MICROALBUMINURIA AND HYPERTENSION

EMMA MARJORIE GRAHAM-CLARKE

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THE UNIVERSITY OF ASTON IN BIRMINGHAM

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A relationship between blood pressure and albumin excretion in hypertensive patients was first suggested by Parving et al[1] in 1974. The range of albumin excretion which they were studying was below that which can be detected by the use of a conventional dipstick test such as Albustix but above the normal ranges which had been determined by Petersen et al[2] and Miles et al[3] in 1969 and 1979 respectively. This range of albumin excretion was termed microalbuminuria. Much work has been carried out on the role of microalbuminuria in predicting diabetic nephropathy, but comparatively little is known about its relationship with blood pressure. In order to determine if certain commonly encountered factors (smoking, alcohol and caffeine intake) had any effect on albumin excretion a questionnaire was conducted on the hypertension clinic population. Urine samples for detection of albumin were also collected from a proportion of those questioned. This study failed to show any relationship between microalbuminuria and smoking, alcohol intake or caffeine intake. A relationship was found between blood pressure and microalbuminuria. This relationship was then investigated further in two studies. The first study took "spot" samples of urine from a large number of patients and looked at the correlation between blood pressure and microalbuminuria. The relationship appeared to be strongest in the group of treated patients, especially those of caucasian treated males. The second study looked at a group of untreated patients who were subsequently started on mono antihypertensive therapy. Their medication doses were increased until their blood pressure was within acceptable limits. This study failed to find any relationship between blood pressure and microalbuminuria either before or after treatment in either of the two treatment groups.

Key words: Hypertension
Blood Pressure
Microalbuminuria
Albumin/Creatinine Ratio

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CHAPTER 1

INTRODUCTION

A number of environmental factors may either raise or depress blood pressure; stress[4,5], obesity[6] and salt[7] intake have all been reported to have an adverse effect on blood pressure control. Other factors (smoking, alcohol intake and caffeine) have been shown to affect blood pressure in clinical studies, however their relevance to the patient is not known. Nicotine by intravenous injection (1-2mcg/kg/min) is known to cause a rise in blood pressure of 5-10 mmHg[8,9] which appears to be due to effects on both the heart rate and peripheral resistance. However, it has been found that long-term smokers in fact have a lower blood pressure in between cigarettes than non-smokers. The effect of alcohol appears to vary according to the quantity ingested[10]. Those with a low intake appear to have a lower blood pressure than those who do not drink alcohol at all. However, those with a moderate to heavy intake show a rise in blood pressure of about 5-10 mmHg. This relationship appears to be linear and the rise affects both systolic and diastolic blood pressure. Caffeine has been shown[4,11] to raise both systolic and diastolic blood pressure by approximately 5mmHg after an oral dose of 3.3mg/kg. It has no effect on heart rate but seems to increase vascular resistance.

A relationship between blood pressure and microalbuminuria has been suggested by various workers[1,12,13]. Albuminuria is usually taken as the point at which albumin may be detected in the urine by tests such as Albustix . The basis of this test is the change of colour which occurs when proteins bind to the indicator tetrabromophenol blue at a constant pH, however, it is not specific for albumin. These tests are capable of detecting the presence of a large amount of protein but become less reliable at detecting 'traces' of albumin (for Albustix this corresponds to the range 50-200mg/l), where other factors may affect the results. for example concentrated urine may give a positive 'trace' result but the excretion rate of albumin may be normal. Microalbuminuria is defined as an excretion rate of albumin which is higher than the rates seen in healthy volunteers but lower than that which can be detected by urine testing sticks such as Albustix .

It was decided to investigate this relationship further to see if it may be of use as a diagnostic test in the hypertension clinic. It is possible that some patients may have a falsely elevated blood pressure simply from the stress caused by attending the clinic. At present there is no satisfactory method of distinguishing these patients from the true hypertensives, and it may be that microalbuminuria could prove to be useful in this area. It is important to evaluate the effect of various environmental factors on microalbuminuria independently of their effect on blood pressure so that if necessary these may be taken into account.

These were investigated by means of a questionnaire, conducted in the hypertension clinic, and urinary albumin samples (Chapter 3 page 40).

Most studies investigating the relationship between albumin excretion rate and blood pressure have tended to involve small numbers of patients and therefore may not represent the true picture in the general population. However, two studies involving larger numbers of subjects have been reported since the present project began[14,15]. The first study was part of a general practice survey of patients who were screened for blood pressure, body mass index and "spot" daytime urine samples. Diabetics, patients with proteinuria and patients with raised serum creatinine levels were excluded. The second study involved a factory screen where randomly selected factory workers were screened for blood pressure and body mass index and were also asked to complete a 24 hour urine save. In both studies most of the subjects were normotensive. No relationship between albumin and blood pressure was demonstrated in either study. However, once the normotensive patients had been excluded in the latter study a relationship became apparent in the remaining few (28) hypertensive subjects which appeared to indicate that as blood pressure rose so did the albumin excretion rate. However, in both studies the hypertensive patients formed only a small minority of the total number of subjects. Therefore, a study has not been conducted using a reasonably large number of subjects to determine if the suspected relationship between

albumin excretion rate and blood pressure can be demonstrated in a hypertensive population.

The next part of this study investigated further the relationship between blood pressure and albumin excretion rate in a large number of both treated and untreated patients randomly selected from the hypertension clinic at the hospital (Chapter 4 page 54). These results were analysed to see if any relationship between blood pressure and albumin/creatinine ratio was found and also how the relationship was affected by differents races or genders. There appeared to be a stronger correlation between microalbuminuria and blood pressure in the treated group but these patients were on a variety of drugs which may affect albumin excretion by a variety of mechanisms. The caucasian, male treated group appeared to have the strongest correlation.

A small sample of patients was also investigated before and after they started monotherapy (Chapter 5 page 70). Two different drugs were used (a beta-adrenoreceptor blocker or an angiotensin converting enzyme inhibitor) which have different modes of action, to see if either class of drug had a specific effect on the relationship.

In summary the aim of the project was to investigate further the relationship between microalbuminuria and blood pressure, with special consideration towards factors that affect blood pressure (including drugs), to see how useful measurement of

microalbuminuria would be as a diagnostic tool in selecting patients with persistently raised blood pressure.

CHAPTER 2

METHODS OF DATA COLLECTION AND SAMPLE TREATMENT

2.1 Data collection for questionnaire

The hypertension clinic at Dudley Road Hospital has a list of about 900 patients. This number stays reasonably constant despite the inclusion of new patients, because patients are regularly discharged from the clinic list or removed from it because of failure to attend. There are usually about 60 patients each week attending the main follow-up clinic with a further 10 to 15 new patients being seen each week in a seperate clinic. The frequency of attendance at the clinics depends on the patient's blood pressure control and may vary from weekly to yearly visits.

Data were collected over a period of ten months from a large proportion of the clinic population, on factors which may affect blood pressure, by means of a questionnaire (Appendix I). All new hypertensive patients completed the questionnaire at their first clinic attendance. At the follow-up clinic there were initially too many attenders, who had not completed a questionnaire, to be seen in one morning. A selection of patients was made, therefore, by taking the record notes of the next patient to be seen at clinic and asking them if they would help to answer a questionnaire. Once a questionnaire had been

completed for that patient the notes were marked to identify them for future use and the next available patient was seen. Initially about a third of the clinic attenders could be seen in one morning, however, as more people completed the questionnaire the choice of patients became limited until only four or five patients were seen in a morning. At this point questionnaire collection at the follow-up clinic ceased.

Each patient selected was asked if they would help with a questionnaire which was designed to assess the effect of various factors that may affect blood pressure. They were then asked the questions as they appeared in the questionnaire. If they answered 'no' to the question 'Do you drink alcohol?' the alcohol intake diary was omitted. Patients who drank decaffeinated coffee or herbal tea were recorded as drinking no tea or coffee, as the aim was to estimate the quantity of caffeine ingested. A note was made on the questionnaire to the effect that the patient drank herbal tea or decaffeinated coffee. A section was included to record any over-the-counter medicines taken by the patient in order to produce a complete picture of the medication the patient was receiving.

The questionnaires were coded and entered onto a computer from which statistical analyses could be made using the Scientific and Profession Statistical System (SPSS).

2.2 Lisinopril/Atenolol Study - Trial Design

Patients attended for a total of five visits over a ten week period. At the initial visit (visit I, week -2) a full screening was carried out, and if the patient's diastolic blood pressure was between 95-130 mmHg, they were admitted into the placebo phase of the trial. Each patient received two bottles, one containing placebo lisinopril 10mg tablets, and one containing placebo atenolol 50mg tablets. The patients were instructed to take one tablet from each bottle at 8.00 hrs each morning. After two weeks (visit II, week 0) they were seen again and if their diastolic blood pressure remained between 95-130 mmHg they were admitted into the study and randomly allocated to one of two groups to receive either active lisinopril or active atenolol. Each patient received two bottles and was instructed to take one tablet from each bottle at 8.00 hrs each morning. For Group A one bottle contained lisinopril 10mg tablets and the other contained placebo atenolol 50mg tablets. For Group B one bottle contained placebo lisinopril 10mg tablets and the other contained atenolol 50mg tablets. After a further two weeks (visit III, week 2) the patient's blood pressure was measured again. If the diastolic blood pressure was 90 mmHg or less the patient remained on the same medication, however, if the blood pressure was 91 mmHg or above then the dose was increased. Group A patients received one bottle containing lisinopril 20mg tablets and one containing placebo atenolol 50mg tablets. Group B received one bottle containing placebo lisinopril 20mg tablets and one

containing atenolol 50mg tablets. Both groups were instructed to take one tablet from each bottle at 8.00 hrs each morning. After a further two weeks (visit IV, week 4) each patient again had their diastolic blood pressure measured. If it was 90 mmHg or below they remained on the treatment they were already receiving. If it was 91 mmHg or above then the dose was increased. Group A patients received one bottle containing lisinopril 40mg tablets and one containing placebo atenolol 100mg tablets. Group B received one bottle containing placebo lisinopril 40mg tablets and atenolol 100mg tablets. Both groups were instructed to take one tablet from each bottle at 8.00 hrs each morning. All patients were seen four weeks later (visit V, week 8) at the end of the study for a full screening and assessment of adverse effects.

2.3 Measurement of Blood Pressure

All blood pressure readings were measured by trained operators using a Hawksley random zero sphygmomanometer. The use of this form of sphygmomanometer helps to eliminate operator bias when more than one reading is taken from the same patient[16]. The patient was seated during measurement of blood pressure. The blood pressure was measured to the nearest 2mmHg and the diastolic pressure was recorded at the 5th Korotkoff phase (disappearence of the sounds).

2.4 Sample Collection and Preparation

The definitive method of determining an albumin excretion rate is by measurement of albumin content in a timed 24 hour urine save. Unfortunately, urine saves tend to be inaccurate and unreliable due to incomplete collection which results in incomplete data. Patients find the process of a 24 hour urine collection socially embarrassing and may not appreciate the importance of complete urine collection. Albumin degenerates if it is stored at room temperature so it is important that the urine save is returned to the laboratory as soon as possible after the save is completed so that it can be stored below -8°C until it can be assayed. Patients may find it difficult to return the urine save to the laboratory due to work or transport problems and may be reluctant to store the urine save in their domestic refrigerator. Compliance with a request for a 24 hour urine save may therefore be poor and for these reasons workers have been looking for alternative methods of predicting raised albumin excretion rates (ie microalbuminuria) which maintain the accuracy of a complete 24 hour urine save whilst encouraging patient compliance.

Timed two or three hour samples are one method that has been studied. They are easier to complete and patients find them more socially acceptable. However, a diurnal variation in albumin excretion has been reported[17] which means that all samples may not be comparable. Timed overnight urine collections are another

method that has been used with some success. They are convenient and acceptable to the patient. The albumin excretion rate has been shown to decrease overnight[17] but this is unlikely to be a problem in a comparative study. Timed collections require organisation in the supply and collection of bottles and patient education on the accurate completion of a save. A solution could be to issue patients with bottles at the clinic visit, to be returned on the next visit. There is obviously a time delay with this procedure and patients may not remember the save if the clinic visits are months apart. In addition other arrangements would have to be made in order to obtain a collection from new patients.

All these methods are time consuming for both the patient and the laboratory staff. Ideally the method should allow a sample of urine to be collected in clinic which could then be assayed in a batch and the results ready as rapidly as possible to enable any necessary theraputic alterations to be made.

Shaw et al[18] compared protein/creatinine ratios in early morning samples, and random samples with 24 hour total protein excretion rates in 91 subjects. The subjects were subdivided depending on the amount of protein excreted daily (the range varied from <150mg/day to >3500mg/day). Patients in advanced renal failure were excluded from the study. They found that a protein/creatinine ratio from a random urine sample was the best means of predicting a 24 hour total protein excretion rate.

Gatling et al[15] conducted a study on 265 diabetic patients to determine if spot urine samples could be used as a method of predicting raised albumin excretion rates. Each patient was asked to collect a timed overnight urine save which was then assayed for albumin and creatinine levels. From the overnight save an albumin excretion rate was calculated which acted as the baseline against which three proposals were tested. The three proposals were 1) a raised random albumin concentration from a spot sample would predict a raised overnight albumin excretion rate, 2) a raised albumin concentration from an early morning sample would predict a raised overnight albumin excretion rate, and 3) a raised albumin/creatinine ratio from an early morning sample would predict a raised overnight albumin excretion rate. They found that an albumin/creatinine ratio on an early morning sample proved to be the best predictor of the overnight albumin excretion rate. It may be that the random albumin samples were altered by the effects of exercise which has been shown to raise albumin excretion[19]. In addition the specimens used to determine the albumin/creatinine ratio and albumin concentrations in the early morning samples were the same as those used to calculate the overnight albumin excretion rate. However, as the albumin/creatinine ratio gave the best prediction of albumin excretion rate this ratio was used in our studies.

Urine samples were collected from patients at their visit to the clinic. Where possible each specimen was divided to provide duplicate samples which were stored in Universal specimen

containers and frozen at -18°C until they could be assayed. Before an assay was performed the samples were allowed to thaw out at room temperature and they were then clarified by centrifugation. An aliquot of each specimen was also sent to Clinical Chemistry to measure the creatinine concentration.

The urine samples obtained were assayed for albumin concentration as described below (page 26). From these measurments an albumin/creatinine ratio could be calculated for each sample using the formula:

$$\frac{\text{Albumin (mcg/ml)}}{\text{Creatinine (mol/l)}}$$

2.5 Assay

A variety of assay methods have been used for the detection of small quantities of albumin. Miles et al[3] developed a single antibody radioimmunassay for the detection of albumin. Anti-human-albumin antibodies were used to precipitate out a solution of a known concentration of radioactive albumin and a known volume of the test sample. The bound albumin was separated from the liquid by microfiltration and the radioactivity of the precipitate was counted. By using samples of known concentration a calibration curve could be obtained and hence the concentrations of the unknown samples found. This method was also used by Pedersen et al[19]. Petersen et al[2] used a radial immunodiffusion method to measure albumin concentrations. This

involved placing small volumes of test and standard solutions into wells of immunodiffusion plates. The solution diffused out and reacted with the antigen present to produce a precipitate, the diameter of which could be measured and used to construct a calibration curve. Both of these procedures took at least 24 hours to perform. Hemminsen and Skaarup[17] used an Auto-Analyzer for measuring specific proteins. Mono-specific antiserum against albumin was mixed with filtered, diluted samples and the light reflectance was measured. This was adapted to give greater sensitivity of about 0.1mg/l.

A commercial kit has been developed by Diagnostic Products UK Ltd which is based on a double antibody radioimmunassay procedure. The detection limit of the basic assay is quoted as 0.9µg/ml, however this can be decreased by using a slightly different procedure. A quantitative assay using an immunoprecipitation method is also being developed which is quoted as giving results in the range 5mg/l to 250mg/l. Other kits may be available.

2.5.1 Method Theory

The assay used is that produced by Diagnostic Products UK Ltd. This assay method ultilises the competitive binding of albumin to albumin antiserum. If albumin labelled with radioactivity and unlabelled albumin are mixed together and albumin antiserum is added, the proportion of labelled albumin bound to the antiserum

will be the same as the proportion of labelled albumin present in the entire mixture. By precipitating out the albumin-antiserum complex from the solution, using an excess of anti-albumin-antiserum gamma globulin to ensure complete precipitation, the activity of the albumin-antiserum complex can be measured using a gamma counter. By comparing the results obtained from known concentrations of albumin it is possible to determine the concentration of albumin in unknown samples. Variations due to background radiation counts and non-specific binding (NSB) of albumin may both occur. This needs to be limited as much as possible and is estimated by a pair of tubes to which no albumin antiserum is added, only the appropiate aliquots of zero calibrator and labelled albumin. These tubes are then assayed in the normal manner. Any radioactivity counted from these tubes is, therefore, caused by either background radiation or the non-specific binding of albumin to the tubes. A second pair of tubes are also assayed which contain the same reagents as the NSB tubes except for the addition of antiserum. This allows for the maximum amount of labelled albumin to be bound to the antiserum and provides a figure for maximum binding (MB). Each calibrator is expressed graphically as a percentage of MB,the calculation being:

$$\text{Percent bound} = \frac{(\text{mean calibrator CPM} - \text{mean NSB CPM})}{(\text{mean MB CPM} - \text{mean NSB CPM})} \times 100$$

(CPM = counts per minute)

A sigmoid curve is produced when a graph is plotted on linear graph paper of the percentage of albumin bound against the

concentration of albumin for each of the calibrators. In the middle range any increase in unlabelled albumin is reflected in a proportional decrease in bound labelled albumin. However, at the extremities of the curve any change in concentration of unlabelled albumin is not reflected by a proportional change in bound labelled albumin. Logit transformation of the percent bound results straightens the sigmoid curve at the ends. It should be noted that both the maximum and minimum values for percent bound are fixed by the MB and NSB results respectively. These set the upper and lower limits for each assay. If the concentrations for the unknown values are to be determined by manual means then the transformation is best acheived using log/logit graph paper which allows direct plotting of the percent bound results. For non-graphical methods the logit of p is defined as:

$$y = \log_e \frac{p}{1-p}$$

By using log/logit graph paper to plot a calibration curve, or by calculating a regression equation (using the method of least squares), it is possible to determine the concentration of albumin in unkown samples.

2.5.2 Method

The kit contains all of the reagents required for the assay, ie Iodine-125 labelled albumin, anti-human albumin antiserum, a

precipitating solution of donkey anti-goat gamma globulin, six albumin calibrators and two albumin controls. The extra equipment required were disposable-tip pipettes, sample counter tubes, test-tube racks, a refrigerated centrifuge, a vortex mixer and a gamma counter.

The kit provides methods for two levels of sensitivity, a basic method and a method allowing greater precision at the lower end of the calibration scale. For the more sensitive assay a larger aliquot of sample (100 microlitres instead of 25 microlitres) was taken and the calibrators were diluted to produce points at the low end of the calibration scale (the scale became 0.5, 1, 2.5, 5, 10, 20 µg/ml instead of 5, 10, 20, 30, 60 µg/ml). Normal ranges of albumin excretion have been determined by other workers[2,3,17,19] using a variety of assay techniques. Peterson et al[2] reported a range of 3.9-24.2mg/24hrs whilst Miles et al[3] found rates between 8-12.6mg/24hrs. Hemmingson and Skaarup[17] found rates of between 1.64-34.2mg/24hrs (median 6.1mg/24hrs) on assaying the 24hr urine samples of 239 apparently healthy volunteers. More recently Pedersen et al[19] determined ranges of 4-15.5µg/min. The mean rate of excretion found in these studies varies from 7µg/min to 10.1mg/24hrs. From these studies it seemed likely that most samples could easily be measured by the basic technique, with the option to repeat the assay using the more sensitive technique if necessary.

