NS	NS NS NS	-		
7	0,0002 0,03 0,02	0,0004 0,04 NS	0,0001 NS NS	0,03 0,03 NS	NS 0,04 NS	0,02 NS NS	-	
8	0,0009 NS 0,001	0,001 NS NS	0,0001 NS NS	0,01 NS NS	NS NS NS	0,04 NS NS	NS 0,0001 NS	-

Top value represent Na^{2+}-K^{+}-ATPase activity, middle value Ca^{2+}-ATPase activity and bottom value Mg^{2+}-ATPase.

Group			Group		
1	=	BHT female	5	=	WHT female
2	=	BHT male	6	=	WHT male
3	=	BNT female	7	=	WNT female
4	=	BNT male	8	=	WNT male
			NS	=	not significant

9.4.3 Correlation studies

9.4.3.1 Correlations between ATPase activity and MAP in the normotensive and hypertensive groups

9.4.3.1 (i) Black group (Table 9.10)

In the normotensive group, there was a significant inverse correlation between erythrocyte Na^+-K^+-ATPase activity and MAP ($r = -0,39$; $p = 0,009$) and between erythrocyte Ca^{2+}-ATPase and MAP ($r = -0.31$; $p = 0.0001$).

In the hypertensive group there was a significant inverse correlation between MAP and all the ATPases (platelet and erythrocyte membrane) ($p = 0,0001$).

9.4.3.1 (ii) White group (Table 9.11)

There were no significant correlations between ATPase activity and MAP in the normotensive and hypertensive white groups.

9.4.3.2 Correlations between ATPase activity and the cations in the normotensive and hypertensive groups

9.4.3.2 (i) Black group (Tables 9.12, 9.13)

In the normotensive group, there were significant positive correlations between:-

- serum Na^+ and erythrocyte Na^+-K^+-ATPase activity ($r = 0,30$; $p = 0,05$)
- serum Na^+ and platelet Ca^{2+}-ATPase activity ($r = 0,40$; $p = 0,03$)
- platelet K^+ and platelet Na^+-K^+-ATPase activity ($r = 0,31$; $p = 0,02$)

There were significant inverse correlations between:-

- serum K^+ and platelet Na^+-K^+-ATPase activity ($r = -0,30$; $p = 0,03$)
- platelet Ca^{2+} and platelet Mg^{2+}-ATPase activity ($r = -0,27$; $p = 0,05$)

In the hypertensive group, there were significant direct correlations between:-

- erythrocyte Mg^{2+} and erythrocyte Mg^{2+}-ATPase activity ($r = 0,30$; $p = 0,04$)
- serum Ca^{2+} and erythrocyte Ca^{2+}-ATPase activity ($r = 0,33$; $p = 0,01$)
- serum Mg^{2+} and erythrocyte Mg^{2+}-ATPase activity ($r = 0,29$; $p = 0,05$)
- serum Mg^{2+} and platelet Mg^{2+}-ATPase activity ($r = 0,28$; $p = 0,05$)

- platelet Mg^{2+} and platelet Na^+-K^+-ATPase activity
  ($r = 0,32$; $p = 0,02$)

Significant inverse correlations included:-

- erythrocyte Na^+ and erythrocyte Ca^{2+}-ATPase activity
  ($r = -0,30$; $p = 0,04$)
- platelet Ca^{2+} and platelet Mg^{2+}-ATPase activity
  ($r = -0,47$; $p = 0,0005$)

9.4.3.2 (ii) White group (Tables 9.14, 9.15)

In the normotensive group, there were significant positive correlations between:-

- erythrocyte K^+ and erythrocyte Ca^{2+}-ATPase activity
  ($r = 0,60$; $p = 0,001$)
- platelet K^+ and platelet Na^+-K^+-ATPase activity
  ($r = 0,38$; $p = 0,05$)
- platelet K^+ and platelet Ca^{2+}-ATPase activity
  ($r = 0,49$; $p = 0,01$)
- platelet Mg^{2+} and platelet Na^+-K^+-ATPase activity
  ($r = 0,41$; $p = 0,03$)

Significant inverse correlations included:-

- serum Na^+ and platelet Na^+-K^+-ATPase activity
  ($r = -0,53$; $p = 0,004$)
- platelet Na^+ and platelet Mg^{2+}-ATPase activity
  ($r = -0,49$; $p = 0,01$)

- serum K^+ and platelet Na^+-K^+-ATPase activity
  ($r = -0,38$; $p = 0,05$)
- platelet Ca^{2+} and platelet Mg^{2+}-ATPase activity
  ($r = -0,53$; $p = 0,006$)

In the hypertensive group, there were significant direct correlations between:-

- serum Na^+ and platelet Mg^{2+}-ATPase activity
  ($r = 0,52$; $p = 0,009$)
- serum K^+ and platelet Ca^{2+}-ATPase activity
  ($r = 0,44$; $p = 0,03$)
- serum Mg^{2+} and platelet Mg^{2+}-ATPase activity
  ($r = 0,48$; $p = 0,01$)

Significant inverse correlations included:-

- erythrocyte K^+ and erythrocyte Ca^{2+}-ATPase activity
  ($r = -0,51$; $p = 0,01$)

Table 9.10: Pearsons correlation coefficients for correlations between cell membrane ATPase activity and MAP in the black group.

	Black group			
	Normotensive blacks		Hypertensive blacks	
	r - value	p - value	r - value	p - value
Erythrocyte membrane				
Na^+-K^+-ATPase	-0,39	0,009*	-0,34	0,01*
Ca^{2+}-ATPase	-0,31	0,02*	-0,46	0,0008*
Mg^{2+}-ATPase	-0,13	0,34	-0,45	0,0007*
Platelet membrane				
Na^+-K^+-ATPase	0,20	0,14	-0,39	0,003*
Ca^{2+}-ATPase	-0,06	0,67	-0,55	0,0001*
Mg^{2+}-ATPase	-0,17	0,12	-0,42	0,001*

* = significant correlation

Table 9.11: Pearsons correlation coefficients for correlations between cell membrane ATPase activity and MAP in the white group.

	White group			
	Normotensive whites		Hypertensive whites	
	r - value	p - value	r - value	p - value
Erythrocyte membrane				
Na^+-K^+-ATPase	-0,01	0,95	-0,26	0,20
Ca^{2+}-ATPase	0,10	0,60	-0,33	0,11
Mg^{2+}-ATPase	0,05	0,77	-0,07	0,71
Platelet membrane				
Na^+-K^+-ATPase	0,09	0,60	-0,26	0,22
Ca^{2+}-ATPase	0,07	0,70	-0,36	0,09
Mg^{2+}-ATPase	-0,16	0,43	-0,26	0,22

Table 9.12: Pearsons correlation coefficients for correlations between the cations and cell membrane ATPase activity in the black normotensive group.

	Erythrocyte membrane ATPase activity			Platelet membrane ATPase activity		
	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A
Serum Na^+	0,30*	-0,04	0,04	0,08	0,40*	0,01
Erythrocyte Na^+	0,07	0,33	0,06	0,06	0,04	0,11
Platelet Na^+	0,10	0,26	0,08	0,12	0,22	0,02
Serum K^+	0,21	0,22	0,02	-0,30*	-0,20	0,06
Erythrocyte K^+	0,04	0,01	0,07	0,02	0,11	0,01
Platelet K^+	0,07	0,08	0,09	0,31*	0,02	0,15
Serum Ca^{2+}	0,12	0,16	23	0,01	0,08	0,13
Erythrocyte Ca^{2+}	0,10	0,05	0,09	0,17	0,06	-0,26
Platelet Ca^{2+}	0,18	0,14	-0,35	0,01	0,04	-0,27*
Serum Mg^{2+}	0,12	0,09	0,03	0,03	0,06	0,18
Erythrocyte Mg^{2+}	0,04	0,01	0,14	0,08	0,10	0,34
Platelet Mg^{2+}	0,23	0,07	0,01	0,07	0,01	0,14

* $p < 0,05$

Na^+-K^+-A = Na^+-K^+-ATPase; Ca^{2+}-A = Ca^{2+}-A = Ca^{2+}-ATPase;
Mg^{2+}-A = Mg^{2+}-ATPase

Table 9.13: Pearsons correlation coefficients for correlations between the cations and cell membrane ATPase activity in the black hypertensive group.

	Erythrocyte membrane ATPase activity			Platelet membrane ATPase activity		
	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A
Serum Na^+	0,09	0,05	0,23	0,02	0,10	0,13
Erythrocyte Na^+	0,02	-0,30*	0,05	0,15	0,01	-0,29
Platelet Na^+	0,08	0,06	0,30	0,24	-0,27*	0,08
Serum K^+	0,07	0,05	0,01	0,02	0,04	0,12
Erythrocyte K^+	0,10	0,10	0,11	0,17	0,12	0,03
Platelet K^+	0,29	0,08	-0,21	0,01	0,14	0,12
Serum Ca^{2+}	-0,11	0,33**	0,07	0,10	0,03	0,12
Erythrocyte Ca^{2+}	0,08	0,03	0,22	0,18	-0,32*	-0,14
Platelet Ca^{2+}	0,08	-0,33	-0,41	0,22	0,12	-0,47**
Serum Mg^{2+}	0,21	0,05	0,29*	0,16	0,13	0,28*
Erythrocyte Mg^{2+}	0,01	0,07	0,30*	0,17	0,21	0,04
Platelet Mg^{2+}	0,18	0,03	0,27	0,32*	0,04	0,10

* $p < 0,05$; ** $p < 0,01$
Na^+-K^+-A = Na^+-K^+-ATPase; Ca^{2+}-A = Ca^{2+}-ATPase;
Mg^{2+}-A = Mg^{2+}-ATPase

Table 9.14: Pearsons correlation coefficients for correlations between the cations and cell membrane ATPase activity in the white normotensive group.

	Erythrocyte membrane ATPase activity			Platelet membrane ATPase activity		
	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A
Serum Na^+	-0,24	-0,38	0,12	-0,53	0,10	0,27
Erythrocyte Na^+	0,23	0,04	0,07	0,13	0,01	0,17
Platelet Na^+	0,02	0,09	0,21	0,24	0,01	-0,49**
Serum K^+	0,12	0,25	0,05	-0,38*	0,15	0,25
Erythrocyte K^+	0,14	0,60**	0,20	0,28	0,22	0,15
Platelet K^+	0,12	0,34	0,31	0,38*	0,49**	0,14
Serum Ca^{2+}	0,21	0,30	0,04	0,28	0,25	0,16
Erythrocyte Ca^{2+}	0,34	0,08	0,02	0,24	0,05	0,15
Platelet Ca^{2+}	0,08	0,02	0,03	0,23	0,30	-0,53**
Serum Mg^{2+}	0,30	-0,25	0,02	0,30	0,04	0,28
Erythrocyte Mg^{2+}	-0,40	0,05	0,16	0,01	0,29	0,14
Platelet Mg^{2+}	-0,41	-0,44	0,31	0,41*	0,07	0,10

* $p < 0,05$; ** $p < 0,01$

Na^+-K^+-A = Na^+-K^+-ATPase; Ca^{2+}-A = Ca^{2+} -A = Ca^{2+}-ATPase; Mg^{2+}-A = Mg^{2+}-ATPase

Table 9.15: Pearsons correlation coefficients for correlations between the cations and cell membrane ATPase activity in the white hypertensive group.

	Erythrocyte membrane ATPase activity			Platelet membrane ATPase activity		
	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A
Serum Na^+	-0,22	0,01	0,23	0,09	0,10	0,52**
Erythrocyte Na^+	-0,28	0,02	0,04	0,02	0,01	0,55**
Platelet Na^+	0,16	0,34	0,23	-0,21	0,06	0,33
Serum K^+	0,07	0,32	0,12	0,26	0,44*	0,11
Erythrocyte K^+	0,15	-0,51**	0,01	0,14	0,17	0,19
Platelet K^+	0,12	0,29	0,24	-0,33	0,03	0,13
Serum Ca^{2+}	0,27	0,01	0,19	0,28	0,11	0,01
Erythrocyte Ca^{2+}	0,09	0,14	0,14	0,09	0,07	0,09
Platelet Ca^{2+}	0,30	0,19	0,40	0,01	0,11	0,04
Serum Mg^{2+}	0,08	0,05	0,14	0,03	0,16	0,48**
Erythrocyte Mg^{2+}	0,19	0,26	0,11	0,17	-0,44	0,11
Platelet Mg^{2+}	0,28	0,08	0,18	0,21	0,02	0,51**

* $p < 0,05$; ** $p < 0,01$
Na^+-K^+-A = Na^+-K^+-ATPase; Ca^{2+}-A = Ca^{2+}-A = Ca^{2+}-ATPase;
Mg^{2+}-A = Mg^{2+}-ATPase

9.4.4 Summary of results

1. Erythrocyte and platelet membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were significantly lower in the black hypertensive group compared to the black normotensive group.
2. There were no significant differences in cell membrane ATPase activity between the white normotensive and hypertensive groups.
3. There were no significant differences in ATPase activity between males and females in the white and black groups.
4. Cell membrane ATPase activity was significantly depressed in the hypertensive group when the black and white groups were combined.
5. Cell membrane ATPase activity was inversely correlated to MAP in the black hypertensive group.
6. MAP was not correlated to ATPase activity in the white group.
7. Comparing the black group to the white group:-
   - activity of platelet membrane Na^+-K^+-ATPase and Mg^{2+}-ATPase was significantly lower in the black hypertensive group compared to the white hypertensive group.
   - erythrocyte Na^+-K^+-ATPase activity was significantly lower in the black hypertensive group compared to the white hypertensive group.

9.5 DISCUSSION

The results of this study demonstrate that black hypertensive patients have intracellular sodium and calcium overload with widespread magnesium depletion. The ion pump activity in erythrocyte and platelet membranes of black hypertensive subjects is significantly depressed compared to their normotensive counterparts. In the white hypertensive patients, although cytosolic calcium and sodium concentrations were elevated, the cell membrane ATPase activity was unchanged. This data suggests racial differences in cellular cation handling and transmembrane ion transport in essential hypertension.

Many techniques for quantifying erythrocyte Na^+-K^+-ATPase activity have been developed. In this study, the classic colorimetric method of measuring the inorganic phosphate released during ATP hydrolysis in the presence of prepared cell membranes was employed. Repetitive determinations performed during the study had an average coefficient of variation of 2,1% for erythrocyte Na^+-K^+-ATPase, 1,3% for erythrocyte Ca^{2+}-ATPase and 2,2% for erythrocyte Mg^{2+}-ATPase. The average coefficients of variation for platelet Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase were 0,9%, 1,7%, 1,4% respectively. The activity levels reported should be viewed as a relative index of pump function in the different groups studied.

In this study, cell membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were investigated in a large homogeneous urbanised black population as well as an urbanised white population. The normotensive and hypertensive and black and white groups were matched for age and body mass. Both platelets and erythrocytes were used as cell models. Platelets were studied because they resemble vascular smooth muscle cells and erythrocytes were studied as a baseline comparison, and to assess whether possible defects are limited to one cell type.

The membrane transport system that controls the intracellular ionic milieu may be defective in essential hypertension and may play a role in the differences between black and white hypertension (Gillim, 1979; Ives, 1989; Hall, 1990). Racial differences in Li^+-Na^+ cotransport, Na^+-K^+ cotransport, Na^+-Ca^{2+} exchange, Na^+-H^+ exchange and Na^+-K^+-ATPase have been reported (Hopp et al, 1986; Tuck et al, 1987; Canessa et al, 1984). Data on ATPase activity in black and white and between normotensive and hypertensive populations are conflicting (Parker et al, 1983; Hopp et al, 1986; Nakamura et al, 1989).

Beutler et al (1983) first postulated an influence of ethnic origin on the Na^+-pump and reported a low activity in Asians compared to non-Jewish whites. In Chinese populations, erythrocyte membrane Na^+-K^+-ATPase and Ca^{2+}-ATPase activities were lower in the hypertensive

individuals compared to the normotensive subjects, and significantly depressed compared to White Dutch subjects (Lin, 1985). Some researchers have failed to demonstrate racial differences in ATPase activity, while others have reported increased ATPase activity in white hypertensive patients (Simon and Engel, 1987; Landin et al, 1991). The results from this study confirm those of Beutler where Na^+ pump activity was significantly depressed in the black hypertensive patients with no changes in activity in the white hypertensive patients. Na^+-K^+-ATPase is responsible for a major part of sodium extrusion and potassium influx into the cell. Reduced pump activity may explain the increased intracellular sodium found in the black hypertensive group. Other mechanisms, besides Na^+-K^+-ATPase, must be responsible for the cytosolic sodium accumulation in white hypertensive subjects.

Intracellular calcium overload and defective calcium binding to the cell membrane have been reported for many cell types from essential hypertensive patients and spontaneously hypertensive rats (Postnov et al, 1979; Buhler and Resink, 1988). Essential hypertensive patients may have a widespread membrane defect that results in cytosolic calcium accumulation (Postnov, 1990). This defect relates to calcium efflux mechanisms and particularly to cell membrane Ca^{2+}-ATPase. The magnesium dependent Ca^{2+}-ATPase pump in the plasma membrane maintains a low intracellular calcium by pumping calcium from the

cytoplasm to the extracellular fluid against the electrochemical gradient. Altered Ca^{2+}-ATPase activity in erythrocyte and platelet membranes of SHR and hypertensive patients have been described, with Postnov et al (1979) reporting decreased activity and Resink et al (1986) increased activity. Although Nakamura et al (1989) reported racial differences in fibroblast calcium regulation, it is unknown whether calcium pump activity differs between black and white hypertensives.

In this study, the black hypertensive group had significantly depressed erythrocyte and platelet membrane Ca^{2+}-ATPase activity with significant correlations between Ca^{2+}-pump activity and intracellular calcium. Calcium pump activity in the white hypertensive group was unchanged. These results suggest that in black hypertension the cytosolic calcium overload may be due to decreased extrusion from the cell because of decreased Ca^{2+}-ATPase activity. The significant inverse correlations between platelet calcium and platelet Ca^{2+}-ATPase activity and between erythrocyte calcium and erythrocyte membrane Ca^{2+}-ATPase activity in the black hypertensive group confirms this suggestion. Other mechanisms, possibly increased intracellular calcium mobilisation or enhanced influx, may be responsible for calcium accumulation in the white hypertensive group.

The role of magnesium in the pathoaetiology of essential

hypertension is unclear. The majority of studies examining the relationship between magnesium and blood pressure have been performed in European and American whites. Decreased serum and erythrocyte magnesium concentrations, with significant inverse correlations between magnesium and blood pressure have previously been reported in South African black hypertensive males (Touyz et al, 1989). There is no available data comparing magnesium status in black and white hypertension. In this study, the black hypertensive patients had widespread magnesium depletion with decreased cell membrane Mg^{2+}-ATPase activity. Mechanisms of cellular magnesium regulation are unknown. Magnesium enters cells via facilitated diffusion and is extruded by an active transport system (Flatman, 1988). The exact function of Mg^{2+}-ATPase is unclear. It probably represents all magnesium dependent ATPases (including Na^{+}-K^{+}-ATPase and Ca^{2+}-ATPase) and may also be involved in transmembrane magnesium transport (Flatman, 1988).

In the black hypertensive group, serum erythrocyte and platelet magnesium concentrations were positively correlated with Mg^{2+}-ATPase activity. These relationships may be explained by the following hypothesis - decreased intracellular magnesium inhibits cell membrane Mg^{2+}-ATPase activity which decreases magnesium efflux, and consequently decreases the serum magnesium concentration.

Causes for depressed ATPase activity in the black hypertensive group may be related to extra- and intracellular magnesium deficiency. Since all ATPases have an obligatory need for magnesium, magnesium depletion results in pump activity depression. This thesis is supported by the fact that ATPase activity was positively correlated to serum and cellular magnesium concentrations. Reasons for magnesium deficiency in the black hypertensive group may be explained on a dietary basis. Blacks consume a low potassium and magnesium diet (Grim et al, 1980). Also the black group as a whole had a significantly higher serum GGT level (marker of alcohol intake) compared to the white group. Excessive alcohol intake is a major cause of magnesium deficiency (Lim and Jacobs, 1972).

Other factors that may affect ATPase activity in essential hypertension include circulating endogenous ATPase inhibitors, decreased cell membrane pump numbers and unknown variables (Hopp et al, 1986; Smith et al, 1988; Hamlyn et al, 1989; Iwaoka et al, 1991).

The effect that magnesium has on the Na^+-K^+-ATPase pump may explain why supplementation with potassium alone, in patients that are both magnesium and potassium-depleted, has no effect on intracellular potassium repletion. Magnesium must also be given to activate the Na^+-K^+-pump so that potassium can be transported into the cell (Fischer and Giroux, 1987). Whang et al (1983) reported four cases

of magnesium and potassium-depleted patients in which optimisation of intracellular potassium repletion occurred only after magnesium supplementation.

This is the first study comparing erythrocyte and platelet Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities between black and white hypertensive patients. Unlike other reports, the black group studied here comprised a homogeneous population with no genetic or ethnic diversity.

In conclusion, the findings here reveal racial differences in transmembrane ion transport. Although cytosolic sodium and calcium overload is common in both black and white hypertensive patients, the mechanisms responsible for this are different. Depressed ATPase activity, probably related to magnesium depletion, appears to be an important cause for the cellular cation changes in hypertensive blacks. Cellular ion alterations in hypertensive whites are not related to ATPase defects. If similar differences occur between the cells studied here and vascular smooth muscle the predisposition of blacks to essential hypertension could be explained.

CHAPTER 10

CELL MEMBRANE CALCIUM BINDING IN ESSENTIAL HYPERTENSION

10. CELL MEMBRANE CALCIUM BINDING IN ESSENTIAL HYPERTENSION

10.1 INTRODUCTION

10.1.1 Cell membrane calcium binding

The divalent cations, specifically calcium and magnesium affect biological membranes in many ways. For example, they influence the membrane properties of permeability, adhesiveness, sensitivity to electrical stimuli and chemical agents and they can act to modify the electrical potential near the surface to decrease membrane lipid fluidity, to cause clustering of certain membrane components and possibly to bridge anionic groups in a single membrane or in two different membranes (Onishi and Ito, 1973; Jacobson and Paphadjopoulos, 1975). These membrane properties play a role in maintaining the internal milieau of the cell. In vascular smooth muscle the above membrane characteristics influence contractility and tone, via changes in the intracellular calcium concentration.

There are two categories of electrostatic interactions between divalent cations and negatively charged surfaces (Bara et al, 1988). The first type is the electrostatic binding of calcium or magnesium to anionic surface moieties (Duffy and Schwartz, 1973). In the second type of association - referred to as screening, the cations remain

mobile being held loosely in a diffuse layer close to the surface (McLaughlin et al, 1971; Nir et al, 1978).

Calcium binding, to the inner or outer cell membrane, influences cell membrane function (Hurwitz et al, 1982). Calcium stabilises the electrically excitable membranes of nerve and muscle. By binding to the outer cell membrane, a positive surface potential is created and the cell depolarises (Bara et al, 1988). Membrane bound calcium also stabilises the potential operated calcium channels and thus transmembrane calcium influx (Romero, 1976). The exact stabilising mechanisms of these channels is unclear, but is probably related to the electrostatic charge of the membrane.

Calcium binding to the cell membrane is a function of both membrane lipids and proteins (Long and Mouat, 1971; Fortner and Manery, 1971; Duffy and Schwartz, 1973). Disturbances in membrane fluidity, phospholipid composition, intrinsic binding constant, and the calcium-binding constituents will affect the binding capacity of calcium to the cell membrane (Orlov and Postnov, 1982; Reznikova et al, 1984). In addition to these intrinsic cell membrane properties, other factors may affect calcium binding, including the concentration of intra- and extracellular free calcium and the presence of other cations (Nir et al, 1978; Bara, 1988). Magnesium competes with calcium for binding sites on the cell membrane (Altura

and Altura, 1985; Witteman and Grobbee, 1990).

Defective calcium binding results in depolarisation and activation of potential operated calcium channels (Robinson, 1984). These abnormalities would give rise to an increased basal calcium influx and consequently increased intracellular calcium (Buhler and Resink, 1988). In vascular smooth muscle, this would result in enhanced contractility and tone which are important factors influencing peripheral resistance and blood pressure (see chapter 1.2.3.1).

10.1.2 Calcium binding and hypertension

Several studies have revealed a number of functional and structural alterations of the erythrocyte membrane in essential hypertension and its experimental analogue, spontaneous hypertension of rats (SHR; Kyoto-Wistar) (Orlov and Postnov, 1982; Cirillo, 1990).

In particular, it has been demonstrated that permeability of the cell membrane for cations is increased and calcium-binding ability is decreased (Devynck et al, 1981a and 1981b; Cirillo et al, 1987; Bing et al, 1987). Altered calcium binding to the inner and outer plasma membranes of erythrocytes, adipocytes, hepatocytes and platelets in essential hypertensive patients have been described (Postnov and Orlov, 1985; Bing et al, 1987; Buhler and

Resink, 1988; Cooper et al, 1989).

Associated with the binding of calcium to the cell membrane is a phenomenon known as 'membrane stabilisation' (Furspan and Bohr, 1988). Elevations of extracellular calcium concentrations above physiological levels causes relaxation of vascular smooth muscle (Webb and Bohr, 1978). This response has been attributed to the membrane stabilising effects of calcium (Webb and Bohr, 1978). Jones and Hart (1975) and Furspan and Bohr (1986) showed that the extracellular calcium concentration alters potassium efflux from vascular smooth muscle cells and lymphocytes. As extracellular calcium levels increase, potassium efflux decreases, i.e. membrane stabilisation by calcium decreases membrane permeability to potassium (Romero, 1976). Holloway and Bohr (1973) demonstrated that the relaxing effect of high calcium concentrations was less effective in vascular smooth muscle from renal, DOCA and genetically hypertensive rats than in their normotensive controls. The impaired ability of calcium to stabilise the membranes was attributed to decreased cell membrane calcium binding (Webb and Bohr, 1978).

The altered calcium binding properties and increased cell membrane permeability appear to be specific features of essential hypertension. This is based on the fact that decreased calcium-binding, alterations in univalent-cation permeability and changes in the erythrocyte membrane

structure have been observed in essential hypertensive patients and SHR, but not in patients with renal hypertension nor in rats with renal or deoxycorticosterone/salt hypertension (Postnov et al, 1979; Orlov et al, 1982).

Not all studies have demonstrated decreased calcium binding to cell membranes of hypertensive subjects (Pernollet et al, 1981; Rapp et al, 1986). Cooper et al (1989) recently reported increased levels of calcium bound to the membrane of platelets of hypertensive compared with normotensive individuals. They also found that membrane-bound calcium was significantly related to blood pressure in correlation and regression analysis, and this relation appeared to be independent of other confounding factors (Cooper et al, 1989).

Postnov et al (1977) using an indirect method of assessing calcium binding reported evidence of altered calcium binding to the outer red blood cell membrane in essential hypertension.

10.1.3 Cell membrane calcium binding in relatives of hypertensive subjects - the genetics of calcium binding

Many abnormalities in cation transport that have been reported in essential hypertensive individuals have also

been observed in their first-degree relatives (Swales, 1983; Bing et al, 1986b). It has been suggested that these abnormalities are genetically influenced markers for processes directly involved in hypertension (Bing et al, 1986b). The altered calcium binding and cellular calcium handling in hypertension may also be due to genetic factors. A number of studies have examined membrane calcium binding and intraleukocyte calcium in subjects with a family history of hypertension and compared them with normotensive controls (Bing et al, 1986a; Bing et al, 1986b). Calcium binding by erythrocyte membranes was significantly reduced in the relatives compared with their controls. The reduction of calcium binding was of a similar magnitude to that observed in patients with essential hypertension; although blood pressure was no higher in the relatives (Bing et al, 1986b; Bing et al, 1987). Experimental studies have also suggested a genetic basis to altered calcium binding and cell membrane permeability in essential hypertension (Furspan et al, 1987).

10.1.4 Factors that may alter cell membrane calcium binding in essential hypertension

Altered calcium binding may be due to a reduction in the number of calcium binding sites, or to affinity alteration (Devynck et al, 1982; Postnov et al, 1979). Devynck et al (1982) have reported fewer calcium binding sites on plasma

membranes of heart, nerve and liver cells from SHR compared to those from WKY. Kwan et al (1979) also demonstrated that the membranes of mesenteric artery cells from SHR bind less calcium than those from WKY.

The alteration in the calcium binding ability of the cell membrane may be located at the inner surface i.e. to the part of membrane-bound calcium that determines membrane permeability to univalent cations (Postnov et al, 1977). This may contribute to the enhanced cell membrane permeability to sodium and potassium observed in hypertensive patients.

Abnormal cell membrane structure may also influence calcium binding. Many cell types including adipocytes, blood cells and nerve cells from hypertensive patients have abnormal membrane structure (Bing et al, 1986b). The fatty acid composition of cell membranes from hypertensive patients may differ to that from normotensive controls (Ollerenshaw et al, 1987). It has been shown that the linoleic acid content of cell membranes in SHRs and in hypertensive subjects is decreased (Naftilan et al, 1986; Nara et al, 1986).

Bing et al (1987) examined calcium binding in relation to erythrocyte membrane composition, and found a negative correlation between the palmitic - linoleic acid ratio and calcium binding. This is consistent with previous studies

which have demonstrated a reduction in the unsaturated fatty acid, linoleic acid, in the erythrocyte membrane of hypertensive subjects (Ollerenshaw et al, 1987). The effects of administering linoleic acid on cell membrane calcium properties have also been examined. Four weeks of linoleic acid given to 13 normotensive controls produced a significant decrease in intracellular calcium probably by altering cell membrane calcium binding (Bing et al, 1987).

Reduced sialic acid content has also been reported in erythrocyte membranes from hypertensive patients (Reznikova et al, 1984). Sialic acid and linoleic acid are important components of the calcium-binding constituents of the cell membrane (Forstner and Manery, 1971). Although the exact membrane component responsible for the altered membrane calcium binding has not been identified, the data suggest that abnormalities in cell membrane structure will alter the interaction of membrane lipids with calcium. At a molecular level, Kowarski et al (1986) found significant reductions of an 'integral membrane calcium-binding protein' in various tissues from SHR as compared to those from WKY.

