PROTEIN CLEARANCES AND RENAL PROTEIN SELECTIVITY

IN THE PROTEINURIAS OF PREGNANCY

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I certify that this thesis entitled "Protein clearances and renal protein selectivity in the proteinurias of pregnancy" is the personal work of Dr. M. D. Simanowitz undertaken in my department.

Signed

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PREFACE

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The aetiology and pathogenesis of preeclampsia remain obscure despite exhaustive efforts to find a common denominator. Hypertension, proteinuria and oedema in pregnancy are still grouped together as pre-eclampsia, a rather unsatisfactory categorisation of a syndrome with innumerable variations in presentation, behaviour and influence on maternal and foetal prognosis. Hypertension remains undefined and the precise significance of proteinuria or "albuminuria" in pregnancy remains uncertain almost 130 years after its discovery.

It is not surprising then that two eminent workers in the field take almost opposing views. F. J. Browne, writing in 1958, claims ". . . we now have all the essential facts bearing on the aetiology of eclampsia and they resemble the pieces of a jigsaw puzzle and only need to be assembled in proper sequence to provide an acceptable explanation of eclampsia". Eight years later, T. N. A. Jeffcoate commenting on Browne's statement says "it remains a question whether all the pieces of the jigsaw have been accurately cut and whether the difficulties in assembling them arise because we are attempting to make them into one picture instead of several."

It was an attempt to examine one aspect of this complex syndrome that this investigation was undertaken. The consistent association of proteinuria with hypertension in the clinical picture of preeclampsia and yet the considerable variability in the development and progress of the disease stimulated a more detailed examination of

the nature of the proteinuria and the pathophysiological processes involved. The advent of sensitive and highly specific immunochemical methods permitted a more critical analysis of individual protein clearance patterns. The considerable advances made by such workers as Hardwicke, Squire, Blainey, Soothill and Cameron studying protein excretion patterns in the nephrotic syndrome suggested that this might be a useful line of approach in pregnancy proteinuria.

In the introduction, the historical development of theories relating to the aetiology of preeclampsia is traced. Modern concepts of proteinuria in general are reviewed with particular reference to their bearing on pregnancy proteinuria. The idea of a differential protein clearance across the glomerular basement membrane is discussed, leading to the development of protein selectivity as an expression of glomerular membrane porosity. It is this feature of the glomerular membrane that has been studied in the present investigation.

Forty-three patients with non-infective proteinuria in pregnancy have been investigated. Presentation of the results takes the form of:

1. Clinical features of all patients studied.
2. Renal function studies in patients with pregnancy proteinuria.
3. Protein clearance studies in pregnancy. This involves the use of radial immunodiffusion. All methods used are fully described.

An attempt has been made to differentiate between patients suffering from preeclampsia and those with essential hypertension. A smaller group of patients with renal disease in pregnancy is also included. Protein excretion patterns in the groups are compared and some differences between groups are noted. The difficulty involved in defining the groups is discussed.

The chief finding in the investigation of pregnancy proteinuria has been a characteristically poorly selective pattern of protein excretion. Thus patterns similar to those found in advanced renal disease are a feature of a condition where proteinuria is transient and the underlying renal pathology generally negligible. Some theories about this contradiction are advanced.

This investigation has been undertaken in the Department of Obstetrics and Gynaecology, Hammersmith Hospital, under Professor J. C. McClure Browne. The method has been continuously supervised by Mr. W. G. MacGregor, Reader in the above department, and by Professor J. R. Hobbs, at the time Senior Lecturer in the Department of Chemical Pathology, Hammersmith Hospital. The work was supported by Grant No. G 969/10/C of the Medical Research Council.

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PART I

INTRODUCTION

INTRODUCTION

Albuminuria in Pregnancy and the early ideas on causation

Ever since the association between albuminuria and eclampsia was noted by Lever in 1843, the kidney has figured in the forefront in the search for aetiological factors. Lever, who was a contemporary of Bright at Guy's Hospital, observed the close similarity in appearance between many of his eclamptic patients and patients with Bright's disease. Examination of the urine was the logical next step. Having fould albumin in nine out of ten eclamptic patients in whom the urine had been examined, he went on to examine the urine of fifty "normal" controls, and (rather surprisingly, since one would have anticipated a proportion of unrecognised preeclamptic patients amongst these) found albumin to be absent in every case. Lever recognised the "transitory nature" of pregnancy proteinuria and concluded that the condition differed from the permanent proteinuria of Bright's disease. In the same month of the same year (1843), Simpson made a similar observation in Edinburgh regarding proteinuria and eclampsia. Whilst also recognising that albuminuria disappeared in those patients who survived, he nevertheless attributed the syndrome of albuminuria and convulsions to underlying Bright's disease. This was a view that persisted for some time despite its obvious inconsistencies. Carl Braun of Vienna endorsed this concept and the chapter dealing with albuminuria and eclampsia in his "Lerbuch" was translated and run as a series of

articles in the Edinburgh Medical Journal (1856-57). In this work, Braun states firmly that eclampsia is a direct result of the uraemia resulting from poorly functioning kidneys. The kidney featured prominently in many subsequent theories on aetiology but the idea of primary renal disease being the origin of the process leading to eclampsia was shortlived. Austin Flint in 1863 stated "When Dr. Bright first published his discovery in 1827 and for some time afterwards, it was supposed that albumin in the urine always denotes disease of the kidneys. It is now well known that albumin in the urine occurs incidentally in a host of affections". A while later Barker (1863) wrote of pregnancy proteinuria "It is now an accepted fact that . . . gestation develops a temporary albuminuria which may disappear soon after puerperal convalescence". In 1861, Leyden described the "Kidney of Pregnancy" which became recognised as a pathological entity, clearly differentiating the disease from Bright's disease. He recognised two syndromes which resulted in albuminuria and eclampsia, one being associated with Bright's disease, the other which he called the kidney of pregnancy in which the lesions were peculiar to this condition.

That the kidneys played an important role in the pathogenesis of the disease remained undisputed. It remained a question of deciding how they became affected. The "ancient" theory of mechanical obstruction causing a back pressure on the ureters (Halbertsma, 1882) or on the renal veins was a popular explanation for a while, albuminuria

being a feature of a congested kidney and interference with normal elimination (a commonly used term at the time). This idea appeared to fit several of the known facts of the disease. Thus the predominance of albuminuria and eclampsia in the primigravida (with her relatively rigid and inflexible pelvic and abdominal musculature), the prevalence of the disease in multiple pregnancy and hydramnios and the frequent development of the condition during labour all tended to suggest that increased mechanical tension might play a part. This idea gained many adherents and even the argument against it that it failed to explain postpartum eclampsia was taken up by Webster (1900) who claimed that the postpartum uterus fills the greater part of the pelvis and could well produce the type of obstruction and back pressure described by Halbertsma. Nevertheless, by 1897, Allbutt was already expressing surprise that some authorities still recognised the "ancient mechanical theory" as the aetiology of albuminuria and eclampsia. By this time, the idea of a circulating toxin was generally recognised as the explanation of a condition which had far more generalised effects than was once supposed. Schmorl (1893) had demonstrated extensive involvement of many organs in the disease, notably the liver, and the idea of a systemic toxin was very acceptable as an agent capable of producing the many manifestations of the disease. The "toxaemic era" commenced with the ideas of auto-intoxication suggested by Bouchard in Paris (1887). The kidney, in susceptible subjects, was thought to be unable to cope with the large increase

in metabolic by-products accumulating in the blood, or, alternatively, was itself damaged by these substances. A vicious circle was set up whereby the increasing renal failure led to increasing accumulation of waste products. One or more of these substances (and many were incriminated and investigated) was capable of causing convulsions in toxic doses. From the concept of auto-intoxication, it was but a short step to the concept of a toxin elaborated by the placenta, a concept highly acceptable since the disease was in fact confined to pregnancy. Young (1914) developed the idea of a placental toxin and it was around this time that the significance of a raised blood pressure as part of the syndrome began to be appreciated. Thus Nicholson (1914) spoke of Young's placental toxin as having profound adrenalin-like effects, capable of raising the blood pressure and causing ischaemic damage to the kidneys.

Blood Pressure and Preeclampsia

The awareness of an association between a raised blood pressure in pregnancy and eclampsia thus postdated the discovery of albumin in the urine in the disease by almost fifty years. Clinical sphygmomanometry only dates back to Basch (1883), prior to whom blood pressure measurements required direct access to the vessel and were not suitable for clinical adaptation. The introduction of the Riva-Rocci sphygmomanometer (1896) which operated by measuring the external pressure required to occlude the radial pulse, simplified the technique considerably and regular studies of blood pressure in man began to appear.

Nevertheless, writing in the British Medical Journal as late as 1905, Martin notes "the reason why blood pressure determinations are not more generally made by English physicians is entirely owing to the fact that they have found the apparatus available unhandy or unreliable". Allbutt (1897) referring to reports of a raised blood pressure in pregnancy, felt that the experienced finger was more accurate than "illusory sphygmographs".

The application of sphygmomanometry to obstetrics followed quickly on the clinical application of Basch's instrument with the first known publication of blood pressure studies in pregnancy (Lebedeff and Perechjakew, 1884). Methods at this stage were inaccurate and of little value. The earliest observation of any real importance was that of Vaquez and Nebecourt in Paris in 1897. They drew attention to the fact that the blood pressure was raised in cases of eclampsia, an observation made also by Wiesner in a communication to the Leipsig Obstetrical Society in 1899.

Vegeler (1907) conducted one of the earliest continuous studies of blood pressure in pregnancy. He recognised the importance of a rise in blood pressure (above the limit of 150 systolic) as being an early sign of toxaemia and instituted treatment in all such cases. Every one of his patients with a raised blood pressure who was discovered and treated early went on to deliver normally. He appreciated that it was "impossible to say how many of these cases might not have gone on to eclampsia if untreated". However, he stressed the important

fact that although a raised blood pressure may not result in eclampsia, it nevertheless carried a bad prognosis for both mother and child. At a time when the general emphasis was on the albuminuria as being the main prodromal sign of eclampsia, he supported the view of Vaquez (1906) which he quoted:

"1. that blood pressure is invariably high in eclampsia,

2. the rise of blood pressure is the best prodromal symptom (of eclampsia) and does not depend on whether albuminuria is present or not,

3. the staying up of the blood pressure is a bad sign."

Cook and Briggs (1903) in an extensive analysis of blood pressure including blood pressures in pregnancy, emphasised the importance of a rise in blood pressure as a sign of severe preeclampsia and claimed that "if albumin be present in the urine during pregnancy, there is practically certain to be some degree of hypertension".

By 1910, Starling was able to write in the Lancet "All authorities are agreed that at any rate in its grosser forms, the toxaemia of pregnancy is accompanied by a high blood pressure." He was convinced that throughout normal pregnancy, blood pressure was unaffected and he was wary of any level above 125 systolic. Slemons and Goldborough (1908) had earlier suggested (on rather scanty evidence) that there was a steady rise in blood pressure in pregnancy. Newell (1915) found that a rise in blood pressure above 130 systolic preceded the appearance of albuminuria in every one of his cases and

went so far as to suggest that albuminuria in the absence of a raised blood pressure was of no significance. Donaldson (1913) was impressed with the high systolic blood pressure in eclamptic patients and patients with albuminuria and was struck by the rapidity with which normal levels of blood pressure were attained following delivery. He suggested that where the pressure failed to return to normal, an underlying renal lesion was probably present. Irving (1915) in a survey of 5,000 consecutive antenatal patients, showed a striking relationship between blood pressure levels and the incidence of albuminuria. Thus, with blood pressure levels between 130 and 140, the incidence of albuminuria was one in 32, rising to an incidence of one in three at levels between 150 and 160 and virtually 100% incidence of albuminuria at levels above 180 systolic. Irving felt that 150 was the danger point, at which level there was a sudden increase in the percentage of cases that developed albuminuria.

Sphygmomanometry as part of the care of the pregnant patient, finds a place for the first time in the second edition of De Lee's textbook of Obstetrics (1915), having been omitted from the first edition (1913). By 1922, De Snoo was able to state categorically that a rise in blood pressure was the earliest sign of preeclampsia and was always present in cases of eclampsia. Mussey and Randall (1924) wrote that "routine interval blood pressure readings beginning early in pregnancy have come to be regarded by many observers as of equal, if not greater, importance than routine urinalysis for albumin".

Finally, Browne (1932 and 1933) rounded off the controversy by a very convincing antenatal study conducted from early on in pregnancy. He was able to show that a rise in blood pressure preceded the appearance of albuminuria in 320 of his patients thought to have preeclampsia, and stressed the value of attention to a rise in blood pressure in the management of preeclampsia.

Thus we see the emphasis shift slowly from the concept of albuminuria and the kidney to hypertension and vascular phenomena in the aetiology and pathogenesis of the preeclamptic and eclamptic syndrome. Undoubtedly, the kidney was in some way incriminated, but any theory as to the causation of preeclampsia had to provide an explanation for the rise in blood pressure associated with the albuminuria. A vascular disturbance would more readily explain the situation.

With the demonstration by Goldblatt (1938) that interference with renal circulation even for a relatively short period of time could result in a considerable and often prolonged rise in blood pressure, an obvious link between the two main features of preeclampsia, hypertension and albuminuria, presented itself. The kidney began again to appear intimately involved in the process, especially after the demonstration by Pickering and Prinzmetal (1938) that the powerful vasoconstrictor substance renin is released from an ischaemic renal cortex in rabbits. Sophian (1953) developed the idea that uterine distension set in motion a utero-renal reflex which shunted the main

renal circulation away from the cortical glomeruli resulting in cortical ischaemia, hypertension and proteinuria. There is no evidence, however, that such a "Trueta shunt" mechanism operates in the pathogenesis of preeclampsia, nor indeed whether it occurs in man at all (Chesley, 1951). Paramore (1929) was more basic, suggesting that a rise in intra-abdominal tension attendant upon gestation created a back pressure, and engorgement of the venules in the renal medulla resulted. These swollen medullary venules caused an obstruction to the outflow of urine from the uriniferous tubules. He contended that eclampsia was an obstructive nephropathy, that the disabilities of the kidney were primary and that eclampsia was an acute uraemia. This in many ways was a reverberation of the ancient theory of mechanical obstruction, carried over into the twentieth century.

The wheel has turned full circle, with many of the old ideas recurring in more sophisticated forms in the literature. Fitzgibbon (1922) claimed that the lesions in preeclampsia were identical to those found in subacute glomerulonephritis and again invoked the concept of inefficient elimination of toxic products by a poorly functioning kidney. Theobald (1931) claimed that albuminuria without hypertension can be explained on the basis of mechanical obstruction to the venous return and the resulting renal congestion. McPhail writes in 1938 "It is believed that toxaemia will not develop if no impairment of renal function exists" and carries on to suggest that the disease originates from the failure of the kidney to excrete the

waste products of an overloaded metabolism. Studd et al., (1969 and 1970) have likened the condition to the nephrotic syndrome. They feel that all the prerequisites for applying this terminology are present. Albuminuria, hypertension and oedema are invariably associated with a hypo-albuminaemia, and a frequent rise in ∝-2-macroglobulin concentration in the serum completes the picture.

The Kidney and Preeclampsia

The role of the kidney in the pathogenesis of preeclampsia thus remains obscure, despite exhaustive attempts to clarify the situation. That renal changes, both physiological and pathological, do occur is supported by considerable evidence, but the absence of a consistent pattern together with the uncertainty regarding their timing in relation to the syndrome creates much confusion.

Early workers studying renal function in pregnancy reflect this confusion in the inconsistency of their findings. Chesley et al. (1940) using diodrast clearances as a measure of renal blood flow found this to be essentially normal in pregnancy. This was mirrored by the absence of any significant alterations in urea clearance and blood urea nitrogen in eclampsia (Chesley, 1939) although he did find a significant retention of uric acid in the disease. This latter finding has been noted by others (Stander and Cadden, 1934, Pollack and Nettles, 1960) but has also been found in males and non-pregnant females with essential hypertension (Cannon et al, 1966). Dill et al. (1942) using inulin and diodrast clearances confirmed the normality

of renal blood flow in preeclampsia, but found a decrease in glomerular filtration rate (G.F.R.). In hypertensive disease in pregnancy, they found a reduction in both G.F.R. and renal blood flow. In a more complex analysis, Corcoran and Page (1941) obtained essentially the same results. Homer Smith (1951) in an extensive review on the subject, concluded that renal function in both normal and hypertensive pregnancy remained unaltered.