Sample counter tubes were labelled in duplicate for the calibrators, total counts (TC), nonspecific binding (NSB), maximum binding (MB) and the samples. An appropiate aliquot was added to the relevant tubes from each of the samples and calibrators. An aliquot of the zero calibrator was added to the NSB and MB tubes, whilst the TC tubes were left empty. 200 microlitres of the radioactive albumin was then added to each tube followed by 200 microlitres of albumin antiserum to each except to the TC and NSB tubes. The contents of the tubes were then intimately mixed using a vortex mixer and were allowed to equilibrate at room temperature for 30 minutes. One millilitre of cold precipitating solution was then added to all of the tubes except TC and the resultant mixture was centrifuged for 15 minutes at 3000g. The supernatent liquid was removed and the tubes containing the remaining precipitate, and the TC tubes, were then counted for one minute using the gamma counter. A calibration curve was estimated as described previously and from this the values of the unknown samples could be determined.

2.5.3 Method Development

A disposible tip micropipette was used for all of the additions of the reagents and the samples. Practice sessions involving transfer and weighing of water showed a minimium error (2.5%) either when the same tip was used throughout or different tips were used. However, it was noted that when water was drawn too rapidly into the pipette, air also entered, and the resultant

volume of water was considerably smaller than it should have been. It was also found that it was extremely difficult to pipette the reagents without getting air into the tip. A solid liquid interface pipette seemed to be the solution, however, care would be needed to prevent radioactive contamination of the equipment. By using a solid liquid interface pipette which had a disposible tip and a disposible plunger any problems associated with radioactive contamination were eliminated, whilst maintaining accurate transfer of the reagents. On one occasion it was discovered that insufficient precipitate had been produced. On investigation it was discovered that the pipette used for the addition of the precipitating fluid had a faulty seal which allowed some of the liquid to escape. As a result the precipitating fluid was no longer in excess and consequently not all of the albumin antiserum complex was precipitated from the solution. The variation between the paired results was as much as 350% of the lower count (a cut off of 10% of the lower result is used to check the validity of the counts). Since then a repeat dispenser has been used for the addition of precipitating fluid and this problem has not recurred.

During several of the early assays it was noted that, when the supernatent liquid was drawn off the precipitate had a tendency to 'float' back into the solution. Consequently, either some precipitate was lost with the supernatent, or insufficient supernatent was drawn off. There were three possibilities for the occurence of this phenomenon. The precipitating solution

needed to be cold to work properly, however the solution was always kept in the refrigerator until just before use and so this did not seem to be the problem. The second possible cause was that the tubes were not spun for long enough at the required force. Access was obtained to a refrigerated centrifuge (Mistral 3000) which could hold all the tubes used in the assay and which could also spin them at the correct speed. This meant that the tubes were not standing for too long before being spun down, nor did they get too hot during the process and so it seemed unlikely that the centrifuge was the source of the problem. The remaining potential source of error lay in the counter tubes used. These were of polystyrene construction which require a mould releasing agent during the manufacturing process. It seemed that the mould releasing agent was preventing the precipitate from adhering to the tube and allowing the supernatent to be drawn off separately. Polypropylene tubes which need no releasing agent in their manufacture were then used which seemed to solve the problem.

Further developments of the techniques used in the assay have meant that the assay can now be performed accurately and relatively rapidly. A repeat dispenser of the Eppendorf type is used for all additions of reagents. This not only speeds up the procedure but also reduces the problems associated with foaming. Radiaoactive contamination is reduced by using diposable barrels for the pipettes. The design of the tubes used previously were flat bottomed which led to problems when removing the

supernatent liquid, because the small amount of precipitate formed was deposited as a thin layer on the base of the tube. There was a slight tendency for the precipitate to tear and be lost, either when decanting the fluid or when aspirating using a pipette. By changing to a smaller tube with a curved base a distinct pellet was formed which allowed the fluid to be decanted off easily using a decanting rack. The use of a decanting rack allowed about forty tubes to be decanted at the same time, which was much faster than aspirating each tube using a pipette, in addition decanting produces a much dryer product. One further minor alteration in the method has been the vortex mixing of the tubes after the addition of the precipitating fluid. This ensures that all the albumin antibody complex is intimatly mixed with the precipitating solution and, therefore, maximises precipitate formation.

2.6 Treatment of Results

To minimise errors when fitting a 'line of best fit' visually a computer program was developed to run on the Amstrad PCW8256 (Appendix II). It converts the CPM readings for the calibration points to the logit of the percent bound (using the equations already given) and then fits a regression line using the method of least squares. As a visual check of the linearity of the calibration points the percent bound readings were plotted against the appropiate concentrations using log-logit paper (Figure 2.1). To obtain the values for the unknown samples the

CPM readings for the duplicate tubes were entered into the computer program which inserted them into the regression equation and then printed the results (Figure 2.2). The program will also produce several quality control parameters such as the 20, 50 and 80 percentage bound intercept values, which may be compared between assays as test of reproducibility. It will also calculate the percentage values of MB and NSB as a function of TC. These measure the performance of the assay and indicate how well the albumin is binding to the antiserum and what proportion is binding to non-specific sites such as the counter tubes.

2.7 Validation of Method

A 1280 Ultrogamma gamma counter was used for measuring the gamma radiation emitted by the Iodine-125 which has a photon energy of 35 KeV. The gamma counter uses a 'window' to define the ranges of wavelength to be counted and so by setting a narrow window around the expected wavelength it is possible to reduce counts from other isotope sources. As the counter ages it may become less accurate. To test the accuracy and reliability of the machine two samples were counted repeatedly and the results analysed. A sample producing a high count rate per minute and a low count rate per minute sample were used. The results obtained are shown in Table 2.3

Figure Number: 2.1
Sample calibration graph, showing the points calculated from the standards, and the line of best fit

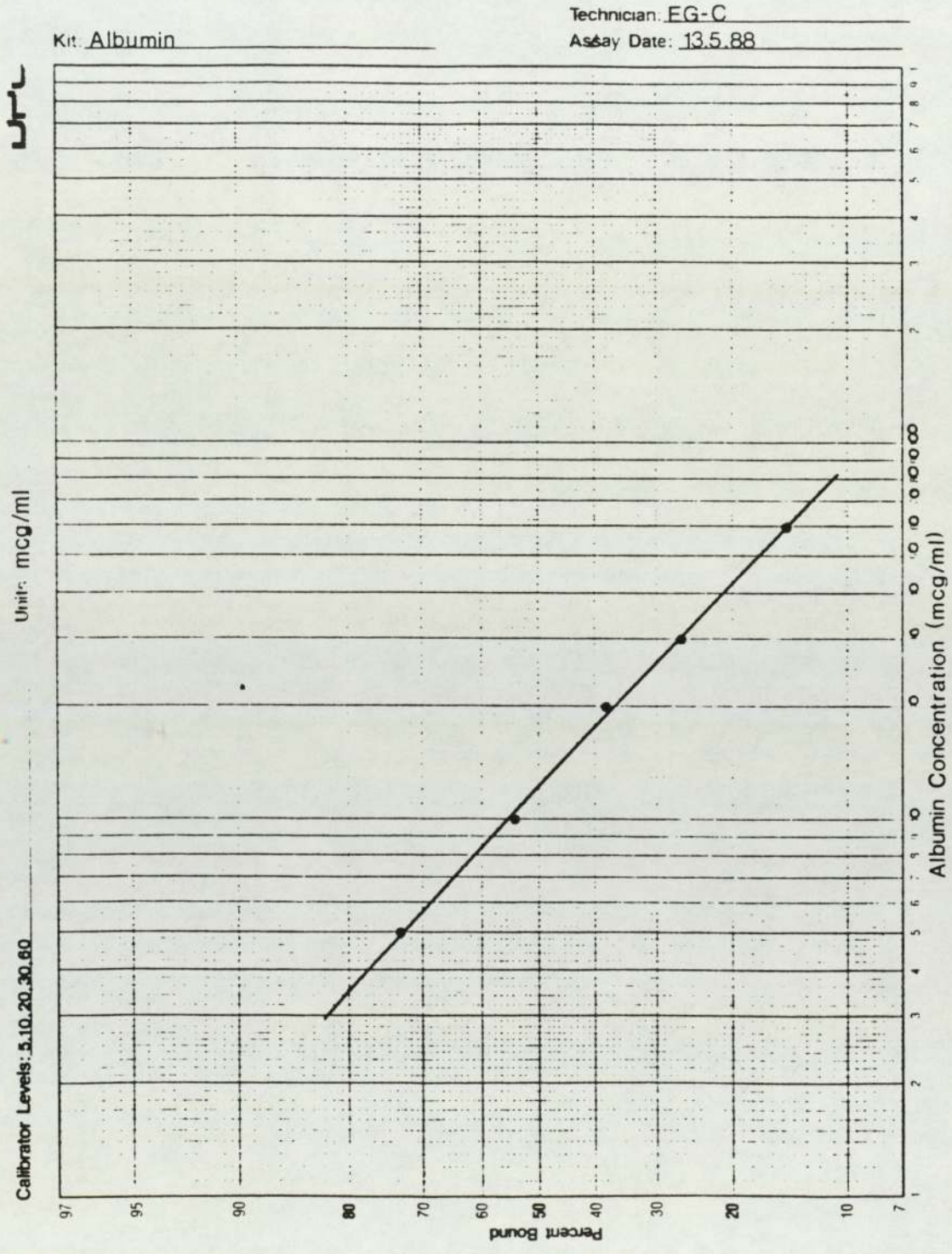


Figure Number: 2.2
Typical printout obtained from the computer for one run of the assay

RESULTS FOR ASSAY RUN ON: 13.5.88

Q.C. Parameters:

Total Counts 73535 cpm.

%NSB 1.86

%MB 71.9

The 20% intercept is 42.47

The 50% intercept is 12.3

The 80% intercept is 3.56

ASSAY RESULTS

The regression equation is

Y= -2.575267 X + 2.806347

Calibration Results

% Bound	Concentration
73.58	5
54.51	10
38.1	20
26.39	30
14.54	60

PATIENT RESULTS

Patient Number	Patient Counts		Albumin microgram/ml
1	28616	29626	11.25
2	39744	39950	5.1
3	42535	42483	4
4	43707	43552	3.57
5	35089	35112	7.41
6	16811	16357	27.64

Table 2.3

	Range	Mean	S.D.	C.V.	Mean ±1S.D.	Mean ±2S.D.
High Count Sample (n=54)	22968 to 23712	23312	171	0.7%	23141 to 23484 (76% inside range)	22969 to 23655 (94.4% inside range)
Low Count Sample (n=34)	3160 to 3403	3281	64.5	1.97%	3216 to 3346 (67.6% inside range)	3152 to 3410 (100% inside range)

(S.D. = Standard Deviation)
(C.V. = Coefficient of Variation)

The graph for both sets of data produced a curve with one main peak. The higher coefficient of variation for the low count sample probably indicates the effect of background radiation on the counter. The results suggest a satisfactory degree of accuracy for the counter.

Each kit is provided with a pair of control solutions assayed by the company. Accompanying each control solution is the statistical data for that control, setting limits within which the control would be expected to fall. By assaying each control solution several times it is possible to compare these results with those obtained by the manufacturer. Ten pairs of tubes were assayed for each control. The results are shown in Table 2.4

Table 2.4

	Mean	S.D.	2 S.D. Range	C.V.
Results obtained from assay				
Control I	12.25	0.5	11.25 - 13.25	4.1%
Control II	28.26	0.9	26.07 - 30.05	3.2%
Results from company				
Control I	12.7	1.42	9.9 - 15.5	11.2%
Control II	28.1	2.81	22.5 - 33.7	10.0%

(2 S.D.Range = Mean±2 S.D.)

The results obtained from the assay are well within the limits given by the manufacturer.

As stated previously a record was kept of the 20, 50 and 80% intercepts for each calibration graph. The concentration values for these points should be similar for each assay, and should indicate the reliability of the method.

Analysing the results obtained from all 25µl assays performed produced the results shown in Table 2.5

Table 2.5

	Mean Mcg/ml	N	Standard Deviation	Coefficient of Variation
20% intercept	43.64	18	3.03	6.95%
50% intercept	11.56	18	1.15	9.95%
80% intercept	3.09	18	0.52	16.9%

Readings taken from either end of the curve are unreliable because of the sigmoid nature of the calibration curve. At the low concentration end, the surplus of radioactive albumin is such that the bound proportion of non-radioactive albumin may bear little resemblance to the actual proportion present. The reverse is also likely to occur at the opposite end of the scale. Therefore a change in albumin concentration, at either end of the scale, may produce a disproportionately small change in the readings obtained for the two sample tubes. In the 25µl assay the lowest calibration point is at 5mcg/ml, therefore the readings for the 80% intercept fall below the calibration points, and this may account for the large coefficient of variation obtained.

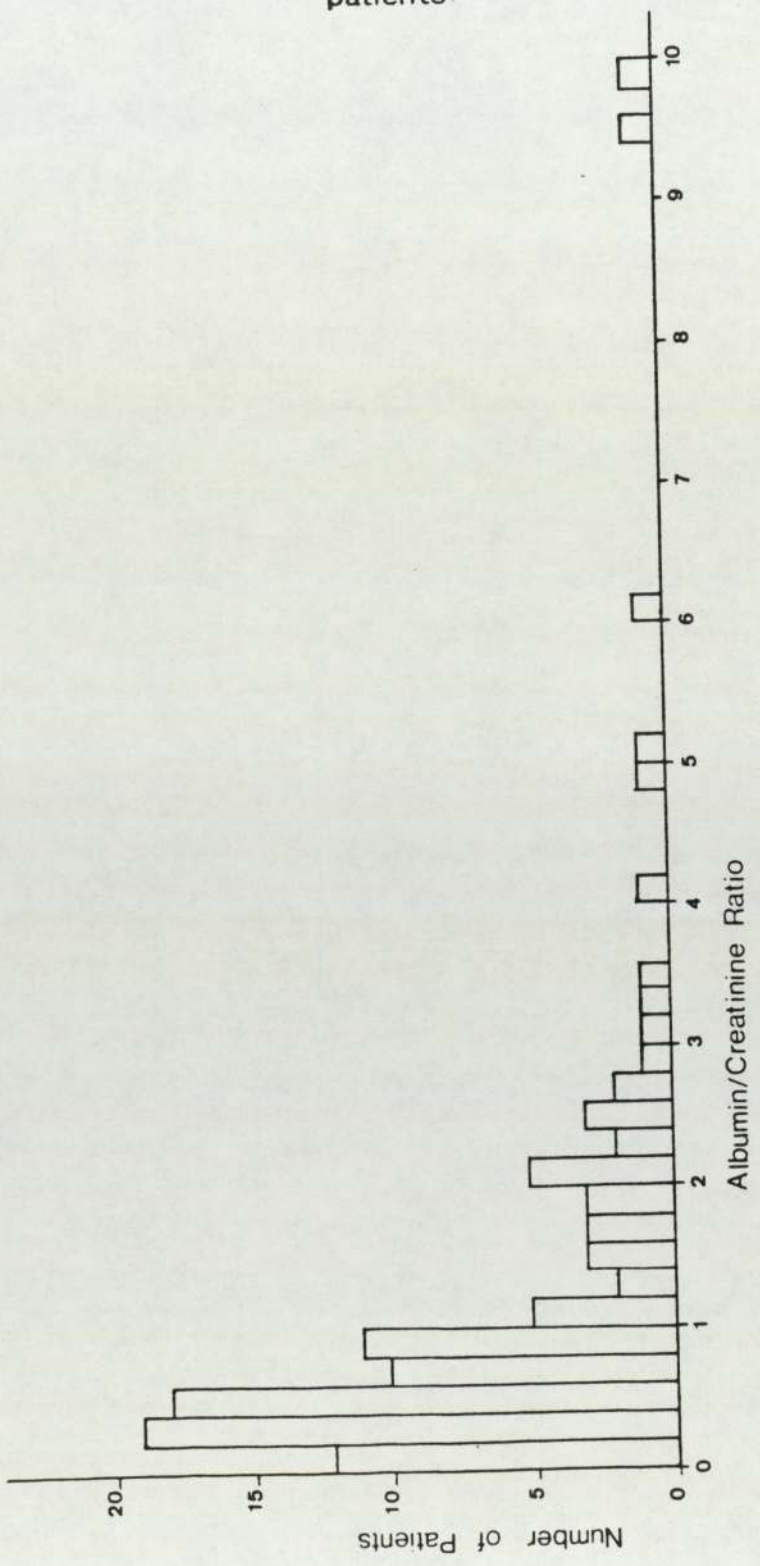
2.8 Expression of Results and Statistical Analyses

Results from the questionnaire are expressed, where appropiate, as the mean value, sample size, and standard deviation. Results from the assay have been expressed as albumin/creatinine ratios as described on page 23.

A frequency plot of albumin/creatinine ratios for normotensive patients (graph 2.6) shows that the distribution has a strong right hand bias. In order to reduce this bias a log scale has been used for the albumin/creatine ratio axis. This right hand bias has also been reported by both Watts et al[20] and Gosling and Beevers[21] in the general population.

Statistical analyses was made using the Student's t-test for paired (or unpaired) data as appropiate. Regression lines and coefficients were calculated using the method of least sum of squares. P values for both t and the regression coefficient were read from statistical tables contained in either Geigy's Scientific Tables 7th Ed or Murdoch and Barnes 2nd Ed. P values less than 0.05 were regarded as statistically significant.

Figure Number: 2.6
Frequency plot of albumin/creatinine ratio for normotensive patients



CHAPTER 3

THE EFFECTS OF VARIOUS FACTORS ON BLOOD PRESSURE AND ALBUMIN EXCRETION SHOWN BY USING A QUESTIONNAIRE

3.1 Results

544 patients completed the questionnaire, with only one person refusing to answer the questions. The analysis of patients seen, by race, sex and treatment (or no treatment), is shown below.

Table 3.1.1

		Treated Hypertensives	Untreated Hypertensives	Total
Men				
Afro-caribbeans	New patients	8	10	18
	Follow-up patients	38	3	41
	Total			59
Caucasians	New patients	38	32	70
	Follow-up patients	76	14	90
	Total			160
Asians	New patients	9	10	19
	Follow-up patients	13	6	19
	Total			38
Total				257

Table 3.1.2

		Treated Hypertensives	Untreated Hypertensives	Total
Women				
Afro-caribbeans	New patients	18	3	21
	Follow-up patients	65	12	77
	Total			98
Caucasians	New patients	46	21	67
	Follow-up patients	77	18	95
	Total			162
Asians	New patients	8	8	16
	Follow-up patients	6	5	11
	Total			27
Total				287

51 patients who completed a questionnaire were also asked to give a urine sample. This represents approximately one tenth of the population screened in the survey. Six patients with frank proteinuria were removed from subsequent analyses. The samples were assayed as described earlier (page 26) and the albumin/creatinine ratios were calculated as described on page 23. The analysis of these patients, by race and sex, is shown below.

Table 3.2

	Women	Men	Total
Caucasian	15	14	29
Afro-caribbean	4	4	8
Asian	4	1	5

The results from the questionnaire were separated into those receiving anti-hypertensive medication and those receiving none. Analyses of the effect on blood pressure (systolic or diastolic) of the various factors (alcohol or caffeine intake or number of cigerettes smoked) was then conducted. This was initially performed on an all or nothing basis, but was then expanded to include ranges of, for example, alcohol intake to see if a moderate intake of the factor affected the blood pressure more or less than a greater or lesser intake. By separating those not on anti-hypertensive treatment from those receiving treatment the effects of the factors on blood pressure could be measured without the additional pharmacological effects of the anti-hypertensive medication obscuring the picture.