Besides calcium, magnesium also binds to the cell membrane (Witteman and Grobbee, 1990). These ions bind to the same site. Because of the competitive binding, changes in the concentration of one ion will affect binding of the other. In essential hypertension, cellular magnesium homeostasis

may be altered (Altura and Altura, 1985a). Changes in extracellular and/or intracellular magnesium levels may thus affect calcium binding to the cell membrane.

10.2 AIMS

The aims of this study were:-

10.2.1 To measure the calcium binding capacity of the outer cell membranes of erythrocytes and platelets in hypertensive patients.

10.2.2 To determine the relationships between cell membrane calcium binding and the cations, and

10.2.3 To assess whether cell membrane calcium binding properties differ between black and white hypertensive patients.

10.3 SUBJECTS AND METHODS

10.3.1 Subjects

Calcium binding to the outer cell membrane of platelets and erythrocytes was measured in 76 hypertensive patients and 78 age, height and weight matched normotensive controls. Details of subject examination and venesection are described in chapter 5.

10.3.2 Materials and methods

10.3.2.1 Isolation of erythrocytes

The heparinised blood was centrifuged at 450 x g for 15 minutes and the plasma aspirated for cation analysis. The remaining erythrocyte sediment (5,0-6,0 ml) was divided into two aliquots - one for cation analysis and membrane preparation and the other for measurement of calcium binding to the cell membrane. These cells were washed with 0,9% NaCl (pH 7,4).

10.3.2.2 Determination of calcium binding to the outer cell membranes of erythrocytes

This method was based on that described by Postnov et al (1977) and determines the amount of calcium released from the outer erythrocyte membrane when exposed to different

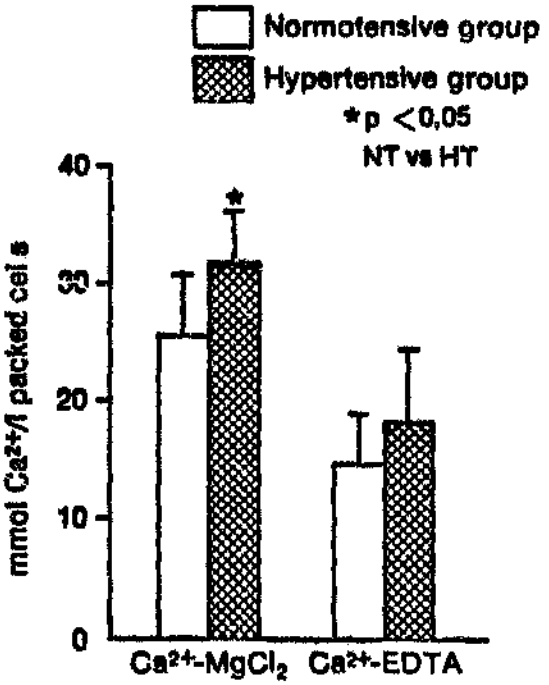
chelating agents ($MgCl_2$ and EDTA). The principles and methods are described in Appendix N.

10.3.2.3 Isolation of platelets

The citrated blood was centrifuged to obtain platelet rich plasma (PRP) (chapter 8.3.2.1 (a)). Aliquots of PRP were used for measuring calcium binding to the plasma membrane. The PRP was centrifuged at 600 x g for 15 minutes at room temperature to obtain a platelet pellet. The platelet pellet was washed three times in a 0,9% NaCl buffer. After the final wash, the pellet was suspended in 1 ml of buffer. A platelet count was performed on the washed platelet suspension and the count adjusted to 1 x 10^8 cells/ml. Determination of calcium binding to the outer membranes of platelets was based on the method of Postnov et al (1977) (Appendix N.b.).

10.4 RESULTS

One hundred and four black subjects (52 normotensive; 52 hypertensive) and 50 white subjects (24 normotensive; 26 hypertensive) were studied. Clinical characteristics of the groups are presented in Chapter 8.



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

Figure 10.1:
Amount of calcium removed from the erythrocytes by $MgCl_2$ and EDTA treatment in the black group.

10.4.1 Black group

10.4.1.1 Calcium binding to the outer cell membrane of erythrocytes:

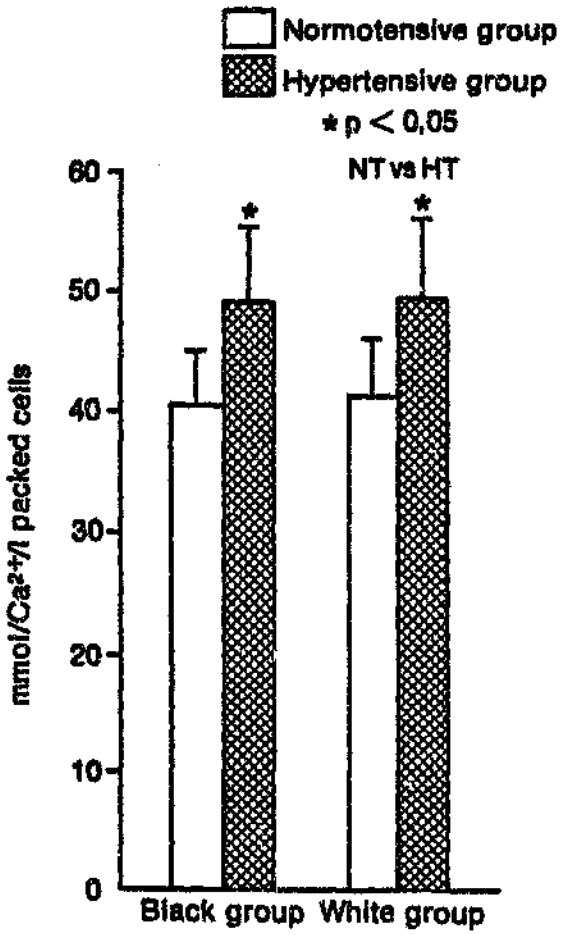
10.4.1.1 (i) Calcium depletion by isoosmotic $MgCl_2$.

In the normotensive group depletion of erythrocytes by isoosmotic $MgCl_2$ resulted in the removal of 27,4 ± 9,1 mmol of calcium (per litre of packed cells) from the outer cell membrane (Table 10.1, Figure 10.1). In the hypertensive group, the amount of calcium removed by $MgCl_2$ was 31,5 ± 8,9 mmol/litre packed cells. This was significantly higher compared to the normotensive group ($p = 0,01$).

10.4.1.1 (ii) Calcium depletion by isoosmotic EDTA

The subsequent treatment of erythrocytes with isoosmotic EDTA solution removed an additional amount of calcium from the erythrocyte membrane. In the normotensive group EDTA removed 14,2 ± 4,3 mmol/litre packed cells and in the hypertensive group, EDTA results in the removal of 17,5 ± 5,6 mmol calcium/litre packed cells. There was no significant difference in the amount of calcium removed by EDTA between the two groups (Table 10.1, Figure 10.1).

The total amount of calcium removed from the outer cell membranes of erythrocytes was significantly greater in the



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

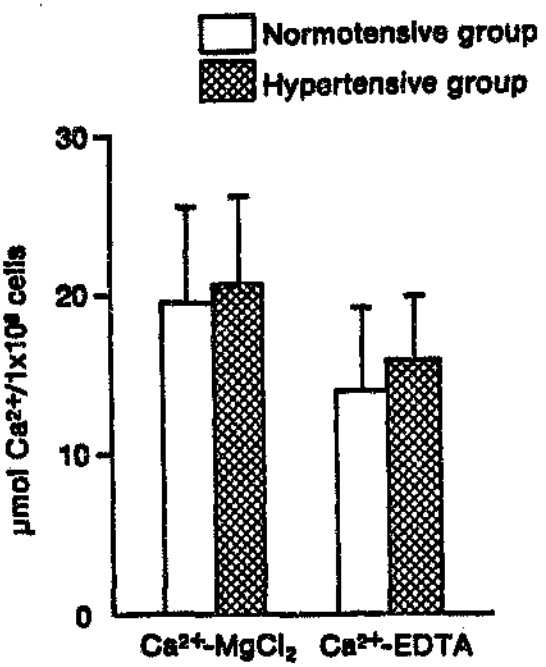
Figure 10.2 :
Total amount of calcium removed from the erythrocytes in the black and white groups (ie. Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA)

Table 10.1: Calcium binding to the outer cell membranes of erythrocytes and platelets:- Amount of calcium removed from the erythrocytes and platelets by $MgCl_2$ and EDTA in the black group.

	Black group		
	Normotensive group	Hypertensive group	
			p value compares normotensive and hypertensive
Erythrocytes (mmol Ca^{2+}/l packed cells)			
Ca^{2+}-$MgCl_2$	27,4 ± 9,1	31,5 ± 8,9	0,01*
Ca^{2+}-EDTA	14,2 ± 4,3	17,5 ± 5,6	0,47
Total Ca^{2+} removed	41,2 ± 6,5	49,0 ± 7,2	0,03*
Platelets (/umol Ca^{2+}/l x 10^8 cells)			
Ca^{2+}-$MgCl_2$	19,1 ± 4,4	20,9 ± 7,0	0,18
Ca^{2+}-EDTA	14,0 ± 5,5	15,6 ± 4,8	0,08
Total Ca^{2+} removed	33,1 ± 4,6	36,5 ± 5,9	0,06

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
Total Ca^{2+} removed = Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA

* = significant difference



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

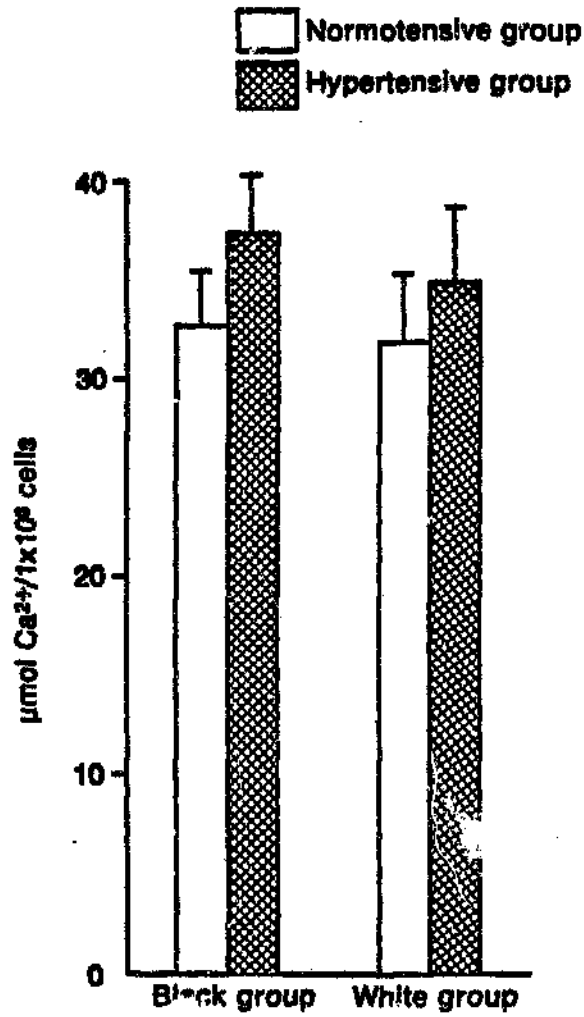
Figure 10.3:
Amount of calcium removed from the platelets by $MgCl_2$ and EDTA treatment in the black group.

Table 10.2: Calcium binding to the outer cell membranes of erythrocytes and platelets:- Amount of calcium removed from the cells by $MgCl_2$ and EDTA in the white group.

	White group		
	Normotensive group	Hypertensive group	
			p value normotensive vs hypertensive
Erythrocytes (mmol Ca^{2+}/l packed cells)			
Ca^{2+}-$MgCl_2$	27,3 ± 5,9	29,4 ± 8,7	0,31
Ca^{2+}-EDTA	15,3 ± 5,9	19,9 ± 5,0	0,01*
Total Ca^{2+} removed	42,6 ± 5,9	49,3 ± 6,8	0,03*
Platelets (/umol Ca^{2+}/l x 10^8 cells)			
Ca^{2+}-$MgCl_2$	21,5 ± 5,8	21,2 ± 6,9	0,90
Ca^{2+}-EDTA	11,3 ± 3,2	13,9 ± 3,8	0,06
Total Ca^{2+} removed	32,8 ± 4,5	35,1 ± 6,4	1,5

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
Total Ca^{2+} removed = Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA

* = significant difference



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

Figure 10.4:
Total amount of calcium removed from the platelets in the black and white groups (ie. Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA).

hypertensive group compared to the normotensive group (Table 10.1, Figure 10.2).

10.4.1.2 Calcium binding to the outer cell membrane of platelets

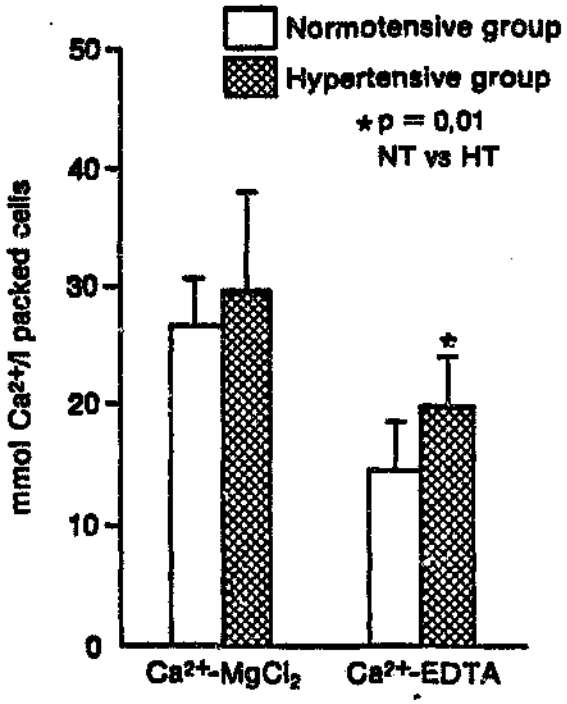
10.4.1.2 (i) Calcium depletion by isoosmotic $MgCl_2$

Depletion of calcium from platelet membranes by isoosmotic $MgCl_2$ resulted in the removal of about 20 umol/1 x 10^8 cells from the outer cell membrane. This amount is approximately the same for the normotensive and hypertensive groups (Table 10.1, Figure 10.3).

10.4.1.2 (ii) Calcium depletion by EDTA

The amount of calcium released by isoosmotic EDTA was similar in the normotensive and hypertensive groups (Table 10.1, Figure 10.3).

The total amount of calcium removed from the outer cell membrane of platelets in the normotensive group was 33,1 $\pm$ 6 umol/1 x 10^8 cells and in the hypertensive group 36,5 $\pm$ 5,9 umol/1 x 10^8 cells. There were no significant differences between the groups (Table 10.1, Figure 10.4).



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

Figure 10.5:
Amount of calcium removed from the erythrocytes by $MgCl_2$ and EDTA treatment in the white group.

10.4.2 White group

10.4.2.1 Calcium binding to the outer cell membrane of erythrocytes:-

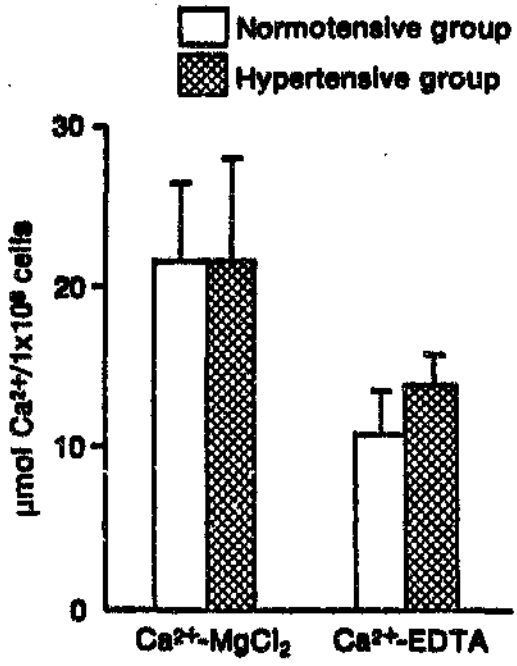
10.4.2.1 (i) Calcium depletion by isoosmotic $MgCl_2$

The amount of calcium removed from the outer cell membrane of erythrocytes when exposed to isoosmotic $MgCl_2$ was similar in the normotensive and hypertensive groups. (Table 10.2, Figure 10.5).

10.4.2.1 (ii) Calcium depletion by isoosmotic EDTA

Depletion of calcium from erythrocytes by isoosmotic EDTA resulted in the removal of 15,3 $\pm$ 5,9 mmol/litre packed cells in the normotensive group, and 19,9 mmol/litre packed cells in the hypertensive group. The amount removed in the hypertensive group was significantly higher compared to the normotensive group (Table 10.2, Figure 10.5).

The total amount of calcium removed from the outer cell membrane of erythrocytes was significantly higher in the hypertensive subjects compared to the normotensive subjects ($p = 0,01$) (Table 10.2, Figure 10.2).



Ca^{2+}-$MgCl_2$ = amount of Ca^{2+} removed by $MgCl_2$
Ca^{2+}-EDTA = amount of Ca^{2+} removed by EDTA

Figure 10.6:

Amount of calcium removed from the platelets by $MgCl_2$ and EDTA treatment in the white group.

10.4.2.2 Calcium binding to the outer cell membrane of platelets:-

10.4.2.2 (i) Calcium depletion by isoosmotic $MgCl_2$

The amount of calcium removed from the outer cell membrane of platelets when exposed to isoosmotic $MgCl_2$ was similar in the normotensive and hypertensive groups (Table 10.2, Figure 10.6).

10.4.2.2 (ii) Calcium depletion by isoosmotic EDTA

The subsequent treatment of platelets with isoosmotic EDTA solution removed an additional amount of calcium from the platelet membrane. There were no significant differences between the normotensive and hypertensive groups (Table 10.2, Figure 10.6).

The total amount of calcium removed from the outer cell membrane of platelets was not significantly different between the normotensive and hypertensive groups (Table 10.2, Figure 10.4).

10.4.3 Combined black and white groups

10.4.3.1 Calcium binding to the outer cell membrane of erythrocytes

Table 10.3: Calcium binding. Amount of calcium released from the outer cell membrane of erythrocytes and platelets by $MgCl_2$ and EDTA in the combined black and white groups.

	Normotensive group	Hypertensive group	
			p value normotensive vs hypertensive
Erythrocytes (mmol Ca^{2+}/l packed cells)			
Ca^{2+}-$MgCl_2$	27,4 ± 8,1	30,8 ± 8,9	0,05*
Ca^{2+}-EDTA	14,5 ± 4,9	18,3 ± 5,5	0,0001*
Total Ca^{2+} removed	41,9 ± 6,5	49,1 ± 7,2	0,01*
Platelets (/umol Ca^{2+}/l x 10^8 cells)			
Ca^{2+}-$MgCl_2$	19,9 ± 6,4	20,9 ± 6,9	0,24
Ca^{2+}-EDTA	13,1 ± 5,0	15,1 ± 5,2	0,06
Total Ca^{2+} removed	33,8 ± 5,8	36,0 ± 6,1	0,06

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
Total Ca^{2+} removed = Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA

* = significant difference

10.4.3.1 (i) Calcium depletion by isoosmotic $MgCl_2$

The amount of calcium removed from erythrocytes when exposed to isoosmotic $MgCl_2$ was significantly higher in the hypertensive subjects compared to the normotensive subjects ($p = 0,01$) (Table 10.3).

10.4.3.1 (ii) Calcium depletion by isoosmotic EDTA

When exposing the erythrocytes to isoosmotic EDTA, the amount of calcium released from the outer membrane was significantly greater in the hypertensive group compared to the normotensive group ($p = 0,0001$) (Table 10.3).

10.4.3.2 Calcium binding to the outer cell membrane of platelets

There was no significant difference in the amount of calcium removed from the outer cell membrane of platelets between the normotensive and hypertensive groups when exposed to $MgCl_2$ and to EDTA (Table 10.3).

When comparing the black and white groups, there were no significant differences for erythrocyte calcium binding (Table 10.4). For platelet calcium binding there were also no significant differences between the black and white hypertensive groups (Table 10.5).

10.4.4 Calcium binding to the outer cell membrane of erythrocytes and platelets in males and females

The amount of calcium removed from the outer cell membrane by isoosmotic $MgCl_2$ and EDTA in the normotensive and hypertensive, black and white, male and female groups are presented in Tables 10.6 and 10.7. In the black and white groups significantly more calcium was removed from the erythrocytes of the male hypertensive patients compared to the female hypertensive patients.

10.4.5 Comparison of calcium binding between the sexes and groups

Significant results comparing calcium binding between female and male, normotensive and hypertensive and black and white groups are presented in Tables 10.8 and 10.9.

10.4.6 Correlation studies

10.4.6.1 Correlations between calcium binding (calcium depletion) and MAP

The only significant correlation between calcium binding and MAP was in the white normotensive group, where the amount of calcium removed by EDTA from erythrocyte membranes was significantly correlated to MAP. There were no other significant correlations between calcium depletion

and MAP in the black and white groups (Table 10.10).

10.4.6.2 Correlations between the amount of calcium depleted from cell membranes and cations

10.4.6.2 (i) Black group (Table 10.11)

In the normotensive group, there were significant positive correlations between:-

- serum Na^+ and erythrocyte Ca^{2+} depletion by EDTA ($r = 0,33$; $p = 0,01$)
- erythrocyte Na^+ and erythrocyte Ca^{2+} depletion by EDTA ($r = 0,36$; $p = 0,001$).

Significant inverse correlations included:-

- serum Mg^{2+} and erythrocyte Ca^{2+} depletion by $MgCl_2$ ($r = -0,35$; $p = 0,01$)

In the hypertensive group, significant direct correlations were found between:-

- platelet Ca^{2+} and platelet Ca^{2+} depletion by EDTA ($r = 0,35$; $p = 0,01$)

Significant inverse correlations included:-

- serum Mg^{2+} and erythrocyte Ca^{2+} depletion by EDTA ($r = -0,30$; $p = 0,04$)
- erythrocyte Mg^{2+} and erythrocyte Ca^{2+} depletion by EDTA ($r = -0,31$; $p = 0,03$)

10.4.6.2 (ii) White group (Table 10.12)

In the normotensive group, significant positive correlations were found between:-

- erythrocyte Mg^{2+} and erythrocyte Ca^{2+} depletion by $MgCl_2$ ($r = 0,50$; $p = 0,009$) and EDTA ($r = 0,44$; $p = 0,02$)
- erythrocyte K^+ and erythrocyte Ca^{2+} depletion by EDTA ($r = 0,41$; $p = 0,03$)
- serum Ca^{2+} and platelet Ca^{2+} depletion by $MgCl_2$ ($r = 0,54$; $p = 0,004$)

Significant inverse correlations included:-

- serum K^+ and platelet Ca^{2+} depletion by $MgCl_2$ ($r = -0,65$; $p = 0,0003$)

In the hypertensive group, there were significant positive correlations between:-

- serum Ca^{2+} and platelet Ca^{2+} depletion by $MgCl_2$ ($r = 0,49$; $p = 0,01$) and EDTA ($r = 0,53$; $p = 0,001$)
- serum Ca^{2+} and erythrocyte Ca^{2+} depletion by $MgCl_2$ ($r = 0,49$; $p = 0,01$)

Significant inverse correlations included:-

- erythrocyte Mg^{2+} and erythrocyte Ca^{2+} depletion by $MgCl_2$ ($r = -0,44$; $p = 0,03$)

Table 10.4: Pairwise-t-test comparisons for calcium binding to the outer cell membrane of erythrocytes between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
BNT	0,01 NS	-		
WHT	NS NS	NS NS	-	
WNT	0,03 NS	NS NS	NS 0,01	-

Top value represents calcium depletion by $MgCl_2$, and the bottom value calcium depletion by EDTA.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 10.5: Pairwise-t-test comparisons for calcium binding to the outer cell membrane of platelets between the black and white groups.
Sig ificant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
BNT	NS NS	-		
WHT	NS NS	NS NS	-	
WNT	NS 0,0004	NS 0,02	NS NS	-

Top value represents calcium depletion by $MgCl_2$, and the bottom value calcium depletion by EDTA.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 10.6: Calcium binding to the outer cell membrane:- amount of calcium removed by $MgCl_2$ and EDTA in the male and female black group.

	Black group			
	Normotensive group		Hypertensive group	
	Females (n = 23)	Males (n = 29)	Females (n = 30)	Males (n = 22)
Erythrocyte (mmol Ca^{2+}/l packed cells)				
Ca^{2+}-$MgCl_2$	28,1 ± 11,35	26,8 ± 7,1	29,9 ± 6,7	33,5 ± 11,2*
Ca^{2+}-EDTA	14,3 ± 3,9	14,1 ± 4,8	17,2 ± 5,8	17,9 ± 5,4
Total Ca^{2+} removed	42,4 ± 9,7	40,9 ± 6,2	47,1 ± 6,4	51,4 ± 9,3*
Platelet (/umol Ca^{2+}/1 x 10^8 cells)				
Ca^{2+}-$MgCl_2$	18,5 ± 8,9	19,7 ± 4,7	20,3 ± 6,6	21,4 ± 7,9
Ca^{2+}-EDTA	14,1 ± 6,5	13,9 ± 4,7	15,3 ± 4,4	15,2 ± 5,5
Total Ca^{2+} removed	32,6 ± 8,1	33,6 ± 4,7	35,6 ± 5,8	37,6 ± 7,4

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
Total Ca^{2+} removed = Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA

*$p < 0,05$ female group versus male group

T. : 10.7: Calcium binding t~ the outer cell membranes of erythrocytes and platelets:- amount of calcium removed by $MgCl_2$ and EDTA in the male and female white group.

	White group			
	Normotensive group		Hypertensive group	
	Females (n = 13)	Males (n = 13)	Females (n = 14)	Males (n = 10)
Erythrocyte (mmol Ca^{2+}/l packed cells)				
Ca^{2+}-$MgCl_2$	28,8 ± 5,2	25,8 ± 6,4	27,6 ± 4,9	32,0 ± 9,6*
Ca^{2+}-EDTA	16,2 ± 5,7	14,5 ± 6,2	20,7 ± 8,2	21,9 ± 4,7
Total Ca^{2+} removed	45,0 ± 5,4	40,3 ± 6,4	48,3 ± 8,0	53,9 ± 9,2*
Platelet (μmol Ca^{2+}/l x 10^8 cells)				
Ca^{2+}-$MgCl_2$	23,8 ± 7,6	20,8 ± 4,4	20,7 ± 8,2	21,9 ± 4,8
Ca^{2+}-EDTA	11,8 ± 3,7	10,8 ± 2,9	13,4 ± 2,7	14,6 ± 6,3
Total Ca^{2+} removed	35,6 ± 7,2	31,6 ± 4,1	34,1 ± 8,1	36,5 ± 6,2

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
Total Ca^{2+} removed = Ca^{2+}-$MgCl_2$ + Ca^{2+}-EDTA

* $p < 0,05$ female group versus male group

Table 10.8: Comparisons for erythrocyte membrane calcium depletion by $MgCl_2$ and EDTA between the male and female, white and black, normotensive and hypertensive groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7	8
1	-							
2	0,01 NS	-						
3	NS NS	0,03 NS	-					
4	NS NS	0,006 NS	NS NS	-				
5	NS NS	0,04 NS	NS NS	NS NS	-			
6	NS 0,02	NS NS	NS 0,05	NS 0,02	0,02 NS	-		
7	NS NS	NS NS	NS NS	NS NS	NS NS	NS NS	-	
8	NS NS	0,01 NS	NS NS	NS NS	NS NS	NS NS	NS NS	-

Top value represents erythrocyte membrane Ca^{2+} depletion by $MgCl_2$, bottom value represents Ca^{2+} depletion by EDTA.

Groups

1 = BHT female
2 = BHT male
3 = BNT female
4 = BNT male
5 = WHT female
6 = WHT male
7 = WNT female
8 = WNT male

Table 10.9: Comparisons for platelet membrane calcium depletion by $MgCl_2$ and EDTA between the male and female, white and black, normotensive and hypertensive groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7	8
1	-							
2	NS NS	-						
3	NS NS	NS NS	-					
4	NS NS	NS NS	NS NS	-				
5	NS NS	NS NS	NS NS	NS NS	-			
6	NS NS	NS NS	NS NS	NS NS	NS NS	-		
7	NS 0,03	NS 0,01	0,02 NS	NS NS	NS NS	NS NS	-	
8	NS 0,009	NS 0,003	NS NS	NS NS	NS NS	NS NS	NS NS	-

Top value represents platelet membrane Ca^{2+} depletion by $MgCl_2$, bottom value represents Ca^{2+} depletion by EDTA.

Groups

1 = BHT female
2 = BHT male
3 = BNT female
4 = BNT male
5 = WHT female
6 = WHT male
7 = WNT female
8 = WNT male

Table 10.10: Pearson's correlation coefficients for correlations between cell membrane calcium depletion by $MgCl_2$ and EDTA and MAP in the black and white normotensive and hypertensive groups.

	Black group		White group	
	Normotensive	Hypertensive	Normotensive	Hypertensive
Erythrocyte				
Ca^{2+}-$MgCl_2$	-0,16	-0,08	0,21	0,18
Ca^{2+}-EDTA	0,03	0,18	0,29*	0,14
Platelet				
Ca^{2+}-$MgCl_2$	-0,03	0,09	0,26	0,17
Ca^{2+}-EDTA	0,09	0,23	0,16	0,12

Ca^{2+}-$MgCl_2$ = amount of calcium removed by $MgCl_2$
Ca^{2+}-EDTA = amount of calcium removed by EDTA
*p = 0,05

Table 10.11: Pearson's correlation coefficients for correlations between cell membrane calcium depletion by $MgCl_2$ and EDTA and the cations in the black normotensive and hypertensive groups.