Bucht and Werko in a much quoted work (1953) used a variety of renal clearance techniques to study renal function in preeclampsia. Earlier, Bucht (1951) had clearly demonstrated a rise in both renal blood flow and G.F.R. in normal pregnancy, and he thus appreciated the necessity of comparing values in preeclampsia with the values found in the same gestational month in a series of normal pregnancies. Furthermore, in this study, they followed up their patients with further clearance studies for periods ranging from four to twenty-four months.

All methods used, which included para-amine hippuric acid (P.A.H.), inulin and creatinine clearances, demonstrated quite clearly a fall in both renal blood flow and G.F.R. when compared with normal pregnancy. Glomerular filtration returned rapidly to normal within two weeks of delivery but renal blood flow, as estimated by P.A.H. clearances, took rather longer to return to normal levels. This rapid return to normal function after delivery, suggested that the deterioration in function probably results from some form of

vascular spasm which disappears in the early puerperium.

Kenney et al. (1950), using diodrast to measure renal blood flow and inulin to measure G.F.R., arrived at similar conclusions observing a clear reduction in both renal blood flow and G.F.R. in preeclamptic patients. They too stressed the importance of using the correct controls for comparison. Assali et al. (1953) used mannitol and P.A.H. clearances and arrived at the same conclusions.

There seems little doubt, then, that the preeclamptic syndrome is associated with some disturbance of renal function. The mechanism by which this might occur will be pursued further in the discussion of the results of this investigation. At this stage it is as well to mention the place of renal biopsy in the development of an understanding of the kidney in preeclampsia.

Initially, attention was directed towards renal morphology in fatal cases. Leyden's "Kidney of Pregnancy" (1881) was a large pale organ, showing fatty degeneration in both glomeruli and tubules. Schmorl's observations were similar (1893). Lohlein (1918) first drew attention to the similarity of the lesion to glomerulonephritis, describing a generalised ischaemia, thickened capillary walls and cellular swelling in the glomeruli. Thenceforth, investigators appreciated that the glomerulus was the seat of the main changes in the condition.

Since the advent of percutaneous renal biopsy (Iversen and Brun, 1951), pathologists have been able to pinpoint the lesion more

precisely. The application of electronmicroscopy has further clarified the situation. The typical lesion of preeclampsia is now recognised as a diffuse swelling of the endothelial cells of the glomerular capillaries (sometimes referred to as capillary endotheliosis) with consequent ischaemic narrowing of the capillary lumina (Spargo et al., 1959, Altchek, 1964, Pollak and Nettles, 1960, Pirani et al., 1961). Early ideas that narrowing of the capillary lumina was due to an actual thickening of the basement membrane, have not been substantiated (Bell, 1932). Thus the lesion is imminently reversible since no permanent damage has occurred (Spargo et al., 1959).

These lesions, though typical, are not consistent. They may be entirely absent in classical cases of preeclampsia. Furthermore, lesions identical to those of preeclampsia may be found in patients who fail to fit the clinical pattern of the disease (McCartney, 1968, Pollak and Nettles, 1960). In any event, renal biopsy is not a practical proposition as either a diagnostic, research or prognostic tool, since it carries a considerable morbidity in pregnancy even in experienced hands (Dennis et al., 1968, Schewitz et al., 1965).

With these concepts in mind, an approach has been made to investigate renal patho-physiological processes by a study of renal protein clearances in cases of pregnancy proteinuria.

Concepts of Proteinuria

There are basically four mechanisms by which protein may appear in the urine:

1. Increased glomerular permeability to protein
2. Diminished tubular reabsorption
3. Excretion of protein not normally present in the plasma
4. Proteins originating in the urinary tract.

The third possibility which was once favoured as the chief mechanism involved (Epstein, 1922, Addis, 1925) has now been largely rejected. With the exception of a group of low molecular weight myeloma proteins appearing in the urine in the myelomatoses, urinary protein is electrophoretically (Hardwicke, 1954, Neale, 1955) and immunochemically (Gitlin and Janeway, 1952) indistinguishable from plasma protein. Small quantities of protein originating in the urinary tract are in fact found in the urine in health (Boyce et al., 1961) but these do not seriously enter into the differential diagnosis of proteinuria. Infective exudates pus and blood when present are generally clinically obvious and clearly originate from the urinary tract.

Urinary albumin has been isolated in its pure form and found to be identical to plasma albumin according to every available test, (Rowe, 1957). In the experimental animal, plasma protein from heavily proteinuric rats when exchanged into normal rats failed to

appear in the urine of the recipient animal (Wakim, 1958). Normal rat protein labelled with I_{131} is excreted at the same rate in nephrotic rats as their own endogenous protein (Spector, 1954). Thus for practical purposes, proteinuria can be considered in general to be a loss in the urine of normal plasma protein. Further evidence to substantiate this in pregnancy will be provided in the description of methodology used in this investigation.

Having established that the protein in the urine comes from the plasma, we are left with two possible sites where an abnormality might result in loss of protein in the urine, the glomerulus and the tubule. At one time it was thought that the glomerular membrane was impermeable to plasma proteins, so that any protein in the urine could only result from increased filtration through the glomerulus. Wearn and Richards (1924), who first succeeded in obtaining glomerular filtrates directly by micropuncture in amphibia, thought that these were protein-free. It now appears that small amounts of protein, variously estimated at between 30 mgs. to 200 mgs. per 100 ml. are present in the glomerular filtrate of mammals (Walker et al., 1941, Dock, 1942, Dirks et al., 1964). Normal urine on the other hand contains not more than 200 mgs. of protein per day, so that a considerable tubular reabsorption of protein does in fact take place (Berggard, 1961, Glass et al., 1963). In keeping with the idea of relative impermeability of the glomerular membrane, however, is the finding that a considerable proportion of this protein in normal

urine is protein of very low molecular weight, present only in low concentrations in the blood (Berggard, 1970).

Morphological evidence suggests pinocytosis as the main mechanism for protein reabsorption (Sellers et al., 1954). Furthermore, clearance studies have suggested that reabsorption of protein from the glomerular filtrate is non-selective so that proteins are present in the urine in the same relative proportions as in the glomerular filtrate. Hardwicke and Squire (1955) arrived at this important conclusion after infusing albumin into nephrotic patients with albuminuria. They found an increase in the globulin clearance rate matching the increased albumin clearance. Petrie et al., (1968) compared the clearances of a series of dextrans with molecular weights approximating those of several plasma proteins. Since dextrans are not reabsorbed by the tubules, their clearance ratios provide a fairly accurate guide to glomerular porosity. They found a close correlation between the range of clearances of the dextrans and the clearances of plasma proteins of equivalent molecular weights. This again indicates that tubular reabsorption of protein must be non-selective and therefore plays little part in the ultimate clearance patterns achieved.

Accepting then, that tubular reabsorption is non-selective, the profile of proteins in the urine must be dependent upon their relative rates of filtration throught the glomerular membrane. In health, the glomerular membrane is relatively impermeable to plasma

proteins. Protein will only begin to appear in the urine in clinically detectable quantities once the tubular "threshold" for reabsorption is exceeded (Rather, 1952). This was shown by studies in dogs (Terry et al., 1948) and man (Waterhouse et al., 1948) by infusion of albumin into healthy subjects. Albumin began to appear in the urine when serum albumin levels exceeded 9.6 to 10.4 gms per 100 ml. in dogs whilst in human subjects a linear relationship was found between plasma albumin levels and rate of excretion.

The glomerular basement membrane has traditionally been regarded as a membrane behaving as though it had a range of pores (Bott, 1941). In diseased states, an increase in glomerular membrane porosity rapidly floods the renal tubules with quantities of protein well above their threshold levels. The quantity of protein appearing in the urine will to some extent depend on the tubular reabsorptive capacity, but the quality of the proteinuria, assuming that reabsorption is non-selective, will be entirely dependent on the degree of glomerular damage and glomerular membrane porosity. The applicability of this concept to the kidney has been tested using low molecular weight dextrans of varying size (Wallenius, 1954) and high molecular weight dextrans (Petrie et al., 1968) and data consistent with the idea obtained.

If one measures the proportion of proteins of different molecular weights in urine, it should be possible to derive some idea as to the nature of the glomerular leak and hence the severity of the

disease process. It would be anticipated that the greater the renal insult, the greater would be the glomerular leak and the higher would be the proportion of large molecular weight proteins in the urine.

This concept has been applied with considerable success in the nephrotic syndrome. Hardwicke and Squire (1955) using paper electrophoresis, demonstrated that smaller plasma proteins were cleared more rapidly than larger proteins in the nephrotic syndrome. With the advent of immunochemical methods, this idea was given greater precision, and Hardwicke and Soothill (1961) obtained data on the clearances of a number of different plasma proteins determined immunochemically. From the differential protein clearances thus noted, they developed the concept of protein selectivity. In essence, this represents the ratio of the clearances of plasma protein of large molecular weight to that of small molecular weight, and provides a useful index of glomerular membrane porosity.

Clearance patterns have been compared with morphological findings and a reasonably close correlation has been found between the selectivity of protein excretion and the underlying renal pathology (Blainey et al., 1960, Cameron and White, 1965, Cameron and Blandford, 1966). Perhaps even more important has been the relationship between protein selectivity in the nephrotic syndrome and the response to steroid therapy (Cameron, 1966, Joachim et al., 1964). Thus, with a highly selective pattern, a fairly good response to steroids may be anticipated, whereas with poor selectivity, response to steroids is inevitably bad.

In pregnancy proteinuria, we have a situation reminiscent of the nephrotic syndrome, in that a group of diseases with differing pathologies may present with an identical clinical picture. The successful application of immunochemical methods to the study of protein clearances in the nephrotic syndrome led to the idea that a similar approach might provide valuable information in proteinuria of pregnancy.

PART II

MATERIAL AND METHODS

MATERIAL AND METHODS

THE PATIENTS

All patients exhibiting proteinuria during pregnancy, whether associated with hypertension or not, fell within the scope of the investigation. An initial examination of the urine both microscopically and bacteriologically was performed in the first instance to exclude urinary infection as a cause of the proteinuria. Wherever infection was present, or any other complicating factor, such as gross haematuria or renal calculi, the patient was excluded from the study.

Patients investigated in this project were drawn primarily from women attending routine antenatal clinics at the Hammersmith Hospital, London, and from emergency admissions from the district.

Additional material was made available by courtesy of the following hospitals in the area:

Queen Charlotte's Maternity Hospital, London

Central Middlesex Hospital, London

Hillingdon Hospital, Middlesex

A further seven patients were gathered by the investigator from the National Maternity Hospital, Dublin, during a short period of residence specifically designed to obtain further material.

Every patient included in the study was seen personally by the investigator, and a full clinical examination conducted at least

once during the investigation. Where indicated, further clinical examinations were performed. Blood pressures were checked from time to time, the remainder of the readings being accepted as recorded by a senior member of the nursing staff. Special care was taken in checking clinical findings in the subsidiary hospitals, and all laboratory investigations on these patients were carried out at the Hammersmith Hospital to ensure consistency in methodology.

Selection of Material

In view of the complexity of control over management where patients are drawn from several hospitals, patients have been excluded from the study where it was thought that adequate personal supervision was lacking. Thus in all instances where basic management deviated significantly from the plan laid down at the Hammersmith Hospital, the cases were not included in the study. Wherever patients have been included where management has differed in any way, this has been clearly stated and any such patients included have been drawn from the Hammersmith Hospital only, where adequate supervision of management was feasible.

Follow-up Patients

All patients in the study were carefully followed throughout their antenatal period, and into the puerperium. Blood pressure and urine were checked and recorded on the fourth puerperal day, and where proteinuria persisted, further samples were taken for immunodiffusion studies.

An attempt was made to see all patients six to eight weeks after delivery and further clinical and biochemical assessments were made. Postnatal follow-up on three patients proved impossible and this small deficiency had to be accepted. Postnatal records of the seven Dublin patients were made available by arrangement with a designated investigator.

In a few instances, follow-up was continued beyond eight weeks.

Management and Investigation of Patients in the Study

All patients exhibiting non-infective proteinuria in pregnancy were admitted to hospital for full assessment and were retained in hospital for a period of time depending on the severity of the condition and the response to treatment.

The basis of treatment in all instances was bed rest and induction of labour.

Bed Rest

Patients were confined to bed in the first instance and response to simple measures was noted. Mobility was then gradually commenced according to the response. Where the severity of the condition warranted it, total bed rest was instituted. Clinical assessment took particular note of blood pressure recordings, urinary protein, weight gain and oedema. Foetal growth was assessed clinically by regular measurement of abdominal girth and by clinical "impression" of foetal size.

Supplementary measures, instituted primarily at the Hammersmith Hospital but also used to a varying extent in the other hospitals, included:

Diet

A normal diet was instituted in most instances and reduced to 1,000 Calories only where specially indicated. "Extra" salt was avoided but no further attempts at salt restriction were made.

Sedation

All patients received a sedative (amylobarbitone 100 mgs) at night. Where indicated, particularly in cases where blood pressure remained raised, additional sedatives were employed during the day, up to amylobarbitone 200 mgs being administered three times per day. Heavier sedation such as morphine 15 mgs was used in the more severe states, particularly prior to induction and during labour.

Antihypertensive Agents

Where severe hypertension was noted in early pregnancy or where patients were known hypertensives, antihypertensive drugs were employed from early on in pregnancy. They were not used where hypertension appeared as a late feature. Methyl-dopa was the drug of choice.

Diuretics

Chlorothiazide (grams 1 daily) was employed mainly as an adjunct to antihypertensive therapy and rarely as a specific method of reducing tissue oedema.

Clinical Observation

Blood Pressure

This was recorded four times daily. A level below $^{140}/_{90}$ was aimed at and where this could not be achieved, the patient was in general retained in hospital at complete bed rest. Where recordings below $^{140}/_{90}$ were regularly noted, mobilisation was commenced and in certain instances patients were permitted to leave hospital and attend antenatal clinics biweekly.

Urine

Ward testing for protein (albustix) was performed every morning and where indicated, more frequently. If this was positive for albumin, a mid-stream specimen of urine was obtained and the test repeated. The amount of albumin was estimated using 3% sulphosalicylic acid and estimating the turbidity formed against a series of standards (page 30).

Weight

Daily weighing was done on all patients, allowance being made for ½ to 1 lb/week increase for foetal growth and other gestational products.

Further routine daily investigations of maternal and foetal condition included temperature, pulse and foetal heart recordings, measurement of abdominal girth and clinical assessment of foetal size.

Laboratory Observation

Twenty-four hour oestriol excretion was estimated three times per week from the 32nd week as a guide to foetal well-being. Cognisance was taken of sequential values, a static or falling level being taken as a warning of possible placental malfunction.

Timing of Delivery - Induction of Labour

Where possible, a gestational age of 38 weeks was aimed at and induction of labour by amniotomy was performed between 38 and 40 weeks in most instances.

Earlier induction was performed in severe hypertensive states where response to treatment was unsatisfactory or where clinical and laboratory observations prompted premature delivery of the foetus to remove it from its increasingly hazardous environment.

Where doubt existed as to the maturity of the foetus, radiographic examination was performed. Use was made of the differential lipid staining technique (Brosens and Gordon, 1966). Increasing numbers of lipid containing cells are found in the liquor as the foetus becomes more mature and these are detected by staining with Nile Blue sulphate.

Caesarean section was readily employed where response to induction and the use of an oxytocin infusion was poor.

No patients were permitted to go beyond 40 weeks gestation.

Foetal weight and condition were noted at birth.

LABORATORY INVESTIGATIONS

Collections

1. 24 hour urine collection

   Wherever possible, patients in the study had a 24 hour urine collection for total protein excretion and creatinine clearance. Urine was collected under 10 ml. toluene used as a preservative. Since all patients were initially confined to bed, 24 hour collections could be controlled and supervised. The first urine passed in the morning was discarded, the collection then commencing to terminate with the inclusion of the early morning urine of the next day.

2. Venous blood was drawn once only during the period of urine collection, sufficient being taken for the following investigations:

   (i) 5 ml. clotted blood for creatinine clearance.

   (ii) 10 ml. clotted blood for estimation of:

   blood urea nitrogen

   blood uric acid

   blood cholesterol

   serum proteins

   (iii) 8 ml. clotted blood for protein immunodiffusion study.