Table 3.3

The table shows the effect of alcohol on both systolic and diastolic blood pressure for all patients answering the questionnaire. The results have been divided into those receiving or not receiving anti-hypertensive medication.

Systolic blood pressure (mmHg)	Number of units of alcohol per week				
	0	1-14	15-28	29+	Total
Anti-hypertensive medication	165.25 ±31.72 233	162.0 ±29.62 126	174.54 ±27.57 26	165.59 ±31.89 17	164.85 ±30.86 402
No anti-hypertensive medication	165.10 ±36.91 69	159.11 ±27.12 45	159.80 ±18.87 10	164.13 ±24.28 16	162.69 ±31.53 140
Total	165.22 ±32.92 302	161.24 ±28.93 171	170.44 ±26.06 36	164.88 ±28.02 33	164.29 ±31.02 542

Diastolic blood pressure (nmmHg)	Number of units of alcohol per week				
	0	1-14	15-28	29+	Total
Anti-hypertensive medication	93.67 ±15.02 233	91.10 ±14.75 126	95.46 ±12.22 26	91.47 ±13.69 17	92.89 ±14.73 402
No anti-hypertensive medication	95.51 ±14.59 69	94.42 ±13.61 45	[illegible] ±12.66 10	95.38 ±11.92 16	95.68 ±13.86 140
Total	94.09 ±14.92 302	91.97 ±14.49 171	97.33 ±12.54 36	93.61 ±12.86 33	93.61 ±14.55 542

Results given as: Mean
Standard deviation
Number

A unit of alcohol was defined as a single measure of a spirit, or half a pint of beer.

Table 3.4

The table shows the effect of caffeine on both systolic and diastolic blood pressure for all patients answering the questionnaire. The results have been divided into those receiving or not receiving anti-hypertensive medication.

Systolic blood pressure (mmHg)	Number of units of caffeine per day				
	0	1-5	6-10	11+	Total
Anti-hypertensive Medication	154.62 ±18.25 13	162.44 ±31.96 222	169.81 ±30.69 139	164.19 ±24.91 27	164.86 ±30.90 401
No anti-hypertensive medication	160.33 ±26.49 6	156.36 ±29.27 69	169.80 ±32.49 55	167.78 ±40.88 9	162.59 ±31.62 139
Total	156.42 ±20.60 19	161.00 ±31.40 291	169.81 ±31.12 194	165.08 ±29.08 36	164.27 ±31.07 540

Diastolic blood pressure (mmHg)	Number of units of caffeine per day				
	0	1-5	6-10	11+	Total
Anti-hypertensive medication	95.85 ±10.21 13	92.76 ±14.83 222	92.80 ±15.61 139	92.41 ±11.05 27	92.85 ±14.73 401
No anti-hypertensive medication	96.33 ±15.20 6	95.20 ±12.76 69	95.56 ±14.71 55	98.67 ±18.14 9	95.62 ±13.89 139
Total	96.00 ±11.57 19	93.34 ±14.39 291	93.58 ±15.37 194	93.97 ±13.17 36	93.56 ±14.56 540

Results given as: Mean
Standard deviation
Number

A unit of caffeine was defined as a cup of tea or coffee

Table 3.5

The table shows the effect of cigarettes smoked on both systolic and diastolic blood pressure for all patients answering the questionnaire. The results have been divided into those receiving or not receiving anti-hypertensive medication.

Systolic blood pressure (mmHg)	Number of cigarettes per day				
	0	1-5	6-10	11+	Total
Anti-hypertensive medication	165.50 ±30.31 329	157.47 ±27.84 15	159.70 ±32.30 27	166.00 ±36.93 31	164.85 ±30.86 402
No anti-hypertensive medication	163.95 ±32.61 110	152.20 ±24.76 10	166.00 ±22.45 6	158.79 ±31.19 14	162.69 ±31.53 140
Total	165.11 ±30.87 439	155.36 ±26.25 25	160.85 ±30.54 33	163.76 ±35.05 45	164.29 ±31.02 542

Diastolic blood pressure (mmHg)	Number of cigarettes per day				
	0	1-5	6-10	11+	Total
Anti-hypertensive medication	92.87 ±15.12 329	92.67 ±13.30 15	91.04 ±10.78 27	94.77 ±14.60 31	92.89 ±14.73 402
No anti-hypertensive medication	95.45 ±13.82 110	97.00 ±12.94 10	102.67 ±10.33 6	93.57 ±16.31 14	95.68 ±13.86 140
Total	93.51 ±14.83 439	94.40 ±13.06 25	93.15 ±11.48 33	94.40 ±14.97 45	93.61 ±14.55 542

Results given as: Mean
Standard deviation
Number

From the results presented in Tables 3.3-3.5 we can see that there was no significant correlation between systolic or diastolic blood pressure and number of units of alcohol per week or units of caffeine per day or cigarettes per day for the patients seen at the clinic. Although from inspection of the results there appears to be a trend showing an increase in systolic blood pressure and caffeine intake this did not acheive statistical significance.

For the results obtained from the patients who gave urine samples, graphs were plotted of systolic blood pressure, diastolic blood pressure, units of alcohol per week, number of cigarettes per day, and units of caffeine per day against the logarithm of the albumin/creatinine ratio (Figures 3.6-3.10). The coefficients of correlation are shown in Table 3.11. Since only a small number of samples were taken, it was not possible to divide the results into those receiving anti-hypertensive medication and those not receiving medication in the manner of the questionnaire data.

Figure Number: 3.6
Scatter diagram to show the relationship between Systolic Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for patients answering the questionnaire and giving an urine sample

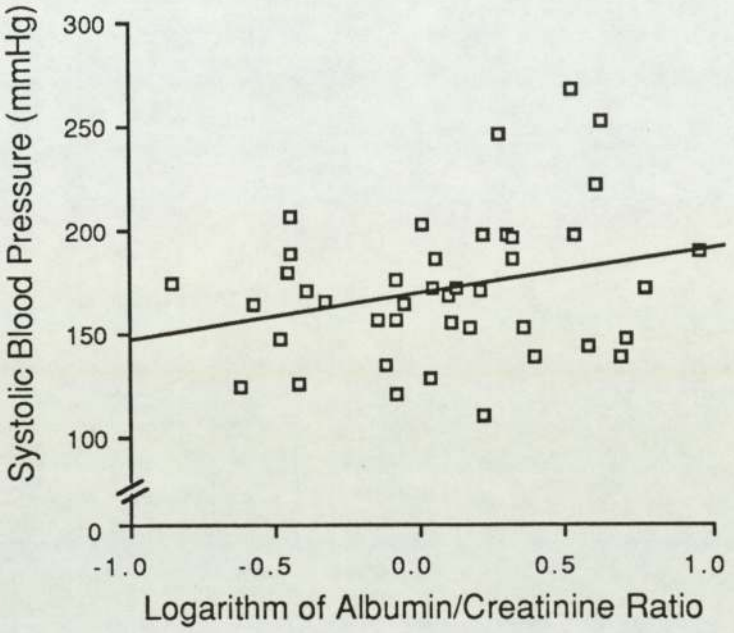


Figure Number 3.7
Scatter diagram to show the relationship between Diastolic Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for patients answering the questionnaire and giving an urine sample

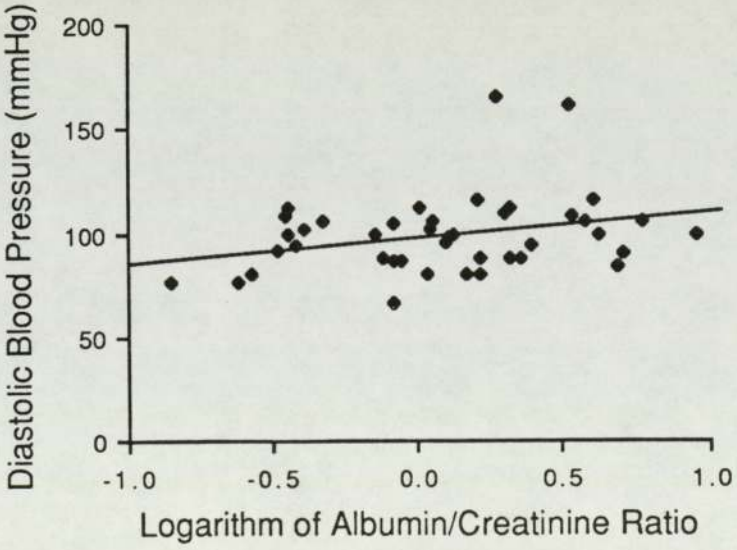


Figure Number: 3.8
Scatter diagram to show the relationship between the average number of cigarettes smoked per day and the logarithm of the Albumin/Creatinine Ratio for patients answering the questionnaire and giving an urine sample

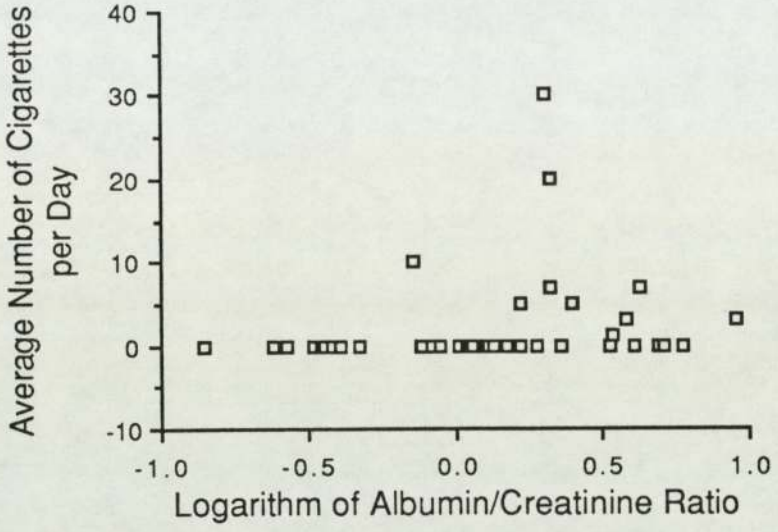


Figure Number: 3.9
Scatter diagram to show the relationship between the average number of cups of tea and coffee drunk per day and the logarithm of the Albumin/Creatinine Ratio for patients answering the questionnaire and giving an urine sample

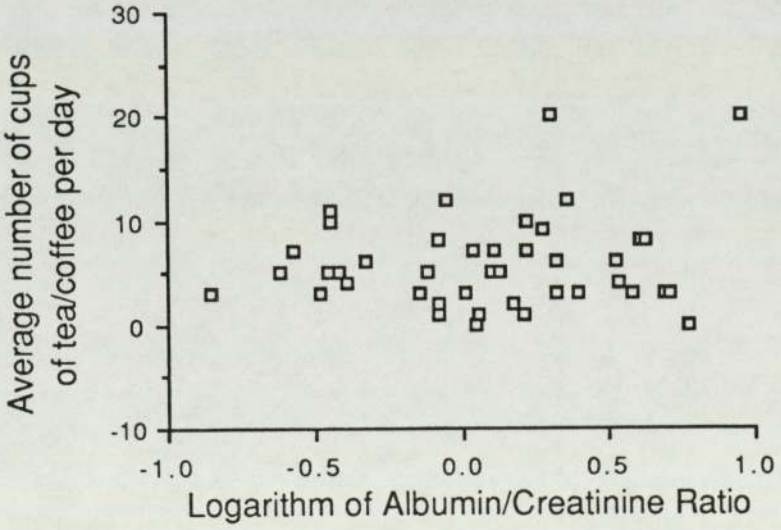


Figure Number: 3.10
Scatter diagram to show the relationship between the number of units of alcohol drunk per week and the logarithm of the Albumin/Creatinine Ratio for patients answering the questionnaire and giving an urine sample

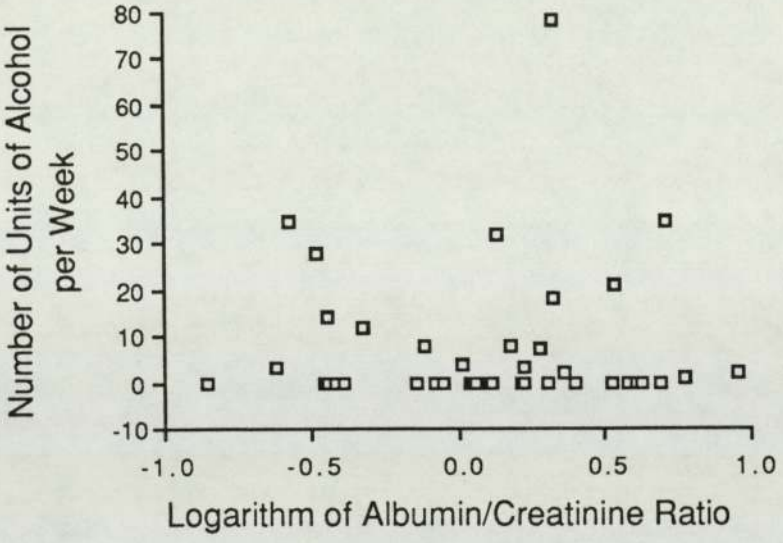


Table 3.11

The following correlation coeffiecents were obtained for the correlation between albumin/creatinine ratio and the various factors under consideration (n = 42):

Systolic blood pressure	Y = 169.75 + 22.10X r= 0.27 (graph 3.6) 0.1<p<0.05
Diastolic blood pressure	Y = 98.01 + 12.58X r= 0.28 (graph 3.7) 0.1<p<0.05
Units of alcohol per week	r= 0.19 (graph 3.8) p>0.1
Number of cigarettes per day	r= 0.14 (graph 3.9) p>0.1
Units of caffeine per day	r=-0.06 (graph 3.10) p>0.1

From the results presented in Table 3.11 and Figures 3.6-3.10 we can see that there was no statistically significant correlation between albumin/creatinine ratio and number of units of alcohol per week or units of caffeine per day or cigarettes per day for the 45 patients who were seen at clinic and gave a urine sample. The strongest correlations ($p<0.1$) were found between the logarithm of the albumin/creatinine ratio and diastolic and systolic blood pressures.

3.2 Discussion

The intention of the questionnaire was to evaluate the extent of the impact that certain factors had on blood pressure in a clinic population, and also the impact that they had on

microalbuminuria. These data are not available and are required in order to assess the suitability of microalbuminuria as a marker of hypertension.

From the results obtained from the questionnaire no significant effect on blood pressure from any of the factors investigated could be found. At first sight this would appear to disagree with the results obtained by other workers.

Previous work has shown that alcohol[10] and caffeine[4,11] intake both result in a rise in blood pressure of about 5-10mmHg compared to the baseline readings. Acute nicotine intake[8,9] (ie during smoking or intravenous injection) causes a rise in blood pressure whilst it appears that the blood pressure in between smoking cigarettes, for smokers, is in fact lower than in non smokers. These studies were however all conducted on healthy normotensive volunteers over relatively short periods of time under controlled circumstances. Data collection for the questionnaire could not be so precisely controlled and is therefore much more variable in nature.

It should be noted that the reported intake of alcohol is likely to be inaccurate. It has been shown[22] that people regularly underestimate their alcohol intake. To date no accurate measurement of alcohol intake has been devised although it seems[23] that a combination of biochemical tests and a questionnaire may prove to be the most reliable. An alcohol

intake diary as used in this questionnaire is easy to complete and gives an idea of alcohol consumption over a week. In some cases the quantity is easy to gauge, for example the person who regularly has one pint in the evening or at a weekend. In most cases however, quantity is far more difficult to judge. This leads to inherent inaccuracies in the data collected. Equally, trying to judge the quantity of tea and coffee drunk in any one day is difficult as the amount will usually vary from day to day. Smokers tend to calculate the amount they smoke from the frequency with which they have to purchase a packet of cigarettes. However, in the clinic environment people are more liable to underestimate their consumption of what they may consider to be 'bad things' in order to appease the doctor, especially if the 'bad thing' concerned is smoking or drinking.

It is therefore not easy to gather accurate data from clinic visits and this may be reflected in the poor correlations found. In the other studies quoted the subjects were given a defined quantity of the substance under investigation in controlled surroundings at set time intervals. For the questionnaires patients were asked to recall their consumption or give an indication of their daily consumption, without any reference to timing, except for alcohol intake. Other factors may have influenced their blood pressure, for example exercise involved in getting to clinic, and stress caused by the clinic visit. It may therefore be that while a factor considered on its own in a controlled enivironment may influence blood pressure, when several factors are combined

together the effects are obscured. In addition the range of blood pressures in the general population is large and can vary from minute to minute for individual people. It is therefore difficult to pick out changes due to a certain factor from amongst the normal variations when considering an uncontrolled population. This may also explain the apparent lack of effect of these factors on albumin excretion. It may be that if each factor were to be studied individually an effect would be found. However, in practice these separate effects would be combined. together with the effects from other factors, for example posture and exercise, which may cancel each other out.

It would seem therefore that the various factors considered may not have a significant effect on albumin excretion, but further work would be needed to clarify this situation, both in normotensive subjects, and also hypertensive subjects.

CHAPTER 4

FURTHER INVESTIGATION OF THE RELATIONSHIP BETWEEN BLOOD PRESSURE AND MICROALBUMINURIA

The results from this previous study (page 40) suggested that there might be a relationship between blood pressure and microalbuminuria. However, the numbers involved in both the earlier cited studies[1,12,13] and this study were relatively small and may, therefore, fail to reflect accurately what actually occurs in the general population. It was, therefore, felt necessary to investigate the relationship between microalbuminuria and blood pressure further using larger numbers of patients to determine whether race or gender influenced the excretion of albumin in urine and whether these factors altered any relationship between microalbumiuria and blood pressure and to determine what effect, if any, drug treatment had on the relationship.

All patients were seen in morning clinics and therefore all the samples collected were morning specimens. Most samples were collected from the 'new patients' clinic and contained a mixture of samples from treated and untreated patients. Records were taken of the blood pressure recorded in clinic, weight, height, and results of the urine dipstick test where possible. Patients with gross albuminuria, shown by two or three 'plusses' on the dipstick, or haematuria, also shown by dipstick reading were excluded from the study.

Contamination of urine with blood leads to grossly raised albumin levels, because the level of albumin in the circulating blood is many times higher than the level normally found in urine. Patients whose urine samples gave a 'trace' (or one 'plus') albumin reading were included. Theorectically, samples giving a one 'plus' reading should be excluded as the albumin level would be expected to fall outside the defined range of microalbuminuria (page 11). However, clinical experience has shown that both the 'trace' and one 'plus' readings are open to operator error. Shaw et al[18] prepared four albumin solutions, in duplicate, and asked 15 people to test them using the dipstick test used here (Albustix). Total agreement between observers on the concentration of albumin present was only found using a solution containing 46mg/l of albumin. When solutions of higher concentration were used the results were spread over three graduations according to the dipstick. In addition, approximately half the observers failed to give the same readings for the duplicate samples.

In practice we found several samples where the assayed albumin concentration varied considerably from the concentration estimated using the dipstick test. The expected ranges for the readings using the Albustix dipstick test would be:

Negative	less than 50mg/l
'Trace' positive	50-200mg/l
'One Plus' positive	mid-point of range 300mg/l
'Two Plus' positive	mid-point of range 1 g/l

However, one sample giving a negative reading was found to contain 70.76mg/l albumin; another sample showing a 'trace' positive reading was found to contain 5.26mg/l albumin, and a sample with a positive one 'plus' reading was found to contain only 30.35mg/l albumin. The observers used to dipstick test the urine were nurses who routinely deal with dipstick testing on a daily basis and would be expected to be familiar with the testing strips.