	Erythrocyte membrane		Platelet membrane	
Variable	Ca^{2+}-$MgCl_2$	Ca^{2+}-EDTA	Ca^{2+}-$MgCl_2$	Ca^{2+}-EDTA
Serum Na^+	0,20 -0,28	0,33** 0,04	0,10 0,19	0,28 0,16
Erythrocyte Na^+	0,05 0,04	0,36** 0,03	0,02 0,15	0,16 0,17
Platelet Na^+	0,11 0,06	0,02 0,04	0,06 0,09	0,03 0,09
Serum K^+	0,16 0,15	0,14 0,14	0,14 0,26	0,19 0,06
Erythrocyte K^+	0,11 0,09	0,15 0,09	0,18 0,14	0,01 0,16
Platelet K^+	0,21 0,28	0,05 0,01	0,14 0,21	0,22 0,27
Serum Ca^{2+}	0,07 0,08	0,04 -0,30	0,03 0,13	0,07 0,22
Erythrocyte Ca^{2+}	0,17 0,11	0,05 -0,27	0,11 0,04	0,06 0,12
Platelet Ca^{2+}	-0,26 0,02	0,11 0,14	0,04 0,13	0,02 0,35**
Serum Mg^{2+}	-0,35** 0,05	-0,26 -0,33*	0,16 0,01	0,12 0,14
Erythrocyte Mg^{2+}	0,04 0,05	0,30 -0,31*	0,28 0,55	0,11 0,16
Platelet Mg^{2+}	-0,31 0,01	0,01 0,06	0,04 0,11	0,07 0,30

Top value represents the black normotensive group and the bottom value the black hypertensive group.

* $p < 0,05$

** $p < 0,01$

Table 10.12: Pearson's correlation coefficients for correlations between cell membrane calcium depletion by $MgCl_2$ and EDTA and the cations in the white normotensive and hypertensive groups.

	Erythrocyte membrane		Platelet membrane	
Variable	Ca^{2+}-$MgCl_2$	Ca^{2+}-EDTA	Ca^{2+}-$MgCl_2$	Ca^{2+}-EDTA
Serum Na^+	0,05 0,02	-0,24 0,04	-0,16 0,04	0,15 0,16
Erythrocyte Na^+	0,10 0,13	-0,10 0,15	0,14 0,15	0,01 0,08
Platelet Na^+	0,09 0,19	0,07 0,22	0,01 0,22	0,27 0,25
Serum K^+	0,03 0,36	0,21 0,06	-0,65** 0,06	0,10 0,08
Erythrocyte K^+	0,23 0,06	0,41* 0,26	0,22 0,26	0,23 0,17
Platelet K^+	0,24 0,03	0,12 0,17	0,09 0,17	0,03 0,18
Serum Ca^{2+}	0,14 0,27	-0,27 0,49*	0,54** 0,49*	0,31 0,53**
Erythrocyte Ca^{2+}	0,13 0,11	0,30 -0,02	0,41 0,02	0,19 0,09
Platelet Ca^{2+}	0,06 0,06	-0,27 0,14	0,01 0,14	0,19 0,11
Serum Mg^{2+}	0,11 0,14	-0,28 0,29	0,17 0,29	0,01 0,18
Erythrocyte Mg^{2+}	0,50* -0,44*	0,44* 0,11	0,06 0,11	0,02 0,11
Platelet Mg^{2+}	0,18 0,17	-0,67** 0,22	0,03 0,22	0,12 0,08

Top value represents the white normotensive group and the bottom value the white hypertensive group.

* $p < 0,05$

** $p < 0,01$

10.4.7 Summary of results

1. In the black group the amount of Ca^{2+} removed from the outer membrane of erythrocytes by $MgCl_2$ was significantly higher in the hypertensive group compared to the normotensive group.
2. The total amount of calcium released from the outer membranes of erythrocytes and platelets was significantly higher in the black hypertensive subjects compared to their normotensive counterparts.
3. In the white group, calcium depletion from erythrocytes and platelets by EDTA was significantly higher in the hypertensive compared to the normotensive group.
4. The total amount of calcium depletion from erythrocytes was significantly higher in the white hypertensive patients compared to their normotensive counterparts.
5. In the combined black and white groups, calcium depletion from erythrocyte membranes was significantly raised in the hypertensive group.
6. In the black normotensive group, calcium depletion from cell membranes was directly correlated to serum Na^{+} and inversely correlated to serum Mg^{2+} and platelet Mg^{2+}.
7. In the black hypertensive group, calcium depletion from cell membranes was directly correlated to platelet Ca^{2+} and inversely correlated to serum Mg^{2+}

and erythrocyte Mg^{2+}.

8. In the white normotensive group, cell membrane calcium removal was directly correlated to erythrocyte Mg^{2+}, erythrocyte K^{+}, and serum Ca^{2+} and negatively correlated to serum K^{+}.
9. In the white hypertensive group, cell membrane calcium removal was directly correlated to serum Ca^{2+} and inversely correlated to erythrocyte Mg^{2+}.
10. In the black and white groups, significantly more calcium was removed from the erythrocyte membranes in the male hypertensive patients compared to the female hypertensive patients.

10.5 DISCUSSION

The results of this study confirm previous reports that calcium binding is altered in essential hypertension. The method used here was based on that described by Postnov et al (1977). It is an indirect assessment of the amount of calcium that is bound to the outer plasma membrane, and this is determined by measuring the amount of calcium that is removed by exposing the intact cells to $MgCl_2$ and EDTA. This method removes 90-95% of the membrane bound calcium (Harrison et al, 1968). A possible small residue of calcium in the membrane, was not determined.

In this study, both the black and the white hypertensive groups exhibited increased calcium release from the

erythrocyte membrane compared to the normotensive groups. In the black hypertensive group the amount of calcium removed by $MgCl_2$ and EDTA was greater than that in the normotensive group. However significant differences were only found for the $MgCl_2$ treated cells. In the white group, the amount of calcium removed by $MgCl_2$ and EDTA was also higher in the hypertensive group compared to the normotensive group. Significant differences however were only found in the EDTA treated erythrocytes. For both black and white hypertensive patients, the total amount of calcium depleted from the erythrocyte membranes was significantly greater than that from the normotensive controls.

There were no significant differences for platelet membrane calcium binding between the normotensive and hypertensive groups. This may be due to the fact that the method used was not sensitive enough for determining the amount of calcium released from platelet membranes, or the platelet count may have been too low to elicit differences. Alternatively, the defects may be present in erythrocyte membranes and not platelet membranes.

The results obtained here for the erythrocyte membranes are the same as those reported by Postnov et al (1977). They showed that the outer membrane of the erythrocytes in hypertensive patients have more calcium available for removal when a chelate type compound acts on the membrane

erythrocyte membrane compared to the normotensive groups. In the black hypertensive group the amount of calcium removed by $MgCl_2$ and EDTA was greater than that in the normotensive group. However significant differences were only found for the $MgCl_2$ treated cells. In the white group, the amount of calcium removed by $MgCl_2$ and EDTA was also higher in the hypertensive group compared to the normotensive group. Significant differences however were only found in the EDTA treated erythrocytes. For both black and white hypertensive patients, the total amount of calcium depleted from the erythrocyte membranes was significantly greater than that from the normotensive controls.

There were no significant differences for platelet membrane calcium binding between the normotensive and hypertensive groups. This may be due to the fact that the method used was not sensitive enough for determining the amount of calcium released from platelet membranes, or the platelet count may have been too low to elicit differences. Alternatively, the defects may be present in erythrocyte membranes and not platelet membranes.

The results obtained here for the erythrocyte membranes are the same as those reported by Postnov et al (1977). They showed that the outer membrane of the erythrocytes in hypertensive patients have more calcium available for removal when a chelate type compound acts on the membrane

and concluded that this may be indicative of altered binding ability of the membranes (Postnov et al, 1977).

Reduced calcium binding to the inner and outer surfaces of plasma membranes of erythrocytes, adipocytes and vascular smooth muscle have been reported in human and experimental hypertension (Orlov and Postnov, 1982; Devynck et al, 1982; Cirillo et al, 1990). This membrane abnormality has been found in essential and genetic hypertension but not in secondary forms of hypertension (Postnov et al, 1979). Thus, altered calcium binding to cell membranes may play a primary role in the pathogenesis of essential hypertension, as the widespread defect in membrane calcium handling is detectable before the onset of established hypertension (Devynck et al, 1982; Van Breemen et al, 1986).

Not all studies have reported decreased calcium binding in hypertension. Cooper et al (1989) demonstrated increased levels of calcium bound to platelet membranes of hypertensive patients compared with normotensive controls. These results suggest a higher cell burden of calcium in hypertensive subjects (Thomson and Scrutton, 1985; Cooper et al, 1989). Some experimental studies have also reported increased calcium membrane uptake in hypertensive rats (Webb and Bhala, 1976; Pernollet et al, 1981; Rapp et al, 1986).

The reported contradictory findings and the fact that

significant differences were found in erythrocyte membranes and not in platelet membranes in this study, suggests that the calcium binding defects in hypertension may not be universal to all cell types.

In this study, the amount of calcium removed from the erythrocyte membranes in the hypertensive group was significantly greater in the males than the females. These results suggest that male hypertensive patients may have more cell membrane defects than female hypertensive patients. Possibly the binding site number may be reduced in males, or there may be greater structural deformity of the cell membranes in males. Similar findings were demonstrated by Cooper et al (1989). They reported that the calcium binding properties of platelet membranes were more pronounced in men than women. These differences were attributed to the fact that the hypertensive females studied were obese (Cooper et al, 1989).

In the white hypertensive group, the amount of erythrocyte membrane calcium depletion by EDTA was significantly, although weakly, correlated to mean arterial pressure. This relationship appeared to be independent of several potentially confounding factors - for example alcohol, weight and age. These results confirm previous studies (Cooper et al, 1989). The white hypertensive subjects showed no significant direct correlations between the amount of calcium removed from the cell membrane and serum

calcium. This may imply that in the presence of increased extracellular calcium concentration, calcium binding increases. The opposite is true for decreased serum calcium concentrations.

In the black group, the hypertensive subjects demonstrated significant inverse correlations between the amount of calcium removed from erythrocyte membranes and magnesium (serum and erythrocyte). These findings suggest that changes in intra- or extracellular magnesium concentrations are associated with changes in calcium binding.

The correlation studies suggest that the mechanisms responsible for altered calcium binding to cell membranes in hypertensive patients may differ between blacks and whites. In whites, calcium may be important, whereas in blacks, the competitive binding properties of magnesium may be important.

Reasons for the altered cell membrane handling in the hypertensive patients may be due to an increase in the total number of calcium binding sites on the outer erythrocyte membrane, or to defective calcium fixation. The changes in the calcium binding ability of cell membranes may be part of a widespread membrane defect.

The consequences of altered calcium binding are related to intracellular calcium. Reduced membrane binding may

increase cell membrane permeability resulting in increased calcium influx (Porzig, 1977; Chan et al, 1983). In hypertensive rats, low membrane calcium binding is associated with a reduction in active calcium extrusion from the cell (Cirillo et al, 1987; Cirillo et al, 1990). Furthermore, reduced binding stimulates the potential operated calcium channels, which results in increased calcium entering the cell (Romero, 1976; Isenberg, 1977; Hurwitz et al, 1982). These processes lead to increased cytoplasmic calcium which, in vascular smooth muscle, causes increased contractility (Van Breemen et al, 1986).

CHAPTER 11

THE EFFECTS OF MAGNESIUM SUPPLEMENTATION ON BLOOD PRESSURE IN SHR AND WKY

11. THE EFFECTS OF MAGNESIUM SUPPLEMENTATION ON BLOOD PRESSURE IN SHR AND WKY

11.1 INTRODUCTION

The role of divalent cations in blood pressure regulation has recently provoked much investigation. The physiological roles for magnesium ions in vascular smooth muscle include the regulation of contractile proteins, sarcoplasmic reticular membrane transport of calcium ions, cofactor in ATPase activities and metabolic control of energy-dependent cytoplasmic and mitochondrial pathways, (Gunther, 1986a; Bara et al, 1988b; Altura and Altura, 1990). In addition, small changes in the external magnesium or cytoplasmic magnesium concentrations have significant effects on cardiac and vascular smooth muscle contractility (Altura and Altura, 1985b). In vitro studies have demonstrated that increasing the extracellular magnesium concentration promotes vasodilation while decreasing the extracellular magnesium concentration promotes vasoconstriction (Altura et al, 1981). These effects are brought about by altering membrane and intracellular organelle binding and transport of calcium, affecting hormone-receptor interactions, regulating electrolyte content and transport, altering resting membrane-generated and action potentials, changing excitation-contraction coupling events and regulating peripheral and cerebral vascular tone and blood flow

(Aikawa, 1981; Altura and Altura, 1985b; Gunther, 1986; Bara et al, 1988; Witteman and Grobbee, 1990).

Dietary, metabolic or drug-induced changes in magnesium levels may play important roles in the aetiology of cardiac and vascular disorders. Although many in vitro investigations have examined the role of magnesium in blood pressure regulation, there are fewer in vivo studies examining this relationship. Normal rats on a magnesium deficient diet exhibit an elevation in blood pressure (Altura et al, 1984). Spontaneously hypertensive rats consuming a high magnesium diet have lower blood pressures compared to SHRs on a normal diet (Luthringer et al, 1988a). Many studies have demonstrated altered magnesium metabolism in genetically hypertensive rats (SHR and Lyon hypertensive rats) (Hsu et al, 1986; Berthelot et al, 1987; Luthringer et al, 1988b).

The possibility that magnesium supplementation may contribute to the prevention of arterial hypertension is controversial (Durlach, 1988b). Some studies have demonstrated a blood pressure lowering effect of magnesium in essential and in experimental hypertension (Dyckner and Wester, 1983; Berthelot and Esposito, 1983; Schultz et al, 1985; Karanja et al, 1987). Other studies however have failed to confirm these findings (Gunther et al, 1984b; Cappuccio et al, 1985).

11.2 AIMS OF THIS STUDY

The aims of this study were -

1. to investigate the effects of magnesium supplementation on blood pressure in the SHR and in the genetically related normotensive Wistar Kyota (WKY) and

2. to determine the effects of magnesium supplementation on sodium, potassium and calcium status in SHR and WKY.

11.3 ANIMALS AND METHODS

11.3.1 Animals

Ten week old male SHR (Wits Animal Unit) and WKY (Wits Animal Unit) rats were studied. The rats were randomly divided into four groups:- Group 1 - WKY (n = 30) receiving standard rat chow (Epol mice cobes and tap drinking water; Group 2 - (n = 30) WKY receiving rat chow and magnesium enriched drinking water; Group 3 - (n = 36) SHR receiving rat chow and tap drinking water and Group 4 (n = 36) - SHR receiving rat chow and magnesium enriched drinking water. Food and water were available ad libitum. The magnesium enriched water contained 650 mg/l $MgCl_2$.

The rats all consumed the same rat chow from the same batch (Batch 4700; number 8203). Epol mice cubes were used. The constituents of the cubes were:- protein 180 g/kg
moisture 120 g/kg
fat 25 g/kg
fibre 60 g/kg
calcium 18 g/kg
phosphorous 7 g/kg
trace elements < 1 g/kg

The rats were all housed in the same room at 22 ± 2°C with a 12 hour light/dark cycle. One hundred and thirty two rats were studied:- 60 WKY and 72 SHR. They were studied for 17 weeks.

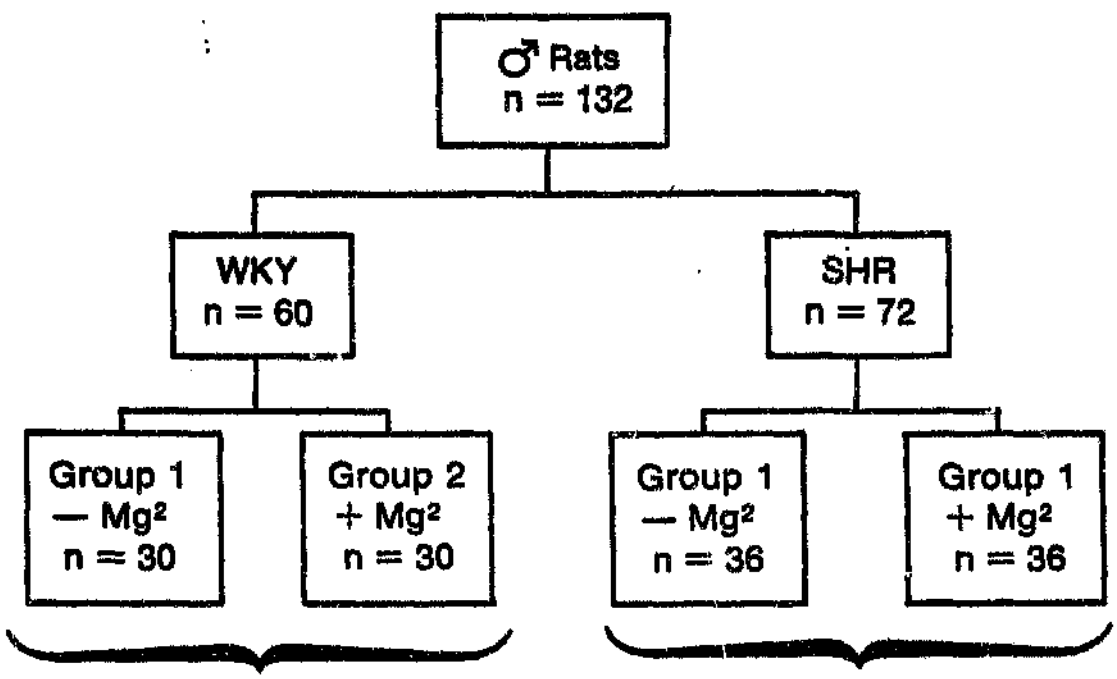
11.3.2 Experimental procedure

Five WKY rats and six SHR from each group were studied weekly for 17 weeks. The following clinical parameters were measured.

1. weight was measured weekly
2. volume of water consumed daily
3. systolic blood pressure was measured weekly

Systolic arterial pressure was determined weekly in unanaesthetised, restrained prewarmed rats. Tail blood pressure was measured by plethysmography (Perks Electronics Plethysmograph, Model 270 and Beckman Dynograph Recorder,

Fig. 11.1 Experimental design



Model R511A). At 4 weekly intervals for 8 weeks and then 3 weekly for 9 weeks, 5 WKY rats and 6 SHRs from each group were killed by exsanguination from the abdominal aorta while under pentobarbital anaesthesia (6 mg/100 g body weight, given by intraperitoneal route) (Figure 11.1). From each animal 3 ml to 5 ml of heparinised aortic blood was obtained for biochemical analysis. The kidneys, heart and a section of the mesentery were removed and fixed in 10% formalin for histological examination.

11.3.3 Biochemical analysis

The blood samples were centrifuged at 450 x g within two hours of collection. The serum was aspirated and kept for biochemical analysis. The buffy coat was discarded and the erythrocyte sediment washed and prepared as described in chapter 8.3.

The following biochemical parameters were measured:- serum and erythrocyte concentrations of sodium, potassium, magnesium and calcium. Sodium and potassium were measured by flame photometry according to methods described in Appendix G. Magnesium and calcium were determined by atomic absorption spectroscopy according to methods described in Appendix G.

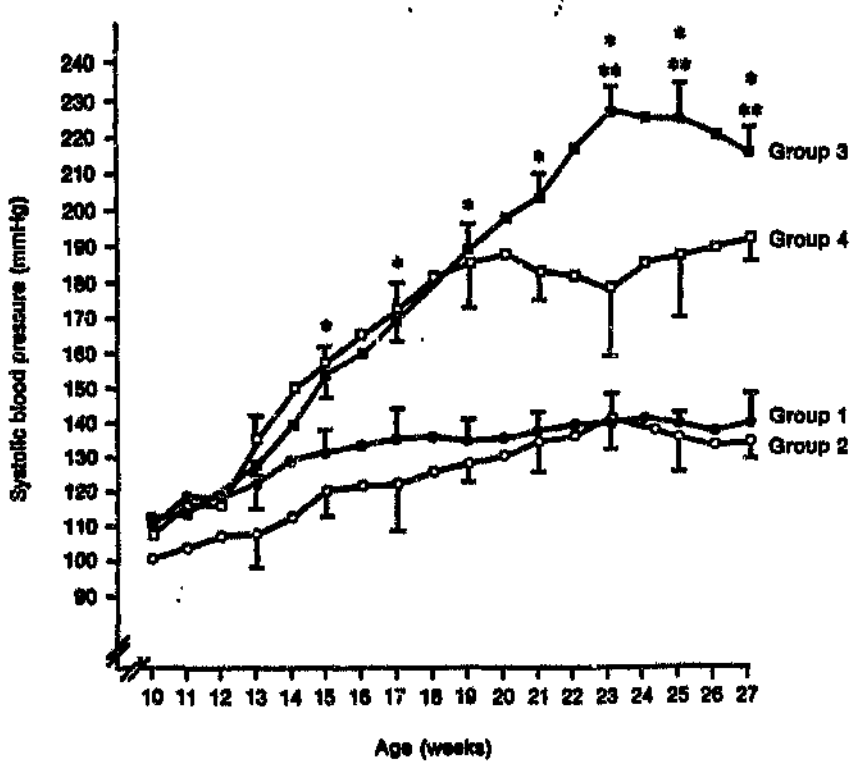


Fig. 11.2 Weekly blood pressures in the 4 groups.
Group 1 = WKY; Group 2 = WKY + Mg^{2+} supplementation;
Group 3 = SHR; Group 4 = SHR + Mg^{2+} supplementation.
* Groups 3 and 4 versus Groups 1 and 2. $p < 0,05$
** Group 3 versus Groups 1, 2, 4. $p < 0,02$

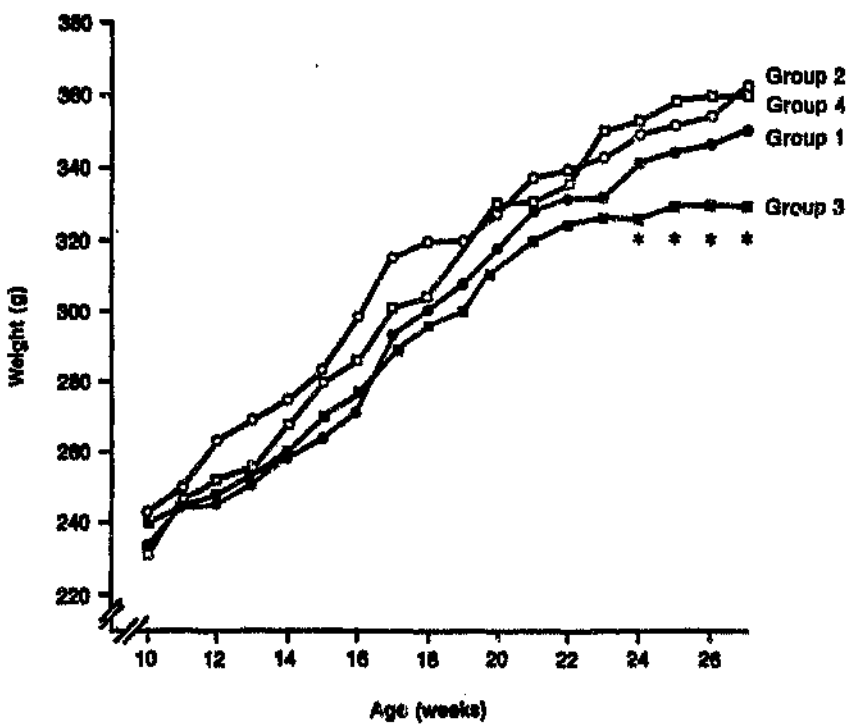


Fig. 11.3 Weekly weights of the 4 groups.
* Group 3 versus Groups 1, 2 and 4. $p < 0,05$

11.3.4 Histological methods

The formalin fixed tissue was embedded in paraplast; the slices were stained with HE, PAS and Acian blue at pH 1,0 or 2,5. Only light microscopic examinations were performed. Histological examinations were performed at the Pathology Department of the South African Institute for Medical Research.

11.4 STATISTICAL ANALYSIS

Values are expressed as means $\pm$ 1SD. Results were compared between the groups at various time intervals using the Students-t-test. A p value less than 0.05 was accepted as significant.

11.5 RESULTS

All groups showed a similar increase in body weight with ageing. From 24 weeks of age, body weight was significantly lower in Group 3 compared to the other groups (Figure 11.2). Drinking water consumption was similar in all four groups throughout the experimental period.

11.5.1 Blood pressure

In all 4 groups, systolic blood pressure (SBP) increased progressively throughout the 17 week experimental period.

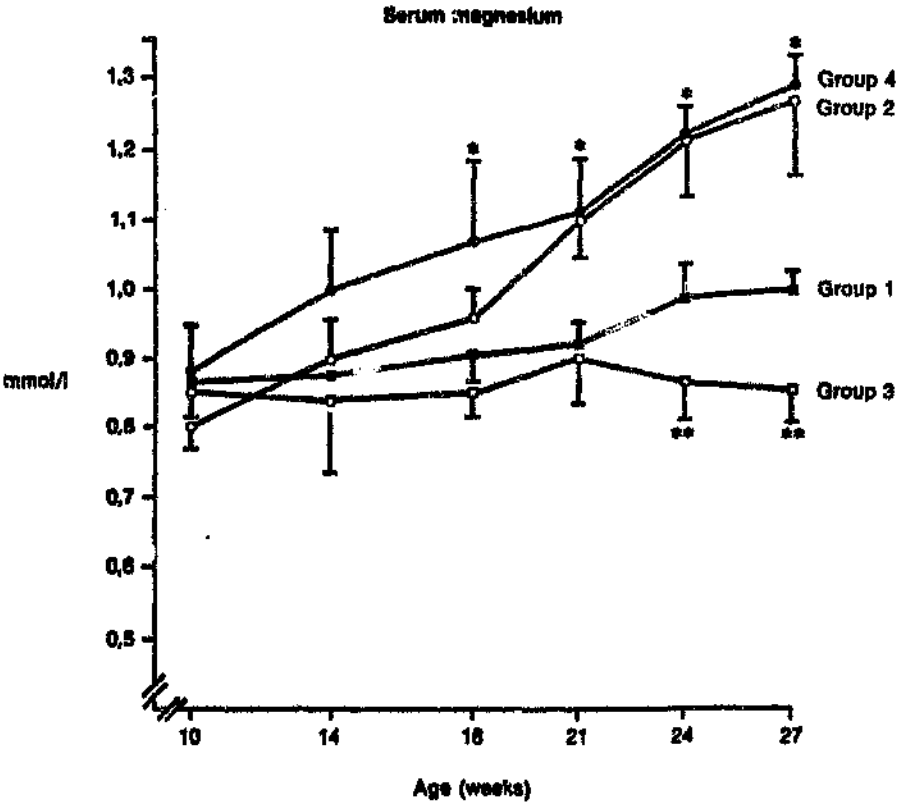


Fig. 11.4 Serum magnesium concentrations in the 4 groups.
* Groups 2 and 4 versus Groups 1 and 3. $p < 0,05$
** Group 3 versus Group 1. $p < 0,05$

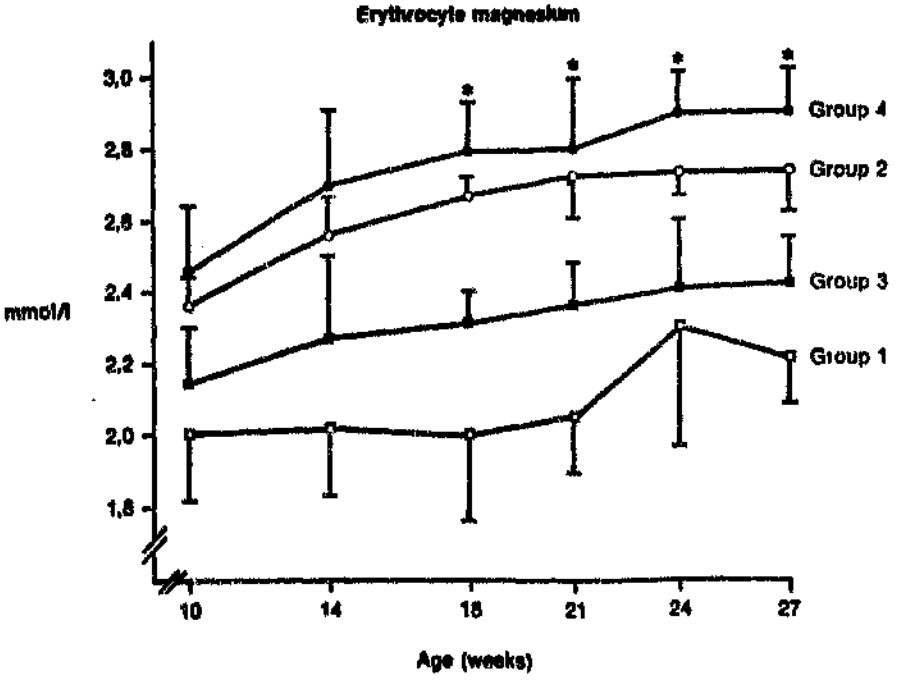


Fig. 11.5 Erythrocyte magnesium concentrations in the 4 groups.
* Groups 2 and 4 versus Groups 1 and 3. $p < 0,05$

At 10 weeks of age, SBP was similar in all 4 groups. From 15 weeks of age, SBP was significantly higher in the SHR groups compared to the WKY groups. At 23 weeks of age, the SBP was significantly higher in Group 3 (the SHR + water group) compared to that in Group 4 (SHR + Mg^{2+} enriched water group). Blood pressure continued to be significantly lower in the magnesium supplemented SHR group compared to the unsupplemented group until the termination of the experiment (27 weeks of age) (Figure 11.3).

11.5.2 Biochemical Data

There were no significant differences in serum sodium, potassium and calcium between the four groups throughout the experiment (Table 11.1). From 18 weeks of age serum magnesium was significantly higher in the WKY and SHR magnesium supplemented groups compared to the unsupplemented groups. From 24 weeks of age, serum magnesium was significantly lower in Group 3 compared to Group 1 (Figure 11.4).