3. A mid-stream specimen of urine, obtained fresh on the same day as blood was taken. This specimen was divided into three parts for:

   (i) bacteriological culture

(ii) microscopic examination.

(iii) immuno-diffusion protein study.

The number of times these investigations were repeated depended largely on the duration of the disease, but wherever possible, immuno-diffusion studies were performed at least every two weeks on "long-stay" patients and more frequently on shorter-stay patients.

Laboratory Methods

Methods for estimating the following substances were adapted for use in a Technicon auto-analyser which was utilised in this study:

blood urea nitrogen

blood uric acid

serum proteins

serum and urinary creatinine

blood cholesterol

Blood Urea Nitrogen

The automated method used here is an adaptation of the carbamido-diacetyl reaction as applied to urea nitrogen (L. T. Skeggs, 1957, W. H. Marsh et al., 1957).

Blood Uric Acid

The method used involves the reduction of a phosphotungstate complex to a phosphotungstite complex, the colour being intensified by the presence of cyanide.

Serum Proteins

Albumin and globulin estimations are made by estimation of total serum proteins by a modification of the biuret method and an estimation of serum albumin alone on the same sample.

Biuret method for estimation of total proteins

(T. E. Weichselbaum, 1946)

This method depends on the formation of a purple coloured complex of copper in an alkaline solution with two or more carbamyl (-CO-NH-) groups. The method has been modified for use in the auto-analyser by J. F. Failing (1960).

Estimation of serum albumin

This is based on the dye binding capacity of albumin with 2-benzoic acid (D. D. Rutstein et al., 1954).

Serum and Urinary Creatinine

Creatinine clearances are obtained by estimation of serum and urinary creatinine using a modification of the procedure described by Folin and Wu in "Practical Physiological Chemistry" (Hawk, Oser and Summerson, 12th ed.). This involves dialysis of the sample diluted with saline and mixing this with saturated picric acid and 0.5 N. sodium hydroxide.

Blood Cholesterol

This estimation is based on the reaction of concentrated sulphuric acid and ferric chloride in acetic acid with steroids having the 5-ene, 3β-ol grouping. Automation of the method

is described by Zlatkis et al. (1953), Zak et al. (1954) and Leffler (1959).

Estimation of total urinary protein

1. Sulphosalicylic acid turbidimetric method

3 ml. of 3% sulphosalicylic acid is added to 1 ml. of urine under investigation. The turbidity produced is read after five to ten minutes against permanent standards (Gallenkamp). If protein concentration is too high to read from the top standard (100 mg. per 100 ml.), the test is repeated with urine suitably diluted.

This test was used as a screening test for all urines studied in order to get an idea of the total protein concentration in the specimen prior to immuno-diffusion studies. A more accurate quantitation of the total urinary protein excreted over 24 hours was achieved using the second method of protein estimation.

2. Biuret method

(Bell and Baron, 1968)

Equal quantities (1 ml.) of urine under investigation are mixed in a centrifuge tube and allowed to stand for at least three hours. The mixture is then centrifuged and the supernatant fluid carefully decanted without loss of precipitate. 0.5 ml. normal sodium hydroxide (N. NaOH) is added to the deposit which is stirred until solution is complete. 1.5 ml. of water is added.

A standard is prepared containing 1 ml. of bovine albumin solution (containing 500 mg. albumin per 100 ml.) and 1 ml. 5 N. NaOH.

A blank is prepared containing 1 ml. distilled water and 0.5 N. NaOH. To each of the three tubes is added 2 ml. of working Biuret reagent and after standing at room temperature for twenty minutes, these are then read in a colorimeter at 540 mμ.

Protein concentration in the unknown urine (in mg. per 100 ml.) is obtained by the equation:

$$\frac{\text{URINE - BLANK}}{\text{STANDARD - BLANK}} \times 500$$

All the foregoing investigations were undertaken in the routine biochemical laboratory. The following investigations including all microscopic examinations and all protein clearance studies were performed personally.

DIFFERENTIAL PROTEIN CLEARANCES AND SELECTIVITY STUDIES

Individual plasma/urine protein ratios have been studied with the object of estimating clearances of a range of plasma proteins of varying molecular weights. These clearance ratios have been expressed as a percentage of the transferrin and albumine plasma/urine ratio, giving an index of renal selectivity for each patient studied (Hardwicke and Squire, 1955, Cameron and Blandford, 1966).

The following plasma protein clearances have been investigated:

Protein	Molecular Weight (Schultze & Heremans, 1966)
Albumin	69,000
Transferrin	90,000
Ig G (7S) Gamma globulin	160,000
$\beta_1{}^A/C$ globulin	250,000
Pseudocholinesterase	300,000
Ig M (19S) Gamma globulin	1,000,000
α-2-macroglobulin	820,000

Fresh $\beta_1 C$ globulin (M.W. approximately 270,000) rapidly undergoes transformation to $\beta_1 A$ globulin (M.W. 240,000) and both of these react in the immunochemical system used. Since there would be little difference in migration in gel for such close molecular weights, the mean of 250,000 is used.

Serum and urine proteins have been estimated immunochemically and pseudocholinesterase has been estimated both immunochemically, and biochemically by the action of the enzyme on a substrate.

The latter four proteins of large molecular weight are present in urine in amounts necessitating concentration of urine in all instances, the degree of concentration varying from 20 to 200 times depending on the total urinary excretion of protein.

Description of the method will be divided into two phases:

(a) Preparation of the blood and urine samples.

(b) Immuno-diffusion technique.

Preparation of Blood and Urine Samples

Blood

Approximately 5 ml. of venous blood is drawn into a plain tube and allowed to stand at room temperature for one to two hours to permit a firm clot to develop.

The sample is then centrifuged at 1,000 r.p.m. for five minutes. Supernatant serum is carefully removed with a Pasteur pipette and placed in a centrifuge tube. This is centrifuged at 3,000 r.p.m. for a further three minutes to remove any remaining cellular elements. The serum is transferred to a 5 ml. storage tube containing sodium azide as a preservative and placed in a refrigerator at -20°C until required.

Urine

A fresh mid-stream specimen of urine is collected, untimed apart from the fact that it is collected on the same day as the blood sample.

Microscopic examination

All urines were examined under both low power (100 times) and high power (400 times) shortly after the collection was obtained.

Where there was evidence of contamination, the specimen was discarded in favour of a fresh one. Where evidence of urinary infection was present in the form of excess leucocytes or bacteria, the patient was eliminated from the study.

Centrifuged deposits (1,000 r.p.m. for three minutes) were examined in addition to uncentrifuged specimens.

Observations were made of all cellular elements, bacteria and in particular, tubular casts, their number and character.

Urine is then filtered through Whatman filter paper into a flask. A preliminary assessment is made of the total protein content of the filtrate, using 3% sulphosalicylic acid in a ratio of one part urine to three parts acid solution. Turbidity is compared to a series of standard protein solutions and a rough estimate of the protein content obtained. This step is essential as it is necessary to have some knowledge of the protein concentration in the urine when preparing for immuno-diffusion, in order to determine to what extent (if any) dilution of the urine is required.

Further ultrafiltration of the urine is achieved using Sartorius membrane filters of pore size 0.8 and 0.2 microns contained in a filter holder (Swinnex-25, Millipore), the urine being forced through under pressure using a syringe attached to the filter holder. Sodium azide is added as a preservative. The sample is divided into two, one aliquot containing approximately 5 ml. of urine being placed in a refrigerator at -20°C for estimation at a later date of albumin, transferrin and Ig G gamma globulin, the remainder, usually 20 ml., being concentrated down 20 to 200 times for estimation of the larger protein molecules.

Concentration of Urine

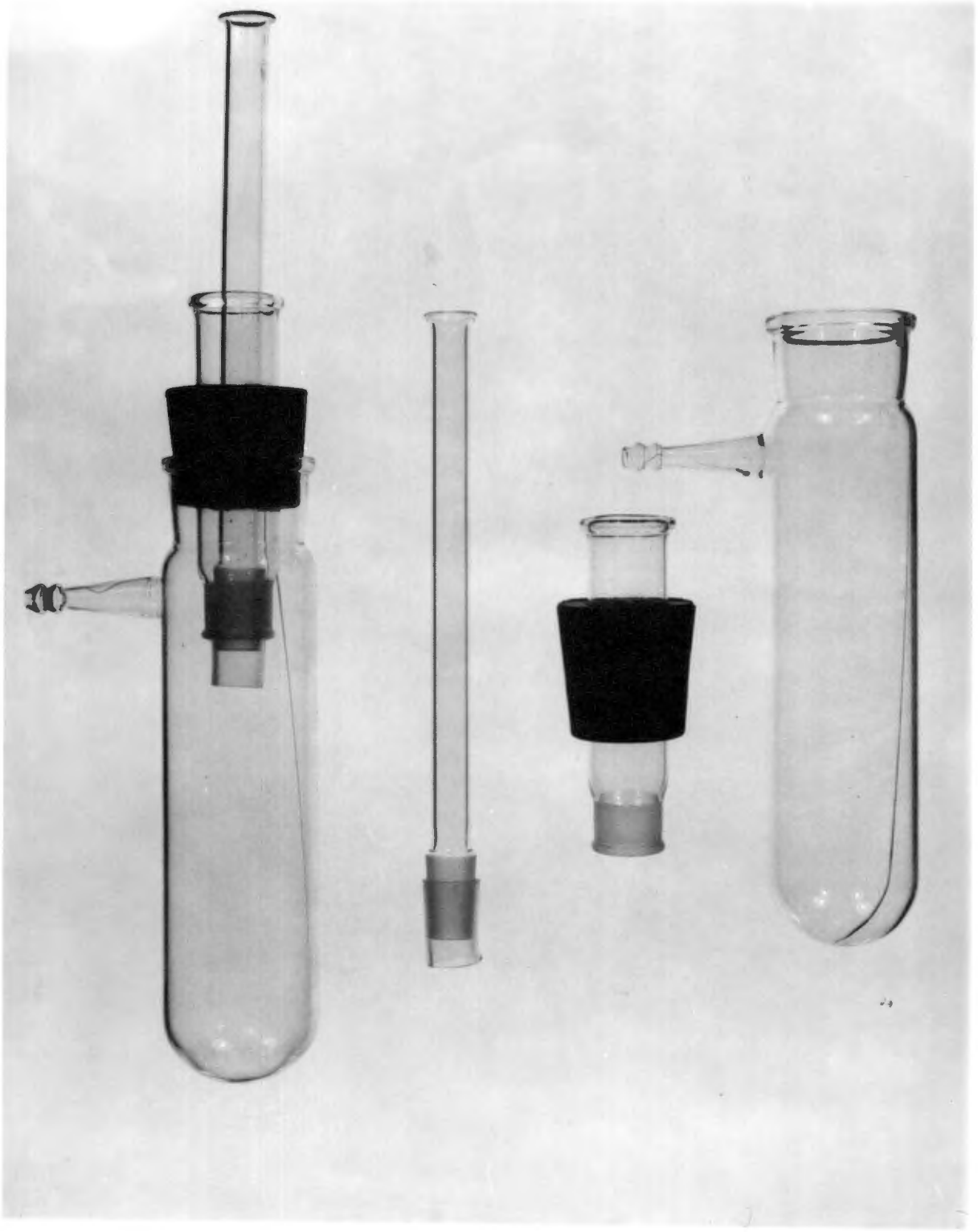
The extent to which the urine is concentrated depends largely on the amount of protein in the urine. A concentration of approximately 10 grams per 100 ml. is aimed at in order to detect small quantities of α-2-macroglobulin and Ig M globulin should they be present.

Apparatus used: Sartorius collodion bags (Sartorius Cat. No. SM 13200)

Glass holders for bags (Sartorius Cat. No. SM 16304)

Vacuum pump

A Sartorius collodion bag is pulled approximately 1 cm. over a plastic sleeve on the inner glass tube of the glass holder.



The inner tube plus bag is then pushed firmly home into an outer glass sleeve, creating a watertight seal. The assembly is placed in a suction flask filled with saline, to which a vacuum pump is applied. A measured quantity of the sample to be concentrated is introduced via the inner glass tube into the collodion bag. Concentration is thus effected by vacuum dialysis.

After concentration, the concentrate is carefully extracted from the bag using a micro-pipette and the walls of the bag are washed down with a small quantity of saline. Ideally, three washings are aimed at. The precise quantity of concentrate plus diluent used for washing are measured and the number of times the sample has been concentrated can thus be estimated.

Recovery of protein using Sartorius collodion bags

A good recovery of protein after concentration is essential if quantitation of individual urinary proteins is to have any relevance. To test the recovery rate of the method, 10 ml. of ^{125}I labelled albumin were introduced into a collodion bag and the solution dialysed by the above method to approximately 0.05 ml. The sides of the bag were then washed down with small quantities of saline and concentrate and diluent were extracted from the bag using a calibrated micro-pipette. By adding diluent very meticulously, the quantity of concentrate

was made up to precisely 0.1 ml., representing a concentration of 100 times. The volume was then reconstituted by adding saline up to 10 ml.

Gamma counts were taken before and after concentration, the latter count being expressed as a percentage of the former. This represents the percentage recovery rate of the method. The procedure was repeated using ^{125}I labelled α-2-macroglobulin. Gamma counts before and after concentration using this method will be found in Appendix I.

The mean percentage recovery of protein using this method of concentration was found to be:

for albumin 92.6%

for α-2-macroglobulin 96.4%

Recovery of protein is thus good using this method, with a recovery of proteins of the molecular weight of albumin of 92.6% and an even better recovery of larger molecular weight proteins. One can reasonably assume that recovery of the proteins with molecular weights falling between these two would be between 92.6% and 96.4%. Since we are only really concerned with recovery rates of the four proteins with molecular weights of 300,000 and above, the high percentage recovery of proteins of this class indicates that this method of concentrating urine is satisfactory for the experiment.

Immuno-diffusion technique

The technique of radial immuno-diffusion as a method of measuring individual proteins in the presence of many others was described in 1963 (Mancini, 1963). Antiserum raised against the particular protein under investigation is mixed in solution with buffered agar in suitable proportions. The fluid containing the unknown protein is introduced into the agar plate together with a series of known standards. The resulting precipitin rings which form from the antigen/antibody complex are proportional in their diameter to the logarithm of the antigen concentration.

The technique followed in this study is a modification of the method outlined by Mancini (1965) and revised by Hobbs (1970). It has been used to measure the specific seven proteins listed in simultaneous blood and urine collections from the patients in the study in order to investigate individual protein clearances.

Antisera

Antisera to the following proteins were raised in rabbits by an immunisation schedule described in Appendix II:

Albumin

Transferrin

Ig G globulin

Ig M globulin

Goat antiserum to α-2-macroglobulin was purchased from Hyland Laboratories, Thetford, Norfolk (List No. 071 - 218).

Commercial immuno-plates impregnated with anti-human complement (C3) in agar (Hyland Laboratories, List No. 085 - 050) were utilised for the measurement of β_1^A/C globulin. A rabbit antiserum against human pseudocholinesterase was purchased from Dakopatts A/S (Denmark) (quantitation of this protein was also conducted by a chemical method - page

Antisera were tested for monospecificity against human serum using immuno-electrophoresis. This will be described under "Validation of the method".

Preparation of the agar

Reagents 1. I.D. agar tablets 0.5 G (Oxoid BR27)

2. Barbiturate buffer pH 8.6 Ionic strength 0.1

9 Grams of sodium diethylbarbiturate

65 ml. of $1/_{10}$ N. hydrochloric acid

0.5 Grams sodium azide (preservative)

Volume adjusted to one litre using distilled water.

A 1% solution of agar is made by dissolving one 0.5 gram agar tablet in 50 ml. barbiturate buffer and heating. The solution is transferred in appropriate quantities to test tubes standing in a controlled temperature water bath. Temperature is maintained thermostatically between 48° to 52°C. (Below 48°C agar mixes badly and above 56°C antisera may be denatured.)

Preparation of antiserum in agar plates

Antiserum is mixed in suitable proportions with agar in a test

tube and the mixture is poured into a 9 cm. Petri dish. The correct concentration of antiserum has to be worked out for each fresh batch of antiserum as the potency varies considerably between batches. In general, the amount of antiserum required varies between a 1% and 10% solution, depending on the type and potency of antiserum, and this is made up in 8 ml. buffered agar. The mixture is then rapidly poured into a 9 cm. plastic Petri dish on a perfectly flat surface (checked by spirit level). A glass slab on a flat work bench was used for pouring the plates so that the surface could be warmed with a flame prior to pouring. Air bubbles are rapidly dispelled by flaming and the agar is allowed to set, then covered, inverted and placed in a refrigerator at 4°C.