The samples collected were treated and assayed as described earlier (page 26)

4.1 Results

The albumin/creatinine ratios of the group studied, subdivided according to race, sex and medication (or none) is shown in Table 4.1

Initially the data were plotted as a scatter diagram of blood pressure (systolic or diastolic) against the logarithm of the albumin/creatinine ratio and the regression lines of Y on X and the correlation coefficients were calculated (Figures 4.3-4.4 and Table 4.5). These regression lines were then compared statistically with those obtained from the previous study to determine if they came from similar populations.

Table 4.1: Albumin/Creatinine ratios in treated and untreated patients subdivided according to race. Results are presented as means ± standard error of mean.

	Treated Patients	Untreated Patients	Total
Men			
Afro-caribbeans	0.38 ±0.24	1.44	0.55 ±0.45
n	5	1	6
Caucasians	2.85 ±3.61	2.80 ±4.76	2.82 ±4.22
n	22	22	44
Asians	1.50 ±1.15	2.76 ±2.37	2.13 ±1.96
n	5	5	10
Total	32	28	60
Women			
Afro-caribbeans	1.04 ±0.87	1.61 ±1.26	1.21 ±1.04
n	9	4	13
Caucasians	2.35 ±2.68	0.82 ±0.71	1.89 ±2.38
n	14	6	20
Asians	0.32 ±0.20	1.86 ±0.50	1.09 ±0.86
n	2	2	4
Total	25	12	37

Table 4.5: Regression line data for results shown in tables 4.3 and 4.4.

Systolic Blood Pressure	$Y = 160.29 + 14.19X$ $r = 0.31$ $0.01<p<0.001$
Diastolic Blood Pressure	$Y = 94.43 + 7.53X$ $r = 0.30$ $0.01<p<0.001$

The data were then divided depending on whether or not the patient was receiving antihypertensive medication. Scatter diagrams of the blood pressure (systolic and diastolic) against the logarithms of the albumin/creatinine ratios were plotted. The regression line of Y on X and the coefficient of correlation were calculated for each graph (Figures 4.6-4.7, Table 4.8).

Table 4.8: Regression line data for results shown in tables 4.6 and 4.7

	Systolic Blood Pressure	Diastolic Blood Pressure
Treated Patients (n=57)	$Y = 158.06 + 17.75X$ $r = 0.36$ $0.01<p<0.001$	$Y = 94.11 + 5.51X$ $r = 0.28$ $0.05<p<0.02$
Untreated Patients (n=40)	$Y = 163.60 + 9.69X$ $r = 0.22$ $p>0.1$	$Y = 96.23 + 8.07X$ $r = 0.32$ $0.1<p<0.05$

Figure Number: 4.3
Scatter diagram to show the relationship between Systolic Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients

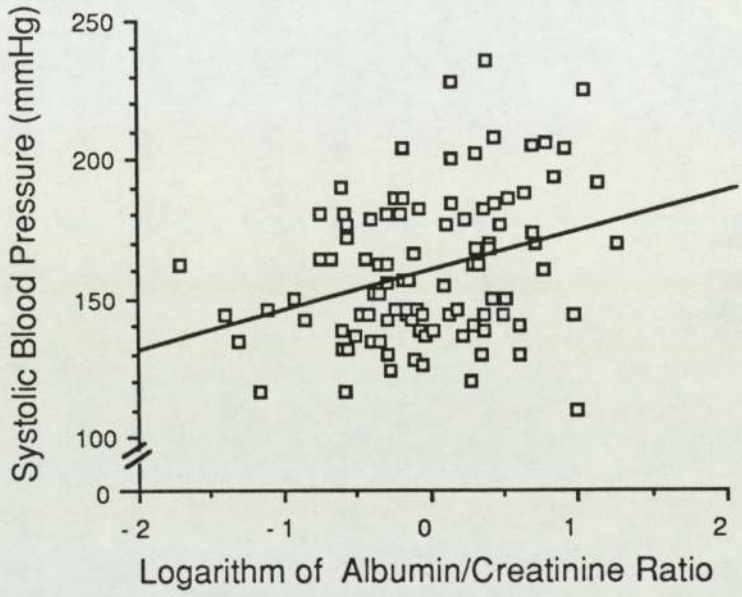


Figure Number: 4.4
Scatter diagram to show the relationship between Diastolic Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients

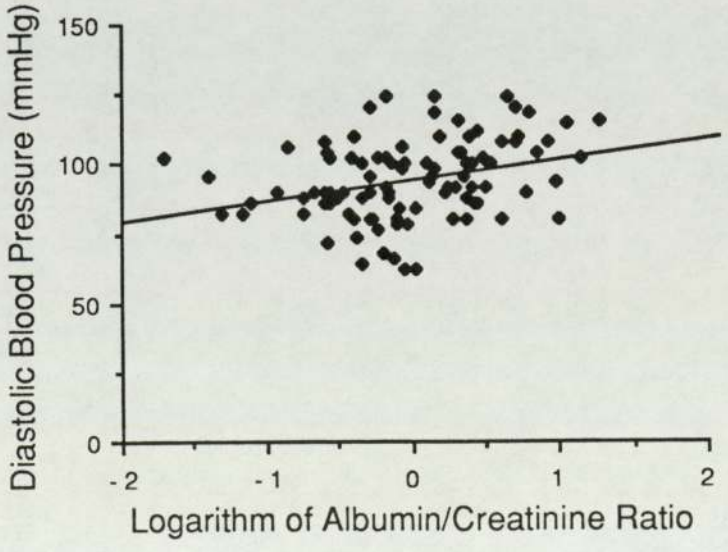


Figure Number : 4.6
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients who were not receiving medication

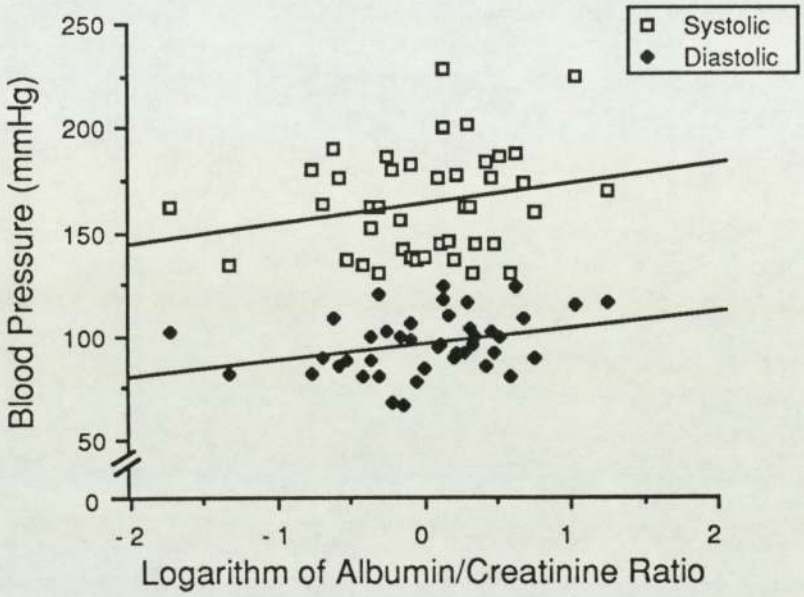
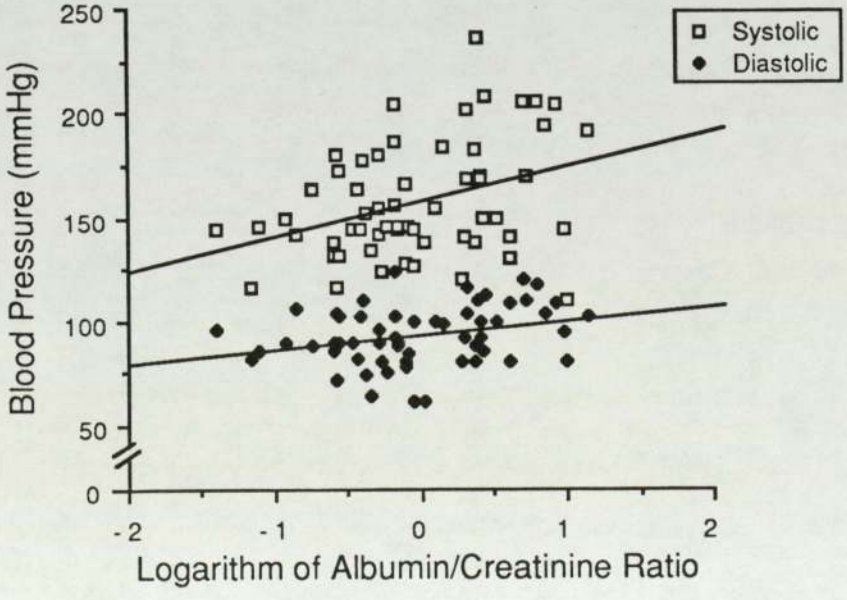


Figure Number: 4.7
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients who were receiving medication



These data were further subdivided according to gender (Figures 4.9-4.12). The following regression lines and coefficients of correlation were calculated:

Table 4.13	Systolic Blood Pressure	Diastolic Blood Pressure
Men		
Treated Patients (n=32)	Y = 158.99 + 17.75X r = 0.42 0.02<p<0.01	Y = 94.11 + 5.51X r = 0.28 p>0.1
Untreated Patients (n=28)	Y = 164.73 + 6.38X r = 0.20 p>0.1	Y = 99.33 + 13.92X r = 0.41 0.05<p<0.02
All Patients (n=60)	Y = 161.45 + 16.63X r = 0.34 p>0.02	Y = 94.30 + 8.11X r = 0.32 p>0.02
Women		
Treated Patients (n=25)	Y = 156.85 + 15.21X r = 0.27 p>0.1	Y = 91.95 + 9.58X r = 0.30 p>0.1
Untreated Patients (n=12)	Y = 160.34 + 6.38X r = 0.28 p>0.1	Y = 99.33 + 5.13X r = 0.34 p>0.1
All Patients (n=37)	Y = 158.09 + 10.24 r = 0.24 p>0.1	Y = 94.53 + 6.79X r = 0.27 p>0.05

Figure Number: 4.9
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all male patients who were not receiving medication

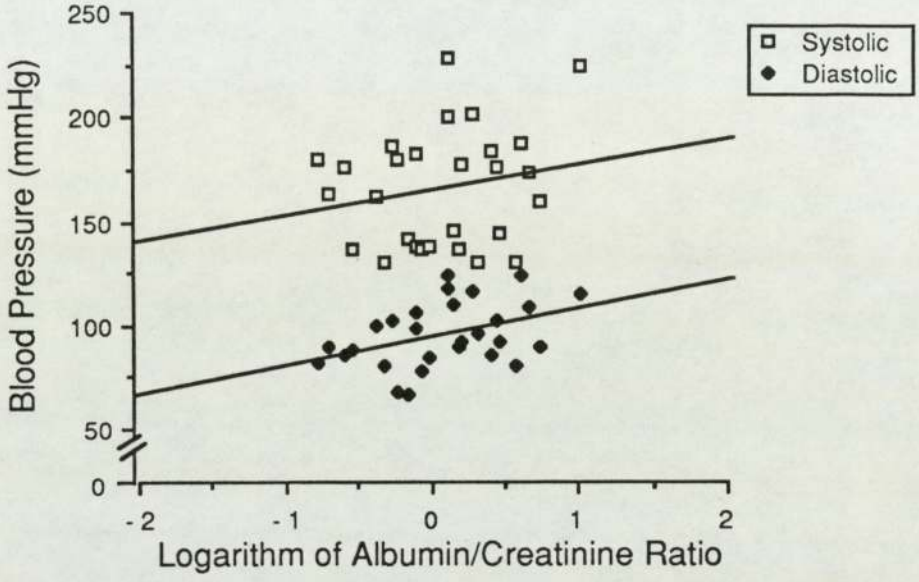


Figure Number: 4.10
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all male patients who were receiving medication

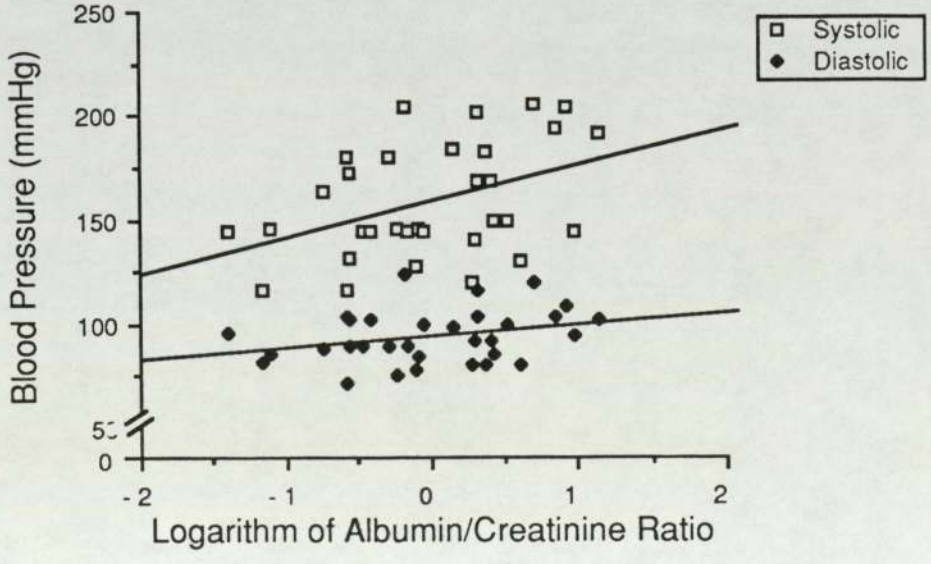


Figure Number: 4.11
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all female patients who were not receiving medication

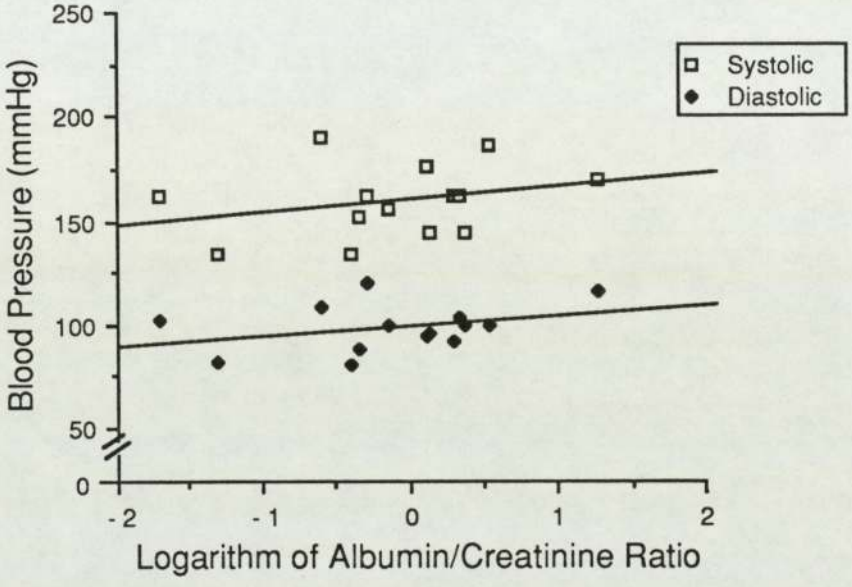
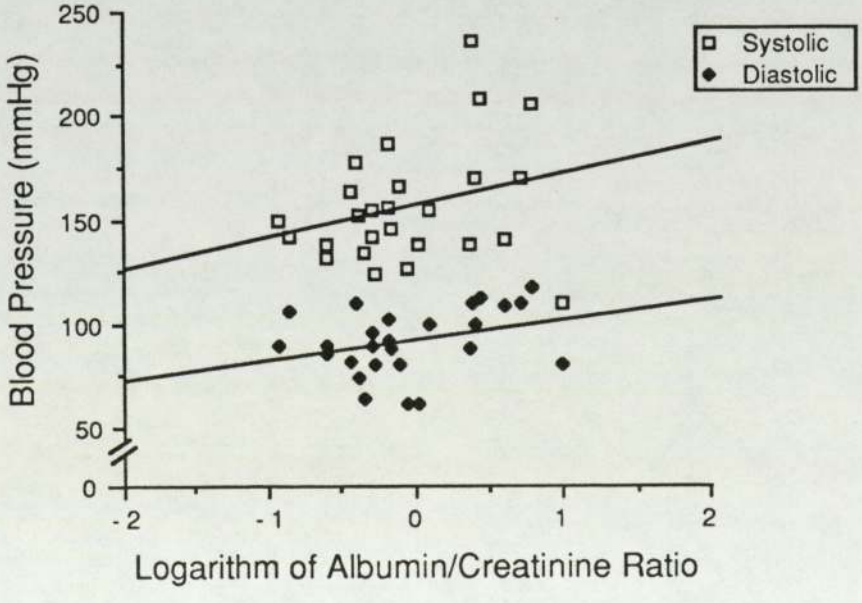


Figure Number: 4.12
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all female patients who were receiving medication



The data were also analysed according to racial group (caucasian or non caucasian). The regression lines and coefficients of correlation are shown in Table 4.18 and Figures 4.14-4.17.

Table 4.18	Systolic Blood Pressure	Diastolic Blood Pressure
Caucasians		
Treated Patients (n = 36)	Y = 151.81 + 17.47X r = 0.36 0.05>p>0.02	Y = 92.43 + 8.24X r = 0.35 0.05>p>0.02
Untreated Patients (n = 28)	Y = 163.98 + 13.10X r = 0.27 p>0.1	Y = 94.59 + 3.58X r = 0.16 p>0.1
Non-caucasians		
Treated Patients (n = 21)	Y = 153.09 + 19.97X r = 0.37 0.1>p>0.05	Y = 89.87 + 0.84X r = 0.03 p>0.1
Untreated Patients (n = 12)	Y = 163.10 + 12.93X r = 0.25 p>0.1	Y = 97.52 + 11.00X r = 0.34 p>0.1

Within each racial group the data were further subdivided according to gender. The regression lines and coefficients of correlation obtained are shown in Table 4.19.