Erythrocyte sodium and potassium were similar in all four groups throughout the experiment (Table 11.2). From 18 weeks of age erythrocyte magnesium was significantly higher in Group 2 (WKY + magnesium supplementation) compared to the other groups. From 16 weeks of age erythrocyte magnesium concentration was significantly elevated in Group 4 (SHR + magnesium supplementation) compared to Group 3

Table 11.1 Serum potassium, sodium and calcium concentrations in the 4 groups (Grp). Values are expressed as mmol/l.

	Serum K^+				Serum Na^+				Serum Ca^{2+}			
Age (weeks)	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4
14	4,1 ± 0,1	4,3 ± 0,4	4,0 ± 0,3	4,2 ± 0,2	142 ± 4	144 ± 3	143 ± 3	144 ± 3	1,9 ± 0,3	2,0 ± 0,3	2,0 ± 0,2	2,4 ± 0,3
18	4,3 ± 0,3	4,1 ± 0,6	3,9 ± 0,2	3,8 ± 0,5	142 ± 3	146 ± 4	141 ± 2	144 ± 4	2,0 ± 0,2	2,4 ± 0,1	2,1 ± 0,2	2,2 ± 0,1
21	4,1 ± 0,1	4,0 ± 0,3	4,2 ± 0,2	3,9 ± 0,6	140 ± 4	138 ± 6	138 ± 5	140 ± 3	1,9 ± 0,2	2,1 ± 0,1	1,9 ± 0,3	2,2 ± 0,2
24	3,9 ± 0,4	4,1 ± 0,2	4,0 ± 0,2	4,0 ± 0,2	141 ± 6	147 ± 7	141 ± 4	140 ± 4	2,0 ± 0,2	1,9 ± 0,3	2,0 ± 0,2	1,9 ± 0,2
27	4,0 ± 0,2	3,9 ± 0,4	4,0 ± 0,3	4,1 ± 0,3	143 ± 5	145 ± 3	140 ± 2	141 ± 3	2,3 ± 0,1	2,0 ± 0,3	2,0 ± 0,2	2,0 ± 0,3

Group 1 = WKY
Group 2 = WKY + magnesium enriched diet
Group 3 = SHR
Group 4 = SHR + magnesium enriched diet

Table 11.2 Erythrocyte potassium, sodium and calcium concentrations in the four groups (Grp) at different ages.

Age (weeks)	Erythrocyte K^+ (mmol/l)				Erythrocyte Na^+ (mmol/l)				Erythrocyte Ca^{2+} (/umol/l)			
	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4
14	93 ± 11	98 ± 8	80 ± 14	89 ± 7	7,6 ± 0,3	6,7 ± 0,4	7,0 ± 0,4	6,8 ± 0,6	0,28 ± 0,02	0,30 ± 0,01	0,33 ± 0,07	0,32 ± 0,03
18	100 ± 14	100 ± 11	88 ± 15	90 ± 5	7,2 ± 0,4	7,0 ± 0,2	7,0 ± 0,5	7,1 ± 0,3	0,32 ± 0,04	0,30 ± 0,02	0,34 ± 0,04	0,33 ± 0,04
21	80 ± 12	93 ± 10	94 ± 6	94 ± 8	7,0 ± 0,4	6,9 ± 0,5	6,8 ± 0,5	7,1 ± 0,3	0,32 ± 0,01	0,34 ± 0,02	0,33 ± 0,02	0,33 ± 0,02
24	91 ± 7	80 ± 15	84 ± 12	90 ± 11	6,7 ± 0,5	7,0 ± 0,1	7,6 ± 0,1	6,9 ± 0,6	0,32 ± 0,02	0,33 ± 0,02	0,39 ± 0,05	0,34 ± 0,01
27	88 ± 10	98 ± 6	100 ± 16	96 ± 10	8,0 ± 0,1	7,8 ± 0,6	8,0 ± 0,2	8,5 ± 0,8	0,33 ± 0,01	0,34 ± 0,03	0,41 ± 0,04	0,36 ± 0,05

Group 1 = WKY
Group 2 = WKY + magnesium enriched diet
Group 3 = SHR
Group 4 = SHR + magnesium enriched diet

(SHR) (Figure 11.5). At 27 weeks of age, erythrocyte calcium was significantly higher in Group 3 compared to the other groups.

11.5.3 Histological findings

In all four groups, microscopic examination of the vessels in the heart, kidney and mesentery showed only vessel congestion. There were no histological differences between the groups.

11.6 DISCUSSION

The results of this study demonstrate that a magnesium supplemented diet reduces blood pressure levels in SHR during the period when hypertension is developing. These findings are similar to previously reported data (Berthelot et al, 1987; Luthringer et al, 1988a; Luthringer et al, 1988b).

The pharmacological concentrations of magnesium used in this study induced significant increases in serum and erythrocyte magnesium concentrations in the WKY and SHR magnesium treated groups. These results confirm that the dietary magnesium was absorbed. The blood magnesium levels only became significantly raised after eight weeks of treatment suggesting that cell (specifically erythrocytes) turnover of magnesium is not an acute phenomenon. The

concentration of magnesium given to supplement the diet in this study was similar to that used by Fisher and Giroux (1987). At 600 mg/kg diet (or 600 mg/l water) the magnesium is well tolerated and absorbed with no adverse gastrointestinal effects. The high magnesium intake was related to a significantly lower intracellular calcium concentration in the SHR-magnesium group compared to the untreated SHR group. There were no associated changes in serum or erythrocyte sodium and potassium concentrations.

The anti-hypertensive effects of magnesium may be related to its interaction with calcium. The results of this study support this thesis. Magnesium is a naturally occurring calcium antagonist, it can displace and compete with calcium at smooth muscle cell membranes and it regulates the Ca^{2+}-ATPase pump (Turlapaty and Altura, 1980; Altura and Altura, 1985b). Also, small changes in extracellular and/or cytoplasmic magnesium concentrations can affect vascular smooth muscle tension and contractility (Altura and Altura, 1985b). These effects may be direct or indirect. In this study, the hypermagnesaemia may have prevented an increase in intracellular calcium concentration in the SHR-magnesium group. In the SHR-untreated group, the increase in blood pressure was closely associated with the increase in intraerythrocyte calcium.

Throughout the experiment, serum magnesium was lower in the SHR group compared to the WKY group. Significance however

was only achieved at 24 weeks of age. These results are similar to previously reported data except that the hypomagnesaemia occurred much earlier in other studies. Lutheringer et al (1988) and Henrotte et al (1985) observed decreased plasma magnesium in rats aged between 6 and 22 weeks. Altura et al (1983) found that SHR and WKY rats had lower plasma magnesium than did Wistar rats (WI) at 17 weeks. Several studies have demonstrated that magnesium metabolism is altered in genetically hypertensive rats (Oasa et al, 1983; Henrotte et al, 1985; Luthringer, 1988). Berthelot et al (1987) reported that SHR have lower serum plasma magnesium levels and less urinary magnesium excretion compared to normotensive WKY and WI rats. Hsu et al (1986) showed that the liver content of magnesium was lower in SHR compared to WKY and that SHR have decreased renal tubular reabsorption of magnesium and calcium. Similar to other studies, erythrocyte magnesium concentrations were not decreased in the SHR studied here. Possibly, the intracellular concentration is being spared at the expense of the extracellular concentration of magnesium.

The effects of dietary magnesium on blood pressure have been extensively debated (Durlach, 1988a; Durlach, 1988b). In man, magnesium deficiency has been associated with hypotension and hypertension (Luthringer et al, 1988; Gunther et al, 1984b; Altura and Altura, 1983). Some studies have demonstrated significant hypotensive effects

of magnesium supplementation, while others have shown no or little effect on blood pressure (Dyckner and Wester, 1983; Schultz et al, 1985; Cappuccio et al, 1985). In this study, serum and erythrocyte magnesium concentrations were significantly increased in rats following dietary magnesium supplementation. Although the present findings demonstrate a blood pressure lowering effect of magnesium in SHR, this was not observed in the normotensive WKY rats. Thus, the hypotensive effects of magnesium are not uniform. Possibly, hypertensive subgroups - particularly those who are magnesium deficient may benefit from magnesium supplementation. Long term population studies are needed to clarify the exact therapeutic effects of magnesium supplementation in hypertension.

SECTION II - MALIGNANT HYPERTENSION

CHAPTER 12

INTRA- AND EXTRACELLULAR CATIONS AND CELL MEMBRANE ATPase ACTIVITY IN MALIGNANT HYPERTENSION

12. INTRA- AND EXTRACELLULAR CATIONS AND CELL MEMBRANE ATPase ACTIVITY IN MALIGNANT AND RENAL HYPERTENSION

12.1 INTRODUCTION

12.1.1 Definitions

Malignant hypertension was first described as a disease entity in 1914 by Volhard and Fahr (Volhard and Fahr, 1947). It is defined by very high blood pressure (a diastolic blood pressure greater than 120 mmHg) and fresh fundal haemorrhages with or without cotton wool spots (soft exudates) and papilloedema (McGregor et al, 1986; Houston, 1989). It is associated with a rapid deterioration in renal function and a poor prognosis if untreated (Kincaid-Smith, 1980; Houston, 1989). Pathologically, the hallmark of the condition is arteriolar fibrinoid necrosis and myointimal proliferation (Kincaid-Smith et al, 1958). These changes can develop acutely and compromise the lumen of small vessels and may be responsible for the rapid development of renal insufficiency (Pickering, 1968; Susin and Mailloux, 1978). The degree of myointimal proliferation parallels the severity and duration of hypertension (Schwartz et al, 1987). In contrast to medial and intimal thickening fibrinoid necrosis is more rapidly reversible with blood pressure control and can resolve within days of treatment (Pickering, 1968; Pitcock et al, 1976).

Prior to the development of effective antihypertensive drugs, the one year survival rate of patients with malignant hypertension was 20%, and the five year survival rate was less than 1% (Kincaid-Smith et al, 1958). With the advent of new potent antihypertensive drugs, early detection, effective lowering of blood pressure and a decline in renal disease the incidence of malignant essential hypertension has decreased significantly (Gudbrandsson et al, 1979; Kincaid-Smith, 1985). Malignant hypertension is now a rare disease in the Western world (Gudbrandsson et al, 1979). It is more common among American negroes than whites and in South Africa it is a cause of considerable mortality and morbidity in the black population (Relman, 1982; Milne et al, 1989).

12.1.2 Pathophysiology of malignant hypertension

Myointimal proliferation, associated with 'onion skin' lesions, develops gradually and may be associated with progressive renal dysfunction (Kincaid-Smith et al, 1958; McCormack et al, 1958). These lesions do not respond to antihypertensive treatment and are responsible for the irreversible component of renal failure of malignant hypertension (McCormack et al, 1958). Arterial fibrinoid necrosis develops acutely and resolves more quickly and completely (Pitcock et al, 1976).

In the early phases of fibrinoid necrosis a critical blood

pressure level causes spasm (an autoregulatory phenomenon) in some segments of blood vessels and overdilation (due to overstretching) in others (Giese, 1976; Mann and Atlas, 1990). Endothelial damage, particularly in the overdilated areas, has been documented (Giese, 1964). Fibrin and other plasma components are deposited into the intercellular space with consequent oedema and thrombus formation (Beilin and Goldby, 1977; Susin and Mailloux, 1978; Kincaid-Smith, 1980). The resultant luminal narrowing contributes to target-organ ischaemia (Mann and Atlas, 1990). On fundoscopic examination, these pathological events appear as retinal haemorrhages and exudates, and have been demonstrated in arterioles of many organs, particularly the renal vasculature (Pickering, 1968; Jones, 1976; Healton et al, 1982).

The process of vascular damage in malignant hypertension is thought to be initiated by chronically elevated arterial pressure (Susin and Mailloux, 1978). Other variables interacting with blood pressure may also play a role (Möhring, 1977). Very few patients with benign essential hypertension progress to the malignant form.

12.1.3 Mechanisms of malignant hypertension

The exact mechanisms responsible for the development of malignant hypertension remain controversial. One school of thought implicates the height of the arterial pressure as

the dominant factor while the other speculates on the existence of a circulating humoral factor that increases permeability of the vessel wall (Beilin and Goldby, 1977; Möhring, 1977).

12.1.3.1 The pressure hypothesis

In animals and humans, hypertension will become malignant when blood pressure increases into a 'critically high range' (Byrom, 1954; Garner et al, 1975; Möhring et al, 1977). Arteriolar necrosis develops rapidly except in those vascular beds distal to a constricted artery which are protected from the consequences of high blood pressure (Wilson and Byrom, 1941). This 'critically high pressure' leads to 'breakthrough of autoregulation' which results when the resistance vessels are no longer able to constrict in response to the increase in blood pressure, but rather give way to dilation (Johansson, 1974). With increasing blood pressure the vessel radius continues to increase until 'blow out' occurs due to the mechanical effect of the pressure (Möhring, 1977). This concept is referred to as the 'pressure hypothesis'.

As a consequence of the vascular dilation and 'blow out' the underlying endothelium becomes stretched and damaged with plasma passing into the vessel wall compressing and destroying smooth muscle cells (Beilin and Goldby, 1977). Fibrin deposition, oedema and serous effusions further

damage and occlude the lumen of small vessels. The consequences of these vascular lesions are tissue ischaemia, petechial haemorrhages and erythrocyte fragmentation as they pass through narrowed vessels (Brain et al, 1962).

Thus, when an arteriole is exposed to a sufficiently high filling pressure, vascular smooth muscle contractility will be overcome and focal dilation and structural damage will result. This is the first and essential change in the development of the pathological lesion of fibrinoid necrosis in malignant hypertension.

However, some hypertensive patients and animals may tolerate very high blood pressures without the development of vascular damage. These findings have led to the proposal that other factors in addition to the high blood pressure are necessary to induce the vascular changes of malignant hypertension.

12.1.3.2 Humoral Factors

12.1.3.2 (i) The renin-angiotensin-aldosterone system

Most patients with malignant hypertension have activation of the renin-angiotensin system with elevation of plasma renin activity and increased aldosterone secretion (Laragh et al, 1972; Russell et al, 1980). Angiotensin-converting

enzyme inhibitors effectively lower blood pressure in many of these malignant hypertensive patients (Case et al, 1981; Biollaz et al, 1983). Pre-existing hyperreninaemia may contribute to the risk of developing malignant hypertension in some patients (Mann and Atlas, 1990). In others hypersecretion of renin is probably secondary to the renal vascular changes induced by severely raised blood pressure (McAllister et al, 1971). Hypersecretion of renin may set into motion a self perpetuating cycle in which increased angiotensin levels enhance vasoconstriction and worsen renal ischaemia, resulting in further increases in renin secretion (Dauda et al, 1973; Kincaid-Smith, 1980). A concurrent pressure natriuresis may occur resulting in intravascular volume depletion and further elevation of renin secretion and sympathetic nervous system activity (Gross et al, 1975; Atkinson et al, 1979). Some studies have demonstrated that saline repletion, with accompanying reductions in plasma renin activity and in aldosterone secretion, temporarily reverses the malignant process (Gross et al, 1975; Möhring et al, 1976).

12.1.3.2 (ii) Other humoral factors

Malignant hypertension may develop in patients with normal or suppressed renin levels (McAllister et al, 1971; Kawazoe et al, 1987). In experimental models, necrotising vascular lesions develop in anephric animals in the absence of renin (Muirhead et al, 1951). These findings suggest that other

factors besides the renin-angiotensin system may be activated. The sympathetic nervous system and/or the neurohypophyseal hormonal system have been implicated. Experimental malignant hypertension has been produced by injection of various vasopressors, including deoxycorticosterone-saline, vasopressin and noradrenaline (Gavras et al, 1975; Möhring et al, 1977; Lohmeier et al, 1984). In deoxycorticosterone malignant hypertension, plasma vasopressin and noradrenaline concentrations are significantly increased (Reid et al, 1975; Möhring, 1977). Also raised levels of angiotensin II, noradrenaline and ADH and reduced levels of vasodilating agents such as kininogens, kinins and prostacyclins have been demonstrated in malignant hypertension (Giese, 1976; Padfield et al, 1981; Ribiero et al, 1983; Ramos, 1984). These data suggest that in those forms of malignant hypertension where renin appears to be important, other vasopressor systems could come into play, thus maintaining high blood pressure.

The quantitative contribution of each of these humoral factors varies in the pathogenesis of vascular damage depending on the natural course of high blood pressure, the interference by other pathological processes and upon the experimental model studied.

12.1.4 Aetiology of malignant hypertension

The exact aetiology of malignant hypertension is unknown.

It may be essential, or it may be secondary to an underlying disease. Kincaid-Smith and colleagues (1958) initially reported that 40% of patients with malignant hypertension had essential hypertension whereas later studies revealed that only 20% had primary hypertension (Yu et al, 1986). Similar findings were reported by Gudbrandsson et al (1979) and Guelpa et al (1984). This decrease in incidence of essential malignant hypertension may be attributable to early detection and effective treatment of essential hypertension prior to the development of the malignant phase. In South African blacks, malignant hypertension is usually essential whereas in whites, it is usually secondary to other diseases (Jhetam et al, 1982; Milne et al, 1989).

12.1.5 'Secondary' malignant hypertension

Renovascular and renal parenchymal disorders are the most common causes of 'secondary' malignant hypertension (Davis et al, 1978; Yu et al, 1986). Other causes that have been documented include phaechromocytoma, renal vasculitis and primary aldosteronism (Gifford et al, 1964; O'Connell et al, 1985; Murphy et al, 1985).

12.1.6 Risk factors predisposing to malignant hypertension

The most preventable and correctable risk factors predisposing to malignant hypertension include smoking,

oral contraceptive pill use and analgesic abuse (Kincaid-Smith et al, 1973; Isles et al, 1979; Pettit and Klatsky, 1983; Yu et al, 1986).

Prognostic indicators which have been suggested include sex, race, aetiologic classification, fundoscopic findings, smoking and renal function (Hood et al, 1970; Gudbrandsson and Snorrson, 1976; Lee and Alderman, 1978; Gudbrandsson et al, 1979; Isles et al, 1979). The most significant prognostic indicator appears to be the initial serum creatinine level. Gudbrandsson et al (1979) reported that if serum creatinine was less than 300 μmol/l, renal function could be preserved or improved. Yu et al (1986) confirmed these findings.

12.1.7 Other aetiological factors in malignant hypertension

12.1.7 (i) The renin-gene

Some investigators have suggested that the hyperreninaemia in malignant hypertension may be a consequence of the expression of a duplicate renin gene. On the basis of this hypothesis, the triggering event leading to development of malignant hypertension would be a 'switching on' of expression of the duplicate gene. The increased renin would then result in changes of malignant hypertension. Experimental evidence supports this hypothesis. Certain

strains of mice have a duplication of the renin gene on chromosome 1 (Mullins et al, 1986). Human studies however have failed to demonstrate this gene amplification (Frossard et al, 1986; Samani et al, 1989). Whether alterations in the renin gene are respo sible for the hyper-reninaemia in malignant hypertens'on still have to be conclusively demonstrated.

12.1.7 (ii) Human leukocyte antigens

Gudbrandsson et al (1981) reported increased T-lymphocyte reactivity against human arterial antigen, higher levels of IgA and IgM antibodies and a higher prevalence of autoantibodies in malignant hypertensive patients compared to a control group. Hilme et al (1989) recently demonstrated that patients with malignant hypertension had significantly elevated secretion of IgG and IgA and a significant positive correlation between systolic blood pressure and secretion of these immunoglobulins. These results suggest that an immunological process may be involved in malignant hypertension. This could either be a primary immunological disturbance or secondary effects due to the vascular damage caused by the very high blood pressure. Johnson et al (1984), however failed to demonstrate immunological alterations in malignant hypertension.

12.1.7 (iii) Thymic atrophy

Chatelain et al (1981) demonstrated marked thymus atrophy in malignant hypertensive rats. These cells showed a reduced mitotic reactivity to the T cell mitogen, concanavalin A. Similar results were obtained in 24-day hypertensive rats when severe lymphopaenia was also found in malignant hypertensive animals.

12.1.7 (iv) Cellular cations and cation transport inhibitors in malignant hypertension

Experimental and human malignant hypertension are characterised by vascular wall sodium depletion and water loss (Dauda et al, 1973; Möhring, 1974; Simon and Altman, 1986). In experimental malignant hypertension, various other cellular electrolyte abnormalities have been described. These include:- hyperkalaemia in one-kidney, one-clip malignant hypertension, hypokalaemia, hypomagnesaemia and intracellular calcium overload in DOCA-salt hypertension and decreased serum phosphate in stroke prone spontaneously hypertensive rats (Simon and Altman, 1986; Ishitobi et al, 1986; Touyz et al, 1991).

The intracellular sodium depletion of malignant hypertension may be due to a circulating humoral factor that is different to that found in benign hypertension. Simon and Altman (1986) provided evidence for a plasma

factor in malignant-renal hypertension that causes inhibition of ouabain-insensitive cation transport of vascular smooth muscle cells with resultant intracellular sodium depletion. This factor has furosemide-like properties that may account for the natriuresis and diuresis that characterise malignant hypertension. The source and identity of this transport inhibitor are unknown.

Altered cellular cation status and defective cell membrane function may play an important aetiological role in benign essential hypertension. The role of these factors in the aetiology of malignant hypertension is unclear.

12.1.8 Malignant hypertension in South Africa

Malignant hypertension is now a rare disease in the Western world (Gudbrandsson et al, 1979). In certain population groups, including urban South African blacks, however, it is still an important cause of morbidity and mortality and a major cause of end stage renal failure (Jhetam et al, 1982; Veriawa et al, 1990). In 1982 the hospital prevalence of malignant hypertension in Johannesburg blacks was 2.2% (Jhetam et al, 1982). A recent study has reported the hospital prevalence to be 0,9% (S.H. James, M.Med (Med) dissertation, University of the Witwatersrand). Seedat and Reddy documented a 7% incidence of malignant hypertension at a hypertension clinic in Durban (Seedat and Reddy,

1974). Malignant hypertension is rare in Johannesburg whites except in those patients with underlying secondary causes, usually bilateral renal parenchymal disease or renal artery stenosis (Milne et al, 1989). In the majority of malignant hypertensive black patients, no underlying cause can be found.

Cardiovascular risk factors that have been associated with malignant hypertension in whites include obesity, hypercholesterolaemia, excessive smoking, alcohol and oral contraceptive use (Houston, 1989). These risk factors are not common in African black malignant hypertensive patients (Jhetam et al, 1982).

Whether malignant hypertension is genetically prevalent in blacks or whether it is a socioeconomic disease due to a low rate of early detection and treatment remains unclear (Freis, 1975; Gudbrandsson et al, 1979; Jhetam et al, 1982).

12.2 AIMS OF THE STUDY

The aims of this study were:-

1. To determine the serum, platelet and erythrocyte sodium, potassium, magnesium and calcium concentrations,

2. To assess cell membrane ATPase activity and

3. To determine the relationships between cellular cations and cell membrane function,

in patients with malignant hypertension

4. In order to test the hypothesis that hypomagnesaemia is associated with hypertension, a group of hypertensive patients with renal failure, who are known to be hypermagnesaemic, was also studied. Results from this group of patients were compared to the essential and malignant hypertensive groups.

12.3 SUBJECTS AND METHODS

12.3.1 Subjects

12.3.1.1 Malignant hypertensive patients

Black patients admitted to the medical wards of an academic hospital were used in this study. All had presented with malignant hypertension according to the definitions described in chapter 12.1.1. Secondary causes of hypertension were excluded on the basis of history, medical examination, hormonal profile, chest x-rays, electrocardiograms and renal ultrasound. Renal angiograms and renal biopsies were not performed in these patients.

Inclusion criteria into the study

1. Severe hypertension with diastolic blood pressure greater than 120 mmHg.
2. Evidence of fresh bilateral retinal haemorrhages and/or papilloedema.
3. Presenting serum creatinine level less than 200 /umol/l.
4. No other systemic illness.
5. No chronic intake of medications.
6. Older than 25 years.

All the patients had had at least one dose of treatment (calcium channel antagonists) prior to entry into this study.

12.3.1.2 Hypertensive patients with renal failure

Black patients attending the Dialysis Unit at an academic hospital were studied. They were well known to the Unit and were dialysed (haemodialysis) at least three times a week. The main cause of renal failure in this group was chronic glomerulonephritis.

Inclusion criteria

1. Blood pressure prior to dialysis greater than or equal to 145/95 mmHg on three separate occasions.

2. Older than 25 years.

3. No other systemic illness.

12.3.1.3 Medical examination

The malignant hypertensive patients were examined in the medical wards. A full medical examination including fundoscopy was performed on each patient by two doctors. Blood pressure was measured in the recumbent position in the nondominant arm using a mercury sphygmomanometer. Two observers made all measurements. Three readings were taken with the radial pulse counted between readings 2 and 3. The mean of the three readings was used for analysis (diastolic blood pressure = Korotokoff Sound V).

The patients with renal failure were examined in the Renal Unit. They were ambulant and visited the Unit for dialysis on an out-patient basis. A medical examination was performed on all the patients. Blood pressure was measured in the seated position according to the methods described in chapter 5.

The subjects completed a general and medical questionnaire, and all gave signed informed consent.

12.3.1.4 Venesection

Venous blood was obtained without cuff compression from the

antecubital fossa. The techniques employed for blood withdrawal and preparation are described in chapter 5.

12.3.2 Methods and materials

Serum, platelet and erythrocyte magnesium, calcium, sodium and potassium concentrations were determined in the malignant hypertensive and renal failure patients according to the methods described in Appendix G.

Erythrocyte and platelet membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were measured according to the methods described in Appendix M.

12.4 RESULTS

12.4.1 Malignant hypertension

12.4.1.1 Descriptive characteristics

Sixteen patients with malignant hypertension were studied. There were 10 females and 6 males. The descriptive characteristics are presented in Table 12.1. Comparing the malignant hypertensive group to the normotensive controls and the benign essential hypertensive group (chapter 8.5; Table 8.1), SBP, DBP and MAP were significantly higher in the malignant hypertensive group. There were no significant differences in age, QI, and pulse between the

three groups. Serum creatinine was significantly higher in the malignant hypertensive patients compared to the normotensive controls ($p < 0,01$) and essential hypertensive subjects ($p < 0,05$) (see Table 8.1).

12.4.1.2 Biochemical data

Complete biochemical data were only available in 11 of the 16 malignant hypertensive patients. The results are presented in Table 12.2. In the malignant hypertensive group, serum sodium, erythrocyte potassium, serum calcium, platelet magnesium and erythrocyte magnesium were significantly decreased compared to the essential hypertensive group (Figures 12.1-12.4). Platelet calcium was significantly higher in the malignant hypertensive group compared to the essential hypertensive group. Compared to the normotensive group, serum sodium, serum potassium, serum magnesium, erythrocyte magnesium and platelet magnesium were significantly decreased and erythrocyte calcium and platelet calcium significantly raised in the malignant hypertensive group. The only significant difference between the sexes was for platelet magnesium which was significantly lower in the females compared to the males (Table 12.3).

Table 12.1 Clinical characteristics of the malignant hypertensive patients.

	Malignant Hypertensive Group		
		Female	Male
n	16	10	6
	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	44 ± 10	38 ± 9	51 ± 6
Height (m)	1,68 ± 0,06	1,67 ± 0,05	1,71 ± 0,06
Weight (Kg)	75 ± 9	77 ± 10	74 ± 8
QI (Kg/m^2)	27 ± 3	27 ± 3	25 ± 3
SBP (mmHg)	188 ± 23	182 ± 27	196 ± 13
DBP (mmHg)	124 ± 24	121 ± 28	128 ± 21
MAP (mmHg)	145 ± 23	142 ± 26	151 ± 19
HR (beats/min)	70 ± 9	67 ± 5	72 ± 12
Serum creatinine (/umol/l)	191 ± 30	198 ± 20	182 ± 33
Serum GGT (U/l)	76 ± 27	50 ± 20	104 ± 38

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; QI = quetelet index.

Table 12.2 Biochemical data in the malignant hypertensive group.

Variable	Malignant Hypertensives	P values	
		vs BHT	vs BNT
	Mean ± SD		
Serum Na^+	134 ± 4,0	0,002*	0,0001*
Erythrocyte Na^+	10,9 ± 2,6	0,93	0,12
Platelet Na^+	1,62 ± 0,5	0,95	0,07
Serum K^+	3,71 ± 0,5	0,56	0,05*
Erythrocyte K^+	77 ± 10,7	0,004*	0,01*
Platelet K^+	4,90 ± 1,56	0,52	0,40
Serum Ca^{2+}	1,87 ± 0,27	0,02*	0,20
Erythrocyte Ca^{2+}	6,00 ± 1,99	0,15	0,003*
Platelet Ca^{2+}	2,02 ± 0,43	0,003*	0,0001*
Serum Mg^{2+}	0,71 ± 0,06	0,87	0,0008*
Erythrocyte Mg^{2+}	1,98 ± 0,28	0,05*	0,0002*
Platelet Mg^{2+}	1,50 ± 0,02	0,05*	0,0001*

Serum and erythrocyte values expressed as mmol/l, erythrocyte Ca^{2+} expressed as /umol/l and platelet values as /umol/l x 10^8 cells.