Determination of the optimal antiserum concentration

It is essential that the correct concentration of antiserum is worked out for each batch of antiserum used. The ideal concentration is that which provides the most satisfactory precipitation rings with the antigen to be tested at the range of antigen concentrations likely to be encountered.

Agar is prepared in barbiturate buffer as above and a series of concentrations of the antiserum to be tested are made up using 5 cm. plates and adding 0.025, 0.05, 0.1 ml. and 0.2 ml. antiserum to 2.5 ml. agar. The appropriate antigen is then introduced (as will be described) and the most suitable working

concentration of the antiserum is selected for subsequent determinations.

Preparation of the plates for the antigens

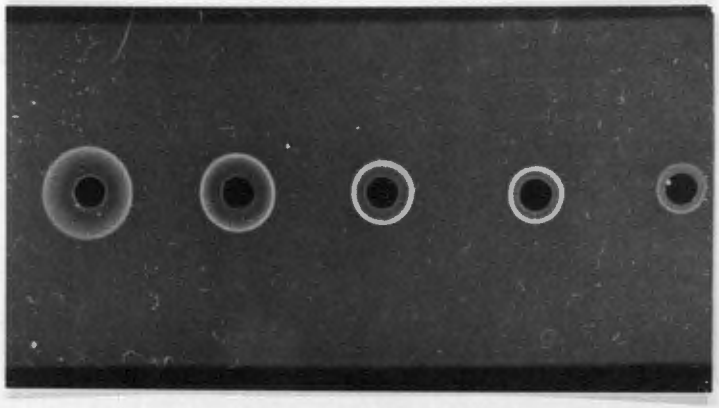
Antigens under investigation are introduced into previously cut wells in the agar plates and allowed to diffuse out into the antibody-containing agar.

Preparation of wells

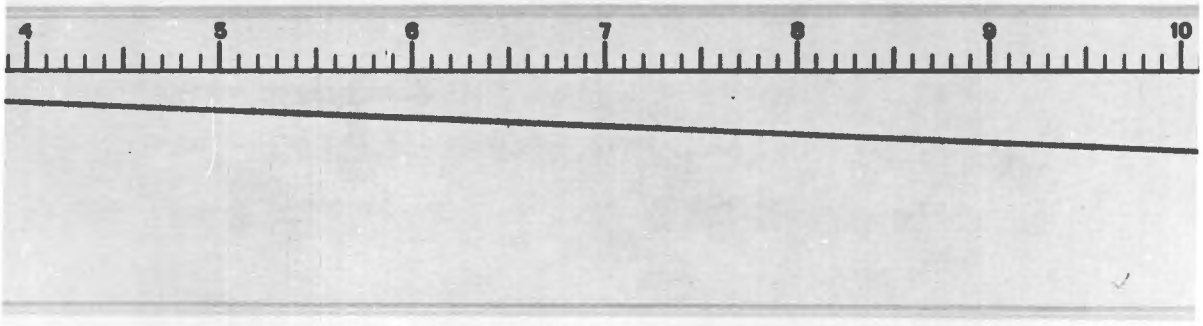
Wells are cut in the agar at suitable distances from each other so that antigen diffusion from one well will not in any way interfere with diffusion from its neighbour. A suitable template is used so that spacing is equal and a pattern planned for subsequent reading. Wells are cut with a 2 mm. diameter stainless steel tube with sharp, keenly-bevelled edges. A Pasteur pipette attached to suction is used to remove the central detached plug from the well.

Filling the wells

The antigens under investigation, suitably diluted, are introduced into the series of wells using a micro-tip Pasteur pipette attached to a mouthpiece. The wells are filled exactly to the brim in each case. Accuracy in filling the wells was achieved by practice and will be discussed under "Precision of the method". An appropriate series of dilutions of known standard antigen are included in the plate and as soon as the solutions have diffused into the agar, the plate is covered,



Precipitin Discs in Antibody-impregnated Agar



Measuring Rule

inverted and kept at room temperature. Albumin, transferrin, and Ig G globulin plates were read after 24 hours and pseudo-cholinesterase, β_1^A/C globulin, Ig M and α-2-macroglobulin plates were read after 48 hours.

Reading of the plates

This was done with the aid of a specially designed reading rule (Hoechst Pharmaceuticals) against a dark ground illuminated viewing screen. The precipitation disc to be read is slid between the two diverging lines of the rule. The rule is calibrated in such a way that when the edges of the ring "kiss" the lines on either side, the reading at that point represents the diameter of the disc.

Plotting the results

Three cycle semi-logarithmic graph paper was used for plotting results (Chartwell graph sheet 4325). Antigen concentrations of the series of standards were plotted on the vertical logarithmic scale against the precipitin disc diameters on the linear horizontal scale. The unknown antigen concentrations were then determined from this graph, corrections being made where appropriate for concentration or dilution of antigen.

Preparation of immuno-diffusion plates for quantitation of individual proteins in serum and urine

Agar plates impregnated with the appropriate antibody at the optimum concentration were prepared for each protein to be

quantitated. Serum and urine were quantitated on the same plate to avoid interplate variation as a source of error. Since the object of the investigation was the determination of plasma/urine ratios for each protein, absolute values of the protein in serum and urine were not critical. All investigations were done in duplicate, and where the difference between two measurements exceeded the coefficient of variation for the method, the results were discarded and the investigation repeated. A similar procedure was adopted for all standards, a duplicate series of dilutions being prepared for each plate.

Since the concentrations of the proteins both in serum and urine varied considerably, appropriate dilutions were prepared where indicated to achieve the most satisfactory precipitation rings. Serum required dilution for the estimation of albumin, transferrin, Ig G globulin, Ig M globulin and ∝-2-macroglobulin. Urine dilution was planned according to the protein under investigation and by an initial estimate of urinary protein concentration by comparing the turbidity produced by the addition of 3% sulphosalicylic acid against a set of known standards.

Albumin Standard

Dried human albumin made up to a 1% solution in saline was used as the standard. Serial dilutions of this standard

were made to provide a range of standards containing from 40 mgs. to 2.5 mgs. albumin per 100 ml.

Plasma albumin

Levels in the patients under investigation varied between 1.5 grams and 4.5 grams per 100 ml. All sera were diluted 1:200 with normal saline, thus providing concentrations which fell well within the range of standards.

Urinary albumin

Levels in the patients under investigation varied between 100 mgs. and 1,500 mgs. per 100 ml. Dilution with normal saline was required in all cases to bring the concentration down to within the range of standards. Dilution varied between 1:4 and 1:40.

Antiserum

Concentration of antiserum in agar varied between 0.4 ml. and 0.6 ml. in 8 ml., depending on the potency of the batch.

Transferrin standard

Purified dried transferrin powder made up to a 1% solution with normal saline was used as the standard. Serial dilutions were made to provide a range of standards containing from 125 mgs. to 3.5 mgs. transferrin per 100 ml.

Plasma transferrin

Levels in the patients under investigation varied between

150 mgs. to 600 mgs. per 100 ml. All sera were diluted 1:8 with normal saline, thus providing concentrations which fell within the range of standards.

Urinary transferrin

Levels varied between 10 mgs. and 350 mgs. transferrin per 100 ml. Most urines required no dilution, but where urinary concentrations above 100 mgs. per 100 ml. were anticipated, the urine was diluted accordingly.

Antiserum

Concentration of antiserum in agar varied between 0.1 and 0.2 ml. in 8 ml. agar.

Ig G gamma globulin standard

Standard human serum (Behringwerke Ag) containing 990 mgs. Ig G per 100 ml. and diluted 1:20 was used as standard. Serial dilutions were made providing a range of standards containing from 100 mgs. to 3 mgs. Ig G per 100 ml. A 100 mgs. standard was achieved by adding the 50 mgs. standard to the same well a second time.

Plasma Ig G globulin

Levels in patients under investigation varied between 300 mgs. and 1,600 mgs. per 100 ml. All sera were diluted 1:20 with normal saline.

Urinary Ig G globulin

Levels varied between 5 mgs. and 100 mgs Ig G globulin

per 100 ml. Most urines required no dilution.

Antiserum

Concentration of antiserum varied between 0.1 and 0.2 ml. in 8 ml. agar.

β_1^A/C globulin standard

Standard human serum (Behringwerke Ag) containing 80 mg. β_1^A/C per 100 ml. was used as a standard. This was diluted with saline to provide a range of standards containing from 80 mg. to 2.5 mg. per 100 ml. A 160 mg. standard was achieved by filling the same well twice.

Plasma β_1^A/C

Levels varied between 50 mg. and 150 mg. per 100 ml. Sera were estimated without dilution.

Urinary β_1^A/C

Urine was estimated for β_1^A/C levels after concentration through a Sartorius collodion bag.

Antiserum

Commercial immunoplates impregnated with β_1^A/C antiserum (Hyland Laboratories) were used for this estimation.

Pseudocholinesterase

This protein though present in only small amounts in serum is nevertheless detectable by immuno-electrophoresis and therefore by radial immuno-diffusion. In estimating clearances of this protein, the patient's serum was used as the

standard, the urine concentration being expressed as a percentage of the plasma concentration. Thus serial dilutions were made of the patient's serum and plotted on a semi-logarithmic graph, using an arbitary figure of 100 as representing the concentration in the serum. The diameter of the precipitation disc produced by the patient's urine could then be read as a percentage of the concentration in her serum.

Antiserum

Concentration of antiserum in agar varied between 0.2 ml. and 0.4 ml. in 8 ml. agar.

Ig M gamma globulin standard

Standard human serum (Behringwerke AG) containing 85 mg. Ig M globulin per 100 ml. was used as a standard. This was diluted with saline providing a range of standards containing from 85 mg. to 5 mg. per 100 ml. A 170 mg. standard was achieved by filling the same well twice.

Plasma Ig M

Levels varied between 80 mg. and 170 mg. per 100 ml., a few sera having concentrations above 170 mg. per 100 ml. and requiring dilution. Otherwise sera were estimated without diluting.

Urinary Ig M

Concentrated urine was quantitated, corrections being made for the amount of concentration.

Antiserum

Concentration of antiserum varied between 0.2 ml. and 0.4 ml. in 8 ml. agar.

α2-macroglobulin standard

Standard human serum (Behringwerke AG) containing 45 mg. α2-macroglobulin per 100 ml. was used as a standard. This was diluted with saline to provide a range of standards containing from 145 mg. to 9 mg. α2-macroglobulin per 100 ml. A 290 mg. standard was achieved by filling the same well twice.

Plasma α2-macroglobulin

Levels varied between 100 mg. to 450 mg. per 100 ml. All sera were diluted 1:2 to bring them within the range of the standards.

Urinary α2-macroglobulin

Concentrated urine was quantitated, corrections being made for the amount of concentration.

Antiserum

Concentration of antiserum varied between 0.4 ml. and 0.5 ml. in 8 ml. agar.

VALIDATION OF THE METHOD

1. Monospecificity of antisera

The measurement of individual proteins in urine and serum using specific antisera depends on the assumption that the antiserum will combine only with the specific antigen to which it has been raised. Cross reaction with other proteins present in these fluids would produce erroneous results. It is thus essential to test all antisera for monospecificity prior to use.

Immuno-electrophoresis

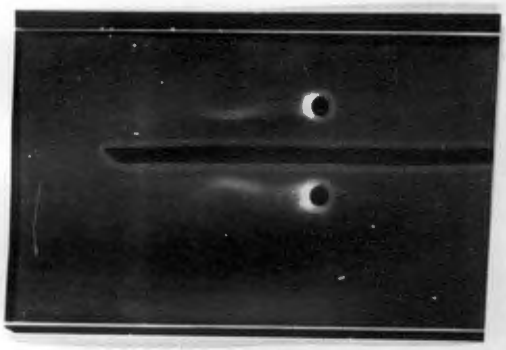
Specificity of the antisera was tested by this method. Human serum is electrophoresed in buffered agar prepared on a microscope slide. Once satisfactory separation of the serum proteins has been achieved, the specific antiserum is introduced to a trough which is cut in the agar parallel to the line of separated proteins. Diffusion is then allowed to proceed at room temperature. A monospecific antiserum will form an antibody/antigen complex with one antigen only, producing a single precipitin line at a characteristic site on the slide. Cross reaction will be detected by more than one precipitin arc.

Apparatus and technique

Buffered agar is prepared as previously described and applied by pipette to alcohol-cleansed level glass microscope slides. Approximately 3 ml. of agar is used per slide. After solidification, holes and troughs are cut in the agar using a commercially

MONOSPECIFICITY OF ANTISERA

I. Immuno-electrophoresis



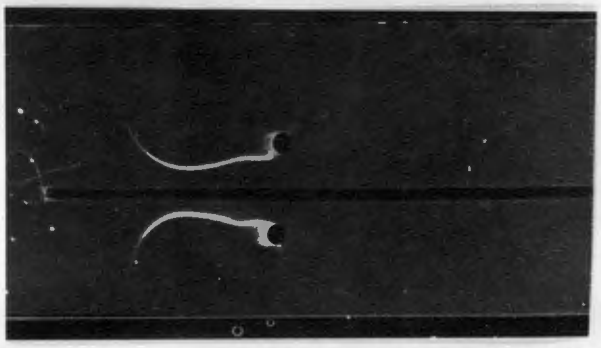
Serum

Serum

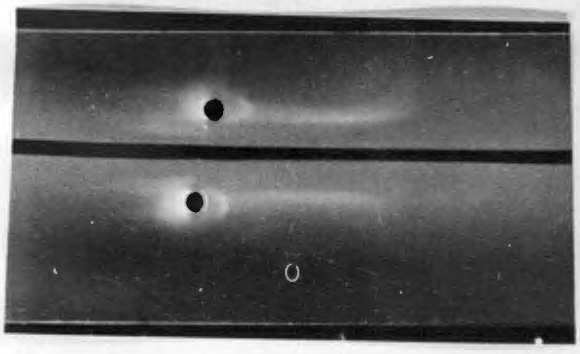
Anti-pseudocholinesterase

Serum

Serum



Anti-alpha-2 macroglobulin



Serum

Serum

Anti-Ig M gamma globulin

manufactured die (L.K.B. Instruments Ltd.). Serum is introduced into the holes as previously described and electrophoresed for approximately 45 minutes. Electrophoresis is carried out in a standard electrophoresis chamber (L.K.B. Instruments Ltd.). The slides are placed across two parallel bars, contact being made with a buffer in the chamber by strips of filter paper at either end of the slide. Voltage between the ends of the slides is 45 volts (6 volts per cm.).

All antisera used in this investigation (with the exception of β_1^A/C) were immuno-electrophoresed to confirm monospecificity. β_1^A/C was supplied in commercially prepared immunoplates and monospecificity was presumed.

2. Reaction of Identity

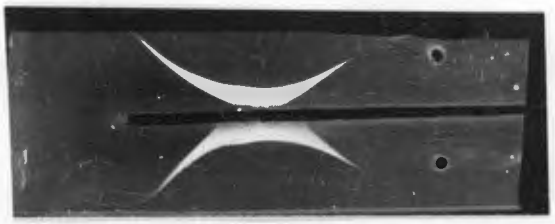
In order to examine plasma/urine ratios of a specific protein, it is necessary to establish that the urinary protein is in fact plasma protein that has been cleared through the glomerulus. Since this is by far the commonest mechanism of proteinuria (page 14), it was reasonable to assume that this is the mechanism operating in pregnancy proteinuria. To confirm this, two experiments were performed to demonstrate a reaction of identity between the serum and urinary proteins being investigated.

(a) Immuno-electrophoresis

This was carried out as before, with simultaneous electrophoresis of serum and concentrated urine from the same patient.