Figure Number: 4.14
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all caucasian patients who were not receiving medication

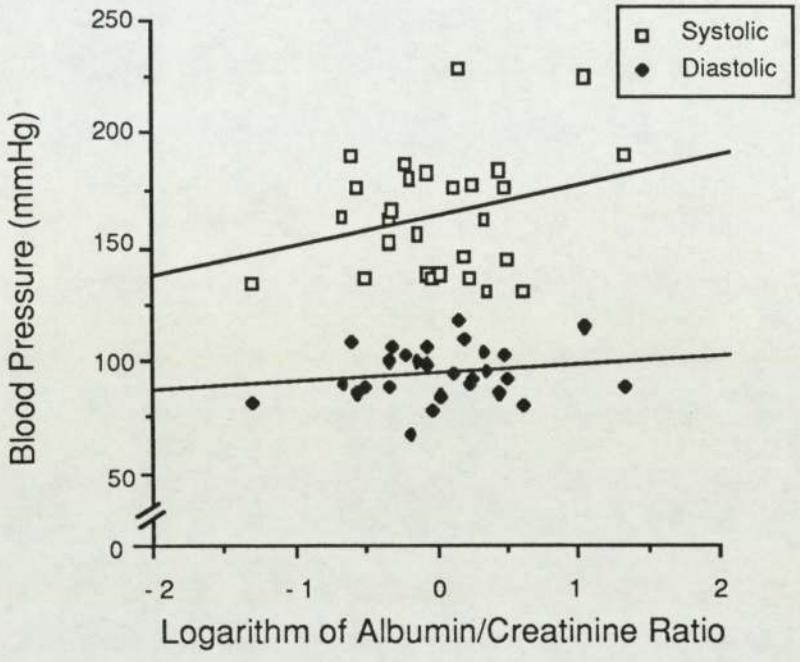


Figure Number: 4.15
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all caucasian patients who were receiving medication

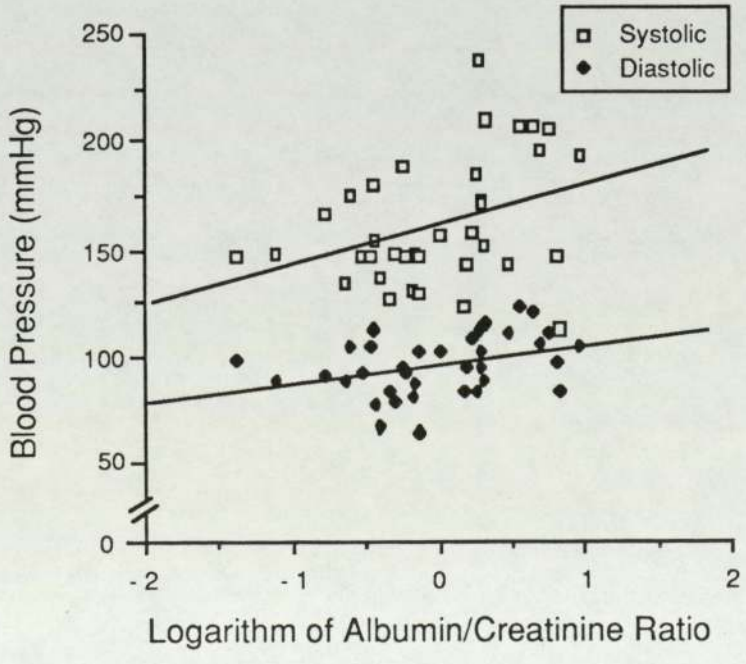


Figure Number: 4.16
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all non-caucasian patients who were not receiving medication

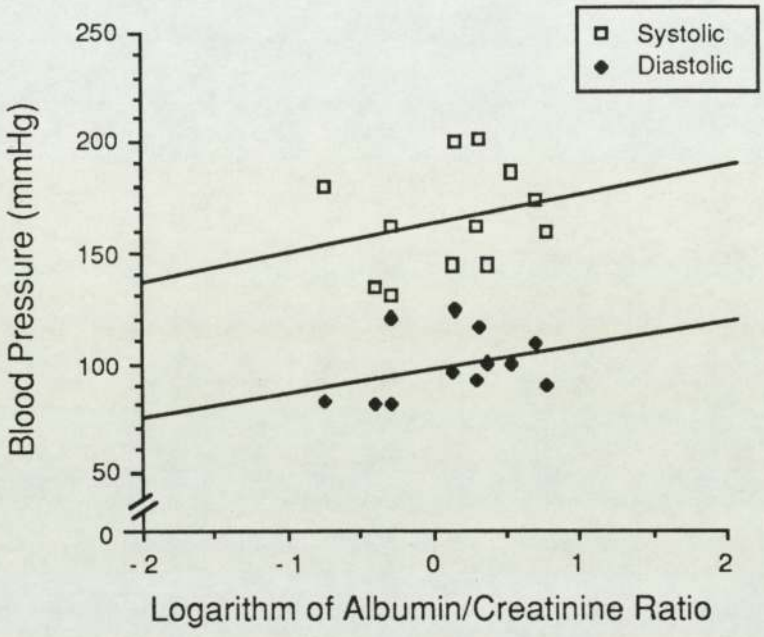


Figure Number: 4.17
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all non-caucasian patients who were receiving medication

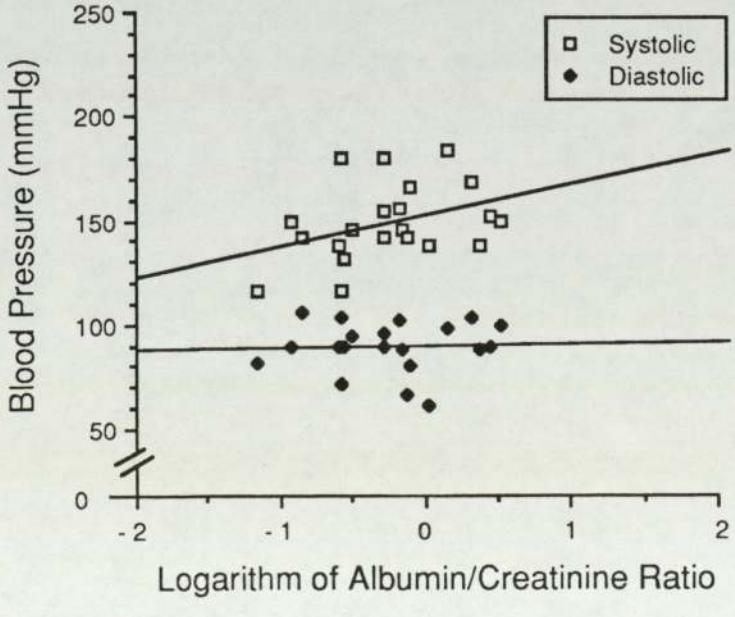


Table 4.19: Regression line data and correlation coefficients for data presented in Figures 4.14-4.17 subdivided according to gender.

	Systolic Blood Pressure	Diastolic Blood Pressure
Afro-caribbeans		
Women (n = 13)	Y = 153.00 + 5.18X r = 0.20 p>0.1	Y = 91.07 - 6.74X r =-0.27 p>0.1
Men (n = 6)	Y = 170.50 + 51.56X r = 0.85 0.05<p<0.02	Y = 105.83 + 30.01X r = 0.86 0.05<p<0.02
Women and Men (n = 19)	Y = 158.23 + 20.28X r = 0.49 0.05<p<0.02	Y = 95.49 + 5.20X r = 0.18 p>0.1
Caucasians		
Women (n = 20)	Y = 161.60 + 12.54X r = 0.24 p>0.1	Y = 93.90 + 12.61X r = 0.46 0.05<p<0.02
Men (n = 44)	Y = 161.13 + 15.22X r = 0.31 0.05<p<0.02	Y = 93.95 + 5.44X r = 0.24 p>0.1
Women and Men (n = 64)	Y = 161.30 + 14.26X r = 0.28 0.05<p<0.02	Y = 93.87 + 7.98X r = 0.33 0.01<p<0.001
Asians		
Women (n = 4)	Y = 147.20 - 5.94X r = 0.63 p>0.1	Y = 95.34 + 7.62 r = 0.88 p>0.1
Men (n = 10)	Y = 164.92 + 6.09X r = 0.14 p>0.1	Y = 96.86 + 9.30X r = 0.45 p>0.1
Women and Men (n = 14)	Y = 160.46 + 6.03X r = 0.15 p>0.1	Y = 96.51 + 9.11X r = 0.50 0.05<p<0.02

The 544 patients who answered the questionnaire represent approximately two-thirds of the total clinic population (page 40). From this data it is possible to depict the racial and gender mix of patients that attend the hypertension clinics at Dudley Road

Hospital. When the data from the questionnaire is compared with the above data it can be seen that there was a smaller proportion of women in this study than would be expected. In addition whilst the racial mix of the female group reflects the racial mix of the clinic population, there is a bias towards caucasians within the male group studied. It is not known why these differences have occurred. It does mean however that the numbers of patients in some of the subdivsions are small and therefore meaningful interpretations of the regression lines and correlation coefficients is not possible. The data are presented for the sake of completeness, however the scattergrams that accompany these results have been omitted.

4.2 Discussion

In the previous study some correlation was found between systolic and diastolic blood pressures and albumin/creatinine ratios. This was with a mixed population of treated and untreated presumed hypertensive subjects using a sample size of just over forty. This present study investigated the relationship between albumin/creatinine ratio and systolic and diastolic blood pressures in a second group of both treated and untreated patients, each with a sample size of about forty. The untreated group of patients could be considered to be pharmacologically pure and therefore any correlation that existed between blood pressure and albumin/creatinine ratio could be expected to be revealed. However, it was found that separating the treated

from the untreated patients failed to significantly alter the correlation coefficients or the slope of the lines. The correlation coefficients between blood pressure and albumin/creatinine ratio remained fairly constant, whether the subject group contained treated, untreated or both treated and untreated subjects. Subdividing the data by gender did not reveal any significant alteration in the correlation coefficients, nor did subdividing the data in relation to race. It was not possible to make any comparisons when the data were subdivided by both sex and race because of the small numbers in some groups.

CHAPTER 5

COMPARISON OF ALBUMINURIA IN PATIENTS TREATED WITH EITHER LISINOPRIL OR ATENOLOL

5.1 Background

Lisinopril is an angiotensin converting enzyme inhibitor (ACEI). It is directly acting, requiring no enzymatic conversion, and structually it contains no sulphydryl group. The sulphyldryl group, which is present in captopril, was thought to be responsible for some of the side effects of captopril. Lisinopril is the lysine analogue of enalaprilate (the active metabolite of enalapril maleate), and in man it has a half life of approximately 12 hours and, therefore, only needs to be given once a day. Minimal protein binding occurs and the drug is excreted unchanged by the kidneys.

Atenolol is a well established cardio-selective beta-blocker, which is widely used in the treatment of hypertension.

Studies[24,25] have shown that a statistically significant fall in both systolic and diastolic blood pressure occurs when lisinopril, in a daily dose of 20 to 40mg, is administered. Daily doses of 1.25mg and 5mg produced no significant change in blood pressure compared to placebo. An anti-hypertensive effect may be achieved with a dose of 10mg of lisinopril each day. The recommended starting dose of atenolol in younger patients with

normal renal function is 100mg a day. However, studies[26,27] have shown that an effective response may be shown with a daily dose of 50mg, and that increasing the daily dose above 100mg failed to increase the anti-hypertensive effect.

A large multicentre study comparing the effects of lisinopril and atenolol has already been conducted and the results published[28]. Lisinopril, in the range of 20 to 80mg each day, or atenolol, in the range of 50 to 200mg each day, and a matching placebo was given to the patients, the dose being increased if the blood pressure failed to respond adequately to the treatment. If the maximum dose of either drug was reached without adequate blood pressure control, hydrochlorothiazide in the range 12.5 to 25mg (non USA participants) or 25 to 50 mg (USA participants) was added to the regime. This study showed that lisinopril was as effective as atenolol in reducing blood pressure, and in fact the reduction in sitting systolic blood pressure achieved by lisinopril was significantly greater than that achieved by atenolol. Significantly more patients on atenolol required additional hydrochlorothiazide therapy, compared to patients receiving lisinopril. Cough and dizziness were seen more frequently in the lisinopril treated group than in the atenolol group, whilst the atenolol group had a significantly higher incidence of chest pain. The occurance of proteinuria (>1g/day) was similar in both groups, but it was shown for two of the lisinopril treated patients that the proteinuria was dose related.

5.2 Study Design

A study was carried out at Dudley Road Hospital as part of a large multicentre trial to compare the effects of lisinopril and atenolol on blood pressure, and to compare the side-effect profile. Ethical approval was received from the District Ethical Committee. Patients over 18 with essential hypertension and a sitting diastolic blood pressure in the range 95-130 mmHg were admitted into the study. Patients with secondary or accelerated hypertension were excluded from the trial, as were patients with congestive heart failure. Patients who had evidence of a myocardial infarction or a cerebral vascular accident within the last six months were also excluded. Patients with renal or hepatic disease or dysfunction or abnormal haematological profile or serum potassium concentration were also excluded. Patients were also excluded if they were already on atenolol, or had suffered side effects to β-blockers or ACEIs. Exclusion also occured if there was any contraindication to atenolol or lisinopril as determined from the appropiate data sheets. Finally, any pregnant woman, or woman of child-bearing potential, including those on hormonal contraceptives was also excluded from entering the trial. The design of the study was double-blind, randomised, with parallel groups, with patients receiving either active atenolol or active lisinopril, and placebo lisinopril or placebo atenolol as appropiate. The dose of both drugs was increased until the diastolic blood pressure remained less than 90mmHg or a maximun dose of 40mg lisinopril or 100mg atenolol was reached.

5.3 Results

Thirty-five patients entered into the placebo phase of the study. Five patients were withdrawn after the placebo phase because their diastolic blood pressure fell above or below the range 95-130mmHg. Therefore, thirty patients entered into the active part of the study, of whom twenty-three completed the study, the remainder being withdrawn due to causes such as non-compliance, failure to attend and side effects. The seven patients who were withdrawn were distributed evenly between the two groups. Unfortunately some patients did not give a urine sample at every visit. This left 16 patients for whom we had results at the beginning and end of the trial.

Three graphs were plotted of blood pressure (systolic and diastolic) and the logarithm of the albumin/creatinine ratio for each patient, against time (graphs 5.1 - 5.3). Graph 5.1 shows the results for all patients in the study, and graphs 5.2 and 5.3 show the results for the patients in each of the two groups.

Figure Number: 5.1
Graph of systolic blood pressure (■), diastolic blood pressure (□) and the albumin/creatinine ratio (●) against time for all patients in the lisinopril/atenolol study

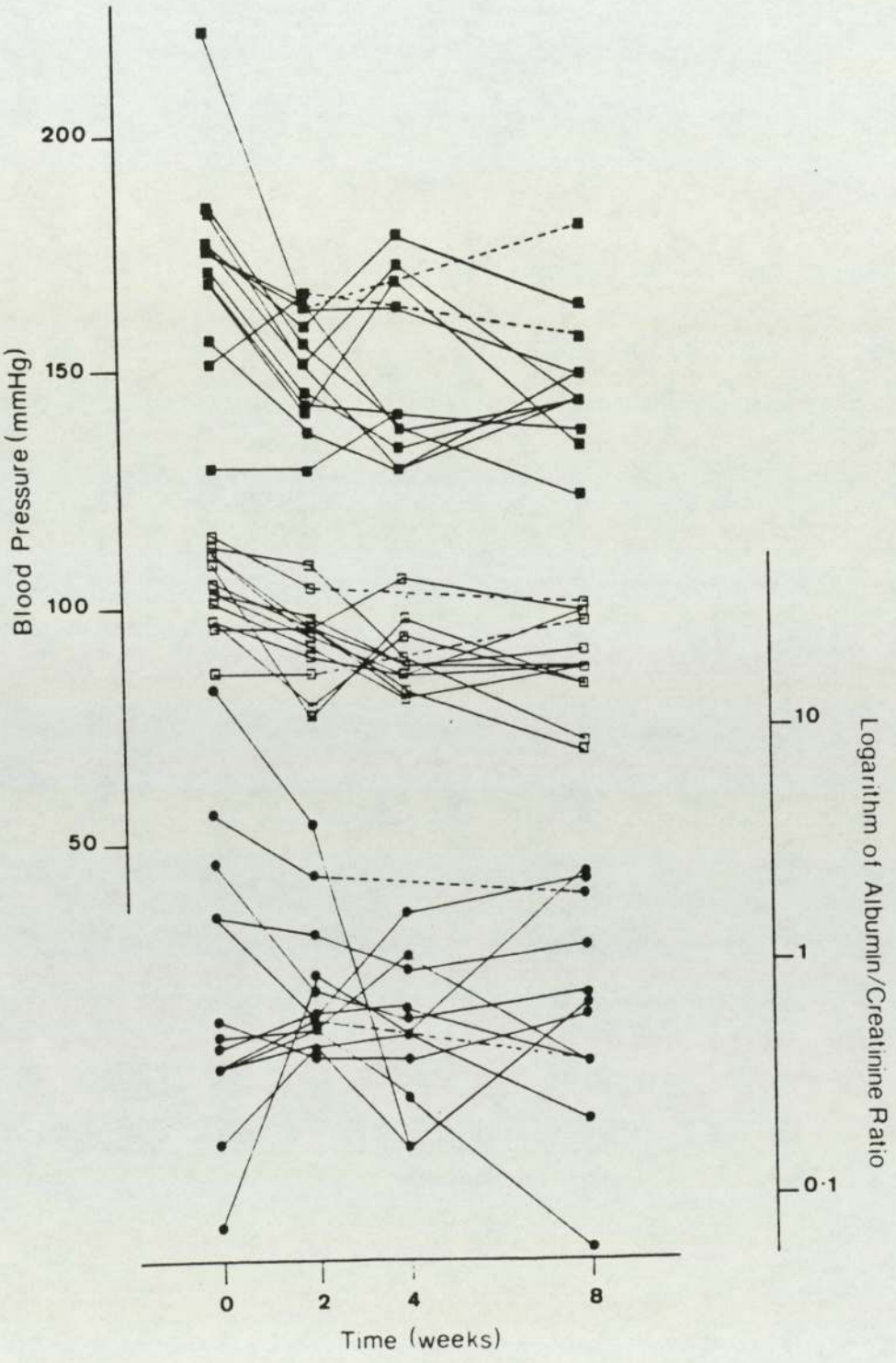


Figure Number: 5.2
Graph of systolic blood pressure (■), diastolic blood pressure (□) and the albumin/creatinine ratio (●) against time for all patients in the lisinopril group