BNT = black normotensive group;
BHT = black hypertensive group
* = significant difference

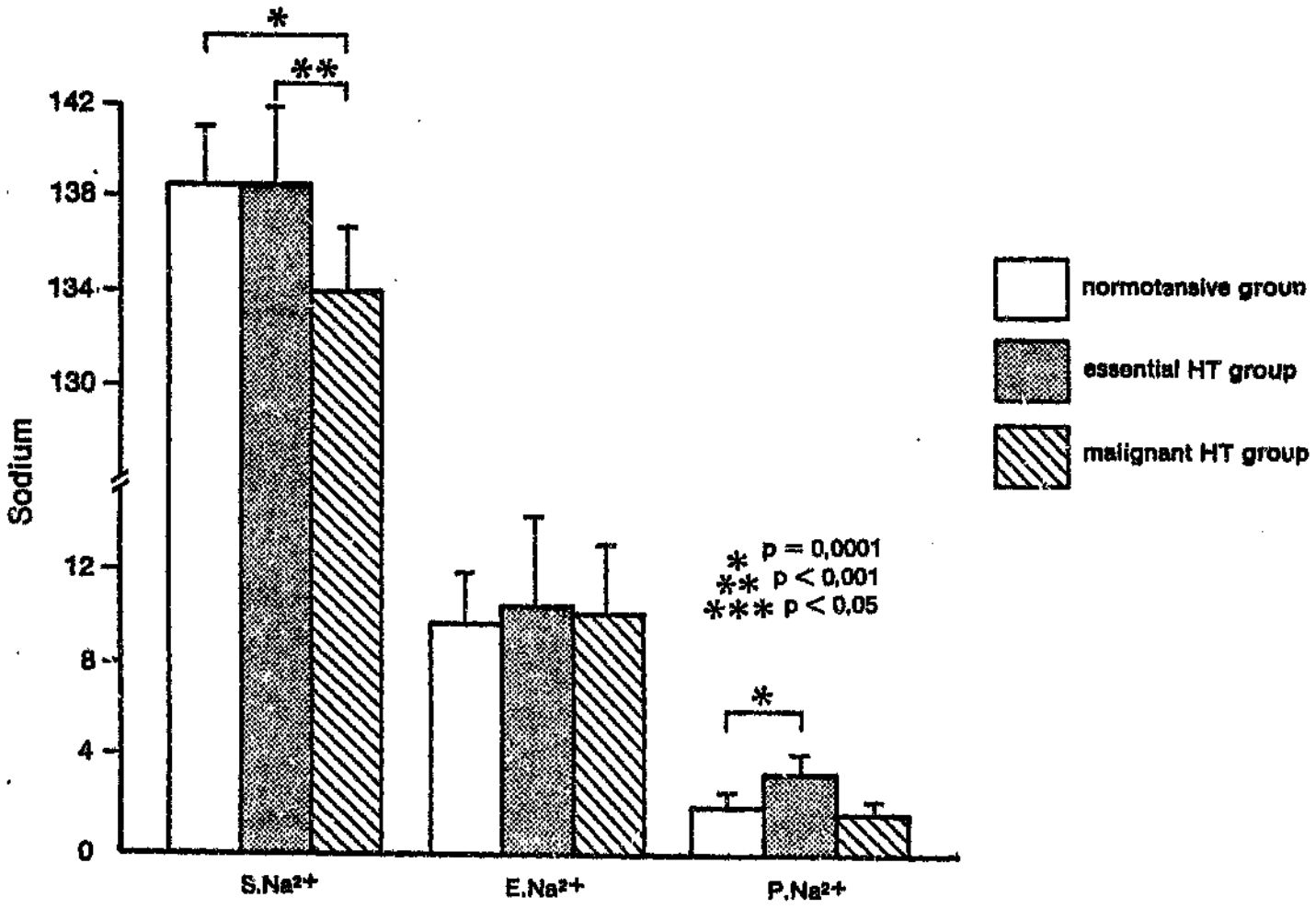


Fig. 12.1. Serum (S), erythrocyte (E) and platelet (P) sodium concentrations in the black normotensive, essential hypertensive (HT) and malignant hypertensive groups.
Serum and erythrocyte values expressed as mmol/l; platelet values expressed as umol/1x10⁸

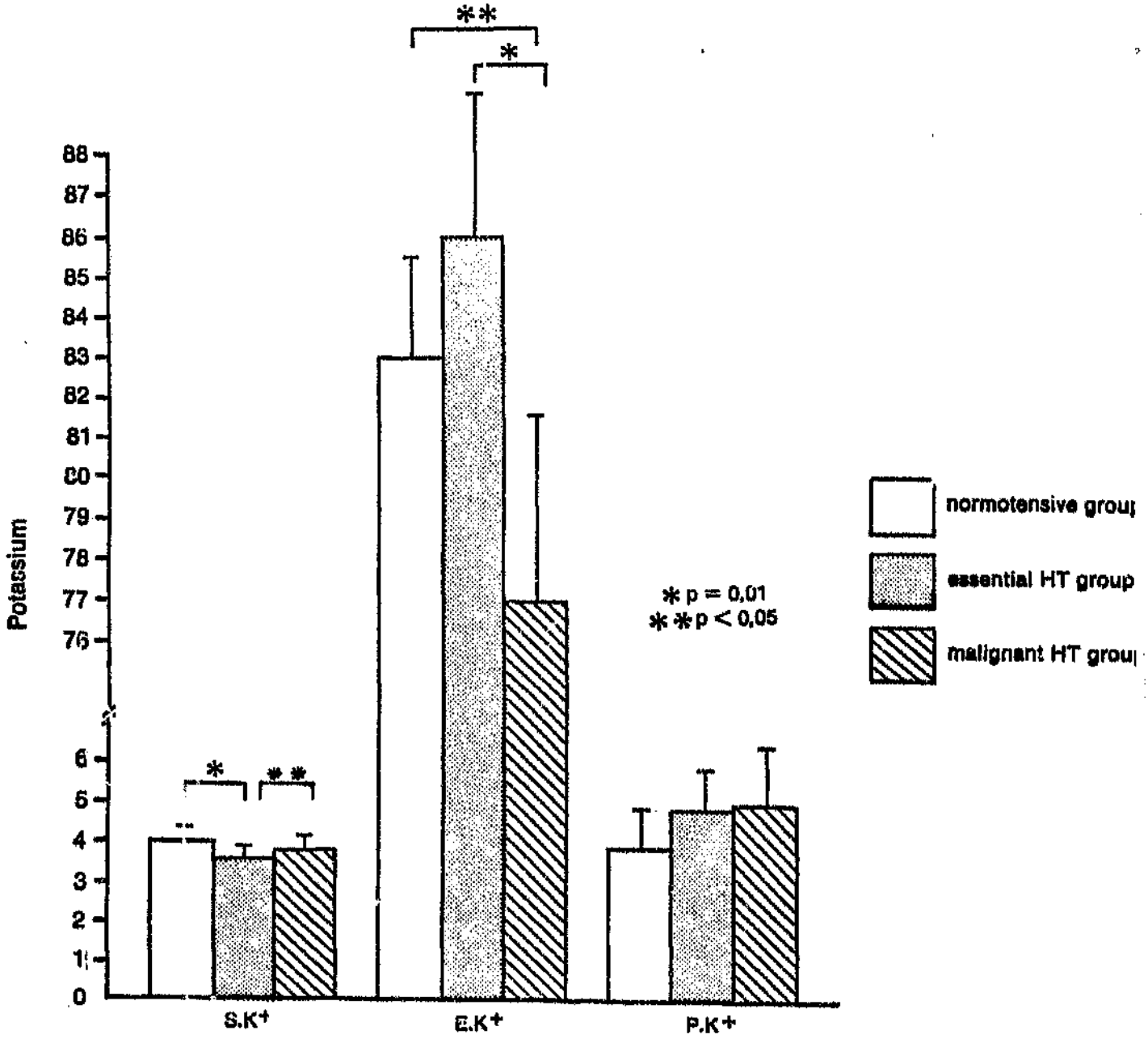


Fig. 12.2. Serum (S), erythrocyte (E) and platelet (P) potassium concentrations in the black normotensive, essential hypertensive (HT) and malignant hypertensive groups.
Serum and erythrocyte values expressed as mmol/l; platelet values expressed as umol/1x10⁸

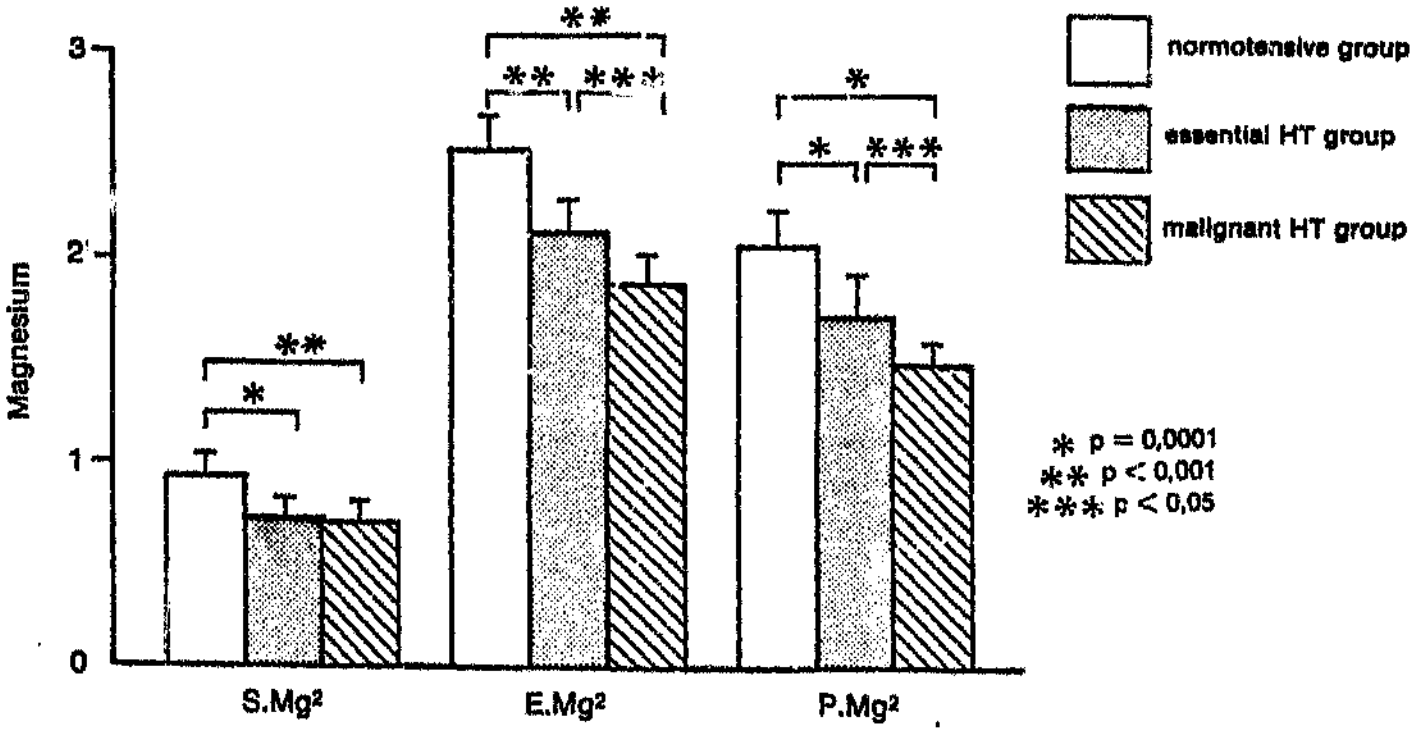


Fig. 12.3. Serum (S), erythrocyte (E) and platelet (P) magnesium concentrations in the black normotensive, essential hypertensive (HT) and malignant hypertensive groups.
Serum and erytnrocyte values expressed as mmol/l; platelet values expressed as umol/1x10⁸

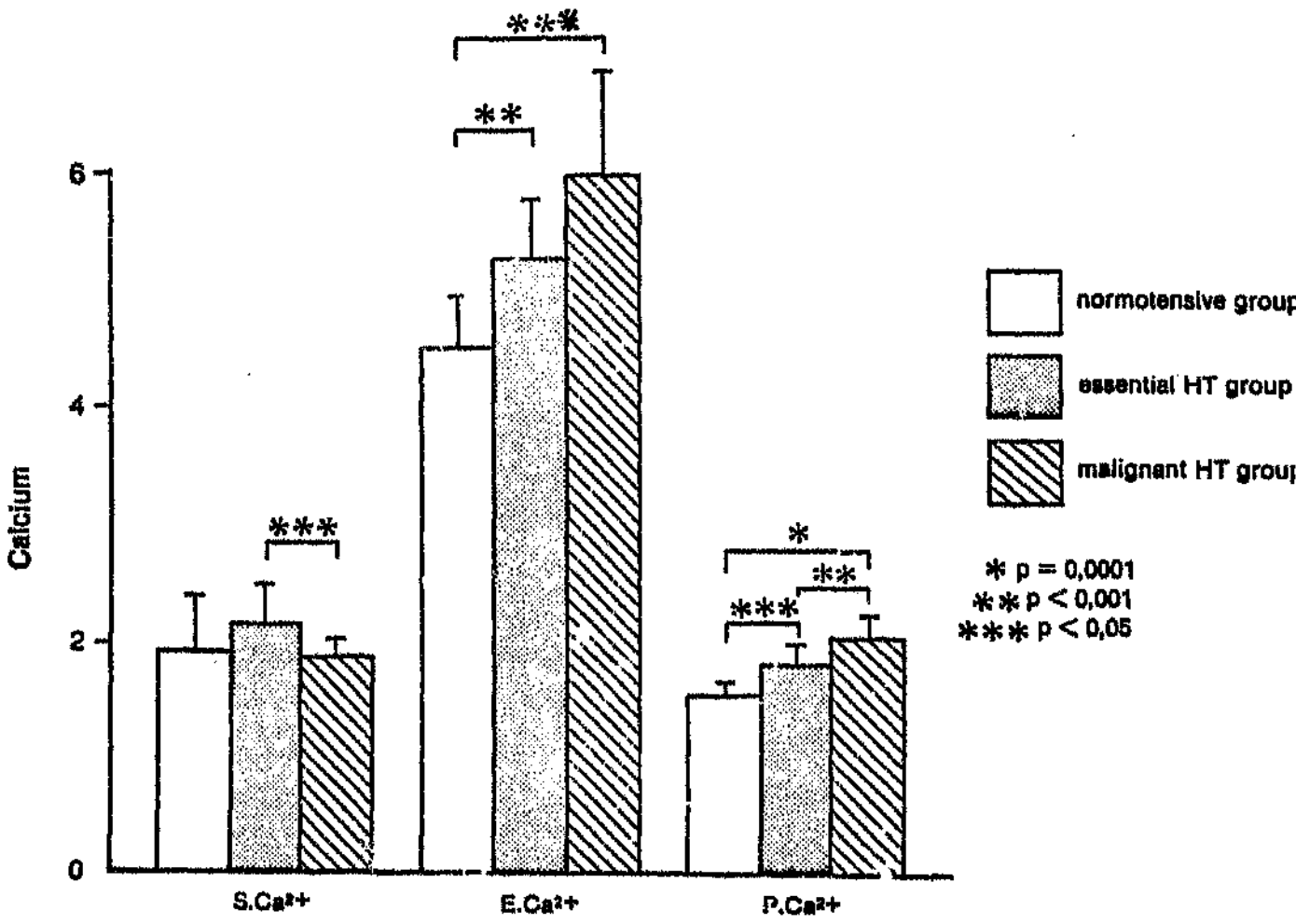


Fig. 12.4. Serum (S), erythrocyte (E) and platelet (P) calcium concentrations in the black normotensive, essential hypertensive (HT) and malignant hypertesive groups.
Serum values expressed as mmol/l; erythrocyte values expressed as umol/l; platelet values expressed as umol/1x10⁸

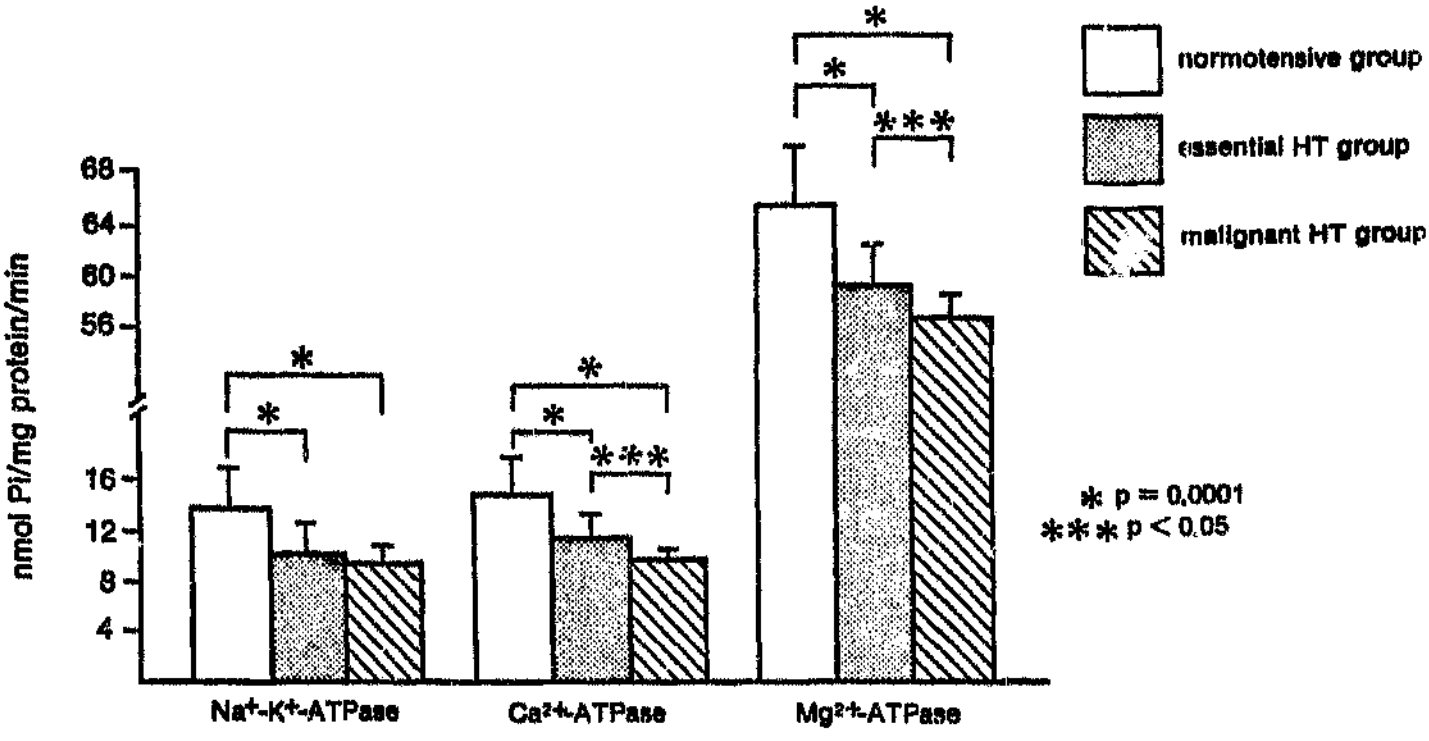


Fig. 12.5 Platelet membrane ATPase activity in the black normotensive, essential hypertensive (HT) and malignant hypertensive groups.

Table 12.3 Biochemical data in the male and female malignant hypertensive group.

	Malignant Hypertensives	
Variable	Female	Male
Serum Na^+	134 ± 3,4	135 ± 5
Erythrocyte Na^+	9,73 ± 1,98	12,4 ± 2,8
Platelet Na^+	1,42 ± 0,29	1,9 ± 0,63
Serum K^+	3,62 ± 0,39	3,8 ± 0,68
Erythrocyte K^+	79 ± 10	76 ± 11
Platelet K^+	4,87 ± 1,66	4,95 ± 1,63
Serum Mg^{2+}	0,71 ± 0,08	0,70 ± 0,04
Erythrocyte Mg^{2+}	2,00 ± 0,35	1,96 ± 0,23
Platelet Mg^{2+}	1,48 ± 0,20	1,63 ± 0,19*
Serum Ca^{2+}	1,86 ± 0,38	1,88 ± 0,04
Erythrocyte Ca^{2+}	5,45 ± 2,65	6,7 ± 0,41
Platelet Ca^{2+}	2,11 ± 0,41	1,92 ± 0,48

Serum and erythrocyte values expressed as mmol/l, erythrocyte Ca^{2+} expressed as /umol/l and platelet values as /umol/l x 10^8 cells.

* $p < 0,05$ males versus females

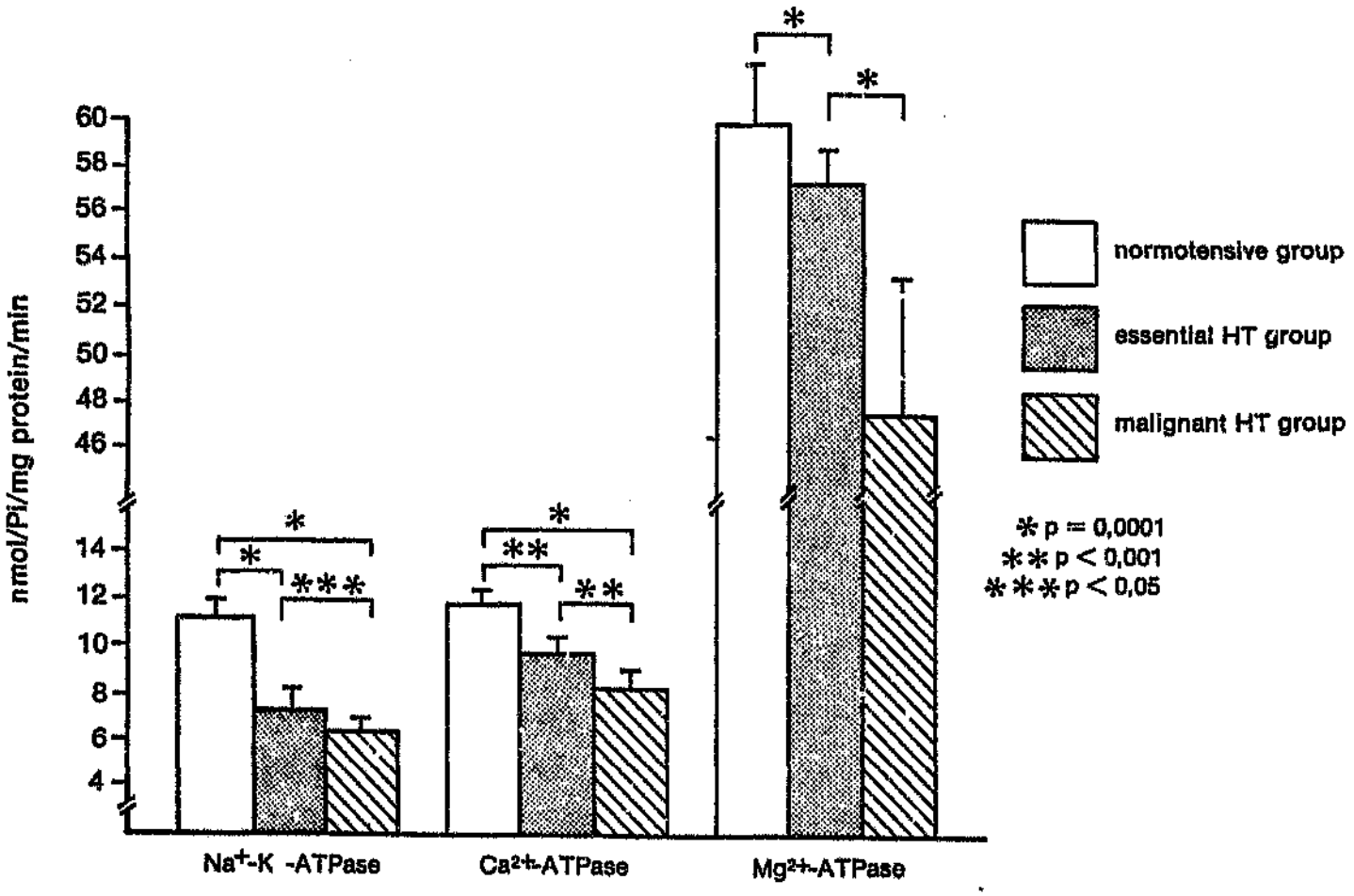


Fig. 12.6 Erythrocyte membrane ATPase activity in the black normotensive, essential hypertensive (HT) and malignant hypertensive groups.

12.4.1.3 Cell membrane ATPase activity in the malignant hypertensive group

Erythrocyte and platelet membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities are presented in Table 12.4. Erythrocyte and platelet membrane activities of all three ATPases studied were significantly depressed in the malignant hypertensive patients compared to the normotensive controls ($p = 0,0001$) (Figures 12.5; 12.6). Erythrocyte membrane Na^+-K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase and platelet membrane Mg^{2+}-ATPase and Ca^{2+}-ATPase activities were significantly lower in the malignant hypertensive patients compared to the essential hypertensive patients ($p < 0,05$) (Table 12.4) (Figures 12.5; 12.6). Cell membrane ATPase activity in the different sexes is presented in Table 12.5. There were no significant differences in ATPase activity between the males and females.

12.4.1.4 Correlation studies

Pearsons correlation coefficients for correlations between MAP and the clinical variables are presented in Table 12.6. Except for SPB and DBP, there were no other significant correlations.

Correlation coefficients for correlations between blood pressure and the cations are presented in Table 12.7.

Serum magnesium, platelet magnesium and serum calcium were negatively and erythrocyte calcium and erythrocyte sodium positively related to MAP.

The only significant correlations between ATPase activity and MAP were for erythrocyte and platelet membrane Mg^{2+}-ATPase activities which were inversely related to MAP (Table 12.8).

Table 12.9 presents the correlations between ATPase activity and the cations. Significant positive correlations were erythrocyte potassium and erythrocyte Ca^{2+}-ATPase ($r = 0,82$; $p = 0,002$); serum magnesium and platelet Ca^{2+}-ATPase are ($r = 0,68$; $p = 0,02$); and serum magnesium and platelet Mg^{2+}-ATPase ($r = 0,53$; $p = 0,05$). Erythrocyte calcium was inversely correlated to erythrocyte Ca^{2+}-ATPase ($r = -0,62$; $p = 0,04$).

Significant positive correlations between the cations in the malignant hypertensive group included serum sodium and erythrocyte potassium ($r = 0,65$; $p = 0,03$). Serum calcium and serum magnesium ($r = 0,64$; $p = 0,03$) and platelet calcium and serum potassium ($r = 0,74$; $p = 0,01$). Erythrocyte magnesium was inversely correlated to erythrocyte sodium ($r = -0,69$; $p = 0,01$) (Table 12.10). Table 12.11 presents the correlations between the cations in the male and female groups.

Table 12.4 Erythrocyte and platelet membrane ATPase activity in the malignant hypertensive group.

Variable		P values	
		vs BHT	vs BNT
	Mean ± SD		
Erythrocyte membrane			
Na^+-K^+-A	6,2 ± 0,9	0,029	0,0001
Mg^{2+}-A	47,9 ± 7,3	0,0001	0,0001
Ca^{2+}-A	8,4 ± 1,9	0,001	0,0001
Platelet membrane			
Na^+-K^+-A	9,4 ± 1,8	0,157	0,0001
Mg^{2+}-A	56,7 ± 6,5	0,002	0,0001
Ca^{2+}-A	9,6 ± 1,1	0,05	0,0001

Na^+-K^+-A = Na^+-K^+-ATPase; Mg^{2+}-A = Mg^{2+}-ATPase; Ca^{2+}-A = Ca^{2+}-ATPase.

BNT = black normotensive group
BHT = black hypertensive group

ATPase activity expressed as nmol Pi/mg protein/min at 37°C

Table 12.5 Cell membrane ATPase activity in the male and female malignant hypertensive group.

Variable	Malignant Hypertensive Group	
	Female	Male
Erythrocyte membrane		
Na^+-K^+-A	6,00 ± 1,09	6,40 ± 0,89
Mg^{2+}-A	49,51 ± 7,50	46,00 ± 7,38
Ca^{2+}-A	8,16 ± 2,22	8,61 ± 1,67
Platelet membrane		
Na^+-K^+-A	9,50 ± 2,16	9,41 ± 1,51
Mg^{2+}-A	57,21 ± 9,78	50,29 ± 7,79
Ca^{2+}-A	9,66 ± 1,03	9,61 ± 1,34

Na^+-k^+-A = Na^+-K^+-ATPase; Mg^{2+}-A = Mg^{2+}-ATPase;
Ca^{2+}-A = Ca^{2+}-ATPase.

ATPase activity expressed as nmol Pi/mg protein/min at 37°C

Table 12.6: Correlation coefficients for correlations between the clinical variables and MAP in the malignant hypertensive group.

	r value	p
Age	0,03	0,91
Height	0,50	0,11
Weight	0,27	0,42
QI	0,04	0,09
SBP	0,88	0,0003
DBP	0,94	0,0001
HR	0,16	0,64

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; QI - quetelet index

Table 12.7 Correlation coefficients between the cations and systolic BP (SBP) diastolic BP (DBP) and MAP.

	Malignant Hypertensives		
	SBP	DBP	MAP
Serum Na^+	-0,21 0,53	0,17 0,60	0,05 0,86
Erythrocyte Na^+	0,54 0,08	0,59 0,05	0,61 0,04
Platelet Na^+	0,33 0,32	0,05 0,87	0,07 0,83
Serum K^+	0,16 0,63	0,13 0,70	0,14 0,66
Erythrocyte K^+	-0,49 0,13	-0,07 0,82	-0,22 0,52
Platelet K^+	0,11 0,76	0,45 0,17	0,35 0,28
Serum Mg^{2+}	-0,55 0,08	-0,59 0,04	-0,55 0,05
Erythrocyte Mg^{2+}	-0,44 0,18	-0,53 0,09	-0,54 0,09
Platelet Mg^{2+}	-0,47 0,10	-0,66 0,02	-0,64 0,03
Serum Ca^{2+}	-0,54 0,09	-0,59 0,05	-0,61 0,04
Erythrocyte Ca^{2+}	0,57 0,04	0,54 0,07	0,55 0,05
Platelet Ca^{2+}	0,16 0,62	0,03 0,90	0,08 0,81

The top value is the 'r' value and the bottom value is the 'p' value.

Table 12.8 Correlation coefficients for correlations between ATPase activity and MAP in the malignant hypertensive group.

Variable	r value	p value
Erythrocyte membrane		
Na^+-K^+-A	0,06	0,86
Mg^{2+}-A	-0,58	0,04*
Ca^{2+}-A	-0,16	0,63
Platelet membrane		
Na^+-K^+-A	-0,07	0,83
Mg^{2+}-A	-0,54	0,05*
Ca^{2+}-A	-0,26	0,43

Na^+-K^+-A = Na^+-K^+-ATPase; Mg^{2+}-A = Mg^{2+}-ATPase; Ca^{2+}-A = Ca^{2+}-ATPase.

ATPase activity expressed as nmol Pi/mg protein/min at 37°C

* = significant correlation

Table 12.9 Correlation coefficients for correlations between ATPase activity and the cations in the malignant hypertensive group.