MONOSPECIFICITY OF ANTISERA AND REACTION OF IDENTITY

I. Immuno-electrophoresis



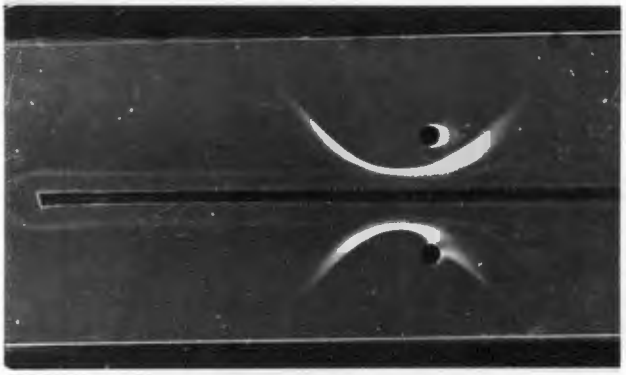
Concentrated Urine

Serum

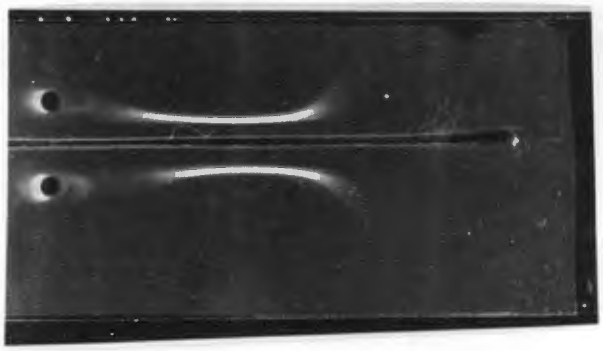
Anti-albumin

Concentrated Urine

Serum



Anti-transferrin



Concentrated Urine

Serum

Anti-Ig G Globulin

Serum and urine were introduced in wells on either side of the central antiserum trough, and equidistant from it. Identically situated precipitin arcs on the serum and urine side of the trough confirm the identical nature of the antigens.

(b) Double diffusion technique

(Gell, 1957, Soothill, 1962)

This is a method by which antigen and antiserum are introduced into wells cut in agar at short distances from each other and allowed to diffuse towards each other. At an optimum antigen/antibody concentration, precipitation occurs resulting in a precipitin line.

Using a specially prepared die containing a central cutting tube surrounded by six equidistant smaller cutting tubes, seven wells are cut in agar prepared in a similar way to that previously described. Antiserum to the protein antigen under investigation is introduced into the central well. Serum and urine under investigation are introduced into four of the surrounding wells, the remaining two wells being filled with pure protein antigen. If the antiserum is monospecific and the protein in the serum and urine are identical to the pure protein antigen, a precipitin hexagon will form, the precipitin lines being identically situated and joining their neighbours exactly, forming an obtuse angle at the junction.

MONOSPECIFICITY OF ANTISERA AND REACTION OF IDENTITY

2. Immunodiffusion

Anti-albumin

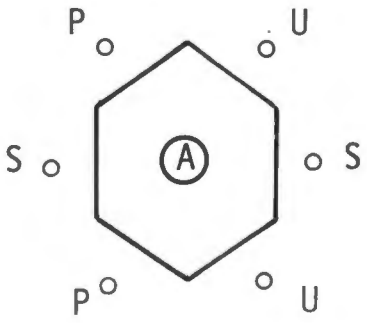
Anti-transferrin

Anti-alpha-2-macroglobulin

Anti-Ig G

Anti-Ig M

Anti-pseudo-cholinesterase



A = Antiserum Well
P = Plasma Well
U = Urine Well
S = Standard Antigen

This experiment was conducted with the sera and urines of three patients in the study, all antisera (with the exception of β_1^A/C) being thus tested. The results again demonstrated the degree of specificity of the antisera used. It also demonstrated that the urinary proteins were in fact immunochemically unaltered plasma proteins which had filtered through the glomeruli. Reaction of identity between pseudocholinesterase in serum and urine was confirmed, but no pure pseudocholinesterase standard was available to confirm that the antigen was in fact pseudocholinesterase. Presumptive evidence that the antiserum was precipitating pseudocholinesterase includes:

(i) the position of the precipitin band in the immunoelectrophoresis strip in the α-2-globulin region where one would expect pseudocholinesterase to lie,

(ii) a similar clearance ratio shown later for pseudocholinesterase and β_1C which have similar molecular weights.

3. Precision and reproducibility

Serum and urine from a patient with heavy proteinuria (200 mg. per 100 ml.) were divided into twelve aliquots each, allowing for twelve separate estimations of serum and urinary protein from the same patient. A further collection of urine from the patient was divided into three parts and concentrations were made for estimation of the larger protein molecules, four estimations being made at three different concentrations.

Detailed results of individual serum and urine values for the six proteins studied will be found in Appendix III. Mean values, standard deviations and the error of the method expressed as the coefficient of variation have been calculated. The error in calculating U/P values and hence protein selectivity has also been assessed.

The error, expressed as the coefficient of variation (standard deviation as a percentage of the mean) for individual readings and for U/P ratios is summarised from Appendix III in the following table:

	Individual Readings		Clearance (U/P ratio)
	Serum	Urine	
Albumin	5.8%	23.3%	9.6%
Transferrin	8.5%	12.3%	11.1%
Ig G globulin	8.8%	12.5%	14.3%
β_1^A/C globulin	11.7%	18.4%	
Ig M globulin	10.1%	-	
α2-macroglobulin	4.2%	24.4%	

Accuracy of measurement was greatest at higher protein concentrations where errors represent a smaller percentage of the total measurement. Thus the error was greater in the quantitation of urinary protein than in quantitation of serum proteins. High precision was obtained in measuring serum albumin levels where

serum levels were around 3,000 mg. per 100 ml. An even smaller error was achieved with α_2-macroglobulin measurements in serum, due to the fact that very little diluting was required. Precision was affected to some extent by concentrating the urine.

U/P ratios were obtained by a series of measurements of serum and urinary proteins in adjacent wells. This technique was employed throughout this investigation to reduce errors which might arise from slight differences in the thickness of the agar. On the whole, filling the wells to the brim rather than introducing a fixed quantity of antigen is self-correcting, since a deeper well containing slightly more antigen will be surrounded by agar gel which contains slightly more antibody, (Hobbs, 1970, Fahey and McKelvey, 1965).

The precision and reproducibility achieved in this investigation compares satisfactorily with other workers in this field. Thus Cameron and Blandford (1966) found the error for individual measurements to be 12% for both transferrin and Ig G globulin. For U/P ratios, their error was as high as 28%. Benster and Wood (1970) measuring serum immunoglobulins in pregnancy found an error of 7.7%. Fahey and McKelvey (1965) recorded an error of 7.5% for Ig G globulin and 10% for other immunoglobulins. Ogg et al. (1968) measuring β_1C levels in serum found an error of 7.2%. Kohler and Farr (1966) found a coefficient of variation of 8.1% for Ig G measurements. Thorn et al. (1967) measuring immunoglobulins and

albumin in umbilical cord serum found coefficients of variation of 5.0%, 7.8% and 6.6% for Ig G, Ig M and albumin respectively.

MEASUREMENT OF PSEUDOCHOLINESTERASE CLEARANCE

Pseudocholinesterase, with a molecular weight of about 300,000 (Schulze and Heremans, 1966), has been included in this investigation of protein clearances to validate the clearance values obtained for β_1^A/C complement (molecular weight 250,000).

The immunochemical method of estimating this protein in serum and urine has been described. A spectrophotometric method for determining pseudocholinesterase activity has been simultaneously employed, urine levels being expressed as a percentage of the serum levels in each case. Once again absolute values have not been regarded as critical, since it was the U/P ratio that was being estimated. The spectrophotometric method described by Gal and Roth (1957) has been used.

The principle of the method of estimation of pseudocholinesterase activity employs the changes produced by the enzymic hydrolysis of choline esters. The method used in this investigation involved the liberation of thiocholine from acetylthiocholine by the action of cholinesterase. The thiocholine thus liberated is oxidised by a dye, 2:6-dichlorophenol indophenol which changes from its blue to its leuco form during the process. The rate of colour change is measured spectrophotometrically at 600 mu. In the presence of excess dye, the rate of colour change will be related to the rate at which thiocholine is liberated and hence to the amount of cholinesterase present (Gal and Roth, 1957).

Method

Chemicals Substrate Acetylthiocholine (Sigma Chemical Co.)

11 mg. substrate was dissolved in 10 ml. distilled water.

Buffers 1. Potassium Buffer (0.1M, pH 7.4)

2.6 grams potassium dihydrogen phosphate (KH_2PO_4)

14.1 grams dipotassium hydrogen phosphate (K_2HPO_4)

Distilled water added to make up 1 litre.

2. Salt mixture

0.1M (5.85 grams) sodium chloride (NaCl)

0.04M (8.1 grams) hydrated magnesium chloride ($MgCl_2 6H_2O$).

Distilled water added to make up 1 litre.

Indicator 2:6-dichlorophenol indophenol (British Drug Houses Ltd., Poole, England.)

Apparatus 1. Beckman DBG Spectrophotometer

2. SANZ 10 microlitre pipette (Beckman Spinco division)

The buffers were stored at 4°C.

Fresh substrate and indicator were prepared for each set of investigations. All investigations were conducted at a fixed temperature of 30°C.

Procedure

The following procedure was employed for the measurement of pseudocholinesterase activity.

Using a Sanz micropipette, 10 microlitres of urine or serum under investigation is added to a cuvette containing 1 ml. of phosphate buffer, 1.75 ml. salt mixture and 0.15 ml. indicator. 0.1 ml. of substrate is added and the cuvette introduced into the spectrophotometer. The O.D. difference between test specimen and a reference specimen (similarly prepared but without the addition of serum or urine) is read after precisely three minutes in the 600 mu light band. This represents a measure of the pseudocholinesterase activity in the sample. An O.D. difference of 0.001 for one minute is regarded as one unit of activity (Gal and Roth, 1957).

Precision and reproducibility

Twenty separate estimations were made of pseudocholinesterase activity in the same serum specimen. A high degree of accuracy was achieved using this method.

Detailed results of the values obtained in the twenty readings will be found in Appendix IV. The error expressed as the coefficient of variation for the method was only 2.24%. In order to detect activity in urine, considerable concentration was required, but as will be seen from the results in the patients studied, cholinesterase clearances correlated well with clearances of $\beta_1{}^A/C$ complement.

Urine/plasma ratios for all proteins included in the investigation have been estimated. Errors in estimating this ratio were minimised by measuring both serum and urine in the same plate. Absolute values for serum and urine measurements were not critical.

Similarly, by expressing each urine/plasma ratio as a percentage of the transferrin and albumin U/P ratios, there was no need to know the urine volume and untimed urine specimens were satisfactory. This removes a large potential source of error (Cameron and Blandford, 1966).

PART III

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Results of the investigation of 43 patients with significant proteinuria in pregnancy are presented in this chapter. These are examined in the first instance as a combined group of patients including all underlying pathologies. A second approach has been attempted, to try to separate the patients according to the probable underlying pathology. Three groups have been recognised for the purpose of this study.

Group 1 Preeclampsia.

Patients who were normotensive (blood pressure (B.P.) less than $^{140}/_{90}$ - see discussion on page 82) in early pregnancy and who developed hypertension (B.P. $^{140}/_{90}$ and above) and proteinuria after the twentieth week of gestation, both symptoms clearing completely by six weeks after delivery.

Group 2 Essential Hypertension.

Patients whose blood pressure readings were $^{140}/_{90}$ and above before the twentieth week of gestation, or who gave a history of essential hypertension before pregnancy. Proteinuria in this group developed after the twentieth week and hypertension generally persisted in the puerperium, raised blood pressure being found six weeks after delivery.

Group 3 Renal disease in Pregnancy.

Included in this group were patients with known renal disease who developed proteinuria during pregnancy, or where heavy proteinuria

early on in pregnancy suggested a probable diagnosis of renal disease. In group comparisons, this group has been excluded because of insufficient numbers. However, a separate discussion on renal disease in pregnancy is included.

Comments on the grouping

In an investigation of this type, difficulties invariably arise with regard to definition, especially when many of the observations in early pregnancy have to be looked at retrospectively. Primarily, this investigation has been a study of proteinuric pregnancies, so that patients fell within the scope of the survey only after proteinuria had developed. It was thus impossible to plan the experiment prospectively and diagnostic criteria had to be decided on retrospectively. These limitations had to be taken into account in attempting any separation into groups at all, and the possibility of some degree of overlap between the two groups, preeclampsia and essential hypertension, must exist.

Further argument in favour of the criteria used here will be advanced in the discussion.

TABLE I

PREECLAMPSIA

NAME	AGE	PARITY	PAST HISTORY		CONDITION IN EARLY PREGNANCY			STAGE OF ONSET IN WEEKS			SEVERITY	
			MEDICAL	OBSTETRIC	GESTATION	URINE (PROTEIN)	B.P.	B.P.	PROTEINURIA	OEDEMA	B.P.	PROTEINURIA (GRAMS PER 24 hrs)
A.W.	25	0	-	-	16 weeks	-	100/70	32	33	-	150/110	0.8
F.S-M	32	0	-	-	13 wks, 17 wks	- -	140/80, 110/70	32	32	32	170/120	2.3
P.F.	24	0	-	Abortion	16 weeks	8.0G	170/100	16	16	16	180/110	8.0
B.M.	20	0	-	-	16 wks, 22 wks	- -	120/80, 110/70	35	35	35	160/125	1.6
E.P.	28	0	-	-	18 weeks	-	120/70	29	31	-	150/110	9.6
M.G.	26	0	-	-	10 weeks	-	95/60	38	38	38	170/105	4.0
A.F.	22	0	-	-	19 weeks	-	130/70	31	35	-	220/140	4.0
M.M.	24	0	-	-	16 wks, 20 wks	- -	120/80, 120/70	32	32	32	180/110	6.4
J.G.	20	0	-	-	16 weeks	-	100/60	40	40	40	170/110	4.2
B.T.	20	0	-	-	14 weeks	-	110/70	36	36	35	150/100	1.4
E.S.	19	0	-	-	13 weeks	-	130/75	30	30	30	175/110	8.0
C.T.	35	0	-	-	12 wks, 16 wks	- -	135/80, 130/60	27	30	31	170/120	9.0
J.T.	27	0	-	Abortion	8 wks, 16 wks	- -	120/70, 110/70	30	32	-	165/110	9.2
S.D.	31	1	-	-	14 weeks	-	115/80	38	39	38	160/120	6.0
M.W.	38	3	-	-	16 weeks	-	105/70	36	37	-	160/90	1.5
L.S.	21	0	-	-	20 weeks	-	115/70	26	26	26	170/110	6.2
K.C.	26	1	-	-	8 weeks	-	110/70	28	30	28	155/110	6.7
M.P.	20	0	-	-	16 weeks	-	120/60	35	35	35	160/120	5.7

TABLE I

PREECLAMPSIA

NAME	GESTATION	DELIVERY ONSET	METHOD	SEX	INFANT WEIGHT	INFANT RESULT	PERCENT.	PUERPERIUM B.P.	PUERPERIUM URINE (PROTEIN)	P.N.C. B.P.	P.N.C. URINE (PROTEIN)
A.W.	34 weeks	-	C/S	F	1785	A	10+	110/90	-	120/80	-
F.S-M	37 weeks	ARM	Spont.	M	2330	A	10-	110/70	-	130/70	-
P.F.	18 weeks	-	Hysterot	-	Hydatidiform Mole			180/110	+	130/70	-
B.M.	36 weeks	ARM	Spont.	M	1980	A	10-	120/80	-	125/80	-
E.P.	36 weeks	ARM	Forceps	M	2605	SB	10+	130/80	-	130/70	-
M.G.	38 weeks	ARM	Spont.	F	2720	A	10+	130/90	-	100/60	-
A.F.	35 weeks	ARM	Forceps	F,F	2040 2040	A,A	10+ 10+	135/90	-	110/80	-
M.M.	36 weeks	-	C/S	M	1700	A	10-	120/70	-	110/70	-
J.G.	40 weeks	ARM	Forceps	M	3600	A	10+	130/80	-	120/80	-
B.T.	37 weeks	Spont.	Spont.	F	2700	A	10+	120/70	-		
E.S.	31 weeks	-	C/S	F	1190	NND	10+	135/75	-	135/75	-
C.T.	31 weeks	-	C/S	F	1647	NND	10+	130/80	-	115/70	-
J.T.	34 weeks	ARM	C/S	M	1670	A	10-	130/90	-	120/70	-
S.D.	39 weeks	ARM	Spont.	M	3766	A	10+	150/100	-	110/75	-
M.W.	38 weeks	Spont.	Spont.	F	2850	A	10+	140/90	-	110/70	-
L.S.	31 weeks	-	C/S	F	1060	NND	10-	140/90	-	105/70	-
K.C.	32 weeks	-	C/S	F	1310	A	10+	130/90	-	130/70	-
M.P.	39 weeks	ARM	Spont.	F	1840	A	10-	110/80	-	110/60	-