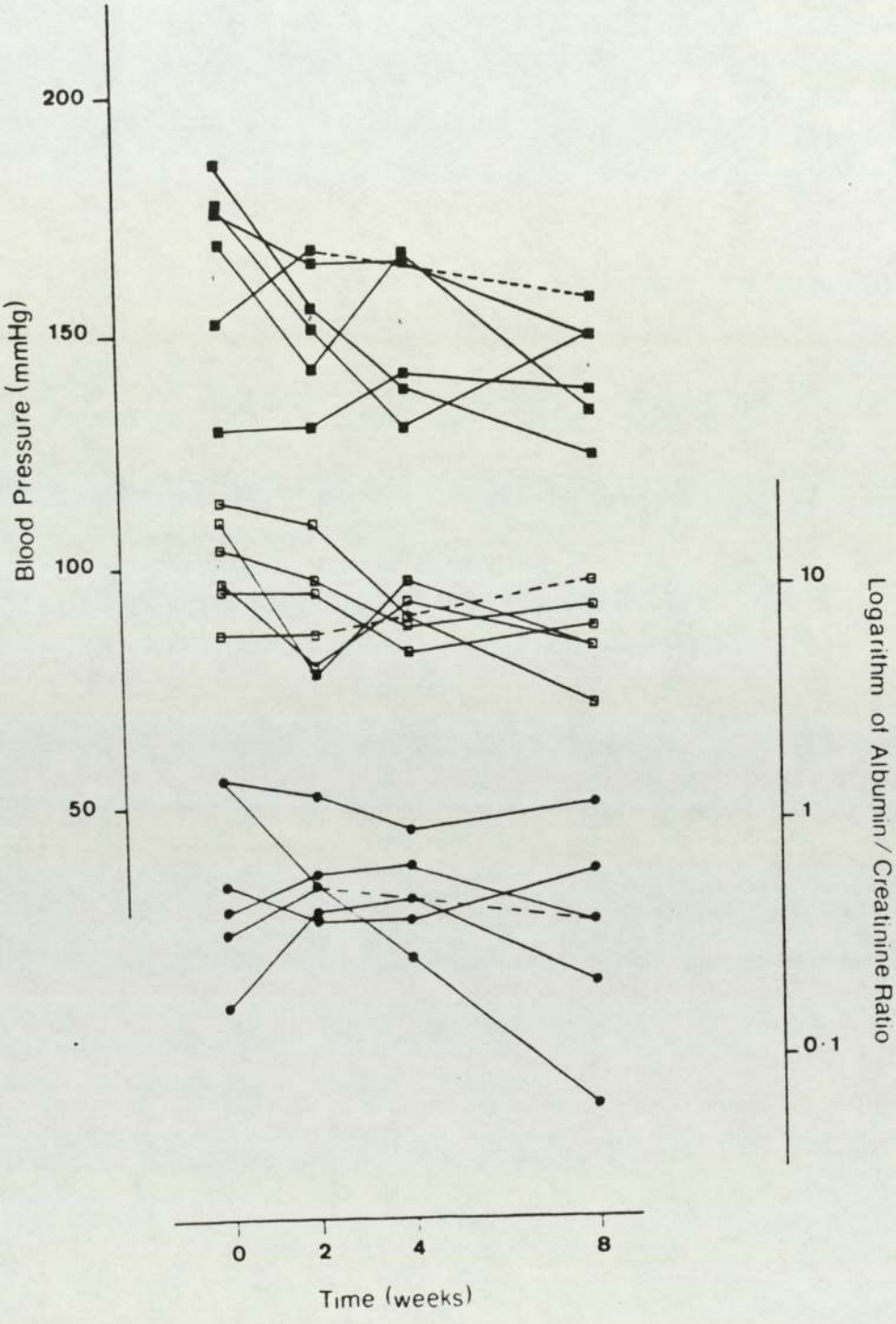
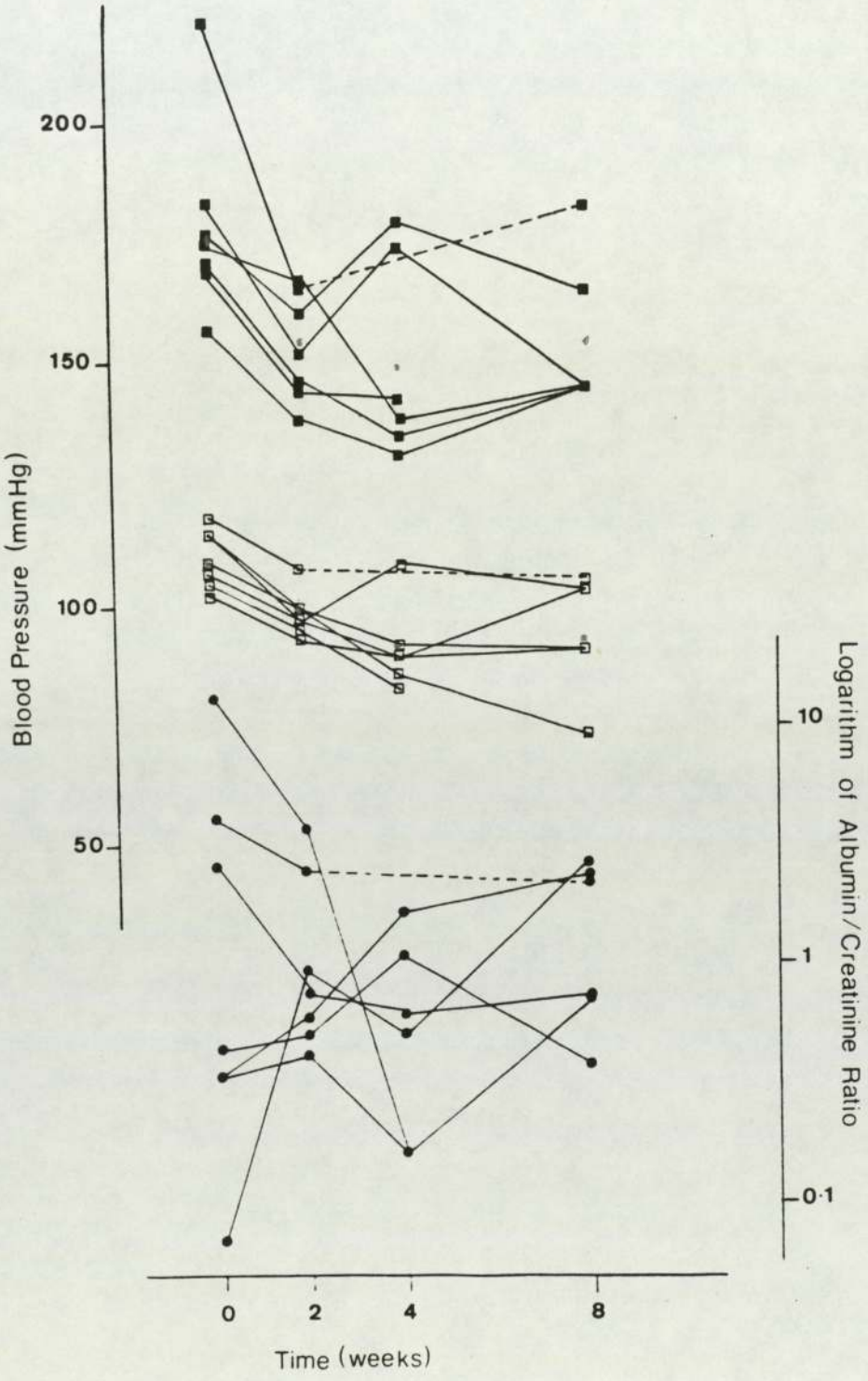


Figure Number: 5.3
Graph of systolic blood pressure (■), diastolic blood pressure (□) and the albumin/creatinine ratio (●) against time for all patients in the atenolol group



Using Student's t-test a statistically significant fall in both diastolic and systolic blood pressures from the beginning to the end of the trial could be shown for all patients who entered into the trial. This was also shown for the atenolol group and, to a lesser extent, for the lisinopril group. The fall in the albumin/creatinine ratio over the trial period was not statistically significant for the study group as a whole or for the atenolol group and was only marginally significant for the lisinopril group.

Table 5.4

	All Patients in Study	Atenolol Group	Lisinopril Group
Systolic blood pressure (mmHg)	t=3.98 0.01>p>0.001	t=4.910 0.01>p>0.001	t=1.775 0.5>p>0.1
Diastolic blood pressure (mmHg)	t=3.519 0.01>p>0.001	t=3.155 0.02>p>0.01	1.804 0.5>p>0.1
Albumin/creatine ratio	t=0.438 p>0.5	t=0.122 p>0.5	t=1.188 0.5>p>0.1

Results of statistical analyses using Student's t-test for paired data, comparing readings taken at the beginning of the study (week 0) with readings taken at the end of the study (week 8)

A t-test was conducted between paired samples at week 0 and week 2 for all patients studied, and also the two individual groups. This revealed that a highly significant fall in blood pressure occured for the whole group during these two weeks (0.001>p for both systolic and diastolic blood pressure). These values were similar for the atenolol group (0.01>p>0.001 for systolic blood pressure and 0.001>p for diastolic blood pressure)

but decreased in the lisinopril group ($0.1>p>0.05$ for systolic blood pressure and $0.05>p>0.02$ for diastolic blood pressure). The change in the albumin/creatinine ratio over the two weeks was only marginally significant in all the groups ($0.5>p>0.1$).

No correlation was found between blood pressure, systolic or diastolic, and albumin/creatinine ratio for the total cohort of patients studied in the trial.

Graphs were plotted of blood pressure (mmHg) against the logarithm of the albumin/creatinine ratio for all patients in the trial at week 0 (Figure Number 5.5) and week 8 (Figure Number 5.6). From these results it can be seen that initially there is no relationship between albumin/creatinine ratio and blood pressure, but that after eight weeks of treatment a relationship appeared to have developed. The patients were then separated into the two groups, those on atenolol and those on lisinopril and graphs were then plotted of blood pressure against albumin/creatinine ratio at the beginning and end of the trial (Figures 5.7-5.10). These showed that there was no correlation in the atenolol group at the beginning of the study and a slight negative correlation in the lisinopril group at the beginning. At the end of the study the atenolol group showed a stronger correlation between blood pressure and albumin/creatinine ratio, than the lisinopril group.

Figure Number: 5.5
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients in the study at Week 0

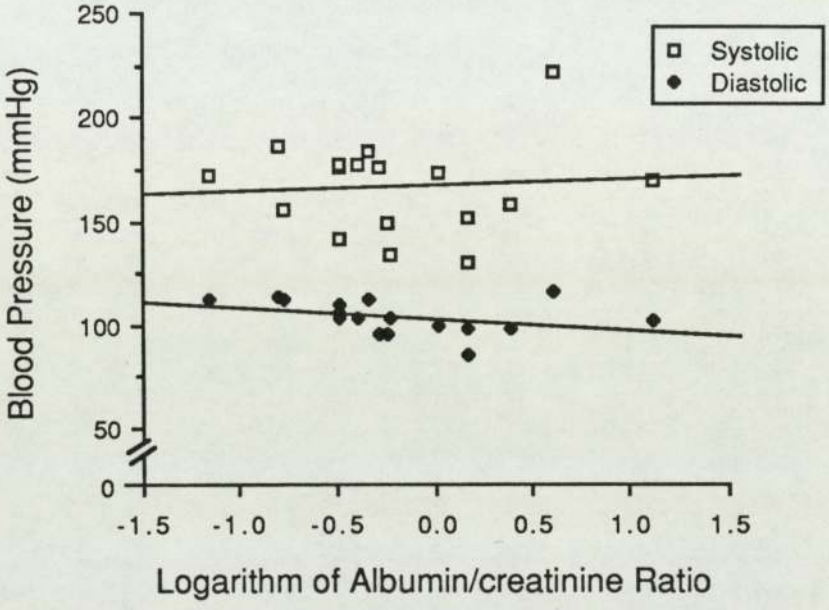


Figure Number: 5.6
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients in the study at Week 8

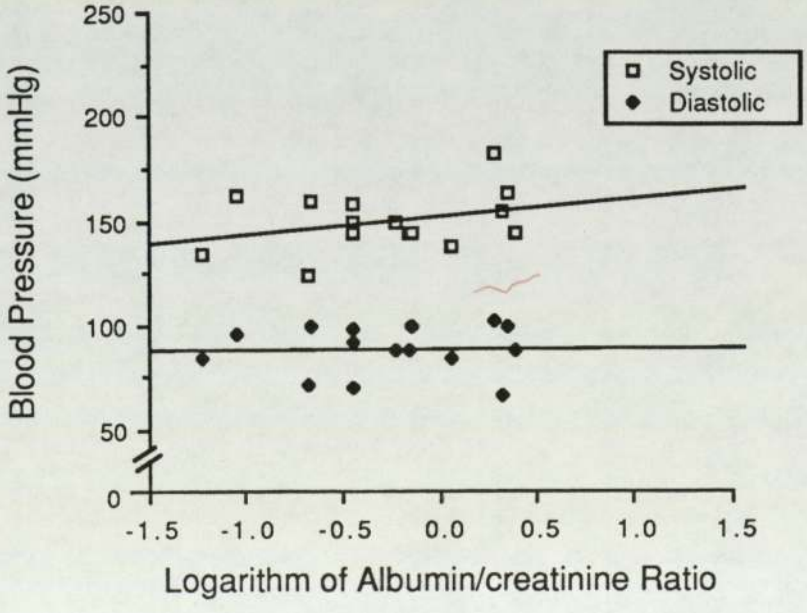


Figure Number: 5.7
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/creatinine Ratio for all patients in the Lisinopril group at Week 0

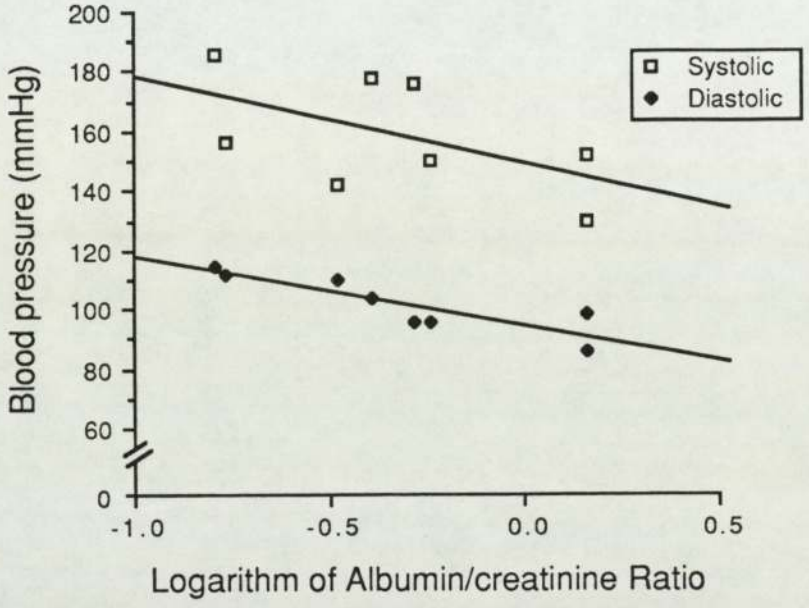


Figure Number: 5.8
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients in the Lisinopril group at Week 8

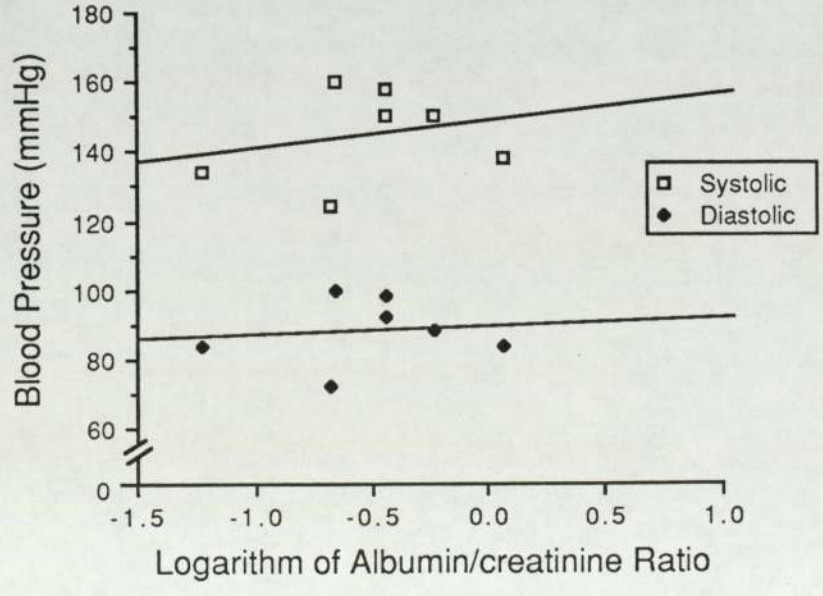


Figure Number: 5.9
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients in the Atenolol group at Week 0

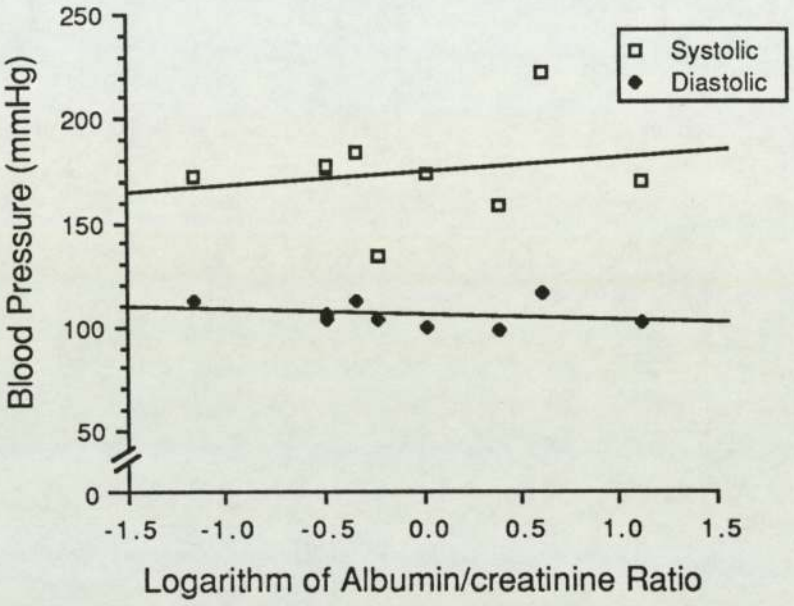


Figure Number: 5.10
Scatter diagram to show the relationship between Blood Pressure and the logarithm of the Albumin/Creatinine Ratio for all patients in the Atenolol group at Week 8

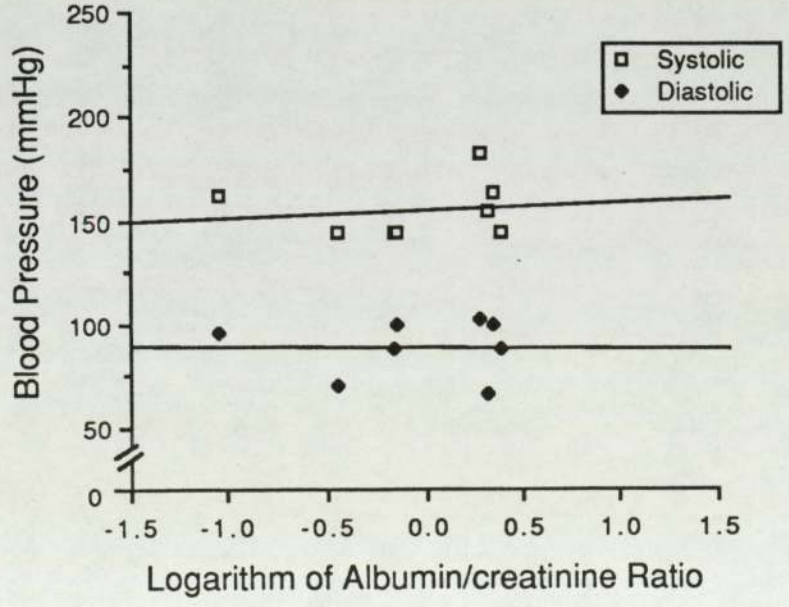


Table 5.11

	Systolic	Diastolic
All Patients		
Week 0	r= 0.02 n=16 p>0.1	r=-0.38 n=16 p>0.1
Week 8	r= 0.40 n=13 p>0.1	r= 0.31 n=13 p>0.1
Atenolol		
Week 0	r= 0.17 n=8 p>0.1	r=-0.29 n=8 p>0.1
Week 8	r= 0.34 n=6 p>0.1	r= 0.37 n=6 p>0.1
Lisinopril		
Week 0	r=-0.67 n=8 p>0.1	r=-0.89 n=8 p>0.1
Week 8	r= 0.24 n=7 p>0.1	r= 0.10 n=7 p>0.1

Correlation coefficients and sample size obtained from the regression analyses between blood pressure and albumin/creatinine ratio for the groups stated above.

From the results in Table 5.4 it can be seen that for the patients studied in this trial atenolol produced a significant fall in blood pressure, whereas lisinopril did not. Overall no correlation was found between blood pressure and albumin/creatinine ratio. However, drug treatment did seem to alter the relationship so that after eight weeks treatment the correlation became more positively inclined.

The study that these results were collected from was part of a much larger multicentre trial. As a consequence the number entered into the trial at Dudley Road Hospital is unlikly to be

sufficient to produce a statistically significant alteration in blood pressure or microalbuminuria. These results can therefore only point to a possible trend which would require further investigation to be confirmed.

5.4 Discussion

In this study untreated proven hypertensive patients were treated with one of two antihypertensive medications (of differing modes of action) in increasing doses until their blood pressure was reduced and maintained at a diastolic blood pressure of 95mmHg or less. Both drugs were used in doses up to their recommended maximum dose given in their product licence (40mg for lisinopril and 100mg for atenolol). Although a fall in blood pressure occurred in both groups only that in the atenolol group acheived statistical significance. There are several possible reasons why the patients in the lisinopril group failed to show a significant fall in blood pressure. From Figures 5.2 and 5.3 it can be seen that atenolol causes a relatively rapid fall in blood pressure compared to lisinopril, hence lisinopril may take longer than 8 weeks to acheive its full antihypertensive action. In addition some patients may prove in clinical practice to require higher doses of lisinopril than those currently recommended. Finally as stated before it may be that there are insufficient numbers in the subdivided groups to reveal statistically significant changes. At the same time the albumin/creatinine ratios for these patients were measured, before, during and after

treatment with the antihypertensive medication. It is of note that no significant change in the albumin excretion rate (as measured by the albumin/creatinine ratio) was found as their blood pressure was reduced despite the use where necessary of the maximum reccomended dose of the drug.