	Erythrocyte membrane ATPase activity			Platelet membrane ATPase activity		
	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A	Na^+-K^+-A	Ca^{2+}-A	Mg^{2+}-A
Serum Na^+	0,03 0,9	0,35 0,28	0,46 0,15	-0,14 0,66	0,51 0,11	-0,29 0,37
Erythrocyte Na^+	0,17 0,61	0,15 0,66	-0,11 0,76	-0,33 0,31	-0,36 0,28	-0,49 0,12
Platelet Na^+	-0,21 0,53	-0,39 0,30	-0,29 0,38	0,22 0,51	0,09 0,77	0,49 0,12
Serum K^+	-0,19 0,55	0,08 0,81	0,29 0,37	0,11 0,75	0,02 0,94	0,33 0,32
Erythrocyte K^+	0,21 0,51	0,82* 0,002	0,01 0,97	-0,34 0,33	0,29 0,39	-0,41 0,20
Platelet K^+	0,05 0,88	-0,31 0,35	0,02 0,96	0,11 0,60	0,42 0,19	0,38 0,25
Serum Mg^{2+}	0,13 0,70	0,07 0,81	0,14 0,66	0,25 0,46	0,68* 0,02	0,53* 0,05
Erythrocyte Mg^{2+}	0,15 0,65	0,01 0,99	0,38 0,25	0,36 0,27	0,16 0,62	0,16 0,63
Platelet Mg^{2+}	0,35 0,29	0,18 0,60	0,03 0,90	0,15 0,66	0,17 0,61	0,25 0,44
Serum Ca^{2+}	0,50 0,11	0,15 0,66	0,14 0,69	0,15 0,65	0,42 0,20	0,35 0,28
Erythrocyte Ca^{2+}	0,01 0,97	-0,62* 0,04	0,27 0,41	0,27 0,41	0,23 0,49	0,37 0,25
Platelet Ca^{2+}	0,20 0,55	0,09 0,79	0,07 0,83	0,05 0,86	0,11 0,71	0,03 0,90

The top value is the 'r' value and the bottom value is the 'p' value.

* = significant correlation

Table 12.10 Correlation coefficients for correlations between the cations in the malignant hypertensive group.

	SN	EN	PN	SK	EK	PK	SMg	EMg	PMg	SCa	ECa	PCa
SNa	-											
EN	0,14 0,68											
PN	-0,31 0,35	-0,04 0,89	-									
SK	-0,12 0,72	0,49 0,12	-0,27 0,41	-								
EK	0,65* 0,03	0,17 0,62	-0,44 0,17	0,16 0,64	-							
PK	0,45 0,16	0,13 0,68	0,09 0,78	-0,26 0,43	0,05 0,87	-						
SMg	0,18 0,59	-0,22 0,51	-0,09 0,78	0,04 0,91	0,36 0,26	0,21 0,54	-					
EMg	0,10 0,74	-0,69* 0,01	-0,13 0,70	-0,39 0,22	-0,16 0,63	-0,14 0,67	-0,05 0,87	-				
PMg	-0,29 0,38	0,03 0,92	0,25 0,46	0,03 0,91	0,06 0,85	-0,52 0,10	0,30 0,36	0,11 0,73	-			
SCa	0,14 0,67	-0,22 0,50	-0,22 0,52	0,07 0,82	0,20 0,55	-0,24 0,47	0,64* 0,03	0,49 0,12	0,51 0,11	-		
ECa	0,02 0,93	0,22 0,51	0,10 0,76	0,48 0,12	-0,51 0,10	0,25 0,45	0,15 0,61	0,11 0,73	0,19 0,57	0,21 0,54	-	
PCa	-0,32 0,33	0,14 0,63	0,28 0,40	0,74* 0,01	-0,19 0,58	-0,23 0,50	0,21 0,51	0,29 0,37	0,23 0,48	0,11 0,75	0,48 0,13	-

Top value represents the r value and the bottom value represents the p value

* = Significant correlation

S = serum; E = erythrocyte; P = platelet; N = Na^+; K = K^+; Mg = Mg^{2+}; Ca = Ca^{2+}

Table 12.11 Correlation coefficients (r value) for correlations between the cations in the male and female malignant hypertensive group.

	SN	EN	PN	SK	EK	PK	SMg	EMg	PMg	SCa	ECa	PCa
SN	-											
EN	-0,71 0,56											
PN	-0,95** 0,28	0,72 0,84*	-									
SK	0,05 -0,31	0,19 0,56	0,09 -0,56	-								
EK	0,56 0,79	0,29 0,77	-0,58 0,36	-0,61 0,18								
PK	0,48 0,42	0,16 0,48	-0,52 0,46	0,68 -0,99**	0,16 -0,04	-						
SMg	0,17 0,39	0,28 0,03	-0,48 0,51	0,09 0,12	0,28 0,56	0,26 0,14	-					
EM	0,51 0,44	-0,94** -0,67	-0,49 0,18	-0,40 0,51	0,19 -0,83*	-0,45 0,44	0,10 -0,71	-				
PM	-0,21 -0,61	0,13 0,20	0,03 0,23	0,77* 0,49	0,52 0,46	-0,52 -0,61	0,49 0,08	0,23 0,08	-			
SCa	0,17 0,38	-0,59 0,97**	-0,39 0,89*	-0,27 0,68	0,22 0,67	-0,30 -0,57	0,74* 0,12	0,62 -0,56	0,66 -0,22	-		
ECa	0,08 0,14	0,06 0,24	0,04 0,33	0,81* 0,14	-0,66 0,16	0,37 0,16	0,28 0,22	0,11 0,09	-0,44 0,44	0,21 0,10	-	
PCa	0,24 0,32	0,15 0,45	0,11 0,36	0,86* 0,84*	-0,77* 0,28	0,31 -0,81	0,29 0,31	0,22 -0,54	0,44 0,17	0,10 0,61	0,96** 0,41	-

Top value represents the females and the bottom value the males.

* $p < 0,05$; ** $p < 0,01$

S = serum; P = platelet; E = erythrocyte; N = Na^+; K = K^+; Mg = Mg^{2+}; Ca = Ca^{2+}

12.4.2 Renal failure patients

12.4.2.1 Clinical characteristics

Six hypertensive patients with renal failure were studied. The clinical characteristics are presented in Table 12.12. These patients had mild-moderate hypertension. There were no significant differences in age, QI and pulse between these patients and the normotensive, essential hypertensive and malignant hypertensive groups (see chapter 8.5; Table 8.1). Serum creatinine was significantly elevated in the renal failure group compared to the other three groups. In the renal failure group, MAP correlated positively with the QI ($r = 0,84$; $p = 0,03$) (Table 12.13).

12.4.2.2 Biochemical data

The biochemical results for the renal failure group are presented in Table 12.14. In this group, serum sodium, erythrocyte calcium and platelet calcium were significantly lower compared to the normotensive and hypertensive groups. Serum potassium, serum magnesium, erythrocyte magnesium and platelet magnesium concentrations were significantly higher in the renal failure patients compared to the other groups.

Table 12.12 Clinical characteristics of the renal failure group.

Total (n)	6
Males (n)	2
Females (n)	4
Age (years)	41 ± 4
Height (m)	1,69 ± 0,08
Weight (Kg)	72 ± 6
QI (Kg/m^2)	25 ± 0,9
SBP (mmHg)	148 ± 18
DBP (mmHg)	98 ± 4
MAP (mmHg)	115 ± 8
HR (beats/min)	70 ± 8
Serum creatinine (/umol/l)	638 ± 121

Table 12.13 Pearsons correlation coefficients for correlations between MAP and the clinical variables.

	r - value	p - value
Age	0,75	0,08
QI	0,84	0,03
SBP	0,99	0,0001
DBP	0,86	0,02
HR	-0,46	0,35

Table 12.14 Biochemical data in the renal failure group. Comparisons with the black normotensive and hypertensive groups.

		P - value	
		vs BNT	vs BHT
Serum Na^+	134 ± 5	0,0002	0,002
Erythrocyte Na^+	11,8 ± 1,9	0,002	0,002
Platelet Na^+	1,46 ± 0,40	0,99	0,19
Serum K^+	4,76 ± 0,30	0,0001	0,0001
Erythrocyte K^+	79 ± 8	0,45	0,05
Platelet K^+	3,83 ± 2,71	0,11	0,08
Serum Ca^{2+}	2,35 ± 0,30	0,01	0,07
Erythrocyte Ca^{2+}	2,31 ± 0,79	0,0001	0,0001
Platelet Ca^{2+}	1,36 ± 0,63	0,0001	0,0001
Serum Mg^{2+}	1,19 ± 0,45	0,0001	0,0001
Erythrocyte Mg^{2+}	2,98 ± 0,65	0,03	0,0001
Platelet Mg^{2+}	2,33 ± 0,38	0,04	0,002

BNT = black normotensive group; BHT = black hypertensive group

Serum and erythrocyte values = mmol/l; erythrocyte Ca^{2+} = /umol/l; platelet values = /umol/l x 10^8 cells.

Table 12.15 Erythrocyte and platelet membrane ATPase activity in the renal failure patients. Comparisons with the black normotensive and hypertensive groups.

Variable		P values	
		vs BHT	vs BNT
	Mean ± SD		
Erythrocyte membrane			
Na^+-K^+-ATPase	9,7 ± 1,8	0,12	0,004
Mg^{2+}-ATPase	68 ± 10	0,0008	0,0001
Ca^{2+}-ATPase	12 ± 2,9	0,31	0,001
Platelet membrane			
Na^+-K^+-ATPase	14,3 ± 4,4	0,68	0,0001
Mg^{2+}-ATPase	70 ± 11,1	0,41	0,02
Ca^{2+}-ATPase	15,6 ± 4,2	0,21	0,0001

Cell membrane ATPase activity expressed as nmol Pi/mg protein/min at 37°C.

BNT = black normotensive group
BHT = black hypertensive group

12.4.2.3 Cell membrane ATPase activity

Erythrocyte and platelet membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in the renal failure group are presented in Table 12.15. Activity of all three erythrocyte and platelet membrane ATPases were significantly higher in the renal failure group compared to the essential hypertensive group. Erythrocyte Mg^{2+}-ATPase activity was significantly higher in the renal failure patients compared to the normotensive controls. There were no other significant differences for ATPase activity between the renal failure and normotensive groups.

12.4.2.4 Correlation studies

There were no significant correlations between MAP and the biochemical variables in the renal failure group.

12.5 DISCUSSION

Malignant hypertension characterised by severe blood pressure associated with rapid deterioration in renal function and specific fundal changes is a rare disease in the Western world today (Gudbrandsson et al, 1979; Kincaid-Smith, 1980). In South Africa, malignant hypertension is rare in whites but is not uncommon in blacks (Seedat and Reddy, 1974; Milne et al, 1989). In blacks, malignant hypertension is usually essential without underlying renal

or other contributory disease. In this study 80% of the malignant hypertensive patients studied had a previous history of essential hypertension.

Although the exact aetiology of malignant hypertension is unknown, a number of variables have been implicated. These include genetic factors, increased angiotensin II, hyperreninaemia, increased ADH and noradrenaline, kininogen and kinin deficiency, decreased prostacyclin and immunological defects (Padfield et al, 1981; Forsberg and Low, 1983; Ribeiro et al, 1983; Ramos, 1984; Samani et al, 1989). Associated risk factors that have been defined include:- race, smoking, drugs (oral contraceptives, corticosteroids, tricyclic antidepressants, sympathomimetics, nonsteroidal anti-inflammatories) and severity of underlying hypertension (Relman, 1982; Tuomilehto et al, 1982; Petitt and Klatsky, 1983; Ram, 1983; Houston, 1989). The patients studied here were all black, 40% were smokers and 10% of the females were taking oral contraceptives. Of the group who had known essential hypertension, only 2 claimed to have been compliant.

The role of cellular cations and cell membranes in the aetiology of human malignant hypertension has not been previously reported. There is extensive data in the literature regarding altered cation status and defective cell membrane function in essential hypertension (Langford and Watson, 1975; Simpson, 1985a; Buhler and Resink, 1988;

Bing, 1987; Postnov, 1990). Whether similar abnormalities occur in malignant hypertension is not known.

Experimental malignant hypertension h been associated with abnormal cellular handling of sodium, potassium and calcium (Gavras et al, 1975; Jones and Hart, 1975; Chan et al, 1983; Dworkin et al, 1990). Simon and Altman (1986) recently identified a circulating furosemide-like inhibitor of ouabain-sensitive cation transport in malignant essential renal hypertension which was different to the cation transport inhibitors in benign essential hypertension. The role of endothelium-derived contracting factor and intracellular calcium have recently been investigated in DOCA-malignant hypertensive rats (Martin et al, 1986; Cordellini et al, 1990). In mineralocorticoid-salt malignant hypertension, acute intravenous magnesium administration lowers blood pressure significantly (Dipette et al, 1987).

12.5.1 Cations in malignant hypertension

The results of this st dy demonstrate several cellular abnormalities in black patients with malignant hypertension. In these patients, serum sodium was significantly lower compared to the normotensive and essential hypertensive subjects. This may be due to the natriuresis that has been described in malignant hypertension (Möhring, 1977). Although previous studies

have reported intracellular sodium depletion in malignant hypertension, the erythrocyte and platelet sodium levels were normal in the patients studied here (Dauda et al, 1973; Möhring, 1974).

Hyper- and hypokalaemia have been documented in human and experimental malignant hypertension (Ishitobi et al, 1986). In the patients studied, serum and erythrocyte potassium levels were depressed. These results relating to sodium and potassium may be due to the hyperreninaemia of malignant hypertension. Since urbanised blacks have been reported to consume low potassium diets, these results may be due to dietary factors (Grim et al, 1980). Platelet potassium may be spared at the expense of potassium deficiency in other cellular compartments.

The involvement of intracellular calcium in the pathogenesis of essential hypertension has been extensively reviewed (chapter 1.3). Increased vascular smooth muscle calcium concentration is associated with enhanced contractility and raised blood pressure (Robinson, 1984). The malignant hypertensive patients had significantly lower serum calcium and significantly raised platelet calcium concentrations compared to patients with essential hypertension. These results suggest that the mechanisms controlling intracellular calcium may be abnormal in malignant hypertension, and more severe compared to the abnormalities in essential hypertension.

The role of magnesium in the pathophysiology of essential hypertension has been investigated (Resnick et al, 1984; Altura and Altura, 1985b; Witteman and Grobbee, 1990). In human essential hypertension, the results have not been consistent (Resnick et al, 1983; Touyz et al, 1989; Kjeldsen et al, 1990). The role of magnesium in human malignant hypertension is unknown. Early studies however reported the successful use of intravenous magnesium to treat severe and malignant hypertension (Blackfan and Hamilton, 1925). The therapeutic hypotensive effects of magnesium sulphate in eclampsia are well known (Conradt et al, 1984). Intravenous magnesium infusion in malignant hypertensive rats significantly decreases blood pressure (Dipette et al, 1988). In the malignant hypertensive patients investigated in this study, serum magnesium, erythrocyte magnesium and platelet magnesium concentrations were significantly lower compared to the normotensive controls. Compared to the essential hypertensive group, the malignant hypertensive group had significantly decreased erythrocyte and platelet magnesium concentrations. These results suggest that intracellular magnesium depletion may be more severe in malignant compared to essential hypertension.

Reasons for the altered magnesium status in malignant hypertension could be related to genetics, dietary factors, ethanol, renal dysfunction and renin. Although the patients all had some degree of renal dysfunction they were

not uraemic. Patients with severe renal failure were excluded from the study. Since there was no significant relationship between serum creatinine and magnesium, and because renal failure causes increased serum magnesium, the altered magnesium status was probably not attributable to renal causes. Serum GGT (an indication of alcohol consumption) was significantly raised in the malignant hypertensive patients. There was a weak correlation between serum GGT and serum magnesium ($r = 0,27$; $p = 0,05$). Also, blacks have a low dietary intake of magnesium (Grim et al, 1980; Steyn et al, 1986). These results suggest that the magnesium depletion may be associated with excessive alcohol intake and deficient dietary magnesium consumption.

The relationship between magnesium and the renin-aldosterone axis has been investigated (Fray, 1977; Dawson, 1984; Cohen, 1988). Resnick et al (1983) demonstrated an inverse relationship between serum magnesium and plasma renin activity in essential hypertension. Studies investigating the effects of magnesium in experimental malignant hypertension have demonstrated that the blood pressure lowering effect of magnesium is dependent on the renin status (Dipette et al, 1988). Malignant hypertension is generally characterised by hyperactivity of the renin-angiotensin system (Giese, 1976). Plasma renin activity was measured in six of the malignant hypertensive patients. Activity was found to be significantly raised. These

findings of hypomagnesaemia and hyperreninaemia in malignant hypertensive patients support those of Resnicks essential hypertensive subjects (1983).

12.5.2 Cell membrane ATPase activity

Several circulating humoral factors have been implicated in the aetiology of malignant hypertension. Some of these increase vascular wall permeability, while others inhibit transmembrane cellular cation transport (Mohring, 1977; Beilin and Goldby, 1977; Simon and Altman, 1986). Although an ouabain-sensitive Na^+-K^+-ATPase inhibitor has been identified in essential hypertension, this factor has not been demonstrated in human malignant hypertension (de Wardener et al, 1987). In rat studies, a digoxin like factor with depressed cell membrane Na^+-K^+-ATPase activity has been reported (Kunes et al, 1985).

In this study, erythrocyte and platelet membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were significantly depressed in the malignant hypertensive patients. The depression of enzyme activity was significantly greater than that in the patients with essential hypertension. Decreased Na^+-K^+-ATPase activity may explain the hyponatraemia. Since Ca^{2+}-ATPase is the major calcium extrusion mechanism, depressed Ca^{2+}-ATPase activity may account for the intracellular calcium overload observed in the malignant hypertensive patients (Bolton,

1986). Mg^{2+}-ATPase depression may be a general marker of decreased activity of all magnesium dependent ATPase systems.

Magnesium is necessary for the normal functioning of the Na^{+}-K^{+}-ATPase and Ca^{2+}-ATPase cellular membrane pumps (Schwartz et al, 1975; Turlapaty and Altura, 1978; Aikawa, 1981). Alterations in extra- and intra-cellular magnesium levels influence cell membrane ATPase activity (Bara et al, 1988; Witteman and Grobbee, 1990). In this study, serum magnesium correlated significantly with platelet Ca^{2+}-ATPase and Mg^{2+}-ATPase activities. These results suggest that the altered cellular magnesium homeostasis may be related to defective cell membrane function in malignant hypertension. Other speculative factors that may play a role in the severely depressed ATPase activity in malignant hypertension include, circulating humoral ATPase inhibitors, structural deformities of the cell wall and unknown variables.

In order to confirm the relationship between cell membrane ATPase activity and magnesium, a group of hypertensive patients with hypermagnesaemia was studied (renal failure patients). In these patients, cell membrane ATPase activity was similar to that in the normotensive group and significantly higher than that in the malignant essential hypertensive groups. These findings support the hypothesis relating altered magnesium status to defective cell

membrane ATPase activity.

Other variables, such as the effects of haemodialysis and renal failure on the structure of cells may also affect cell membrane integrity and structure. This was a preliminary study examining six patients only.

Larger studies investigating the effects of different dialysis techniques and severity of renal failure on cellular function are needed.

The results of this study suggest that cellular cations and cell membrane function are altered in malignant hypertension. These defects may be more severe in the malignant than in the benign phase of hypertension.

It is unclear at what stage these cellular changes occur in the transition from the benign to the malignant forms of hypertension. In order to study this aspect an animal experiment was performed (see chapter 13).

CHAPTER 13

CATIONS AND MUSCLE MEMBRANE ATPase ACTIVITY IN DOCA-SALT SHR

13. CATIONS AND MUSCLE MEMBRANE ATPase ACTIVITY IN DOCA-SALT SHR

13.1 INTRODUCTION

Alterations of cellular ion content and transport have been associated with experimental and human hypertension (Jones, 1973; Swales, 1982). Most experimental studies examining cellular handling of ions have been performed in SHR (Simpson et al, 1984a; Beierwaltes et al, 1982). SHR have normal plasma Na^+ and K^+ levels but increased Na^+ content of their thymocytes, erythrocytes and various cardiovascular tissues (Jones et al, 1981; Furuta, 1977; Simpson et al, 1984b). These findings may be related to defective transmembrane transport mechanisms. There is extensive documentation in the literature regarding altered ion permeability in tissues of the SHR (Postnov and Orlov, 1984; Van der Ven and Bohr, 1983; Postnov et al, 1976). Sodium and K^+ permeability in SHR erythrocytes, leukocytes visceral and vascular smooth muscle is increased (Postnov and Orlov, 1984; Van der Ven and Bohr, 1983). Abnormalities in Na^+ and K^+ ion fluxes across erythrocyte membranes of patients with essential hypertension as well as in several strains of hypertensive rats have been reported (De Mendonca et al, 1980). Studies in human and SHR erythrocytes have shown defective membrane ion binding capacity as well as altered Na^+ pump activity (Postnov et al, 1977; Aoki et al, 1976). Many studies have documented

decreased Na^+ pump activity compared to WKY of similar age (De Mendonca et al, 1984; Chen and Lin-Shiau, 1986). Others have failed to show this difference (Jones AW, 1973; Friedman, 1979).

Recently attention has focused on cellular Ca^{2+} metabolism and hypertension (Bing et al, 1987). SHR have higher urinary Ca^{2+} output, higher parathyroid hormone levels, lower serum ionised Ca^{2+} and increased intracellular (erythrocyte, platelet) levels of Ca^{2+} than WKY (McCarron et al, 1981; Oshima et al, 1990). Defective cell membrane function results in cytosolic Ca^{2+} overload. This may be related to deficient Ca^{2+}-ATPase activi[illegible]ostnov et al, 1984). Decreased membrane Ca^{2+}-ATPase [illegible]ivity and altered cell membrane Ca^{2+} binding have been reported in various tissues in SHR (Vezzoli et al, 1985). However, in SHR of Okamoto-Aoki strain erythrocyte permeability to Ca^{2+} was increased with increased Ca^{2+}-ATPase activity (Chan et al, 1983). Cellular Ca^{2+} metabolism is very closely linked to Mg^{2+} (Iseri and French, 1984). A number of Mg^{2+} defects have been described in SHR, including hypomagnesaemia, decreased renal tubular reabsorption of Mg^{2+} and decreased intracellular (erythrocyte, leukocyte, muscle) Mg^{2+} concentrations (Hsu et al, 1986; Berthelot et al, 1987).

Whether these cation and transport changes seen in SHR play a role in more severe forms of hypertension is unknown. Some studies have been performed on DOCA-malignant

hypertension and have shown increased cell membrane permeability with significant increases in cellular total K^+, Ca^{2+}, Mg^{2+}, Na^+ and Cl^- (Jones and Hart, 1975). In DOCA-hypertension vascular smooth muscle leakiness is increased and sodium pump activity is suppressed (Jones, 1974; Songu-Mize et al, 1982). In DOCA-salt uninephrectomised rats, no erythrocyte membrane abnormalities were observed (Chan et al, 1983). However, these rats did not develop malignant hypertension. A circulating furosemide-like inhibitor of ouabain insensitive cation transport has been identified in malignant experimental renal hypertension (Simon and Altman, 1986).

A number of different experimental forms of malignant hypertension have been described (Sesko et al, 1984; Gavras et al, 1975). DOCA-salt imposition in SHR is an effective, reliable and simple method of inducing experimental malignant hypertension (Sesko et al, 1984). This form resembles the clinical syndrome in humans (Kincaid-Smith, 1980). It is therefore a preferable model for study when examining mechanisms involved in the pathophysiology of malignant hypertension. Little is known of the cellular changes that occur in the transition from benign to malignant hypertension. In this study the role of cellular ions and their transport in the development of malignant hypertension was studied. DOCA-salt SHR were used as a model for malignant hypertension (experimental group) and

untreated SHR as a model for benign hypertension (control group). SHR were used in both groups so that the control and experimental rats had the same predisposition to developing spontaneous hypertension. The combination of DOCA plus salt (saline) is necessary to induce malignant hypertension. The control SHR were not given saline drinking water as they served as the genetic baseline for hypertension.

The aims of this study were 1) to assess at what stage malignant hypertension develops in SHR treated with DOCA-salt and to determine the transition phase from benign to malignant hypertension; 2) to examine the cellular cations and transport functions that occur as hypertension progresses from the benign to the malignant stages. Cell (muscle) membrane Mg^{2+}-ATPase, Ca^{2+}-ATPase and Na^{+}-K^{+}-ATPase activity as well as extra- and intracellular (erythrocyte and muscle) Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} levels were studied in SHR and DOCA-salt SHR. In order to assess the causal role of the various changes that occur they were studied from the beginning of the rise in blood pressure. Thus, this was a time sequence study in 10 week old rats, where the variables were measured weekly in both groups for fourteen weeks.

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13.2 MATERIALS AND METHODS

13.2.1 Animal preparation

Male, 10-week old SHR fed on standard rat chow were randomly divided into two groups: (1) Control group (n=50) were given unlimited tap drinking water, (2) DOCA-salt group (n=70) were given unlimited 1% NaCl drinking water. At weekly intervals the DOCA-salt treated group received 30 mg/Kg DOCA (dissolved in benzyl alcohol and arachis oil) subcutaneously (Wade, 1979). Control rats received identical volumes of sterile saline subcutaneously. At 10 weeks of age, 8 control SHR and at weekly intervals for 14 weeks after the start of the weekly injections, 5 DOCA-salt and 3 control SHR were studied. Under general anaesthetic (intraperitoneal sodium pentobarbitone (60 mg/Kg)), the left common carotid artery was exposed, cannulated and connected to a Statham P23DP pressure transducer (Viggo Spectramed, California). Saline and sodium heparin (1000 U/20 ml) were infused at 1.68 ml/hr. After a 30 minute equilibration period, systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mean of 3 consecutive replications) were measured directly by a Statham BP monitor connected to a Statham 4 channel monitor scope and chart recorder SP 200. Mean arterial pressure (MAP) was calculated from the formula: MAP = DBP + 1/3 (SBP-DBP). 3-5 ml of heparinised carotid blood was collected and centrifuged immediately. The rats were killed with

intraperitoneal pentobarbitone (150 mg/Kg). The vastus lateralis muscles were dissected and frozen in liquid nitrogen. Specimens of each kidney were fixed in mercuric chloride and formaldehyde.

13.2.2 Biochemical Analysis

Serum, red blood cell (RBC) and muscle (M) Na^+, K^+, Mg^{2+} and Ca^{2+}, serum urea, creatinine and uric acid and muscle Na^+ - K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+} ATPase were measured in the rats. Serum urea, creatinine and uric acid were determined by an automated enzymatic method. Na^+ and K^+ were measured by standard flame photometry and Mg^{2+} and Ca^{2+} by atomic absorption spectroscopy (Appendix G). Haemoglobin was measured by Coulter Counter (Coulter electronics). RBC measurements were based on the method of Fortes-Mayer and Starkey (Fortes-Mayer and Starkey, 1977). The separated erythrocytes were divided into 2 aliquots and were washed three times with iso-osmolar NaCl (0.9%) for Mg^{2+} and Ca^{2+} measurements and with iso-osmolar $MgCl_2$ (112 mM) for Na^+ and K^+ measurement. The washed cells were lysed with distilled water. All values were corrected for volume concentration of erythrocytes by comparing the sample haemoglobin with that of the original whole blood corrected to a packed cell volume of 100%.

13.2.2.1 Muscle preparation for cation measurements

Cation muscle content was measured according to the method of Dorup et al (Dorup et al, 1988). Samples of the thawed muscles were weighed (10-20 mg) and homogenized in 2 ml of 5% trichloroacetic acid using a tight fitting Potter homogeniser (Braun, Melsingen, West Germany). The homogenate was centrifuged at 900 x g for 15 minutes. The clear supernatant was further diluted with 2 volumes of redistilled water. This dilution was used for determination of Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} concentrations. Muscle content of the cations is expressed as mol/g wet weight.

13.2.2.2 Isolation of muscle membranes for ATPase studies

Isolation of muscle membranes was based on the method described by Samahara and Gergely (Samahara and Gergely, 1966). Muscle samples (20-40 mg) were homogenised for 2-5 minutes using a tight fitting Potter homogeniser with 5 volumes of a solution containing 0.25 M sucrose, 30 mM histidine, 5 mM EDTA and 0.2% deoxycholate (ph = 7.0). The homogenate was centrifuged for 20 minutes at 600 x g. The sediment was discarded and fractions were obtained by successive centrifugation at 10,000 x g for 20 minutes and then at 30,000 x g for 30 minutes. The sediments were rehomogenised in 1 ml of 0.2 M sucrose, 30 mM histidine and 1 mM EDTA pH, 7.0. The protein concentrations of muscle

membranes were determined colorimetrically according to a modified method of Lowry (Hartree, 1972) using bovine serum albumin as the standard.

13.2.2.2 (i) Assay for Mg^{2+} ATPase and Ca^{2+} ATPase in muscle membranes

The basal Mg^{2+} ATPase and Ca^{2+} ATPase activities were based on the methods described by Niggli et al (Niggli et al, 1979). The spectrophotometric coupled enzyme system was replaced by colorimetric quantitation. Membrane preparations were assayed in duplicate for ATPase activity in a buffer containing 120 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 20 mM N-tris (hydroxymethy-2-aminoethane-sulfonic acid-NaOH, pH 7.5 at 37°C) and either 0.1 mM $CaCl_2$ or 1 mM EDTA. According to the formula $[Ca^{2+}_{free}]^2 + [Ca^{2+}_{free}]([EDTA_{total}] - [Ca^{2+}_{total}] + K_D) - K_D[Ca^{2+}_{total}] = 0$, the free Ca^{2+} concentration in the muscle preparation was less than 1 nM. The reaction was initiated by adding 50 /ul membrane suspension (80-120 /ug protein) to 450 /ul incubation buffer that had been prewarmed for 5 minutes at 37°C (final incubation volume, 500 /ul). The incubation was continued at 37°C for 15 minutes. Reactions were terminated by adding 0.5 ml of 10% trichloroacetic acid (TCA). Inorganic phosphate released from ATP was measured by the colorimetric method described in Appendix L.