TABLE I (Cont'd)

ESSENTIAL HYPERTENSION

NAME	AGE	PARITY	PAST HISTORY		CONDITION IN EARLY PREGNANCY			STAGE OF ONSET IN WEEKS			SEVERITY	
			MEDICAL	OBSTETRIC	GESTATION	URINE (PROTEIN)	B.P.	B.P.	PROTEINURIA	OEDEMA	B.P.	PROTEINURIA (GRAMS PER 24 hrs)
J.S.	26	0	-	-	16 wks, 18 wks	- -	130/95, 130/90	16	30	-	150/120	4.6
B.M.	21	0	-	-	14 wks, 18 wks	- -	130/90, 140/95	14	36	-	170/120	3.7
N.M.	26	0	-	-	14 weeks	-	140/90	14	28	26	170/110	14.4
U.K.	29	1	-	PET/IUD	14 wks, 18 wks	- -	120/90, 130/90	14	28	28	180/130	15.0
P.P.	39	0	-	-	16 wks, 18 wks	- -	110/90, 120/80	16	28	28	190/115	8.0
E.B.	29	0	-	-	8 weeks	- -	130/90	8	32	28	180/140	8.0
J.R.	42	4	H/T	4 N.D'S	17 wks, 21 wks	- -	110/90, 130/100	17	35	-	220/125	1.0
M.M.	26	0	-	-	17 weeks	-	130/90	17	34	34	160/95	3.3
H.D.	30	1	-	-	7 weeks	-	145/95	7	30	-	160/100	4.8
S.K.	32	5	H/T	PET			E M E R G E N C Y				170/110	11.2
M.K.	20	0	-	-	12 weeks	-	140/90	12	31	31	170/110	6.5
N.R.	35	0	-	-	8 weeks	-	140/100	8	29	30	220/120	3.0
D.S.	29	0	-	-	11 weeks	-	170/100	11	34	-	180/110	5.0
W.A.	29	2	H/T	PET	11 weeks	-	170/120	11	37	-	170/120	3.9
E.G.	23	1	-	-	16 weeks	-	130/90	16	33	33	210/120	24.0
D.H.	33	1	-	-	16 weeks	-	130/90	16	31	31	160/105	10.5

TABLE I (Cont'd)

ESSENTIAL HYPERTENSION

NAME	GESTATION	DELIVERY ONSET	METHOD	SEX	WEIGHT	INFANT RESULT	PERCENT.	PUERPERIUM B.P.	PUERPERIUM URINE (PROTEIN)	P.N.C. B.P.	P.N.C. URINE (PROTEIN)
J.S.	31 weeks	Spont.	Spont.	M	906	A	10-	130/90	+	125/95	-
B.M.	37 weeks	ARM	Forceps	M	2150	A	10-	130/90	-	140/90	-
N.M.	32 weeks	Spont.	Spont.	F	1200	IUD	10-	170/110	-	-	-
U.K.	30 weeks	-	C/S	M	850	NND	10-	140/90	-	180/120	-
P.P.	31 weeks	-	C/S	M	1150	A	10-	145/100	-	120/90	-
E.B.	34 weeks	ARM	Forceps	F	2240	A	10+	150/100	-	145/95	-
J.R.	36 weeks	-	C/S	M	2400	A	10+	120/80 (on M.Dopa)	-	-	-
M.M.	36 weeks	ARM	Forceps	F	1640	A	10-	140/90	-	140/90	-
H.D.	35 weeks	-	C/S	M	1840	A	10-	120/80	-	140/90	-
S.K.	34 weeks	Spont.	Spont.	M	2040	A	10+	140/100	-	140/100	-
M.K.	36 weeks	ARM	Spont.	M	2640	A	10+	170/110	-	150/100	-
N.R.	30 weeks	-	C/S	M	915	NND	10-	170/110	-	170/110	-
D.S.	36 weeks	ARM	Spont.	F	2050	A	10-	150/90	-	140/90	-
W.A.	38 weeks	ARM	Spont.	F	2340	A	10-	140/110	-	140/100 (on M.Dopa)	-
E.G.	34 weeks	ARM	Forceps	F	2020	SB	10+	140/80	+	140/80 (on M.Dopa)	-
D.H.	34 weeks	ARM	Spont.	M	1620	NND	10-	120/70	+	150/90	-

TABLE I (Cont'd)

RENAL DISEASE

NAME	AGE	PARITY	PAST HISTORY		CONDITION IN EARLY PREGNANCY			STAGE OF ONSET IN WEEKS			SEVERITY	
			MEDICAL	OBSTETRIC	GESTATION	URINE (PROTEIN)	B.P.	B.P.	PROTEINURIA	OEDEMA	B.P.	PROTEINURIA (GRAMS PER 24 hrs)
S.B.	22	0	Nephritis	-	16 weeks	+++	120/70	-	20	27	130/80	8.0
M.N.	34	2	-	-	10 weeks	+ +	110/70	34	16	30	150/90	7.5
P.C.(1)	22	0	-	-	10 weeks	+ +	120/70	34	10	-	140/90	3.8
P.C.(2)	23	1	-	Prot.	14 weeks	-	110/70	32	24	-	130/90	4.6
F.R.	28	1	Nephrotic	Prot.	16 weeks	-	110/70	34	29	34	170/115	5.5
L.F.	25	2	-	Prot.	18 weeks	+ +	140/80	-	18	-	140/80	8.5
R.F.	38	6	Nephrotic	Prot.	16 wks, 20 wks	+ +	110/75, 120/60	24	16	16	210/110	13.2

UNCLASSIFIED TOXAEMIA

M.G.	29	0	-	-	20 weeks	-	110/70	-	28	38	130/70	1.3
C.B.	20	0	-	-	13 weeks	-	105/50	-	33	-	130/80	2.5
M.P.	40	0	-	-	16 wks, 22 wks	-	130/80, 160/90	22	34	34	170/115	3.0

TABLE I (Cont'd)

RENAL DISEASE

NAME	GESTATION	DELIVERY ONSET	METHOD	SEX	WEIGHT	INFANT RESULT	PERCENT.	PUERPERIUM B.P.	PUERPERIUM URINE (PROTEIN)	P.N.C. B.P.	P.N.C. URINE (PROTEIN)
S.B.	37 weeks	ARM	Spont.	M	2690	A	10+	110/70	+ +	110/70	+++
M.N.	39 weeks	Spont.	Spont.	F	2150	A	10-	125/70	+ +	120/90	+ +
P.C.(1)	36 weeks	Spont.	Spont.	F	1940	A	10-	120/70	+	120/70	+
P.C.(2)	39 weeks	Spont.	Spont.	M	2400	A	10-	110/70	+	120/70	+
F.R.	34 weeks	ARM	Spont.	F,F	1640 2020	A,A	10+	140/70	+	100/60	-
L.F.	35 weeks	ARM	C/S	M	2320	A	10+	120/80	+	110/70	-
R.F.	25 weeks	Hysterotomy						160/90	+ +	130/90	+ +

UNCLASSIFIED TOXAEMIA

M.G.	40 weeks	ARM	Spont.	M	3510	A	10+	110/70	-	120/70	-
C.B.	34 weeks	Spont.	Spont.	F	1450	IUD	10-	110/70	-	110/70	-
M.P.	35 weeks	-	C/S	M	2350	A	10+	130/90	-	150/90	-

TABLE I - ABBREVIATIONS

Percent.	=	Birth weight in relation to 10th percentile for gestational age
ARM	=	Artificial rupture of membranes
Spont.	=	Spontaneous onset of labour
		Spontaneous vaginal delivery
C/S	=	Caesarean section
Hysterot.	=	Hysterotomy
Forceps	=	Forceps delivery
F	=	Female infant
M	=	Male infant
A	=	Live birth
SB	=	Stillbirth
IUD	=	Intra-uterine death
N.D'S	=	Normal deliveries
H/T	=	Essential hypertension
PET	=	Preeclampsia
Prot.	=	Proteinuria
P.N.C.	=	Postnatal Clinic

CLINICAL FEATURES OF PATIENTS IN THE STUDY

The clinical features of all patients in the study are summarised in Table I. They are grouped according to the above definitions.

Eighteen patients satisfied the criteria of Group 1. One patient (P.F.) developed the syndrome in early pregnancy on the basis of an hydatidiform mole. The rapidity of onset, absence of past history and return to normal after hysterotomy suggested she be included in this group.

Sixteen patients satisfied the criteria of Group 2.

Six patients in the study were either known to have or presumed to have underlying renal disease giving rise to proteinuria. One of these patients became pregnant twice during the course of the investigation.

A further three patients defied categorisation according to the above criteria and thus remained unclassified.

Detailed clinical histories of selected patients from each group are included in the Appendix.

Age

The mean age of all patients in the study was 27.5 years. Mean age of the preeclamptic group was 25.4 (S.D. 5.6) and of the hypertensive group, 29.3 (S.D. 6.0). This difference in mean age is significant at the 5% level ($P = <0.05$). The slightly higher mean age in the hypertensive group is in keeping with the higher incidence

of essential hypertension in older women. Master et al. (1950) found a slight mean elevation of diastolic blood pressure in normal women with increasing age. In a survey of primigravid patients in thirteen hospitals in Great Britain and Ireland, the blood pressure of patients under the age of 30 was found to be remarkably constant at twenty weeks gestation (MacGillivray, 1961a). There was a slight but significant rise in B.P. between ages 30 to 35 and a greater rise after the age of 35. Browne (1961) found a mean B.P. at age 15 to 30 of $^{124}/_{73}$, rising to $^{127}/_{77}$ in patients over 35.

Parity

The figures reflect the prevalence of the disease in primigravid patients. Excluding patients with renal disease, which if anything is more common in multiparous patients, 27 of the 37 patients were primigravid, an incidence of 73%. (Primigravida represented only 32.2% of all patients delivered at the Hammersmith Hospital in 1969 - Hammersmith Hospital Report, 1969). In the preeclamptic group, fifteen of the eighteen patients were primigravid (83.3%) whereas in the hypertensive group, nine of the sixteen patients were primigravid (56.3%).

Past History

A past history of renal disease was obtainable in four of the six patients with proteinuria in early pregnancy. Of the other two, one gave a history of proteinuria in previous pregnancies while the other patient, who was included in the series during two successive pregnancies, had heavy proteinuria during both pregnancies. None of these six patients had any previous foetal complications.

In the hypertensive group, three patients had more than one previous pregnancy. All three gave a history of essential hypertension. None of these patients had proteinuria in previous pregnancies and there were no foetal complications. One patient (U.K.) had a previous intra-uterine death on the basis of severe "preeclampsia" during her only other pregnancy. The remaining twelve patients, three of whom had one previous pregnancy, gave no past history of hypertensive disease in any form.

The preeclamptic group was unremarkable as regards past history. Two patients had spontaneous first trimester abortions (P.F. and J.T.). One patient had three previous uncomplicated pregnancies and two patients each had one uncomplicated pregnancy.

Blood pressure in early pregnancy and stage of onset of condition

The stage of gestation when the patient was first seen is noted alongside the earliest blood pressure recording available. In most instances patients were seen well before the twentieth week of gestation. Where indicated, further recordings in early pregnancy are tabulated.

In all groups, the stage of gestation at which a raised blood pressure ($\frac{140}{90}$ and above) was first noted is tabulated. The stage of gestation at which proteinuria or oedema were detected is similarly recorded.

Severity of condition

Under this heading are recorded the highest antenatal blood pressure levels for each patient. Highest levels reached of twenty-four hour urinary protein are recorded. It will be seen that practically every case can be regarded as severe. This was virtually implicit in the criteria required to be satisfied for inclusion in the study. Thus every patient had significant proteinuria, being less than 2 grams per day in only six of the 43 patients. With the exception of two patients with renal disease, blood pressures were above $^{140}/_{90}$ in every case. In 36 of the 43 patients, levels above $^{160}/_{110}$ were recorded, and in 14 cases, pressures rose above $^{180}/_{120}$.

Two patients developed eclampsia (F.S-M. and E.G.). In both cases, this followed immediately on delivery of the infant.

Termination of pregnancy prior to the 28th week had to be performed in one patient (R.F.) because of uncontrollable hypertension.

Labour and Delivery

The stage of gestation together with the method of delivery are recorded. Onset of labour was spontaneous in nine of the 44 pregnancies. Surgical induction of labour was performed in 21 instances, nineteen ending in vaginal delivery and two in Caesarean section. There was a total of thirteen Caesarean sections out of 41 viable pregnancies, (31.7%).

Foetal outcome

This is discussed in detail elsewhere (page 102).

TABLE II

LABORATORY INVESTIGATIONS

PREECLAMPSIA

NAME	URINE MICROSCOPY (presence or absence of granular casts)	BLOOD UREA (mg. per 100 ml.)	BLOOD CHOLESTEROL (mg. per 100 ml.)	BLOOD URIC ACID (mg. per 100 ml.)	CREATININE CLEARANCE (ml. per minute)
A.W.	-	30	195	5.6	122
F.S-M	-	27	180	6.0	87
P.F.	+	34	195	7.7	64
S.D.	+	27	300	6.2	104
B.M.	-	23	280	7.7	47
E.P.	+++	19	185	5.2	73
M.G.	-	17	205	7.4	84
A.F.	-	64	185	9.7	63
M.M.	+	24	230	7.0	60
J.G.	-	32	295	7.1	-
B.T.	-	25	238	6.1	106
M.W.	-	29	250	6.5	-
E.S.	-	25	175	7.2	64
C.T.	-	24	280	7.0	129
J.T.	+	29	215	9.1	98
L.S.	+	42	240	7.3	65
K.C.	-	30	238	9.2	83
M.P.	+	28	190	6.5	47

TABLE II (Cont'd)

LABORATORY INVESTIGATIONS

HYPERTENSION

NAME	URINE MICROSCOPY (presence or absence of granular casts)	BLOOD UREA (mg. per 100 ml.)	BLOOD CHOLESTEROL (mg. per 100 ml.)	BLOOD URIC ACID (mg. per 100 ml.)	CREATININE CLEARANCE (ml. per minute)
J.S.	++	35	225	7.4	58
B.M.	++	20	265	7.7	54
N.M.	+	34	255	6.1	78
U.K.	+	35	218	7.2	84
E.B.	++	24	235	5.4	83
J.R.	+	21	283	4.8	74
M.M.	-	29	252	7.3	-
H.D.	+	25	170	7.2	-
S.K.	-	22	315	6.5	70
M.K.	+	31	260	5.3	46
N.R.	+	30	206	6.9	73
D.S.	+	31	206	7.4	-
W.A.	++	26	280	7.4	-
E.G.	+	23	185	6.5	130
D.H.	++	25	180	7.1	84

TABLE II (Cont'd)

LABORATORY INVESTIGATIONS

RENAL DISEASE

NAME	URINE MICROSCOPY (presence or absence of granular casts)	BLOOD UREA (mg. per 100 ml.)	BLOOD CHOLESTEROL (mg. per 100 ml.)	BLOOD URIC ACID (mg. per 100 ml.)	CREATININE CLEARANCE (ml. per minute)
R.F.	+	21	165	4.5	86
S.B.	+	25	285	3.8	92
M.N.	+	27	420	4.6	148
P.C.(1)	+	28	185	6.9	80
P.C.(2)	+	28	180	6.7	76
L.F.	+	28	280	7.8	90
F.R.	+	30	230	7.0	94

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M.P.	+	25	-	6.2	85
C.B.	+	28	-	8.9	54
M.G.	+	28	230	6.9	55

Puerperium and Postnatal period

Blood pressures and urinary protein level are recorded for the fourth day following delivery and again six weeks after delivery. This is discussed in considering longitudinal studies of the patients (page 85).

Renal function

In order to get some idea of renal function in the patients studied, three investigations were carried out. An estimation of blood urea nitrogen and blood uric acid was made at the time of study in all patients. An estimation of glomerular filtration rate (G.F.R.) by endogenous creatinine clearance was conducted whenever possible. None of these tests provide an acceptable estimation of renal function in an individual patient, unless renal impairment is well advanced. However, when compared to a control group of normal pregnant patients of similar ages and gestational stages, smaller alterations in function as a group can be detected.