However the main purpose of the study was to lower blood pressure in newly diagnosed hypertensive patients by different pharmacological methods and to determine the effect that this had on the albumin/creatinine ratio. Despite achieving a statistically significant fall in blood pressure for the whole group studied no significant change in the albumin/creatinine ratio was found.

CHAPTER 6

GENERAL DISCUSSION

The present study has further investigated the relationship between blood pressure and microalbuminuria which was first reported by Parving et al in 1974[1]. These workers found a linear relationship such that a higher blood pressure was associated with a high albumin excretion rate. Since then relatively little work has been published concerning blood pressure and microalbuminuria although the role of microalbuminuria in diabetic nephropathy has been extensively investigated.

6.1 Microalbuminuria as a Marker of Kidney Damage

In the glomerulus there appears to be three barriers preventing the excretion of macromolecules such as albumin[29]. The first is the endothelial cell of the capillary. Plasma may escape from the capillary through fenestrations in the endothelial cell layer. This leakage will be limited by the size and number of the fenestrations. Diffusion then occurs across the basement membrane, which is thought to consist of a network of glycoproteins and proteoglycans with water filled pores. The permeability of the basement membrane will depend on the size, structure and charge of these molecules. The final barrier are the epithelial cells of the glomerulus. These appear to contain

filtration slit diaphragms of about the same size as albumin molecules. They are negatively charged by the presence of sialic acid and as a result the passage of negatively charged molecules such as albumin is reduced. Those molecules which are prevented from entering the tubules are engulfed by pinocytosis and metabolised by the cell.

Albumin is a negatively charged, medium sized plasma protein with a molecular weight of approximately 68,000[32]. As a result of the barriers mentioned above, only a small quantity of albumin is normally filtered through the glomerulus, and reabsorption of some of the filtered albumin then occurs in the proximal tubules. β_2-microglobulin is a low molecular weight protein which is freely filtered through the glomerulus and almost completely reabsorbed into the proximal tubular cells where it is metabolised. If tubular damage is present then the level of β_2-microglobulin in the urine will rise. Thus in a healthy adult human being less than 50mg of albumin a day is excreted in the urine[2]. The excretion of more than 0.15g of protein a day is usually indicative of disease, whilst the manifestation of large quantities of protein (more than 5g a day) in urine is diagnostic of the nephrotic syndrome. In these cases it would seem likely that the appearance of albumin in the urine simply arises from glomerular damage (which may be confirmed by renal biopsy) allowing greater passage of larger molecular weight proteins from the capillaries. It is thought that the reabsorptive capacity of the tubules is working at a near maximal rate in normal conditions

and that it is therefore easily exceeded by this rise in albumin filtration leading to the presence of albumin in the urine. Petersen et al[2] supported earlier work which suggested that proteinuria, consisting mainly of albumin, indicated glomerular damage whereas proteinuria consisting mainly of β_2-microglobulin indicated tubular damage. By conducting assays for β_2-microglobulin and albumin on the same samples, it has been shown[1,2,13,19,30] that there is no association between β_2-microglobulin concentration and the increase in albumin concentration supporting the idea that the increase in albuminuria is unlikely to be of tubular origin.

There is evidence that a number of factors may influence albumin excretion. Studies to elucidate the effect of exercise[19], posture and diurnal ryhthm[31] upon microalbuminuria have been reported. Both of these studies have involved small numbers of subjects. Montagna et al[31] studied the effects of posture and time of day on albumin excretion in six normal subjects without renal disease. Three consecutive samples of urine were collected from each subject, each of 12 hours duration. The first period was during the day when the subjects were mobile but were not permitted to do any exercise or sit except for mealtimes. For the following two periods, overnight and daytime, the subjects were recumbant. They found that the night-time collection period produced the lowest levels of albumin, whilst the highest levels were found in the mobile, daytime collection. The recumbant daytime collection was intermediate between these two groups. This suggests that

in healthy people there may be diurnal change in albumin excretion and that mobility may also affect the excretion of albumin.

Pedersen, Mogensen and Larsen[19] studied the effects of exercise on albumin and β_2-microglobulin excretion rates in young hypertensive patients and normotensive control subjects. They also measured renal plasma flow (RPF) and glomerular filtration rate (GFR). The patients were divided into three groups. Group 1 contained nine untreated hypertensive patients, group 2 contained eight hypertensive patients treated with propranolol and group 3 contained the ten normotensive controls. The study was divided into four consecutive periods. The first and last sections were control periods lasting 40 minutes each. The second and third periods lasted 20 minutes each during which exercise was performed on a bicycle ergometer, load 75W and 100W respectively. The excretion rates of albumin and β_2-microglobulin in the initial control period were similar in all three groups. In group 3 no significant change was found in the albumin and β_2-microglobulin excretion rates during exercise, whilst in group 2 the albumin excretion rate (AER) only increased during the period of heaviest exercise. However, group 1 showed a significantly raised AER in the second period compared to both groups 2 and 3 and a significantly raised AER in the third period compared with group 3. β_2-microglobulin excretion rates did not alter significantly throughout the trial for group 2. It was found to decrease during the period of heaviest exercise

for group 1. RPF and GFR were decreased during exercise for groups 1 and 2 but only decreased during heavy exercise for group 3. The measured blood pressures were elevated during exercise, being highest in group 1 and lowest in group 2. In summary, AER appear to rise in untreated hypertensives during any exercise, whilst in treated hypertensives this rise is only seen during periods of strenuous exercise. This study suggests that although a patient with mild hypertension seems to have a normal renal function at rest, exercise might have the ability to reveal underlying renal disease (cf group 1).

The present work has extended this published work, to investigate the effects of smoking, caffeine and alcohol intake on blood pressure and microalbuminuria which have not been previously published. The influence of diurnal rhythm, posture and exercise was reduced as far as possible by using the albumin/creatinine ratio, determined from spot, morning urine samples, as a measure of albumin excretion to enable the effects of caffeine, alcohol intake and smoking to be investigated. Although both caffeine and alcohol have been reported to cause a slight rise in blood pressure[4,10] and long term smoking may cause a reduction in blood pressure[8], in the earlier studies there was no evidence of any association between blood pressure and caffeine or alcohol intake or smoking. It is thought that caffeine may exert its effect by either causing vasoconstriction, or increasing cardiac rate and contractility although studies have failed to elicit a definite mechanism[4,11]. It appears that the rise

in blood pressure with chronic alcohol intake is probably due to the withdrawal response which activates the renin-angiotensin system and the sympathetic nervous system[10]. No consistent changes with acute alcohol intake have been reported. The major component of tobacco smoke that is most likely to affect the cardiovascular system is nicotine which stimulates the nicotinic receptors of the autonomic nervous system resulting in an increase in peripheral resistance and hence a rise in blood pressure[8,39]. So although an acute intake of nicotine causes a rise in blood pressure, long-term smokers in fact have lower blood pressures, although the mechanism is unknown. If caffeine and alcohol intake, and smoking affect blood pressure then they may also affect albumin excretion, which has been linked to blood pressure. From the results obtained in the present studies it would appear that these factors do not alter the albumin excretion rate significantly. It may be that the clinic population used for the present study was relatively "clean living" and that had a population that drank and smoked more been studied a relationship may have appeared. In practice the relationship that appeared was between both systolic and diastolic blood pressures and albumin excretion which appeared to confirm the work of Parving[1].

6.2 Microalbuminuria as a Marker of Endothelial Permeability

The reported rise of the albumin excretion rate with a rise in blood pressure[1,12,30] may be causally related and could be

explained by an increase in the capillary pressure causing more plasma to be forced through the basement membrane of the glomerulus into the nephron. If this were the case, it might be expected that there would be a linear relationship between any changes in blood pressure and corresponding changes in the albumin excretion rate. A further extension of this idea may be that the effects of the increased filtration rate need not be confined to the vasculature of the kidney, but may also occur in the rest of the vascular system. Parving (1974)[30] showed that the transcapillary escape rate (TER) of albumin increased during acute angiotensin II induced hypertension and was also raised during untreated benign hypertension. He showed a significant correlation between the TER of albumin and blood pressure (systolic, diastolic and mean) but failed to find a similar correlation in the normotensive control group. Patients with benign hypertension who were treated with a beta adrenergic blocking drug showed a reduction in both blood pressure and TER. Further work in 1977 by Parving et al[32] supported this finding. They showed that the TERs of both albumin and Immunoglobulin G (IgG) were raised in hypertensive patients compared with normotensive subjects (when mean blood pressures were measured). Albumin has a molecular weight of about 68000 and IgG a molecular weight of about 160000. The pore size in the glomerulus is similar in size to an albumin molecule. If the pore was slightly stretched then a molecule the size of albumin would be expected to slip through easily, increasing the size of the pore should therefore have little further effect on the

passage of an albumin sized molecule whereas larger molecules, say the size of IgG would now be able to pass freely. If the increased protein excretion found in hypertension is due to 'stretching' of the pores in the endothelium of the glomerulus, a higher TER of IgG compared to the TER of albumin would be expected. However, when the ratio of TER_{IgG}/TER_{Alb} in normotensive and hypertensive subjects was compared the rise was found to be similar for both proteins indicating that the increased escape rates were due to mechanisms other than an increase in pore size.

An alternative concept of plasmatic vasculosis was proposed by Lendrum[33]. He proposed this hypothesis in order to explain the microscopic changes that he found in the arteriolar walls of hypertensive diabetic kidneys caused by the deposition of fibrin. He suggested that the increased blood pressure forced plasma through the arteriolar walls. This would lead to deposits of fibrin being formed within the arteriolar walls hence allowing passage of plasma proteins across the basement membrane. This process was then manifested in the kidneys by the appearance of albumin in the urine.

Van Liew and Feld (1980)[34] carried out work in spontaneously hypertensive rats (SHR) to study the excretion rate of albumin, creatinine clearance and kidney damage throughout the lifespan of the rat. The SHR were developed to provide analogues of human essential hypertension. The group of SHR used was

genetically matched with Wistar Kyoto (WKY) rats as normotensive controls. The authors reported that although the creatinine clearance remained similar throughout life in the SHR, protein excretion, including albumin, rose as the rats grew older. They also found that kidney damage occured, initially as cast formation in the inner cortical areas, progressing to sclerosis, hyalinization, fibrosis and occluding of tubules and capillary loops. Sufficient kidney function remained to maintain the glomerular filtration rate at normal levels. The same measurements in the WKY rats were unchanged during the life of the rats. It is of note that the changes found in the SHR occured after the rise in blood pressure, and presumably were as a result of the raised blood pressure and not the cause of it.

It may in fact be that the actual mechanism behind a raised albumin excretion is a combination of these two processes. Initially the rise in blood pressure would force out larger molecules into the glomerulus. As this blood pressure is maintained, or even increased further, damage may then occur to the walls of the capillaries allowing further albumin loss. It has been postulated that the changes seen at the kidney (by whatever causative mechanism) reflect events that are occuring throughout the body in the small blood vessels. Retinal changes such as exudates which are seen in moderate and severe hypertensives could therefore be caused by the same mechanisms that results in raised albumin excretion.

6.3 The Relationship Between Blood Pressure and Microalbuminuria

If the first hypothesis, that a rise in blood pressure forced albumin out through the glomerulus as a direct result of the rise in pressure, was correct it might be expected that blood pressure and albumin excretion rates would be very closely related, and if the blood presure was reduced rapidly that there might be a rapid reduction in albumin excretion. If however the cause of the the rise in albumin excretion was endothelial damage caused by the raised blood pressure, then a reduction in blood pressure would not produce a corresponding and immediate reduction in albumin excretion. It may also be that any correlation between blood pressure and albumin excretion may be less pronounced, because the rate of albumin excretion would depend on a consistently raised blood pressure which need not be the current measured blood pressure.

Christensen[13] showed that patients who had a recent rapid rise in blood pressure also had elevated albumin excretion rates. He studied albumin and β_2-microglobulin levels in patients with severe essential hypertension (diastolic $\geq$135mHg) who had been admitted untreated to hospital for blood pressure reduction. Blood pressure was monitored regularly and urine saves taken for albumin and β_2-microglobulin levels. Frusemide, and diazoxide or dihydralazine were used to bring the blood pressure down initially, conventional oral treatment was then used. It was

found that the AER were grossly elevated before treatment but had dropped to within normal levels for most patients after about 3.5 days, at which point the blood pressure had also been markedly reduced. β_2-microglobulin levels were moderately elevated before treatment and were reduced to normal after treatment in most patients. Both the β_2-microglobulin and AER were transformed logarithmically and correlated with the diastolic, systolic and mean blood pressures. Positive correlation was found between the AER and the diastolic, systolic, and mean blood pressures, and also the β_2-microglobulin excretion rate and the diastolic, systolic and mean blood pressures. However, no correlation was found between the β_2-microglobulin and AER. This suggests that the increased AER are caused by raised blood pressures, especially as in benign essential hypertension the β_2-microglobulin rates remain at normal levels[1,19,30]. The raised blood pressure was of such short duration that morphological changes in the kidney would have been unlikely to have occured and thus caused the rise in albumin excretion rates, hence these results support the suggestion that an elevated blood pressure results in an increase in albumin excretion by increasing the filtration rate through leaky glomerula with the protein reabsorption facilities being exceeded leading to raised levels in the urine.

Parving et al[1] looked at the AER in eight control subjects, and benign essential hypertensive patients, both successfully (14 patients) and insufficiently (18 patients) treated. They also

measured the β_2-microglobulin excretion rates, which were similar for all the groups studied. The AER was significantly elevated in the insufficiently treated group compared with both the control group and the effectively treated group. Five of the insufficiently treated group had markedly elevated levels, but even when these were separated from the rest of the group the levels were still found to be higher than normal. The lack of change in the β_2-microglobulin levels indicates that the change in albumin excretion is unlikely to be due to tubular damage leading to decreased reabsorption of albumin. It may be more likely due to the increased blood pressure found in the ineffectively treated patients.

Pedersen and Mogensen[12] showed that treating essential hypertensives to lower their blood pressure resulted in a drop in their albumin excretion rates. They studied 20 essential hypertensive patients before and after 4 to 8 weeks of treatment with alprenolol and hydralazine. They measured blood pressure, AER, GFR and RPF. Half of the patients studied failed to respond adequately to therapy. The AER dropped after treatment in nearly all the patients, but the rates were higher before and after treatment in the insufficiently treated group. No significant change was found in the GFR before and after treatment but a significant drop in RPF ($p<0.05$) was found. Correlation was found between the AER before treatment and the systolic and diastolic pressures during treatment ($r=0.616$, $p<0.01$ and $r=0.665$, $p<0.01$ respectively). Weaker correlation was found

between AER during treatment and the systolic and diastolic blood pressures during treatment ($r=0.509$, $p<0.05$ and $r=0.539$, $p<0.02$ respectively). No correlation was found between AER before treatment and blood pressure before treatment. They concluded that a low AER before treatment was started indicated that the patient would respond well to antihypertensive therapy.

It seems from these various studies that in the essential hypertensive patient the rise in urinary albumin concentrations is related to blood pressure, but is not necessarily a direct result of the raised blood pressure. It may be that a lower albumin excretion rate indicates that less damage has occured to the capillary walls and that either the patient has had only a mildly raised blood pressure for a while or that the rise in blood pressure has only been recent, or that the blood pressure is insufficient to push the albumin through the glomerular barriers. It may be that elevated blood pressure results in more severe glomerular damage, or more widespread arteriolar damage, which can be gradually repaired when the cause of the damage is removed. All these results suggest that the relationship between blood pressure and albumin excretion rate may depend on a variety of mechanisms which interelate such that at different stages in the development of hypertension different mechanisms dominate.

6.4 Intrepretation of the Results of the Present Study

The present study can be conveniently divided into three parts: the questionnaire, the study of treated and untreated hypertensives, and the lisinopril and atenolol study.

The excretion of albumin appears subject to a wide number of variables such as posture and exercise. Blood pressure is equally dependent on a range of factors, but the practical importance of these various variables for the clinic and study patient is unknown. It was important to try and assess their impact on blood pressure and albumin excretion before proceeding further so that suitable steps could be taken if necessary to eliminate or measure their effect. From the results of the questionnaire conducted on 544 patients it was not possible to corroborate the reported effects caused by caffeine and alcohol intake, and smoking on blood pressure. In a subset of 42 patients it was also found that no relationship occured between these factors and the albumin/creatinine ratios. However, the results did indicate the existence of a relationship between blood pressure and microalbuminuria. It seems that in practice the effects of smoking, caffeine and alcohol intake on blood pressure are negligible as they are on the albumin/creatinine ratio, and no special provision may need to be taken to reduce their impact.

A larger sample of treated and untreated hypertensive patients (100) was then investigated with the aim of confirming this apparent relationship between blood pressure and albumin excretion. The previously reported studies had all involved small numbers of patients. The aim of this present study was to see whether any correlation existed in a hypertensive clinic population and also how strong it was. By using large numbers of patients it was also hoped to see what effect gender and race may have on the correlation. There was a correlation between blood pressure and albumin/creatinine ratio for the whole group studied ($0.01>p>0.001$). It was found that in the treated group a relationship between blood pressure and albumin excretion rate did seem to exist. However, these patients were on a variety of antihypertensive medications ranging from monotherapy with, for example, a beta-blocker (usually atenolol) to complex multiple therapy involving drugs such as clonidine, reserpine and minoxidil. Studying the group of untreated patients alone in order to remove any influence of drug therapy on the relationship between blood pressure and albumin/creatinine ratio, it was found that the correlation was less pronounced compared to that obtained from the treated group. Analysing the data by presence or absence of treatment, race and/or gender failed to either strengthen or weaken the correlation. Overall a relationship was apparent between blood pressure and microalbuminuria for the combined group of treated and untreated hypertensives. The correlation coefficient remained approximately 0.3 throughout. This correlation coefficient is in

contrast to those reported previously in the literature. Parving et al[1] found correlation coefficients of 0.6 or greater, using a subject group containing both normal and hypertensive subjects. When Gosling and Beevers[21] studied a subdivision of their total population they showed a correlation coefficient of 0.4 between mean arterial blood pressure and albumin excretion rates in those subjects whose blood pressure was above 140/90. However, the number of subjects in this subdivision was extremely small. A number of patients in the present study were normotensive (either through treatment, or because they were classed as borderline hypertensives), but it was decided against subdividing the data further. Firstly any correlation thus obtained would be less meaningful because of the reduced numbers of patients and secondly the relationship between blood pressure and albumin/creatinine ratio in a hypertensive population (treated, untreated and borderline) was being investigated. It is hoped to continue studying the group of approximately 30 borderline hypertensive patients for some time to see if a relationship between albumin/creatinine ratio and blood pressure develops.

The final section of this study was the closer investigation of the relationship between blood pressure and albumin/creatinine ratio in a small number of untreated proven hypertensive patients, before and during treatment. These patients were taking part in a large multicentre study investigating the antihypertensive action of lisinopril, a new angiotensin converting enzyme inhibitor, compared with an established beta-blocker (atenolol).

Unfortunately the number of patients entering and completing the trial was small (23) and insufficient to produce statistically significant results. Each patient completed a two week placebo phase before treatment was initiated with either lisinopril or atenolol. The dose of the drug was then titrated over the next month to provide optimum blood pressure control, and the patient then remained on this dose for a further month.