The Mg^{2+} ATPase activity was estimated from assays

performed in the absence of $CaCl_2$. The Ca^{2+} ATPase activity was determined by subtracting activity measured in the absence of $CaCl_2$ from the activity measured in the presence of $CaCl_2$.

13.2.2.2 (ii) Assay for Na^+ - K^+ ATPase activity in muscle membranes

Na^+ - K^+ ATPase activity was based on the method described by Ringel et al (Ringel et al, 1987). Duplicate assays were performed. Methods were similar to the measurement for Mg^{2+} and Ca^{2+} ATPase activity, except that the buffer contained 50 mM N-tris (pH 7.2 at 37°C) 100 mM NaCl, 20 mM KCl, 3 mM ATP, 5 mM $MgCl_2$. The ouabain-insensitive ATPase activity was determined in the presence of 2 mM ouabain. The Na^+ - K^+ ATPase activity was taken as the difference between the enzymatic activity measured in the assay medium without ouabain and that in the assay medium containing ouabain.

13.3 STATISTICAL ANALYSIS

The data were analysed with the Mac SS computer program (Statsoft, Tulsa, Oklahoma). Values are given as group means $\pm$SD and n equal number of rats. Where two groups were compared, and the sample sizes were large enough, the Students t-test was applied. If the sample sizes were small and the central limit theorem was not applicable, the

TABLE 13.1. CLINICAL CHARACTERISTICS OF THE SHR AND DOCA GROUPS

Week of Rx		1-6	7-8	9-10	11-12	13-14
Age (weeks)	10	11-16	17-18	19-20	21-22	23-24
n						
SHR	8	18	6	6	5(1f)	6
DOCA (70)		21	8	9(3d)	9(8d)$^{+}$	9(3d)$^{+}$
SBP (mmHg)						
SHR	180±24	189±24	225±24	248±19	247±23	211±20
DOCA		210±40	232±35	252±21	253±14	265±16**
DBP (mmHg)						
SHR	132±30	151±28	162±22	159±13	169±10	146±25
DOCA		141±30	158±24	173±16*	175±10	191±5**
MAP (mmHg)						
SHR	148±27	164±18	183±22	189±13	195±13	180±15
DOCA		164±20	183±27	199±19*	202±10	214±9**
HR (beats/min)						
SHR	355±28	364±34	355±31	360±45	355±31	343±37
DOCA		341±32	316±56	363±36	385±22	328±36
WEIGHT (g)						
SHR	260±24	290±30	318±22	324±24	374±42	399±43
DOCA		280±24	302±25	325±49	289±27*	325±45*

SBP = systolic blood pressure; DBP = diastolic blood pressure
MAP = mean arterial pressure; HR = heart rate. Mann Whitney U test or students t-test compares groups.

* $p<0.05$ ** $p<0.001$ $(d)^{+}$ = number of rats that died

Mann-Whitney U-test was applied. Analysis of variance (ANOVA) for repeated measures was applied in order to study 1) differences between groups; 2) changes during the experimental period (time); 3) interaction between group and experimental period (time). When the parameters were grouped into groups of ions, data were analysed by MANOVA.

The level of significance was taken as a p value less than 0.05.

13.4 RESULTS

13.4.1 Clinico-pathological parameters of SHR and DOCA rats (Table 13.1)

Of the 50 SHR and 70 DOCA-SHR, 1 SHR and 14 DOCA rats died during the course of the study. Features of cerebral oedema and congestive cardiac failure were found at post mortem examination. The final number of rats studied was 49 SHR and 56 DOCA.

13.4.1 (i) Blood Pressure (BP)

SBP and DBP increased progressively in both groups. There were no significant differences in SBP between the groups until 13 weeks of treatment, when SBP became significantly higher in the DOCA group. From week 10 of treatment, DBP was significantly higher in the DOCA group compared to the

TABLE 13.2. ANALYSIS OF VARIANCE FOR REPEATED MEASURES; ONLY SIGNIFICANT RESULTS ARE PRESENTED; NS = NOT SIGNIFICANT

	Comparison between groups		Change during experimental period (time)		Interaction between group and time	
	f	p	f	p	f	p
MAP	3,19	0,05	19,70	0,0001	3,00	0,05
SNa^+	5,65	0,017	19,62	0,0001	NS	NS
RNa^+	NS	NS	5,91	0,008	NS	NS
SK^+	16,67	0,0001	3,98	0,02	NS	NS
RK^+	NS	NS	3,98	0,02	NS	NS
SMg^{2+}	8,24	0,005	NS	NS	NS	NS
RMg^{2+}	6,5	0,012	NS	NS	NS	NS
MMg^{2+}	NS	NS	NS	NS	4,81	0,011
SCa^{2+}	NS	NS	3,3	0,04	NS	NS
RCa^{2+}	NS	NS	37,8	0,0001	NS	NS
MCa^{2+}	NS	NS	3,4	0,05	NS	NS
Na^+-K^+A	17,5	0,0001	NS	NS	NS	NS
Mg^{2+}-A	15,4	0,0002	NS	NS	NS	NS
Ca^{2+}-A	6,17	0,015	NS	NS	NS	NS

S = serum; R = red blood cell; M = muscle; A = ATPase

SHR group. SHR BP stabilized from 18-20 weeks of age.

13.4.1 (ii) Weight

With increasing age, both groups progressively gained weight, but from 11 treatment weeks DOCA rats failed to thrive and were significantly lighter than SHR (Table 13.1).

13.4.2 Histologic Findings

From 0-12 weeks of treatment the histological features in the two groups were similar. From 13 to 14 weeks of treatment, fibrinoid necrosis was present in glomeruli and small arteries of all rats from the DOCA group.

Using the clinical and pathological features, three developmental phases of malignant hypertension were defined: (1) benign phase (rats 10-16 weeks old; BP similar in both group), (2) premalignant phase (rats 17-22 weeks old; DOCA BP significantly increased; failure to thrive; deaths); (3) malignant phase (rats 23-24 weeks old; DOCA BP severe; deaths; fibrinoid necrosis).

Significant results obtained by analysis of variance are shown in Table 13.2. Blood pressure differed between the groups and with time and there was significant interaction between the groups and time. Serum Na^+ and K^+ were

TABLE 13.3. MULTIPLE ANALYSIS OF VARIANCE FOR REPEATED MEASURES; ONLY SIGNIFICANT RESULTS ARE PRESENTED; NS = NOT SIGNIFICANT

	Comparison between groups		Change during experimental period (time)		Interaction between group and time	
	f	p	f	p	f	p
Na^+	3,27	0,04	11,2	0,0001	NS	NS
K^+	5,67	0,002	NS	NS	NS	NS
Mg^{2+}	6,79	0,004	NS	NS	2,69	0,016
Ca^{2+}	NS	NS	12,2	0,0001	NS	NS
ATPase	10,41	0,0001	NS	NS	3,48	0,002

significantly different in the groups and changed with time. Red blood cell Na^+ and Ca^{2+} and serum and muscle Ca^{2+} differed with time. Serum and red blood cell Mg^{2+} and all the ATPases were dependent only on grouping. Significant results obtained by MANOVA are presented in Table 13.3.

13.4.3 Biochemical Parameters

13.4.3.1 Sodium (Na^+)

In SHR and DOCA rats, serum Na^+ progressively decreased with time. There were no significant differences in serum Na^+ between the groups (Fig 13.1a). RBC Na^+ was similar in both groups in the benign and premalignant phases. Peak concentrations occurred in the premalignant period. In the malignant phase, RBC Na^+ was significantly higher in the DOCA group compared to the SHR group (Fig 13.1b). Muscle Na^+ was not detected in either group until the malignant phase when low Na^+ concentrations were measured in the DOCA rats.

13.4.3.2 Potassium (K^+)

Serum K^+ was consistently and significantly lower in the DOCA group compared to the SHR group (Fig 13.2a). RBC K^+ increased with time. There were no significant differences between groups (Fig 13.2b), except at treatment week 6. In

the benign phase, DOCA rats had significantly lower muscle K^+ concentrations compared to SHR (Fig 13.2c).

13.4.3.3 Magnesium (Mg^{2+})

Throughout the development of malignant hypertension the DOCA group had significantly lower serum Mg^{2+} concentrations compared to the SHR group (Fig 13.3a). In the premalignant phase, the DOCA group had significantly lower RBC Mg^{2+} compared to the SHR group (Fig 13.3b). There is an inverse pattern of both serum and RBC Mg^{2+} concentrations over the three developmental phases in the two groups.

13.4.3.4 Calcium (Ca^{2+})

In both groups serum Ca^{2+} progressively decreased until 10 weeks of treatment when the concentrations increased. At 8 treatment weeks serum Ca^{2+} was significantly lower in the DOCA group than the SHR group (Fig 13.4a). In the DOCA and SHR groups, RBC Ca^{2+} had a U-shaped distribution where it decreased from the benign to the premalignant phase and then progressively increased. In the malignant phase, RBC Ca^{2+} was significantly higher in the DOCA rats compared to the SHR (Fig 13.4b). Muscle Ca^{2+} also had a U-shaped trend over time for both groups.

In the late premalignant and malignant phases, DOCA muscle

Ca^{2+} levels were significantly higher than SHR muscle Ca^{2+} concentrations (Fig 13.4c).

There were no significant differences of haemoglobin, serum urea, creatinine and uric acid between the two groups in the three developmental phases (Table 13.4). Due to technical problems (clotting of the washed RBC pellet); RBC cations could not be measured until week 6 of treatment.

13.4.4 Enzyme Studies

In the premalignant and malignant phases Na^{+} - K^{+} ATPase activity was significantly lower in the DOCA group compared to the SHR group (Fig 13.5a). In the benign phase there were no significant differences in Mg^{2+} - ATPase activity between the two groups, but in the premalignant and malignant phases enzyme activity was significantly reduced in DOCA rats compared to SHR (Fig 13.5b). DOCA Ca^{2+} - ATPase activity in the premalignant phase was significantly lower in the DOCA rats compared to SHR (Fig 13.5c).

There was no significant differences in the basal values for the different variables between the SHR and DOCA groups.

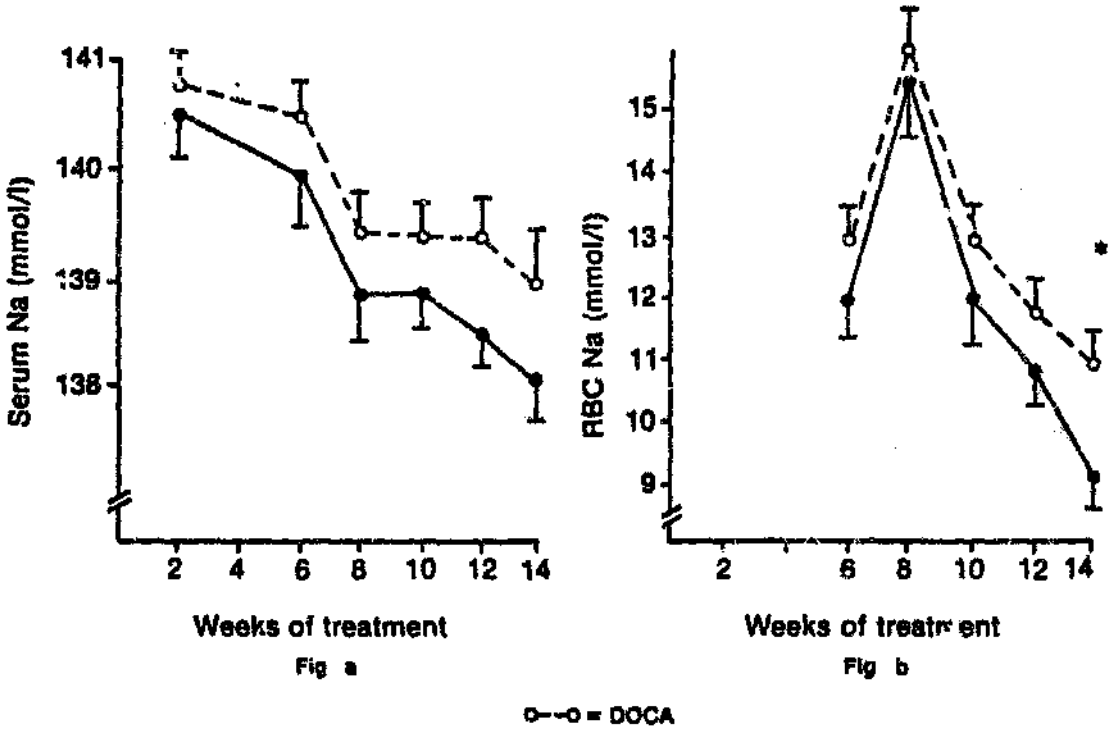


Figure 13.1. Serum and red blood cell (RBC) Na^+ in the DOCA and SHR groups. Weeks of treatment refers to DOCA administration. Mann-Whitney U test compares groups. *$p < 0,05$

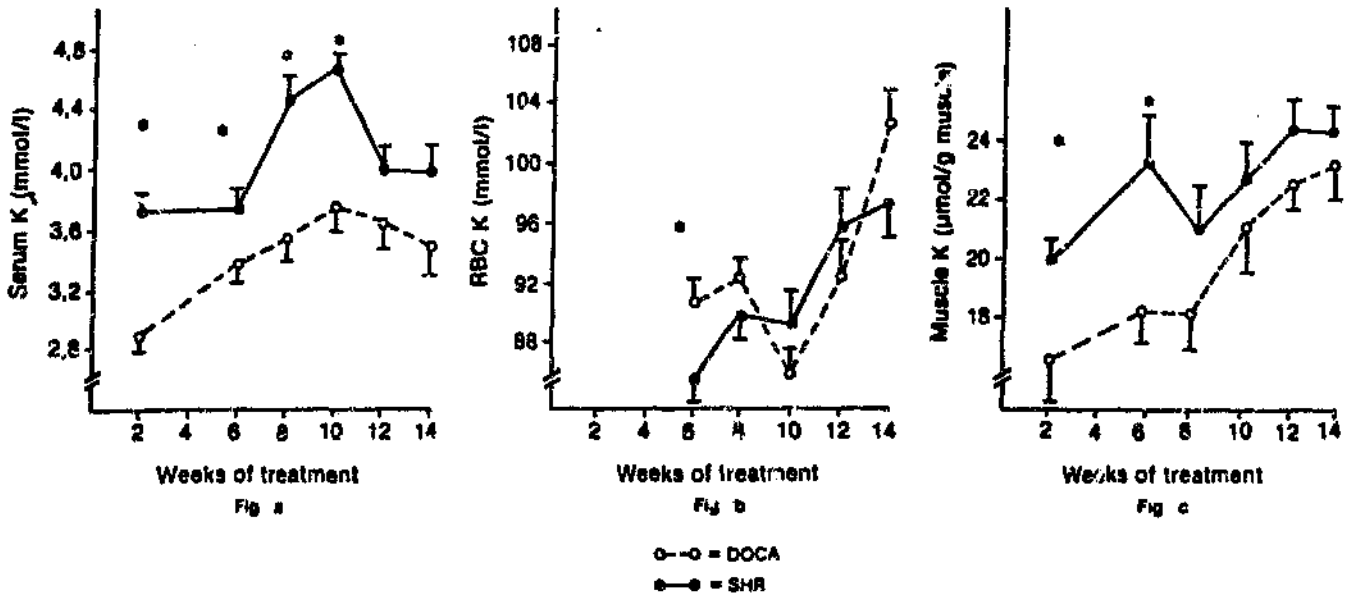


Figure 13.2. Serum and red blood cell (RBC) and muscle K^+ in the DOCA and SHR groups. Weeks of treatment refers to DOCA administration. Mann-Whitney U test compares groups. * $p < 0,05$

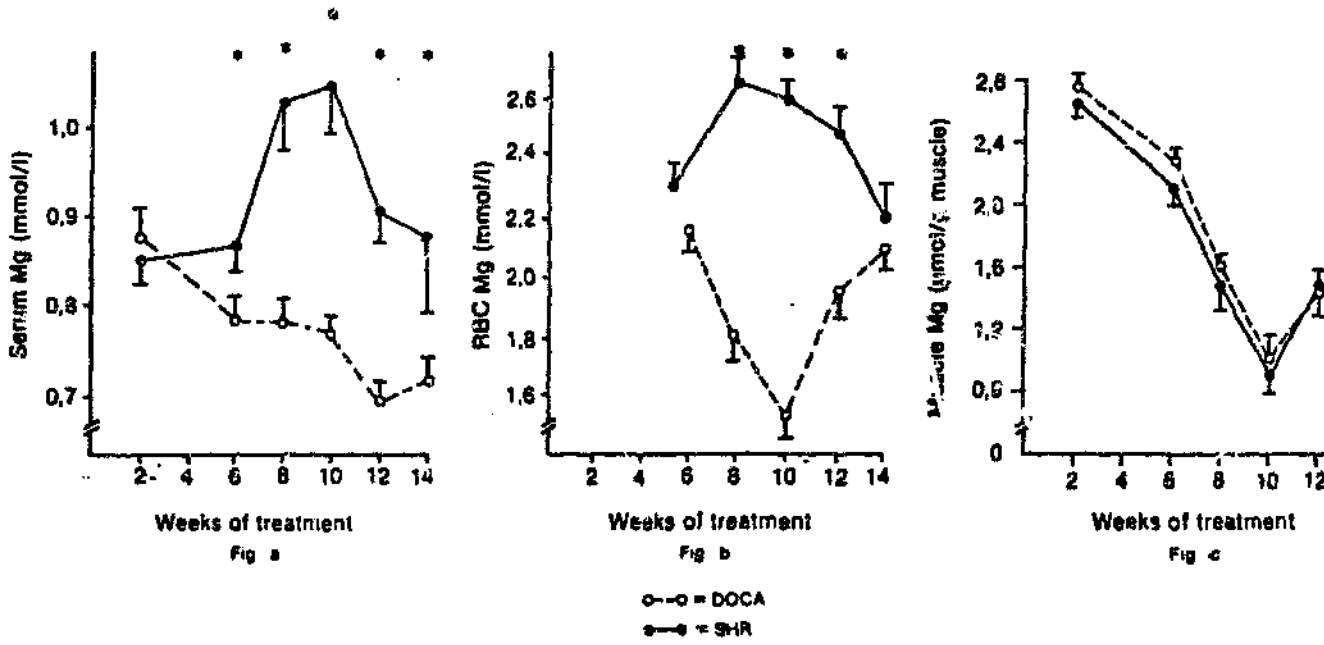


Figure 13.3. Serum, red blood cell (RBC), and muscle Mg^{++} in the DOCA and SHR groups. Weeks of treatment refers to DOCA administration. Mann-Whitney U test compares groups.

* $p < 0,05$

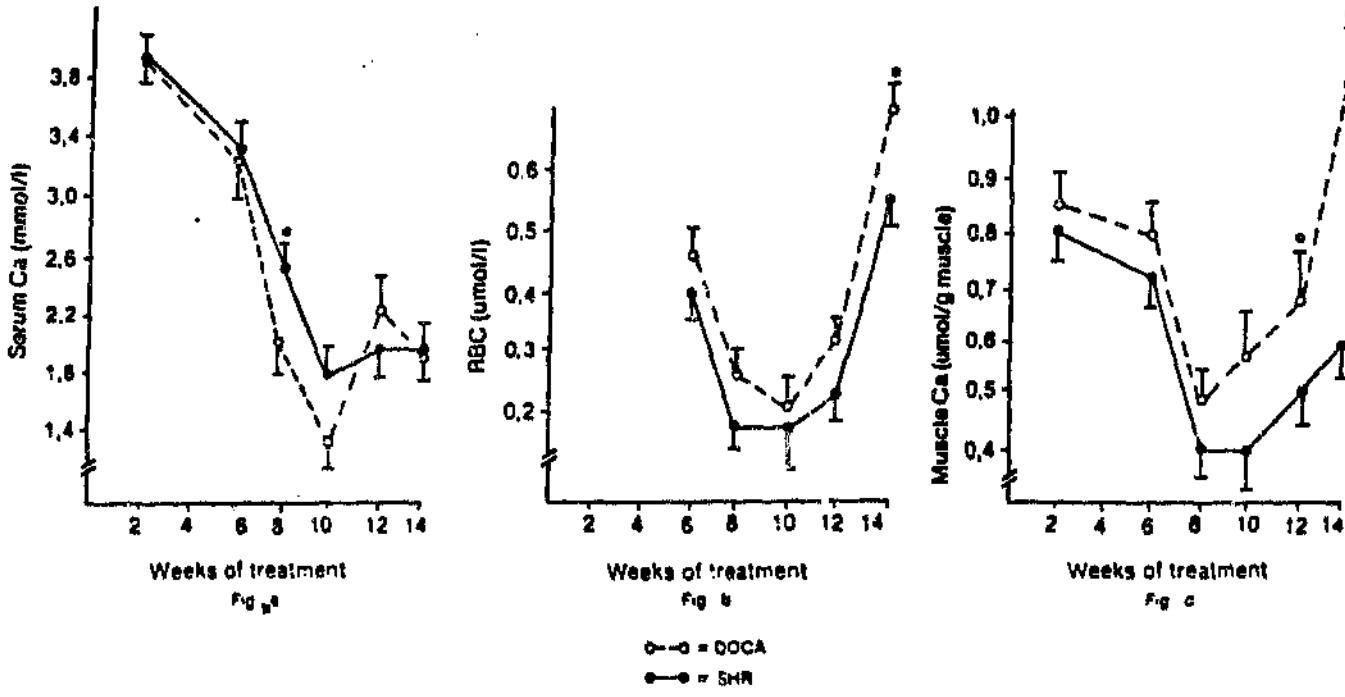


Figure 13.4. Serum, red blood cell (RBC) and muscle Ca^{++} in the DOCA and SHR groups. Weeks of treatment refers to DOCA administration. Mann-Whitney U test compares groups.

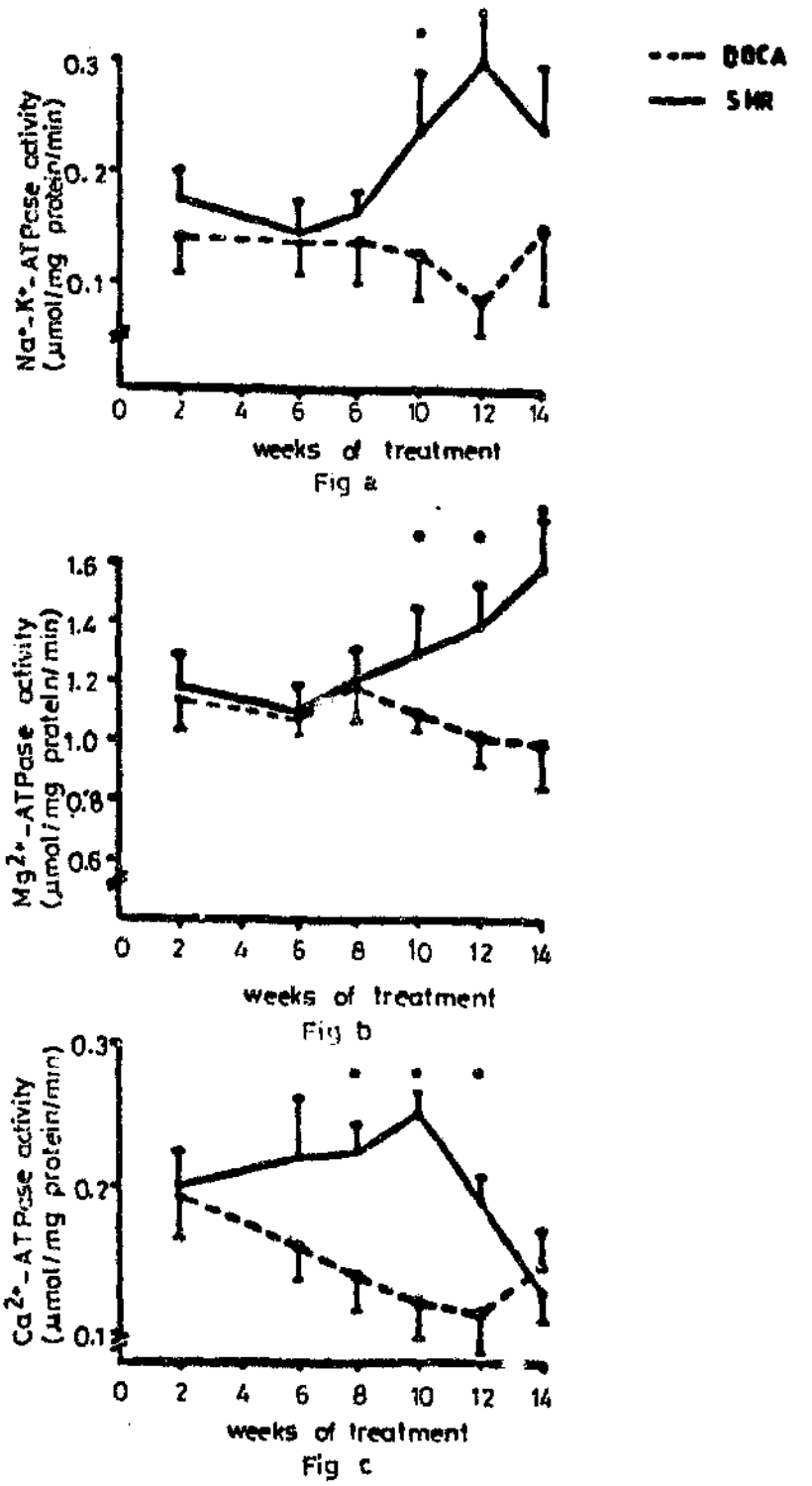
• $p < 0,05$

TABLE 13.4. SERUM UREA, CREATININE AND URIC ACID AND HAEMOGLOBIN LEVELS IN THE TWO GROUPS

Phase	Benign	Premalignant	Malignant
Treatment weeks	1-6	7-12	13-14
Serum urea (mmol/l)			
SHR	6,1+1,2	6,0+1,7	8,2+3,1
DOCA-Salt	7,2±2,5	7,3±2,6	6,3±0,8
Serum creatinine (µmol/l)			
SHR	37+7	40+4	49+5
DOCA-Salt	42±12	45±6	55±12
Serum uric acid (mmol/l)			
SHR	0,06+0,03	0,04+0,02	0,05+0,02
DOCA-Salt	0,07±0,04	0,04±0,01	0,07±0,03
Haemoglobin* (g/100 ml)			
SHR	-	-	16,7+2,8
DOCA-Salt	-	-	16,4±2,0

* Haemoglobin was only measured during the malignant phase

Figure 13.5. Muscle Na^+- K^+ ATPase, Mg^{++}- ATPase and Ca^{++}- ATPase activities in the DOCA and SHR groups. Weeks of treatment refers to DOCA administration. Mann- Whitney U test compares groups. * $p < 0,05$.



13.4.5 Correlation Studies

Correlation studies between variables were performed in the benign (treatment weeks 1-6 combined), premalignant (treatment weeks 7-12 combined) and malignant (treatment weeks 13-14 combined) phases for the two groups. During the benign phase in the SHR there were significant inverse relationships between serum Na^+ ($r = -0.59$), red blood cell Na^+ ($r = -0.70$), serum Ca^{2+} ($r = -0.65$), muscle Mg^{2+} ($r = -0.46$) and MAP. In the DOCA group, red blood cell K^+ ($r = 0.67$) was positively and serum Ca^{2+} ($r = -0.63$) and red blood cell Mg^{2+} ($r = -0.49$) negatively correlated with MAP. In the premalignant phase for the SHR, MAP was inversely correlated with serum Na^+ ($r = -0.42$) and serum Mg^{2+} ($r = -0.55$) and positively correlated with muscle Ca^{2+} ($r = 0.35$) and red blood cell Ca^{2+} ($r = 0.40$). In the DOCA rats, red blood cell Na^+ ($r = -0.39$) and serum Mg^{2+} ($r = 0.40$) were negatively and serum K^+ ($r = 0.34$), muscle K^+ ($r = 0.34$) and muscle Ca^{2+} ($r = 0.34$) were positively correlated with MAP. During the malignant phase in the SHR MAP was inversely correlated to serum Mg^{2+} ($r = -0.60$), serum Na^+ ($r = -0.56$) and serum K^+ ($r = -0.55$). In the DOCA group, serum Na^+ ($r = -0.86$), muscle K^+ ($r = -0.54$), red blood cell Mg^{2+} ($r = -0.40$) and Ca^{2+} - ATPAse ($r = -0.76$) were inversely correlated to MAP.

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13.5 DISCUSSION

The experimental model of malignant hypertension in this study resembles the clinical syndrome in humans (Houston, 1989). Malignant hypertension in the DOCA treated rats developed suddenly and was characterised by severe hypertension, failure to thrive and death. Histological examination of the kidneys revealed fibrinoid necrosis confirming the clinical diagnosis. Malignant hypertension developed after 13 weeks of treatment. In rats given DOCA by implant and in DOCA treated uninephrectomised rats malignant hypertension develops earlier (Gavras et al, 1975; Kincaid-Smith, 1980). In malignant 2 kidney Goldblatt hypertension there is haemoconcentration, increased osmolality and increased plasma vasopressin (Mohring et al, 1978). The exact mechanisms involved in the development of malignant hypertension in experimental SHR remains obscure. Factors which have been implicated include altered haemodynamic responses, sympathetic overactivity and enhanced vascular reactivity (Page, 1987a). Many studies have reported that in order to elicit malignant hypertension in DOCA-SHR, salt feeding is an essential adjunct to DOCA (Page, 1987b). Reasons for the salt dependence are unclear but may be related to the renin-angiotension system (Goodwin et al, 1969). In DOCA-salt SHR malignant hypertension, there is anaemia, renal dysfunction and hyperuricaemia (Gavras et al, 1975). In this study, the DOCA-salt SHR had normal haemoglobin, serum

urea, creatinine and uric acid levels, despite fibrinoid necrosis occurring after 13 weeks of treatment. This suggests that renal function was still intact for the duration of the study and that the pathological features precede the biochemical characteristics of renal failure. The vehicle for DOCA administration was benzyl alcohol and arachis oil. The effects of these agents on blood pressure are unknown, but are currently being tested.