Results of laboratory investigations of all patients are listed in Table II. This includes the above three tests, together with an estimation of blood cholesterol in each patient. The results of the urine microscopy performed at the time urine was collected for clearance studies are also listed. The symbols refer to the presence or absence of granular casts in the urine.

The mean values for these tests are compared in Table III with mean values for twenty normal pregnancies of similar gestational

TABLE III

RENAL FUNCTION IN PREGNANCIES COMPLICATED BY HYPERTENSION AND PROTEINURIA
A COMPARISON OF MEAN VALUES COMPARED WITH VALUES IN 20 NORMAL PREGNANCIES

	NORMAL PREGNANCY	HYPERTENSIVE CONDITIONS	STUDENT 't'	SIGNIFICANCE
BLOOD UREA	20.8 mgs. per 100 ml. (± 3.8)	28.4 mgs. per 100 ml. (± 7.9)	4.01	P = < 0.0005
BLOOD URIC ACID	5.8 mgs. per 100 ml. (± 1.2)	6.9 mgs. per 100 ml. (± 1.1)	3.48	P = < 0.001
BLOOD CHOLESTEROL	240.25 mgs. per 100 ml. (± 35 mgs.)	231.5 mgs. per 100 ml. (± 40.3 mgs.)	0.67	P = > 0.2
CREATININE CLEARANCE	105 ml. per min. (± 24.8)	78.5 ml. per min. (± 22.9)	4.27	P = < 0.0005

ages. The difference in the mean values between the two groups has been examined using the Student "t" test in each case. It will be seen that the two main measurements of renal function, blood urea and creatinine clearance, are significantly altered in patients with hypertension and proteinuria. Blood uric acid is also significantly raised but there is no statistical difference between serum cholesterol levels in the two groups.

The importance of comparing renal function studies in pre-eclampsia with normal pregnant patients at similar gestational ages has been stressed (Bucht and Werko, 1953, Kenney et al., 1950, MacCartney, 1968). Comparison with values in non-pregnant patients or with values found following delivery fail to recognise the now well-established fact that in normal pregnancy there is an hypertrophy of renal function, with G.F.R. increasing at times by over 50% and returning rapidly to pre-pregnant levels following delivery (Bucht, 1951, De Alvarez and Bratvold, 1958). The latter group found that the increase in G.F.R. was not maintained beyond the second trimester, approaching non-pregnant levels near term. Confusion as to changes in renal function as pregnancy progresses have been clarified by serial studies of renal function in normal women during pregnancy and the puerperium (Sims and Krantz, 1958). This study served to confirm the increase in glomerular filtration rate and renal plasma flow (R.P.F.) in normal pregnancy and the resulting lowering of the normal range of values for blood urea and creatinine. R.P.F. was found to have

increased by the fifteenth week of gestation, being maintained throughout pregnancy, with a gradual decrease before term. G.F.R. remained raised throughout pregnancy, with return to normal levels in the puerperium. These changes are reflected in an increased creatinine clearance and a lowering of serum creatinine, together with a fall in blood urea.

Decreased renal function in preeclampsia toxaemia is now well established, much of the evidence having been covered in the introduction. The findings of this study largely confirm this. G.F.R. as estimated from endogenous creatinine clearance was significantly reduced in the patients studied when compared to a control group, and blood urea and serum uric acid were significantly raised. Whilst the most accurate measure of G.F.R. can be achieved by inulin clearance studies, clearance of endogenous creatinine closely approximates this (Miller and Winkler, 1938, Doolan et al., 1962). Sims and Kranz (1958) and Bucht (1951) found that the inulin/creatinine clearance ratio approached unity in the last few weeks of gestation, so that for all practical purposes, G.F.R. can be satisfactorily and more simply estimated using creatinine clearance.

Blood urea levels in preeclampsia and hypertension are generally raised when compared to normal pregnancy. Again levels in the individual case are of little value. Riedel (1963) reviewed 744 patients with preeclampsia who had had blood urea estimations performed by the same technician over a period of ten years. Although

only eight of the 744 patients had serum levels above 40 mg. per 100 ml., there was nevertheless a statistically significant difference between blood urea levels in groups of graded severity and between preeclamptic patients and a group of normal controls. Stander and Cadden (1934) reported a rise in serum uric acid in preeclampsia out of proportion to the rise in blood urea, which they felt was not increased in these conditions. However, they compared blood urea levels to levels in non-pregnant patients. De Alvarez and Richards (1954) also found blood ureas within normal limits in preeclampsia, but many of their cases were very mild with no proteinuria. Caldwell and Lyle (1921) in an extensive investigation of blood chemistry in pregnancy, reported a fall in blood urea in normal pregnancy. In hypertensive pregnancies, blood urea and uric acid levels were found to be elevated, the latter more so than the former. Widholm and Kuhlback (1965) found an elevation of uric acid values correlating with the severity of the condition. Furthermore, toxaemic patients who developed renal failure were found to have hyperuricaemia far in excess of the degree of nitrogen retention.

Some authors have tried to differentiate between renal function in preeclampsia and renal function in essential hypertension. Karjalainen and Widholm (1968) found an increase in blood urea and fall in creatinine clearance which was confined to preeclamptic pregnancies. Values in patients with essential hypertension were within normal limits. Lancet and Fisher (1956) found serum uric acid

levels consistently raised in preeclampsia but frequently normal in hypertension. They also noted a fall in uric acid levels consistent with improvement in the patient's condition. Chesley (1950) and Chesley and Valenti (1958) found urea and uric acid clearances reduced in preeclampsia but normal in patients with essential hypertension. In hypertensive patients where preeclampsia was "superimposed" however, clearances were reduced as in preeclampsia. They also observed a reduction in uric acid clearance far greater than the reduction in urea clearance and believed this was due to increased uric acid reabsorption via the tubules in preeclampsia (Chesley and Williams, 1945, Chesley, 1950). This suggestion is disputed by Hayashi (1956) who found uric acid clearances to parallel creatinine clearance. Reduction in G.F.R. and hence urea and uric acid clearances is the explanation offered by most authors for a rise in blood urea and uric acid in preeclampsia (Schaffer et al., 1943, Bonsnes and Stander, 1946).

In the present investigation, serum uric acid and urea levels were significantly raised above the mean values for normal pregnancy as a whole. No significant differences were found between patients with preeclampsia and those with hypertension (see page 81). This may be explained in part by the fact that cases in both groups were only investigated after the development of proteinuria. It could thus be postulated that the hypertensive patients are in fact examples of "superimposed toxaemia", behaving much as the preeclamptic patients

do (Chesley, 1950). On the other hand, workers who found differences between renal function in preeclampsia and hypertension admit to considerable overlap, and no diagnostic assistance can really be gained from renal function studies.

Urinary Deposit

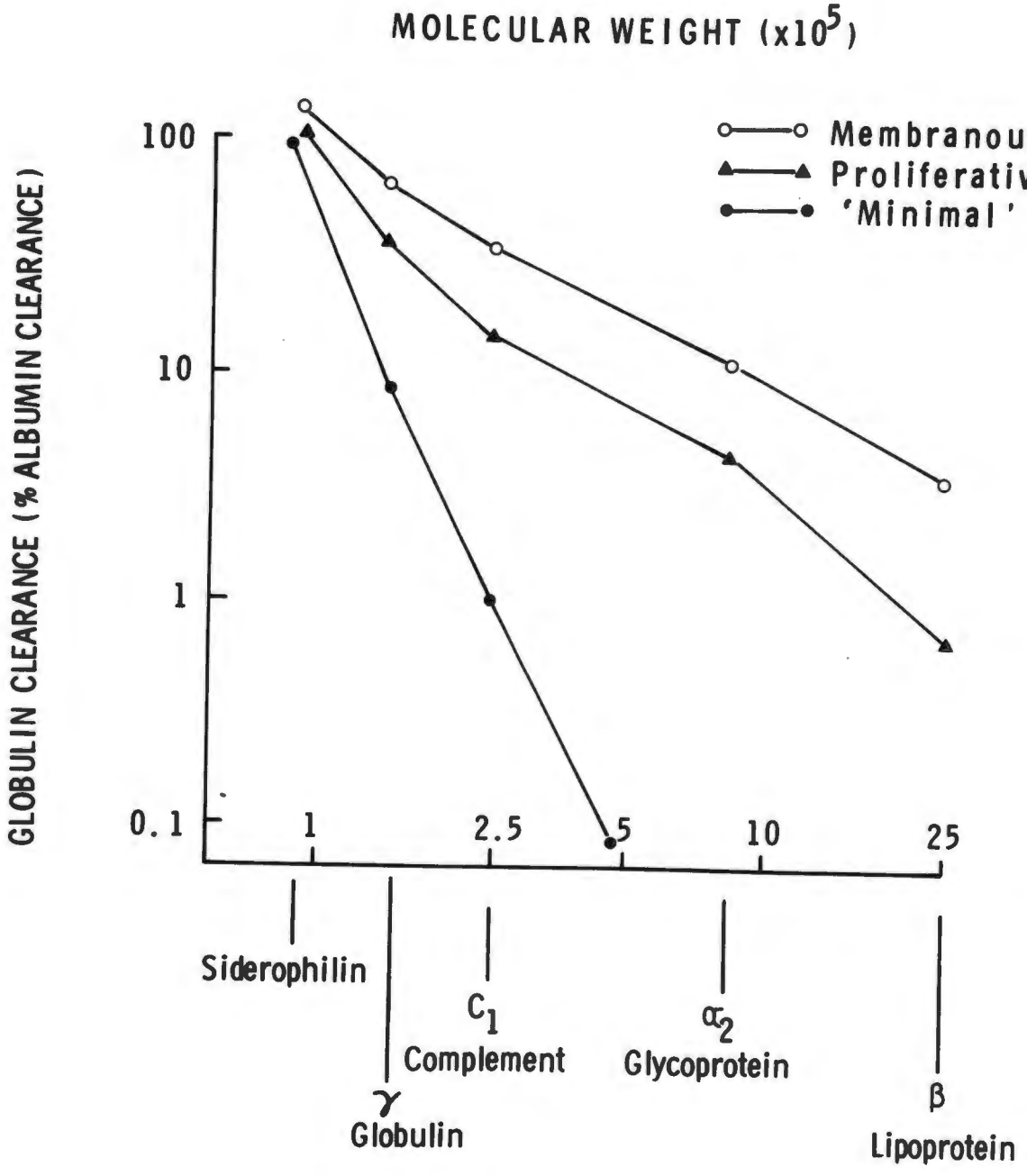
Examination of urinary deposit has not been a great help. De Alvarez and Richards (1954) claimed that red cells and casts were not usually seen in the urine of preeclamptic patients. This is not borne out in the present study, where hyaline and granular casts were a frequent finding in all three categories. There was some relationship to the level of proteinuria, casts being more numerous in the heavy proteinurias. In the hypertensive group, granular tubular casts were present in the urine in thirteen of the sixteen cases. In the preeclamptic group these were present in seven of the eighteen patients. All six patients with renal disease had granular casts in the urine.

RENAL PROTEIN CLEARANCES AND PROTEIN SELECTIVITY

In the introduction, some of the concepts that led up to the idea of protein selectivity being an expression of glomerular membrane permeability have been discussed. Critical to this concept must inevitably be the acceptance that tubular reabsorption of protein is non-selective, so that the spectrum of proteins in the urine bears a directly proportionate relationship to the proteins in the glomerular filtrate. Thus the profile of urinary proteins is dependent upon the porosity of the glomerular basement membrane and is largely unaffected by other influences. Evidence to support this thesis has been presented in the introduction and further confirmation will be forthcoming in the results of the present study.

Hardwick and Squire (1955) first suggested the relationship between the relative clearances of proteins of different molecular weight and the type of renal disease. They studied the clearances of four globulin fractions in proteinuric patients, expressing each clearance as a percentage of the albumin clearance. A total of 44 patients with various kinds of renal disorder were investigated.

Using paper electrophoresis they were able to show that the rate of clearance of a particular protein from the serum is dependent upon its molecular weight and that this assumes a fairly constant ratio in a particular patient when expressed as a percentage of the albumin clearance. Differential staining after paper electrophoresis was used to determine which protein fraction predominated in each



From Hardwicke and Soothill (1961)

Fig. i

protein band, since paper electrophoresis alone fails to differentiate proteins according to molecular weight.

At this early stage, differences in protein excretion patterns in various forms of renal disease were difficult to interpret. Greater precision was achieved by the introduction of immunochemical methods, which permitted fairly accurate measurement of individual plasma globulins.

Applying this method to the earlier studies of protein clearances in the nephrotic syndrome, Blainey et al. (1960) and Hardwicke and Soothill (1961) were able to confirm the relationship between protein clearance and molecular weight, which when plotted on a double logarithmic scale, approaches linearity. In addition, by comparing protein clearance patterns with histological patterns found at renal biopsy, they were able to demonstrate a relationship between the selectivity of protein excretion and type of renal pathology. Thus in patients with extensive glomerular membrane damage, a marked relative permeability to larger protein molecules was apparent. In contrast, patients who showed histologically minimal lesions, had a highly selective type of protein excretion, with the glomerular filter successfully retaining most of the larger protein molecules.

Joachim et al. (1964) investigated the relationship between protein selectivity and renal pathology further. They were able to confirm the association, and attempted to derive some form of selectivity index as an expression of glomerular permeability. The slope

FROM JOACHIM ET AL (1964) HIGH AND LOW SELECTIVITY REGRESSION SLOPES

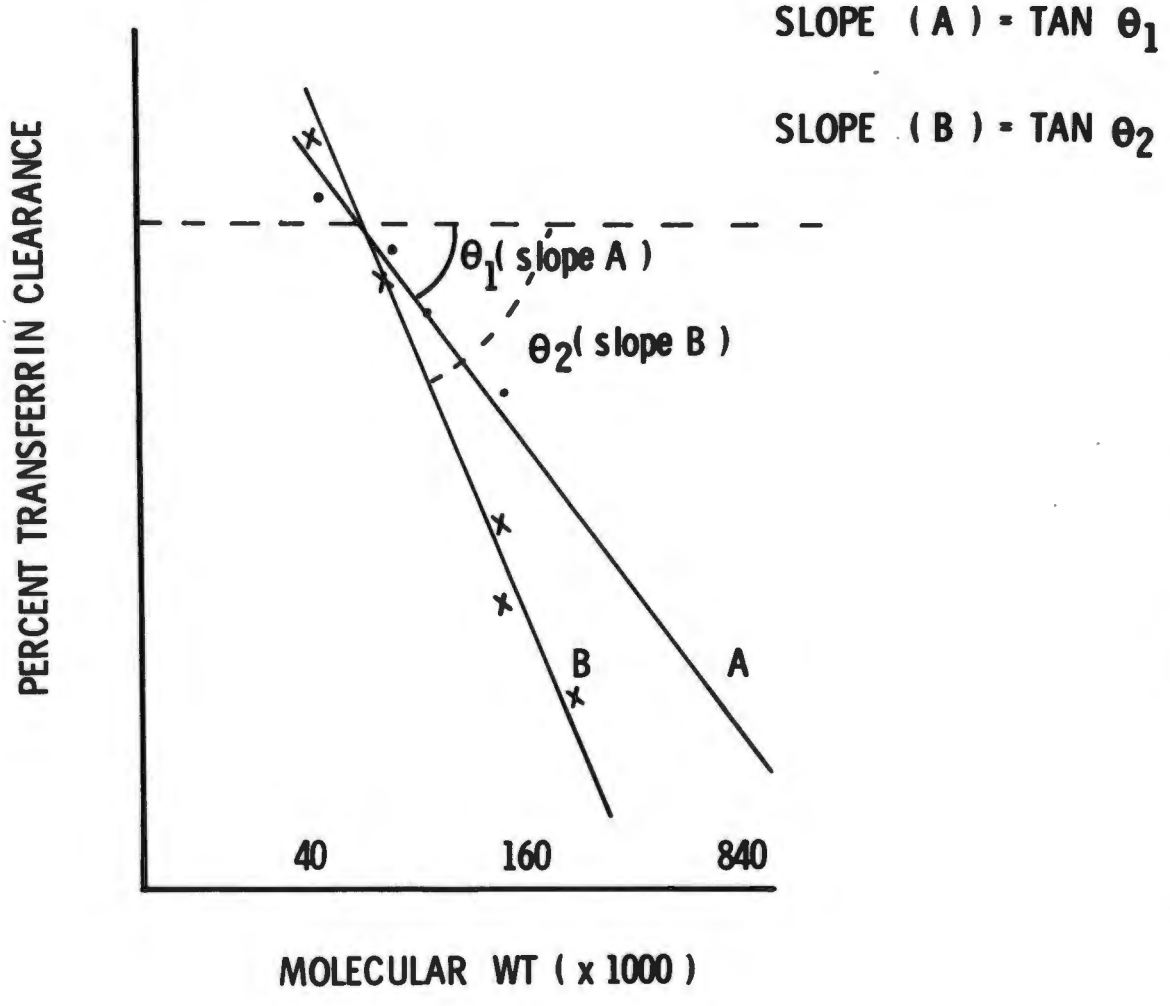


Fig. ii

formed by the regression line of protein clearance (expressed as a percentage of transferrin clearance) on molecular weight when plotted on a double logarithmic scale was found to be a useful measurement (Figure ii). The angle (θ) derived from the slope (K), the tangent of θ, is a more convenient way of expressing selectivity.