It seems likely that the main effect of ACE inhibitors on blood pressure was primarily from their action on the renin-angiotensin system[39]. Renin, released from the juxtaglomerular apparatus, causes the conversion of angiotensinogen to angiotensin I. Angiotensin I is then converted to angiotensin II by the action of angiontensin converting enzyme (ACE). Angiotensin II causes arterial vasoconstriction and release of aldosterone. This results in sodium and water retention and vasoconstriction leading to increased blood pressure. The rise in blood pressure, extracellular volume and angiotensin II exert a negative feedback effect on renin release and in this way normal circulating volumes and pressures can be maintained. ACEI's block the action of ACE and so result in vasodilatation and sodium and water excretion which causes a drop in the circulating volume and so blood pressure drops. However evidence has since suggested that ACEI's can reduce blood pressure without influencing the plasma renin system. It is known that ACE is identical to kinase II, the enzyme responsible for the degradation of bradykinin. ACEI's may therefore interact with the

prostaglandin system allowing vasodilation of peripheral blood vessels, however this area has not yet been fully explored[40]. It has been suggested that as well as the plasma renin system there may also be renin systems within certain tissues such as the brain and blood vessel walls where ACEI's may also exert an effect[41,42]. Again this area requires much further investigation to reveal its relevance to blood pressure control. It therefore seems that the blood pressure lowering action of ACEI's is much more complex than originally thought.

Zatz et al[35] studied the haemodynamic and morphological changes in the kidneys of diabetic rats. They used adult Munich-Wistar rats which were divided into three age and sex matched groups. The first two groups were made diabetic by the use of a single injection of Streptozotocin and were then maintained on a dose of insulin sufficient to keep them in a state of moderate hyperglycemia (between 200 and 400 mg/dl blood glucose levels). One group of the diabetic rats was also given enalapril in the drinking water throughout the duration of the study. The third group acted as a control group. After 4-6 weeks approximately half the rats underwent micropuncture studies. Arterial pressure, GFR, and glomerular plasma flow rate were among the parameters measured. Blood pressure measured both by the tail cuff and directly from the femoral artery was found to be similar in both the control and the diabetic groups but was significantly lower in the diabetic group treated with enalapril. They also found that the mean arterial pressure was significantly lower in

the enalapril treated diabetic rats compared with the control and the diabetic groups. The diabetic rats as a whole had a higher GFR than the normal group, the enalapril only slightly lowering the rate. A similar result was found in respect to the glomerular plasma flow rate. The glomerular transcapillary hydraulic pressure difference (PD) was found to be markedly raised in the diabetic rats compared with the control group, except for those rats treated with enalapril where PD was normalised. In addition to these micropuncture studies the remaining rats were monitored for 14 months for blood glucose levels, blood pressure and 24hr AER. At the end of 14 months the rat kidneys were fixed and sections taken for microscopic examination and staining. The total weight of the kidney was found to be higher in both groups of diabetic rats compared to the control group. A similar percentage of morphological changes were seen in the control group and the diabetic group treated with enalpril (1.10±0.18 and 0.67±0.20 respectively). The remaining diabetic group had a far higher incidence of changes (6.06±1.20). Blood pressure was found to be decreased in the enalapril treated group compared with both the diabetic and control groups. Albumin excretion was markedly elevated in the diabetic group (111±22) compared with both the control (25±6) and the enalapril treated (18±3) groups. This suggested that the increased PD contributes to the morphological changes found in the kidney and the increased albumin excretion. As enalapril decreases PD, so the AER and the frequency of morphological changes are decreased.

Kelleher et al[36] studied the effect on AER of enalapril in diabetic and non-diabetic hypertensives. AER and blood pressure was measured before treatment and after stabilisation on enalapril. For both the diabetic patients and the essential hypertensive patients they discovered a relationship between pretreatment systolic blood pressure and AER. They found an overall reduction in AER with a corresponding fall in blood pressure. However, the fall in AER was not significant but there was a significant relationship between the pretreatment AER and the change in AER during treatment. The higher the pretreatment AER the greater the fall during treatment.

The antihypertensive action of the β-adrenoreceptor antagonists probably results mainly from their action in blocking the β_1-receptors, rather than the β_2-receptors. Stimulation of the β_1-receptors causes an increase in the rate and force of contraction of the heart, blocking these receptors will therefore lead to a decreased cardiac output and hence a lower blood pressure. Pedersen, Mogesen and Larsen[19] used propranolol to lower blood pressure in their study (page 88) whilst Pedersen and Mogensen[12] used a combination of alprenolol and hydralazine (page 96). Both studies showed alterations in the albumin excretion rate and blood pressure between treated and untreated patients. However, Pedersen and Mogensen also showed that with treatment there was no significant change in the glomerular filtration rate.

It had been anticipated that at the end of eight weeks treatment in the present study that the confirmed hypertensive patients would have shown a significant drop in blood pressure. This proved to be the case when the total number of patients was studied. However, when these patients were divided into their respective groups (treatment with lisinopril or atenolol) then it appeared that only those patients treated with atenolol had acheived a statistically significant fall in blood pressure and that occurred within two weeks of starting treatment. No correlation was found between blood pressure and albumin/creatinine ratio for the whole study period or at the beginning or end of the trial for the whole group and the two subdivisions. These findings were contrary to the findings from the other two parts of the present study namely the questionnaire and the large sample study. It would seem that the most likely explanation is that there were not enough subjects studied.

Three possible mechanisms relating blood pressure to albumin excretion can be suggested. The first, that a rise in blood pressure causes the filtration pore to stretch therefore allowing passage of larger molecules does not seem to be supported by Parving's work with TER's of albumin and IgG[32]. The present study is unable to confirm or disprove this theory. The next theory suggests that the rise in blood pressure causes a rise in the glomerular transcapillary pressure which then forces albumin and other large molecules through the barriers into the glomerulus. If this was the actual mechanism then a close

relationship between blood pressure and albumin excretion would be expected with fluctuations in blood pressure being mirrored by changes in the albumin excretion rate. It may possibly be that a certain pressure in the renal blood vessels needs to be reached before albumin excretion starts to rise (which could explain the findings obtained by both Cruickshank[14] and Gosling and Beevers[21] in normotensive populations) and that after a certain pressure has been reached the excretion rate of albumin is at a peak. Practically a strong correlation between blood pressure and albumin excretion rate would be expected. The correlation in the first two parts of the present study was similar but not as strong as may have been expected if blood pressure and albumin excretion were directly connected. The final theory is an extension of the hypothesis first put forward by Lendrum[33] that a rise in blood pressure forces molecules such as fibrin and albumin to be deposited in the cell walls of the blood vessels thereby leading to damage and hence leakage into the glomerulus. The correlation between blood pressure and albumin excretion would not just depend on blood pressure, but other factors such as how long the blood pressure has been raised and to what levels. Reduction of the blood pressure may allow the cell wall to heal and regain its previous properties. This theory could then explain the consistent but weak correlation found in the present study in a range of hypertensive patients of differing duration of raised blood pressure. It is most likely that the mechanism between blood pressure and albumin excretion is a combination of the above

theories, with the initial rise in blood pressure forcing albumin into the glomerulus and also into the cell wall. The rise in albumin excretion would then be maintained by the damage done to the cell wall.

The first part of this study investigated the influence of certain everyday factors on blood pressure and albumin/creatinine ratio (which had previously been shown to be a reliable method for estimation of albumin excretion[14,15,18] and was convenient for the patient) to determine their relevance to the clinic patient. As a result of this study no correlation was found between blood pressure or albumin/creatinine ratio and smoking, caffeine and alcohol intake. The only correlation that appeared was between blood pressure and albumin/creatinine ratio. It was decided to investigate this relationship further in two different ways. Firstly by studying a large number of hypertensive patients, both treated and untreated. Some of these patients were normotensive, either as a result of treatment or because they were classed as borderline hypertensives. The findings from this study supported the earlier findings of a weak but consistent correlation. This correlation was present in both the treated and (to a lesser extent) the untreated group despite the range of blood pressures. It likely that the treated group of hypertensive patients have had a longer duration of high blood pressure which could have led to an increase in kidney damage, supporting the theory of cellular wall damage caused by raised blood pressure.

The third part of the study involved taking proven untreated hypertensive patients and treating them with one of two pharmacologically different antihypertensive medications until their blood pressure was controlled within acceptable limits, or the maximum recommended dose of the drug had been reached. If the excretion of albumin depends on the intra-glomerular pressure differential then a greater fall in the albumin excretion rate would be expected with an ACE inhibitor (lisinopril) than with a ß-adrenoreceptor antagonist (atenolol) because of their differing modes of action in reducing blood pressure. If on the other hand blood pressure caused an increase in the excretion of albumin as a result of mechanical damage to the endothelial wall then any fall in albumin excretion linked to a fall in blood pressure should be independent of the mode of action of the drug. Unfortunately there was no significant fall in blood pressure in the lisinopril group, although the atenolol group did show a fall in blood pressure with treatment. Neither group showed a significant correlation between blood pressure and albumin/creatinine ratio however the relationship between blood pressure and albumin/creatinine ratio did seem to change over the eight weeks of treatment to become more positively inclined. This change was similar for both the lisinopril and atenolol treated groups suggesting that albumin excretion is less dependent on the intra-glomerular pressure differential than on the postulated damage to the cell walls. Ideally a larger group of patients should be followed on monotherapy for a longer period of time to see if any further differences occur. The

present results would therefore seem to support the hypothesis that a raised blood pressure causes damage to the arteriole endothelium, hence allowing passage of albumin and other solutes through.

It has been postulated that the role of microalbuminuria in hypertension could be twofold, firstly to allow detection of changes in kidney function at an earlier stage than detectable using serum creatinine levels (the current marker of kidney damage), and secondly to predict those patients who will require antihypertensive therapy. The main problem that has been found using serum creatinine levels in practice is that the rise only occurs after the kidney has been extensively damaged. It would be useful for the clinician to have an easily performed, non-invasive test which could detect early stages of hypertension induced kidney failure, allowing more intensive antihypertensive therapy and hopefully helping the patient to retain the use of his kidneys, rather than having to undergo dialysis or renal transplant. It is also found that the blood pressure of many patients, who present to the physician with mild hypertension, settles with time. The repeated clinic visits required to discover that this is the case are time consuming for both patient and doctor and a test which may pinpoint which patients would require, and benefit, from treatment would help enormously. This present study has confirmed that a relationship does appear to exist in a population drawn from a hypertension clinic. However the relationship appears too weak to be of any practical use in

the clinic at the moment. Follow up of the borderline hypertensive patients may reveal a developing relationship which could be of use. Unfortunately the mechanism linking albumin excretion and blood pressure remains to be fully elucidated, although these results suggest that it appears to be partially dependent on damage occuring to the endothelial cell walls.

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APPENDIX I

Format of Questionnaire

OUTPATIENTS QUESTIONAIRE

NAME |_____| HOSPNO |_____| TRIAL NO: |_____|

DOB |___/___/19__| AGE |___| RACE |___| SEX |___|

HEIGHT |_____| Cms. WEIGHT |_____| Kgs. OCCUPATION |_____|

DATE |___/___/19__|

	Name	Dose
DRUGS		

SMOKER ? NO |___|

YES |___| How many cigs/day |___|

EX |___| When did you stop? |_____|

Do you smoke a pipe? yes |___| no |___|

If yes, how many oz's/week? |___|

How many cups of tea do you usually drink per day? |___|

How many cups of coffee do you usually drink per day? |___|

Pulse |____| BP |_____/_____| Blood taken |___|

Do you drink any alcohol? Yes |___| No |___|

1

RETROSPECTIVE DIARY

NAME _____ TRIAL NO: _____

DATE ___/___/19__ TIME _____ DAY _____

DRINKING IN THE LAST 7 DAYS

	TYPE: Beer/Spirits/Wine/Port Wine	How much	Units/day
MONDAY			
TUESDAY			
WEDNESDAY			
THURSDAY			
FRIDAY			
SATURDAY			
SUNDAY			
		TOTAL	

Day and approximate time of last drink: Day _____ _____

24 hr clock.

ANALGESIC QUESTIONNAIRE

NAME: _____ UNIT NO: _____

DATE: _____

1) Which pain killers do you take?

	Daily	Weekly	Monthly	Other
_____	___	___	___	___
_____	___	___	___	___
_____	___	___	___	___
_____	___	___	___	___

2) Do you take anything for stiff joints or rheumatics?

NO___ ___YES_____

3) Has your doctor given you any advice on what painkiller to take?

YES _____

NO _____

4) Has any pharmacist/chemist given you advice on what painkiller to use?

YES _____

NO _____

5) Do you take any indigestion mixtures ___YES_____NO_____

Do you know what it is called _____

How often do you take it?

Daily ___ Weekly ___ Monthly ___ Other ___

DSI Disc 11

Computer program to calculate albumin concentrations.

```
10 REM ** ALBUMIN **
20 REM ** A PROGRAM TO CALCULATE RESULTS FROM THE
30 REM ** DPC ALBUMIN DOUBLE ANTIBODY KIT
40 CLEAR
50 PRINT CHR$(27) + "E" + CHR$(27) + "H"
60 QUIT=1: DIM X(8), PcBound(8), Y(8)
70 PRINT "Please enter the date the assay was run"
80 INPUT "in the form dd.mm.yy";date$
90 L=LEN(date$)
100 IF L>8 OR L<6 THEN PRINT "Wrong format": GOTO
80
110 FOR X=1 TO L
120 L$=MID$(date$,X,1)
130 IF L$="." OR L$="," OR L$="/" GOTO 150
140 NEXT
150 DAY$=LEFT$(date$,X-1): MONTH$=MID$(date$,X+1,(
L-3)-X): YR$=RIGHT$(date$,2)
160 day=VAL(day$): month=VAL(month$)
170 IF day=0 THEN PRINT "Too few days": GOTO 80
180 IF day>31 THEN PRINT "Too many days": GOTO 80
190 IF month=0 THEN PRINT "Too few months": GOTO 8
0
200 IF month>12 THEN PRINT "Too many months": GOTO
 80
210 IF YR$<"87" THEN PRINT "Wrong year": GOTO 80
220 PRINT CHR$(27) + "E" + CHR$(27) + "H"
230 PRINT "PLEASE INDICATE WHICH ASSAY YOU WISH TO
 ANALYSE"
240 PRINT "For 25 microlitre ASSAY PRESS  S"
250 PRINT "FOR 100 microlitre ASSAY PRESS  L"
260 INPUT pick$
270 IF pick$ <> "s" AND pick$ <> "l" THEN GOTO 230
280 FOR count=1 TO 8
290 READ x(count)
300 x(count)=LOG10(x(count))
310 NEXT
320 PRINT "INPUT TOTAL COUNTS"
330 GOSUB 1000
340 PRINT
350 total=mean
360 PRINT "INPUT NSB COUNTS":FLAGA=1
370 GOSUB 1000
380 PRINT
390 NSB=mean
400 PRINT "INPUT MB COUNTS":FLAGA=0
410 GOSUB 1000
420 PRINT
430 MB=mean-NSB
440 PRINT "INPUT CALIBRATION COUNTS"
450 IF pick$="s" THEN start=4 : finish=8 ELSE star
t=1 : finish=6
460 FOR count=start TO finish
470 GOSUB 1000
480 IF mean=0 THEN GOTO 530
490 PcBound(count)=(mean-NSB)/MB
500 sumy=sumy + LOG(PcBound(count)/(1-PcBound(coun
t)))
510 sumxy=sumxy + (LOG(PcBound(count)/(1-PcBound(c
ount)))*x(count))
515 sumx=sumx + x(count)
520 sumxx=sumxx + (x(count)^2)
```

```
530 tally=tally + 1: PRINT
540 y(count)=PcBound(count)*100
550 NEXT
560 beta=(sumxy - sumx*sumy/tally)/(sumxx - sumx*s
umx/tally)
570 alpha=sumy/tally - beta*sumx/tally
580 GOSUB 1500
590 WHILE quit=1
600 FLAG=1
610 PRINT "INPUT PATIENT COUNTS"
620 GOSUB 1000
630 p=(mean - NSB)/MB
640 p=LOG(p/(1-p))
650 albumin=10^((p-alpha)/beta)
660 patno=patno + 1
670 IF FIRST<>-1 THEN GOSUB 2000
680 WEND
690 END
1000 INPUT "First count"; first
1010 IF first=0 THEN mean=0: x(count)=0: tally=tal
ly-1: RETURN
1020 IF first=-1 THEN mean=1000: quit=0: RETURN
1030 INPUT "Second count"; second
1035 IF FLAGA=1 THEN GOTO 1050
1040 IF ABS(first - second)>0.1*MIN(first,second)
THEN GOSUB 2500
1050 mean=(first+second)/2
1060 IF mean - NSB>=MB AND FLAG=1 THEN LPRINT TAB(
17-AA)patno+1; TAB(37-BB)"RESULT GREATER THAN 100%
": LPRINT:first=-1: mean=MB
1070 RETURN
1500 LPRINT "RESULTS FOR ASSAY RUN ON:" day$; ".";
 month$ ;"."; yr$
1510 LPRINT
1520 LPRINT "Q.C. Parameters:"
1530 LPRINT
1540 LPRINT "Total Counts"; ROUND(total,0); "cpm."
1550 LPRINT
1560 LPRINT "%NSB" SPC(9) ROUND(100*NSB/TOTAL,2)
1570 LPRINT
1580 LPRINT "%MB" SPC(10) ROUND(100*MB/TOTAL,2)
1590 LPRINT
1600 LPRINT "The 20% intercept is"; ROUND(10^((LOG
(0.25)-alpha)/beta),2)
1610 LPRINT
1620 LPRINT "The 50% intercept is"; ROUND(10^((LOG
(1)-alpha)/beta),2)
1630 LPRINT
1640 LPRINT "The 80% intercept is"; ROUND(10^((LOG
(4)-alpha)/beta),2)
1650 LPRINT: LPRINT: LPRINT "ASSAY RESULTS": LPRIN
T
1660 LPRINT "The regression equation is"
1670 LPRINT
1680 LPRINT ; "Y= "; beta; "X +"; alpha
1690 LPRINT
1700 LPRINT "Calibration Results"
1710 LPRINT
1720 LPRINT TAB(13) "% Bound"; TAB(30) "Concentrat
ion"
1730 FOR count=start TO finish
```

```
1740 Y$(count)=STR$(Y(count)): X$(count)=STR$(10^x
(count))
1750 check$=Y$(count)
1760 GOSUB 3000
1770 EE=x
1780 check$=x$(count)
1790 GOSUB 3000
1800 FF=x
1810 LPRINT TAB(18 - EE) ROUND(Y(count),2); TAB(38
 - FF) ROUND(10^(x(count)),2)
1820 LPRINT
1830 NEXT
1840 LPRINT "PATIENT RESULTS"
1850 LPRINT
1860 LPRINT TAB(10)"Patient Number"; TAB(30)"Patie
nt Counts"; TAB(50)"Albumin microgram/ml"
1870 LPRINT
1880 RETURN
2000 patno$=STR$(patno): first$=STR$(first): secon
d$=STR$(second): albumin$=STR$(albumin)
2010 check$=patno$
2020 GOSUB 3000
2030 AA=x
2040 check$=first$
2050 GOSUB 3000
2060 BB=x
2070 check$=second$
2080 GOSUB 3000
2090 CC=x
2100 check$=albumin$
2110 GOSUB 3000
2120 DD=x
2130 LPRINT TAB(17-AA)patno; TAB(37-BB)first; TAB(
44-BB)second; TAB(57-DD)ROUND(albumin,2)
2140 LPRINT
2150 RETURN
2500 PRINT "The difference in your counts is great
er than 10%.  Please enter the more realistic valu
e"
2510 INPUT first
2520 second=first
2530 IF FLAG=1 THEN LPRINT TAB(13)"The following r
esult is calculated from one count only"
2540 RETURN
3000 FOR x=1 TO LEN(check$)
3010 IF MID$(check$,x,1)="." THEN GOTO 3030
3020 NEXT
3030 RETURN
4000 DATA 0.5,1,2.5,5,10,20,30,60,
4010 REM ** BY STEVE AND EMMA **
```