Little is known about cations and cell membrane function in DOCA-salt SHR malignant hypertension. Unlike other experiments where variables have been assessed at the beginning and end point of a study, this was a time sequence study where the variables were examined weekly from the benign through to the malignant phase of hypertension. Results from this study indicate that alterations in cellular cations and ion transport may play a role in DOCA-salt SHR malignant hypertension.

13.5.1 Potassium

Throughout the experiment the DOCA-salt SHR had persistent hypokalaemia probably due to K^+ renal wasting related to DOCA. Red blood cell K^+ was significantly higher in the DOCA-salt group only in the early phases of treatment. These results confirm others where serum K^+ was decreased and intracellular K^+ increased in steroid induced hypertensive rats (Jones and Hart, 1975; Whitworth et al,

1990). Muscle K^+ however showed opposite trends where K^+ was lower in the DOCA-salt group during the benign phase. These conflicting results may be due to the fact that DOCA influences muscle K^+ metabolism before it affects red blood cell K^+. Throughout the experiment intracellular K^+ progressively increased in both groups. During the benign phase K^+ was the only cation that was significantly different between the two groups, suggesting that of the ions, K^+ metabolism is the first to be affected by DOCA.

13.5.2 Sodium

Much research has focussed on Na^+ in human and experimental hypertension (Kaplan, 1990; Soltis and Bohr, 1989; Simon, 1989). In this study serum and red blood cell Na^+ concentrations were consistently higher in the DOCA-salt rats. The only significant Na^+ difference between the groups occurred in the malignant phase of hypertension where red blood cell Na^+ was elevated in the DOCA-salt SHR. Muscle Na^+ was not detected in either group until the malignant phase when low concentrations were measured in the DOCA-salt group. These findings suggest that intracellular Na^+ increases when BP becomes severe, confirming other studies (Jones and Hart, 1975; Jones et al, 1981). The elevated intracellular Na^+ may be related to decreased electrogenic Na^+ pump activity. In this study Na^+-K^+ ATPase activity was significantly depressed in the premalignant phase suggesting that the pump function

defects precede the cellular overload of Na^+ which only becomes evident in the malignant phase. In DOCA-salt uninephrectomised rats the development of malignant hypertension is closely related to Na^+ accumulation (Whitworth et al, 1990). Increased intracellular Na^+ may result in cellular swelling with injury and may manifest pathologically as cell disruption and fibrinoid necrosis.

13.5.3 Calcium

Altered cellular Ca^{2+} metabolism may be closely linked to the pathogenesis of hypertension. Recent reports have indicated a number of abnormalities in Ca^{2+} metabolism in SHR (Bukoski, 1990; Porsti et al, 1990). However, there is a paucity of data regarding Ca^{2+} in DOCA-malignant hypertension. An early study reported increased total Ca^{2+} in DOCA-salt uninephrectomised rats (Whitworth et al, 1990). The same researchers demonstrated that the increased reactivity of vascular smooth muscle in these rats was associated with changes in its dependence on Ca^{2+} for contraction (Whitworth et al, 1990). Ca^{2+} supplementation alleviates the development of mineralocorticoid hypertension in rats (Yang et al, 1989a). In severely hypertensive rats (stroke-prone rats) Ca^{2+} and phosphorous metabolism were abnormal with persistent hypocalcaemia and hypophosphataemia (Ishitobi, 1986). This study demonstrates that with time serum Ca^{2+} decreases in both groups. There were however no significant differences

in serum Ca^{2+} between the SHR and DOCA-salt rats. With aging red blood cell and muscle Ca^{2+} increased in both groups and were significantly higher in the DOCA-salt SHR during the malignant phase. Since intracellular Ca^{2+} overload is a major determinant of increased vascular tone and contractility, the cytosolic Ca^{2+} accumulation seen in the malignant phase of our study may play a contributory role in the severe elevation of blood pressure. Causes for the increased cellular Ca^{2+} could be associated with depressed Ca^{2+}-ATPase activity and increased intracellular Na^{+}. According to Blaustein, intracellular Ca^{2+} is linked to the Na^{+} gradient across the membrane through a Ca^{2+}-Na^{+} exchange mechanism (Blaustein, 1977). Thus the small increase in DOCA muscle Na^{+}, possibly due to decreased Na^{+}-pump function and cell disruption, may contribute to the elevated intracellular Ca^{2+}.

13.5.4 Magnesium

Although Na^{+} and K^{+} and more recently Ca^{2+} and phosphorous metabolism have been extensively studied in SHR, little is known of Mg^{2+} metabolism in SHR and less is known of Mg^{2+} in DOCA-malignant hypertension. Clinical and epidemiological studies examining Mg^{2+} and hypertension have been conflicting. Normal, increased and decreased serum Mg^{2+} have been reported in human hypertensive patients compared to normotensive controls (Cappuccio et al, 1985; Rinner et al, 1989). Intracellular Mg^{2+}

(leukocytes, erythrocytes and muscle) concentrations have been found to be decreased in hypertensive patients (Touyz et al, 1989; Dorup et al, 1988). In vitro experiments studying blood vessels of different species have demonstrated that increased extracellular Mg^{2+} promotes vasodilation while decreased Mg^{2+} results in vasoconstriction (Altura et al, 1981). A few in vivo studies have examined the role of Mg^{2+} in blood pressure regulation (Berthelot and Esposito, 1983; Altura et al, 1984). Normotensive rats on a Mg^{2+} deficient diet exhibit increased blood pressure compared to rats on a standard diet (Altura et al, 1984). SHR have decreased renal tubular reabsorption of Mg^{2+} with lower serum Mg^{2+} concentrations compared to age matched WKY (Hsu et al, 1986; Berthelot et al, 1987). In DOCA salt rats oral administration of Mg^{2+} lowers BP significantly but not in normotensive rats (Hattori et al, 1991). A recent study examining haemodynamic responses to Mg^{2+} in DOCA-salt low renin and two kidney one clip renovascular hypertension showed that Mg^{2+} administration lowered BP significantly in DOCA-salt rats but not in renovascular hypertensive rats (Dipette et al, 1988). These results suggest that the blood pressure lowering effect of Mg^{2+} is related to the renin-angiotensin system.

The most significant and consistent findings in this study related to Mg^{2+}. Throughout the experiment serum and red blood cell Mg^{2+} were lower in the DOCA-salt SHR compared to

the control rats. During the premalignant phase serum and red blood cell Mg^{2+} levels were significantly decreased in the DOCA-salt group. Although muscle Mg^{2+} decreased steadily in both groups, there were no significant differences in muscle Mg^{2+} between the two groups. This may be attributed to the fact that there is tight intracellular control and binding of Mg^{2+} in myocytes (Somlyo and Somlyo, 1981).

In this study, of all the cations studied, Mg^{2+} correlated most consistently with blood pressure. After 16 weeks of age, there was a significant inverse correlation between BP and serum Mg^{2+} in the DOCA-salt SHR. In the malignant phase, blood pressure was inversely correlated to intracellular Mg^{2+}. These findings support other experimental studies which suggest that Mg^{2+} plays a role in the pathogenesis of hypertension. A recent study however negate this relationship (Kjeldsen et al, 1990). It should be stressed that correlation studies only examine relationships between variables and therefore these are not necessarily causally associated.

13.5.5 Ion transport defects

The cation changes which have been reported in this study may be related to defects in transmembrane ion transport.

13.5.5.1 Na^+-K^+ ATPase

The best defined Na^+ transport system is the ouabain sensitive Na^+ pump (Na^+-K^+ ATPase) which mobilises Na^+ out of and K^+ into the cell (Hilton, 1986). In this study Na^+-K^+ ATPase was consistently lower in the DOCA-salt SHR and significantly depressed in the premalignant phase. The pump defects precede the intracellular Na^+ changes suggesting that there is a delay in cause and effect of Na^+ and K^+ active transport in the DOCA-salt SHR. Our results confirm others which have shown that in tail arteries of DOCA-salt hypertensive rats ouabain sensitive $^{86}Rb^+$ uptake was suppressed (Songu-Mize et al, 1984; Pamnani et al, 1978). The time related changes of the Na^+ pump in the DOCA-salt group in our study are similar to those previously reported where Na^+-K^+ ATPase activity was significantly lower during the prehypertensive (premalignant) period in DOCA rats compared to controls (Songu-Mize et al, 1984). During the malignant phase, Na^+ pump activity was positively correlated to muscle Mg^{2+}. In DOCA treated hypertensive pigs and rats electrogenic Na^+ pump activity was enhanced (Webb, 1982; Jones, 1981). In these studies the Na^+ pump activity was assessed indirectly by measuring the K^+-induced relaxation of isolated vascular segments (Webb, 1982). These conflicting results may be due to technical differences where ATPase activity is measured directly in some studies and indirectly in others. Exact reasons for the discrepancies need to be resolved.

13.5.5.2 Ca^{2+} - ATPase

The major active cellular Ca^{2+} extrusion pathway is by the Ca^{2+} pump of the plasma membrane (Irvine, 1986). Inhibition of this pump results in raised intracellular Ca^{2+} and ultimately increased muscle tone and contractility (Sugiyama et al, 1990). A number of human and experimental studies have reported inhibition of this pump with intracellular Ca^{2+} accumulation (Vezzoli et al, 1985; Sugiyama et al, 1990; Buhler and Resink, 1988). Some studies however have reported elevated Ca^{2}-ATPase activity in human hypertensive patients (Resink et al, 1985). In this study except in the malignant phase Ca^{2+}-ATPase was significantly lower in the DOCA-salt SHR compared to the SHR. In the SHR Ca^{2+}-ATPase activity remained stable until the rats were 20 weeks old when the pump activity progressively declined. In this group intracellular Ca^{2+} was highest when Ca^{2+} pump function was lowest. With aging, in both groups Ca^{2+}-ATPase was inversely correlated to muscle Ca^{2+} and positively correlated to serum Ca^{2+}, and red blood cell Mg^{2+}. In the SHR group, there was a positive correlation between Ca^{2+} pump activity and serum Mg^{2+} and muscle Mg^{2+}.

13.5.5.3 Mg^{2+}-ATPase

Mg^{2+}-ATPase may promote Mg^{2+} entry into cells (Flatman, 1984). After 6 weeks of DOCA-salt treatment Mg^{2+}-ATPase

activity was significantly decreased. In the SHR group Mg^{2+} ATPase activity progressively increased throughout the experiment. With aging there was a positive correlation between serum Mg^{2+} and Mg^{2+}-ATPase in both groups. Causes for the lower cell membrane ATPase activity in the DOCA-salt SHR are unknown. A number of factors have been implicated as ATPase inhibitors in hypertension (Kramer et al, 1985). Since ATPase is Mg^{2+} dependent, changes in Mg^{2+} status will affect ATPase activity (Aikawa, 1978). In this study there was persistent hypomagnesaemia (despite normal muscle Mg^{2+}) in the DOCA-salt SHR with a significant correlation between serum Mg^{2+} and ATPase activity in the malignant phase. The fact that the DOCA-salt rats were hypomagnesaemic yet their muscle Mg^{2+} levels did not differ from the control group suggests that muscle Mg^{2+} concentrations may be kept stable at the expense of serum Mg^{2+}. ATPase function may be more sensitive to serum Mg^{2+} changes than to intracellular Mg^{2+} changes. Significant correlations between Mg^{2+} and ATPase activity in the SHR group confirms that cell membrane pump function is Mg^{2+} related.

In conclusion, this study shows that malignant hypertension develops in DOCA-salt SHR. In these rats there are a number of cation changes with functional membrane related abnormalities. These membrane defects may be associated with serum Mg^{2+} deficiency. The abnormalities occur in the early stages of severe hypertension and may play an

important aetiological role in the pathogenesis of malignant hypertension.

CHAPTER 14

GENERAL DISCUSSION AND CONCLUSIONS

14. GENERAL DISCUSSION AND CONCLUSIONS

Essential hypertension as a disease entity has been recognised for only a century since Mahomet began to systematically measure blood pressure and define a group of patients whose hypertension could not be attributed to pre-pre-existing renal disease (Mahomet, 1881). Before this Schaarschmidt described the existence of spastic conditions of the vascular bed for which no cause could be found (Backer, 1953). Since then the definitions and measurements of hypertension have been refined. Although hypertension can be successfully treated today with large studies demonstrating a benefit in terms of morbidity and mortality from therapy, the exact cause of this disease remains unknown (Dustan et al, 1958; MRC Working Party, 1985). Blood pressure is affected by many genetic and environmental factors and their complex interactions. The elucidation of the pathophysiological mechanisms of essential hypertension remains one of the most difficult challenges in cardiovascular medicine.

14.1 Cations and cell membranes in hypertension

With modern technology, cellular and subcellular processes in the pathophysiology of hypertension are now being investigated. The primary pathogenetic lesion underlying essential hypertension is the presence of a widespread plasma cell membrane defect (hypertensive 'membranopathy')

(Postnov, 1990). This abnormality is associated with:-

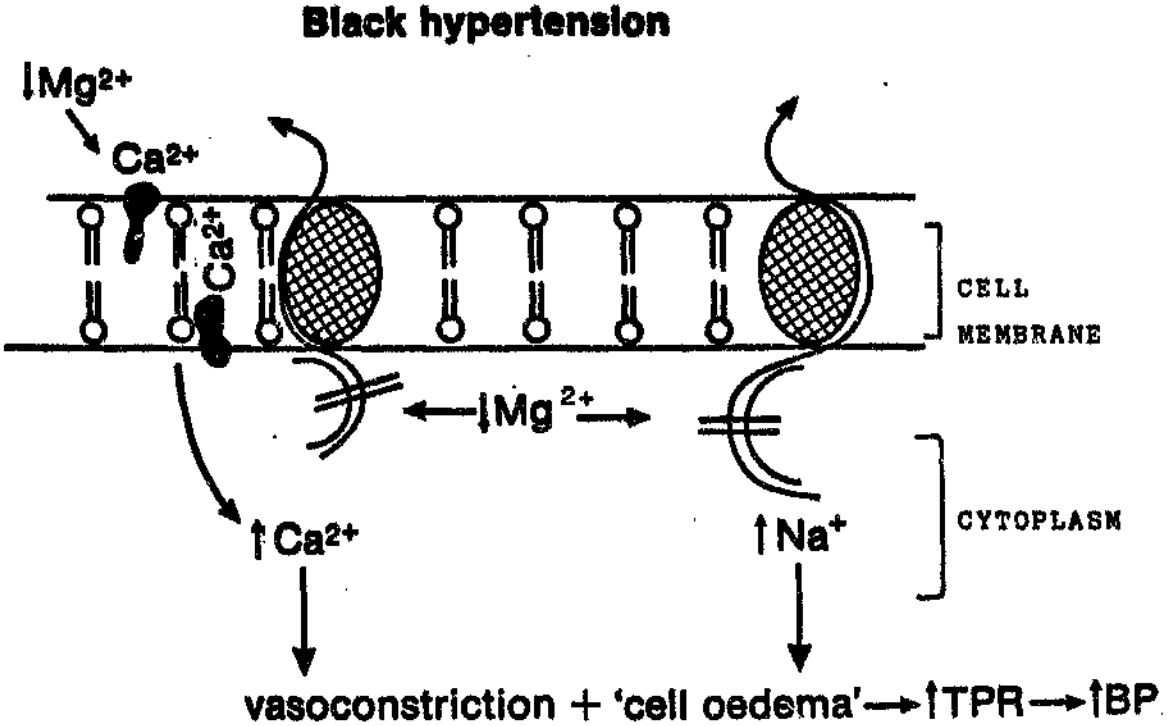
1. altered ion transport function of the cell membrane,
2. ultrastructural changes and
3. physico-chemical alterations

These defects result in changes in the cell's internal milieu with insufficient membrane control over intracellular calcium. The major pathologically significant consequences are increased intracellular calcium and sodium concentrations. In vascular smooth muscle increased cytosolic free calcium is a major determinant of vascular tone and contractility. Increased intracellular sodium influences the cell water volume and size and may affect the lumen diameter of resistance vessels. Raised intracellular calcium and sodium may thus affect total peripheral resistance and blood pressure.

Membrane abnormalities that may result in intracellular calcium accumulation include increased calcium influx rate, decreased cell membrane calcium binding and altered Ca^{2+}-ATPase activity (Postnov and Orlov, 1985). Raised cytosolic sodium may be due to increased sodium influx, increased cell membrane permeability to sodium and decreased efflux via the sodium pump (Hilton, 1986).

This study demonstrates that patients with essential hypertension have increased intracellular sodium and calcium concentrations. These findings are independent of

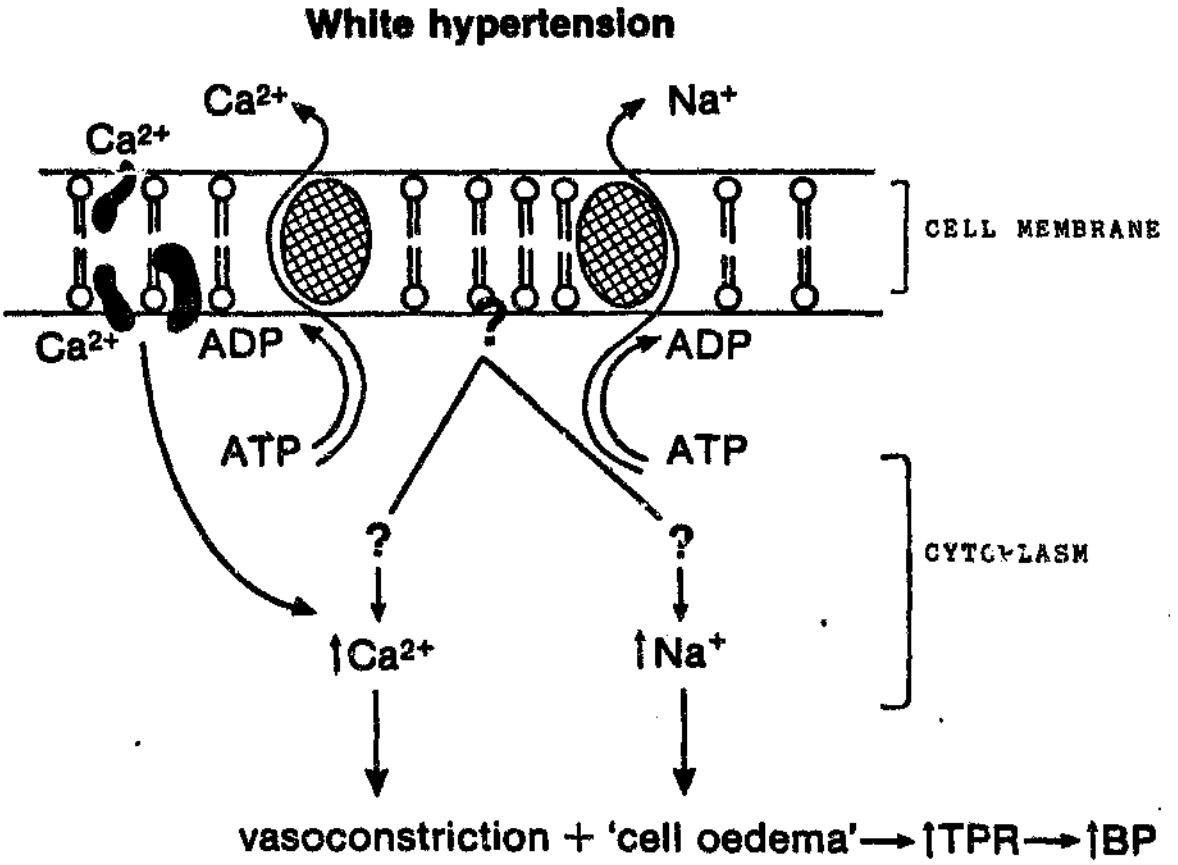
Figure 14.1: Cell membrane defects and associated cellular events in black hypertension.
TPR = total peripheral resistance



race. The mechanisms responsible for these cellular changes in hypertension however may differ between blacks and whites. In the white hypertensive group the cell membrane transport pumps were normal, whereas in the black group, cell membrane ATPase activity was significantly depressed. (Figures 14.1; 14.2). Decreased Ca^{2+}-ATPase activity results in increased intracellular calcium and depressed Na^{+}-K^{+}-ATPase activity leads to increased cytolic sodium. Thus, the mechanisms responsible for the cellular abnormalities in the black hypertensive group are probably related to defective active transport across the cell membrane. These patients also had significant magnesium depletion. Since the Ca^{2+}-ATPase and Na^{+}-K^{+}-ATPase pumps are magnesium dependent, the depressed activity in the black hypertensive patients may be due to magnesium deficiency (Figure 14.1).

Altered calcium binding to the outer cell membrane may also influence intracellular calcium levels. In both the black and white hypertensive groups, the raised intracellular calcium may have been due to defective calcium binding to the cell membrane. In the black hypertensive patients calcium binding was significantly related to magnesium.

Figure 14.2: Cell membrane defects and associated cellular events in white hypertension.



In this study, a subgroup of hypertensive patients with a specific biochemical profile has been identified. These patients are middle aged blacks who demonstrate:-

1. increased intracellular sodium and calcium,
2. depressed cell membrane ATPase activity,
3. altered cell membrane calcium binding,
4. decreased extra- and intracellular magnesium. Reasons for magnesium deficiency in these patients may be attributable to dietary factors, effects of alcohol and possibly intrinsic abnormalities of magnesium metabolism (e.g. increased urinary magnesium wasting)
5. inverse correlations between magnesium and ATPase activity and between magnesium and calcium binding.

These findings strongly suggest a physiologic relationship between cellular magnesium metabolism and blood pressure regulation, and pathophysiologically suggests that intracellular magnesium depletion is common in a subgroup of hypertensive patients. These proposals are supported by previous in-vitro and whole animal studies and by certain clinical observations.

Altura et al (1985) in a series of studies demonstrated for a variety of vascular beds in different species that magnesium levels strongly influence vascular tone and vascular responsiveness to pressor agents. Furthermore, dietary magnesium depletion in rats decreases serum

magnesium levels, decreases the luminal diameter of peripheral resistance vessels and increases blood pressure (Altura et al, 1978). Also varying dietary magnesium levels in spontaneously hypertensive rats enhances or depresses the development of high blood pressure when animals are fed low or high magnesium containing diets respectively (Berthelot and Esposito, 1983). The results from the rat study here confirm others that magnesium loading retards the development of hypertension.

Clinically, magnesium was first shown to lower blood pressure in malignant hypertensive patients in 1925 (Blackfan and Hamilton, 1925). Since then studies have confirmed that magnesium supplementation may have hypotensive effects especially in high renin, magnesium deficient hypertensive patients and hypertensive patients taking diuretics (Resnick et al, 1983; Dyckner and Wester, 1988). Recent studies have demonstrated that in subgroups of hypertensive patients, total and free intracellular magnesium levels are reduced and intracellular magnesium correlates inversely with blood pressure (Resnick et al, 1984; Touyz et al, 1989). A study on white hypertensive adults failed to demonstrate consistent magnesium deficiency (Kjeldsen et al, 1990). These data confirm the results of this study, that magnesium deficiency occurs in black hypertension but not in white hypertension.

14.2 How does intracellular magnesium depletion result in increased peripheral resistance?

The mechanisms of how magnesium affects total peripheral resistance are related to the effects on cellular calcium and sodium metabolism. Intracellular calcium content and distribution are closely related to magnesium. Magnesium binds competitively with calcium to the cell membrane, it is a weak antagonist of calcium entry into vascular smooth muscle, it stimulates sarcoplasmic reticulum calcium influx, and is a vital cofactor for Ca^{2+}-ATPase (Aikawa, 1981; Witteman and Grobbee, 1990). Alterations in magnesium homeostasis will thus alter cellular calcium metabolism. Magnesium deficiency, as demonstrated in the black hypertensive patients here, results in cell membrane Ca^{2+}-ATPase depression and altered calcium binding. The net result of these defects is raised intracellular calcium with consequent increased peripheral resistance and blood pressure.

Magnesium deficiency may also influence peripheral resistance and blood pressure by its affects on cellular sodium metabolism. The major sodium extrusion pathway across cell membranes is via the Na^+-K^+-pump. Magnesium is an absolute requirement for Na^+-K^+-ATPase (Danting et al, 1983). Flatman and Lew (1979) demonstrated that magnesium concentrations in the physiological range influences sodium and potassium transport. By altering magnesium

concentrations sodium and potassium transport are affected (Ellory et al, 1983). In this study the black hypertensive patients had significantly decreased cell membrane Na^+-K^+-ATPase activity probably due to magnesium depletion. The resultant intracellular sodium accumulation causes increased intracellular water content, oedema of the vessel wall with encroachment on the lumen and finally an increase in peripheral resistance and blood pressure.

Results from this study have also demonstrated that the severity of hypertension may be related to the extent of the cellular abnormalities. In both the human and experimental malignant hypertension studies, the cellular defects were more severe in the malignant than the benign phase. Although the earliest studies relating magnesium and hypertension were reported in malignant hypertensive states, there is very little data in the literature on the role of ions and magnesium in particular, in the pathogenesis of malignant hypertension. The findings in this study on malignant hypertension, have not been previously reported.

Essential and malignant hypertension are associated with cellular abnormalities. The end point of these abnormalities i.e. raised intracellular sodium and calcium concentrations appear to be uniform and unrelated to race. The mechanisms responsible for these defects however are different. In black hypertension, cellular magnesium

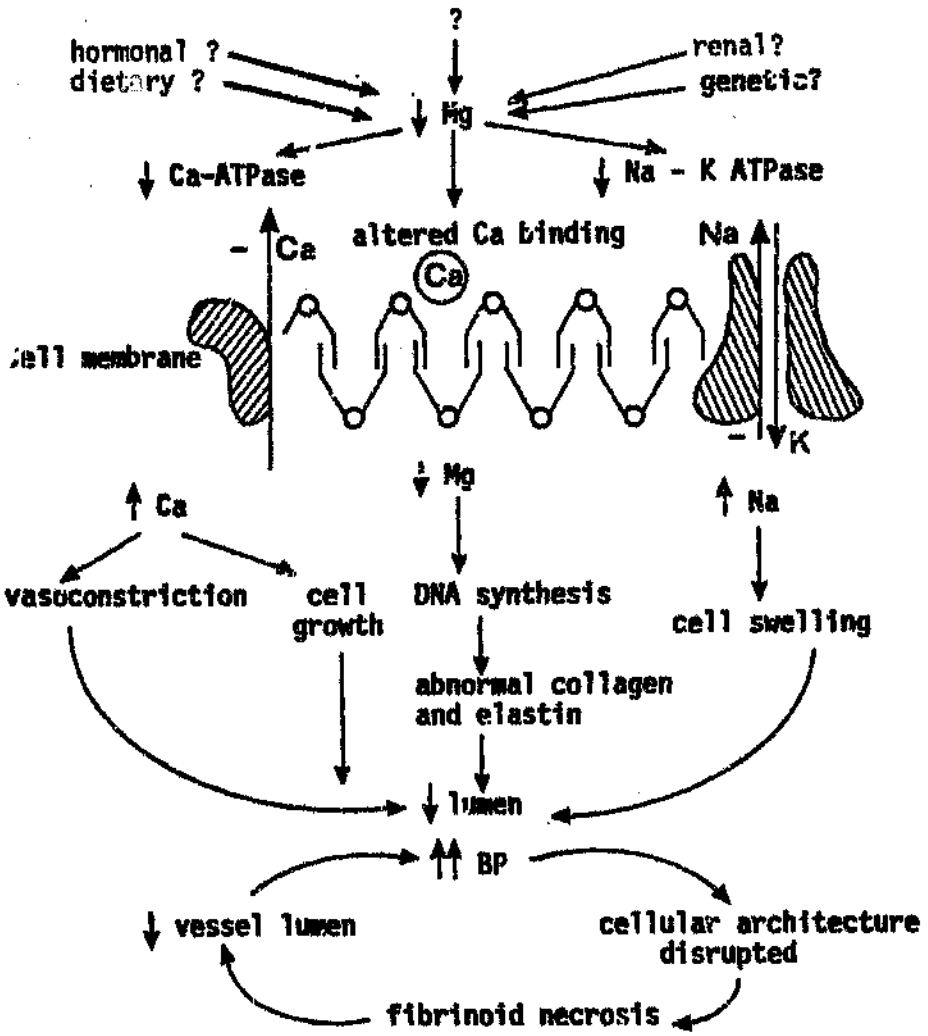


Figure 14.3: Hypothetical role of magnesium in malignant hypertension.

related abnormalities are important. The cause of increased cytosolic sodium and calcium levels in white hypertension is unclear.

14.3 Conclusions

1. In essential hypertension, irrespective of race, intracellular calcium and sodium concentrations are increased.
2. In black hypertension, raised intracellular calcium and sodium are due to altered calcium binding and decreased activity of cell membrane Ca^{2+}-ATPase, Na^{+}-K^{+}-ATPase and Mg^{2+}-ATPase (Figure 14.1).
3. In black hypertension, the cell membrane defects are probably related to magnesium deficiency (Figure 14.1).
4. Other mechanisms are responsible for the intracellular findings in white hypertension (Figure 14.2).
5. Malignant hypertension is associated with severe cell membrane defects. It is proposed that as the biochemical abnormalities worsen, structural changes develop resulting in the characteristic pathological lesion of fibrinoid necrosis (Figure 14.3).
6. Experimental studies confirm that magnesium may play an important role in the pathophysiology of essential benign and malignant hypertension. The initial theory that magnesium may be important in hypertension was a general hypothesis, where all hypertensive patients

were thought to have an abnormality in magnesium metabolism. The results of this study did not confirm this hypothesis, as it was found that only the black hypertensive group had magnesium-related abnormalities.

It is finally proposed that a subgroup of subjects who have a genetic predisposition to high blood pressure will develop hypertension when exposed to specific environmental factors, such as magnesium deficiency. Not all hypertensive patients will be magnesium deficient and not all magnesium deficient patients will be hypertensive. The practical importance of this study is that a magnesium-related subgroup of hypertensive patients has been identified, and these patients may well benefit from magnesium supplementation.

CHAPTER 15

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