On the basis of the 48 nephrotic patients they studied, they considered a θ angle of 67° and above as being highly selective. The average selectivity for their patients lay between 54° and 63°. Simon et al. (1967) considered a θ angle of 70° as being the dividing line between high and low selectivity. Cameron (1966) also regarded selectivities of 70° and above as being highly selective while values below 60° he called non-selective.

This method of arriving at protein selectivity is both cumbersome and open to error. Selectivity expressed as the slope of the regression line of the clearances of five proteins on their molecular weights must inevitably rely upon the best straight line available for the data. A reasonably linear relationship is essential and deviation from linearity in the clearances of one or more of the proteins can significantly alter the selectivity index. An alternative approach has been suggested in which only two protein clearances of different molecular weights are studied (Cameron and Blandford, 1966). In this situation, the selectivity index is expressed by the ratio of the clearance of the larger protein to the clearance of the smaller protein. Using Ig G globulin (M.W. 160,000) and transferrin

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BLOOD UREA	20.8 mgs. per 100 ml. (± 3.8)	28.4 mgs. per 100 ml. (± 7.9)	4.01	P = < 0.0005
BLOOD URIC ACID	5.8 mgs. per 100 ml. (± 1.2)	6.9 mgs. per 100 ml. (± 1.1)	3.48	P = < 0.001
BLOOD CHOLESTEROL	240.25 mgs. per 100 ml. (± 35 mgs.)	231.5 mgs. per 100 ml. (± 40.3 mgs.)	0.67	P = > 0.2
CREATININE CLEARANCE	105 ml. per min. (± 24.8)	78.5 ml. per min. (± 22.9)	4.27	P = < 0.0005

TABLE IV

PROTEIN CLEARANCES AS A PERCENTAGE OF TRANSFERRIN CLEARANCE

PREECLAMPSIA

NAME	ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLIN. IMM.	PSEUDOCHOLIN. CHEM.	α-2-M	Ig M	SLOPE (K)
B.M.	92	100	23	-	-	3.1	0.4	-	-2.3609
S.D.	99	100	21	3.8	2.9	4.2	0.8	0.5	-2.0805
M.G.	68	100	23	2.8	3.2	2.8	0.8	0.7	-1.9429
A.F.	97	100	35	8.7	-	10.2	1.2	1.2	-1.8019
J.G.	122	100	25	2.5	-	3.1	1.0	0.5	-1.9429
B.T.	100	100	26	-	-	-	-	-	-
M.P.	96	100	26	8.3	-	6.1	1.0	0.8	-1.9178
A.W.	106	100	21	-	-	-	-	-	-
K.C.	105	100	21	2.5	5.5	3.5	1.3	0.6	-2.0364
E.S.	91	100	26	4.8	6.1	4.6	1.3	0.7	-1.8959
M.M.	117	100	21	2.8	-	2.2	0.9	0.8	-1.9928
P.F.	112	100	24	6.5	7.8	6.5	1.6	1.3	-1.7468
F.S-M.	97	100	19	5.6	3.5	5.8	0.6	-	-2.1954
L.S.	95	100	19	4.2	5.0	3.9	0.6	0.3	-2.2167
E.P.	107	100	25	3.1	3.3	2.8	1.2	1.2	-1.8513
J.T.	106	100	24	6.5	8.4	7.4	1.0	-	-1.9669
C.T.	93	100	24	4.5	4.4	6.9	1.3	0.9	-1.8378
M.W.	103	100	24	-	-	-	-	-	-

TABLE IV (Cont'd)

ESSENTIAL HYPERTENSION

NAME	ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLIN.		α-2-M	Ig M	SLOPE (K)
					IMM.	CHEM.			
M.M.	116	100	29	-	-	9.0	1.1	-	-1.9321
N.R.	91	100	36	-	-	6.1	0.5	-	-2.2162
D.S.	70	100	47	7.2	8.0	10.8	1.5	0.9	-1.7775
W.A.	120	100	30	-	-	11.4	1.1	-	-1.9149
U.K.	106	100	16	4.0	5.4	4.3	1.0	1.6	-1.7650
B.M.	83	100	46	13.6	8.7	9.9	1.6	1.2	-1.7395
J.R.	110	100	22	-	-	5.0	-	-	-2.2158
D.H.	97	100	27	9.2	6.7	7.0	0.9	0.9	-1.9149
P.P.	97	100	26	-	-	-	0.7	0.4	-2.1421
E.B.	92	100	29	2.8	2.4	3.4	0.6	1.2	-1.9619
M.K.	94	100	34	7.8	6.6	8.2	1.0	1.0	-1.8791
N.M.	107	100	42	10.6	10.3	17.9	3.6	3.0	-1.4506
S.K.	93	100	32	3.7	6.1	9.2	0.9	0.8	-1.9365
J.S.	95	100	32	4.0	5.6	3.8	1.2	1.8	-1.7813
H.D.	101	100	37	3.3	3.5	7.6	1.5	1.3	-1.7242
E.G.	85	100	50	8.8	12.1	12.1	4.5	3.1	-1.3387

TABLE IV (Cont'd)

RENAL DISEASE

NAME	ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLIN.		α-2-M	Ig M	SLOPE (K)
					IMM.	CHEM.			
F.R.	108	100	36	10.2	6.8	9.1	1.7	1.6	-1.7098
L.F.	98	100	22	11.0	6.7	8.0	2.2	2.3	-1.5305
M.N.	120	100	28	4.6	4.6	5.8	6.5	3.9	-1.2908
P.C.(1)	118	100	20	6.9	6.6	7.4	-	1.7	-1.6973
P.C.(2)	90	100	21	5.4	-	5.2	1.0	1.2	-1.8042
S.B.	104	100	26	3.6	4.4	7.9	1.0	1.5	-1.8007
R.F.	96	100	40	18.0	-	20.0	-	3.2	-1.3269

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M.G.	100	100	28	-	8.2	8.9	1.6	2.2	-1.6142
C.B.	93	100	-	-	-	-	-	-	-
M.P.	66	100	26	-	-	4.2	-	1.2	-1.7109

(M.W. 90,000) as their two proteins of differing molecular weights, Cameron and Blandford were able to demonstrate a good correlation between protein selectivity and renal morphology on microscopy. An Ig G globulin clearance of less than 10% (0.1) of the transferrin clearance was a feature of nephrotics whose renal glomerular changes on histology were minimal. Where definite histological disease was apparent, Ig G clearances were generally above 20% of the transferrin clearance, rising above 30% (0.3) in the more advanced forms of renal pathology. This simplified method of arriving at a selectivity index has been fully endorsed by other workers (Simon et al., 1967). This latter group were able to show a close correlation between Cameron's Ig/transferrin clearance ratio, and selectivity expressed as the slope of the regression line of clearance on molecular weight.

Protein selectivity in Pregnancy as determined by the above two methods

In the present investigation, serum and urine proteins have been studied in patients with pregnancy proteinuria and individual protein clearances have been determined for a range of seven proteins of varying molecular weights. The urine/plasma ratios for each protein studied in every patient are to be found in the Appendix. Clearances expressed as a percentage of the transferrin clearance are listed in Table IV. Transferrin is given the value 100%. From these ratios, the regression line of protein clearance on molecular weight has been plotted and the slope of the line determined for each patient. This provides a measure of protein selectivity according to the method

TABLE V

PROTEIN SELECTIVITY

PREECLAMPSIA

NAME	Ig G/TRANS.	K(TAN θ)	θ	S.E.(K)
B.M.	0.24	-2.3609	67.0°	0.12
S.D.	0.21	-2.0805	64.3°	0.16
M.G.	0.21	-1.9429	62.8°	0.22
A.F.	0.35	-1.8011	60.9°	0.10
J.G.	0.29	-1.9429	62.8°	0.22
B.T.	0.26	-	-	-
M.P.	0.26	-1.9178	62.5°	0.08
A.W.	0.21	-	-	-
K.C.	0.21	-2.0364	63.8°	0.24
E.S.	0.26	-1.8959	62.2°	0.15
M.M.	0.21	-1.9928	63.4°	0.25
P.F.	0.22	-1.7468	60.2°	0.12
F.S-M.	0.19	-2.1954	65.5°	0.11
L.S.	0.19	-2.2167	65.7°	0.11
E.P.	0.24	-1.8513	61.6°	0.27
J.T.	0.23	-1.9669	63.0°	0.10
C.T.	0.25	-1.8378	61.4°	0.16
M.W.	0.23	-	-	-

TABLE V (Cont'd)

ESSENTIAL HYPERTENSION

NAME	Ig G/TRANS.	K(TAN θ)	θ	S.E.(K)
M.M.	0.32	-1.9321	62.6°	0.06
N.R.	0.36	-2.2162	60.6°	0.14
D.S.	0.53	-1.7775	60.6°	0.16
W.A.	0.27	-1.9149	62.4°	0.07
U.K.	0.16	-1.7650	60.5°	0.21
B.M.	0.46	-1.7395	60.1°	0.10
J.R.	0.21	-2.2158	65.7°	0.08
D.H.	0.30	-1.9149	62.4°	0.08
P.P.	0.28	-2.1421	65.0°	0.09
E.B.	0.29	-1.9619	63.0°	0.27
M.K.	0.35	-1.8791	62.0°	0.10
N.M.	0.40	-1.4506	54.6°	0.18
S.K.	0.33	-1.9365	62.7°	0.18
J.S.	0.32	-1.7833	60.7°	0.24
H.D.	0.37	-1.7242	59.7°	0.15
E.G.	0.53	-1.3387	53.2°	0.18

TABLE V (Cont'd)

RENAL DISEASE

NAME	Ig G/TRANS.	K(TAN θ)	θ	S.E.(K)
F.R.	0.34	-1.7098	59.7°	0.10
L.F.	0.22	-1.5305	56.9°	0.12
M.N.	0.31	-1.2908	52.2°	0.30
P.C.(1)	0.18	-1.8042	61.0°	0.15
P.C.(2)	0.18	-1.6972	59.5°	0.14
S.B.	0.25	-1.8007	60.9°	0.21
R.F.	0.41	-1.3269	53.0°	0.07

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M.G.	0.29	-1.6142	58.2°	0.10
C.B.	0.45	-	-	-
M.P.	0.26	-1.7109	59.7°	0.17

described by Joachim et al. (1964). Examples of the regression lines are shown in Figure iii in which the line with the steepest slope in the series is compared with the smallest slope found.

Regression slopes and angles determined for 40 of the 44 pregnancies are listed in Table V (in four patients, data on the excretion of the larger protein molecules were not sufficiently adequate to permit the determination of selectivity by this method). The standard error (S.E.) of the estimates of clearance for each slope is included in the table. Selectivity as expressed by the ratio of Ig G clearance to the mean clearances of transferrin and albumin is similarly listed.

Comparison of the two methods

Deviation from linearity appears to be a fairly common finding in protein excretion in pregnancy. This is most marked in the macroglobulin protein range, where protein excretion is often in excess of what would be anticipated. This is illustrated in the regression lines shown in Figures iv and v. The "best" straight line under such circumstances can be but a poor expression of the excretion pattern. Furthermore, in arriving at the regression line for the data in each case, equal value is apportioned to each protein clearance. Thus Ig G clearance is given an equal value to β_1C, pseudocholinesterase, α-2-macroglobulin and Ig M clearances in the derivation of the equation. These last four proteins can only be measured in urine after considerable concentration (see page 33) carrying an error in measurement

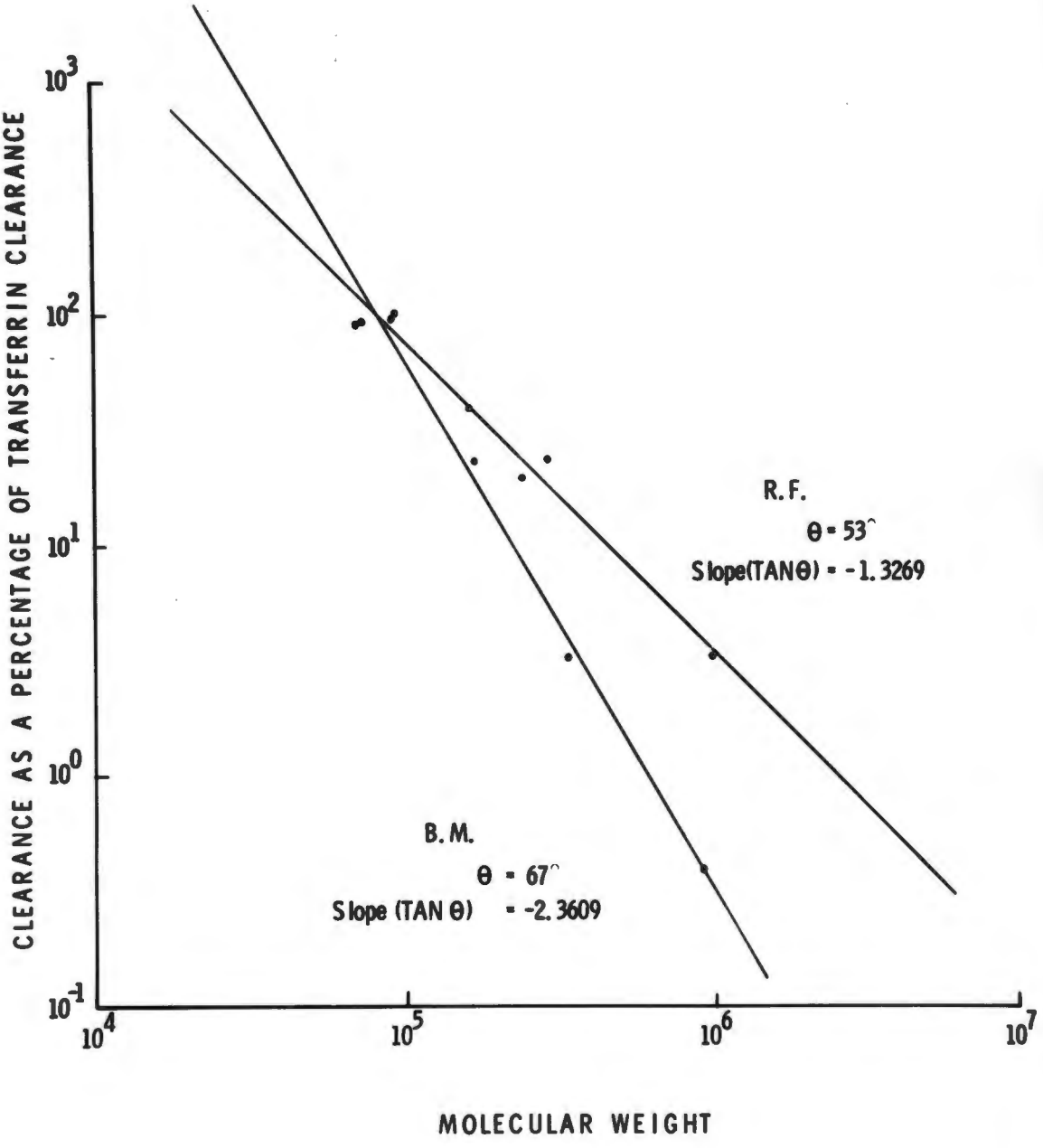
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^{-1}
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT
R.F.
θ = 53°
Slope(TANθ) = -1.3269
B.M.
θ = 67°
Slope (TAN θ) = -2.3609

Fig. iii

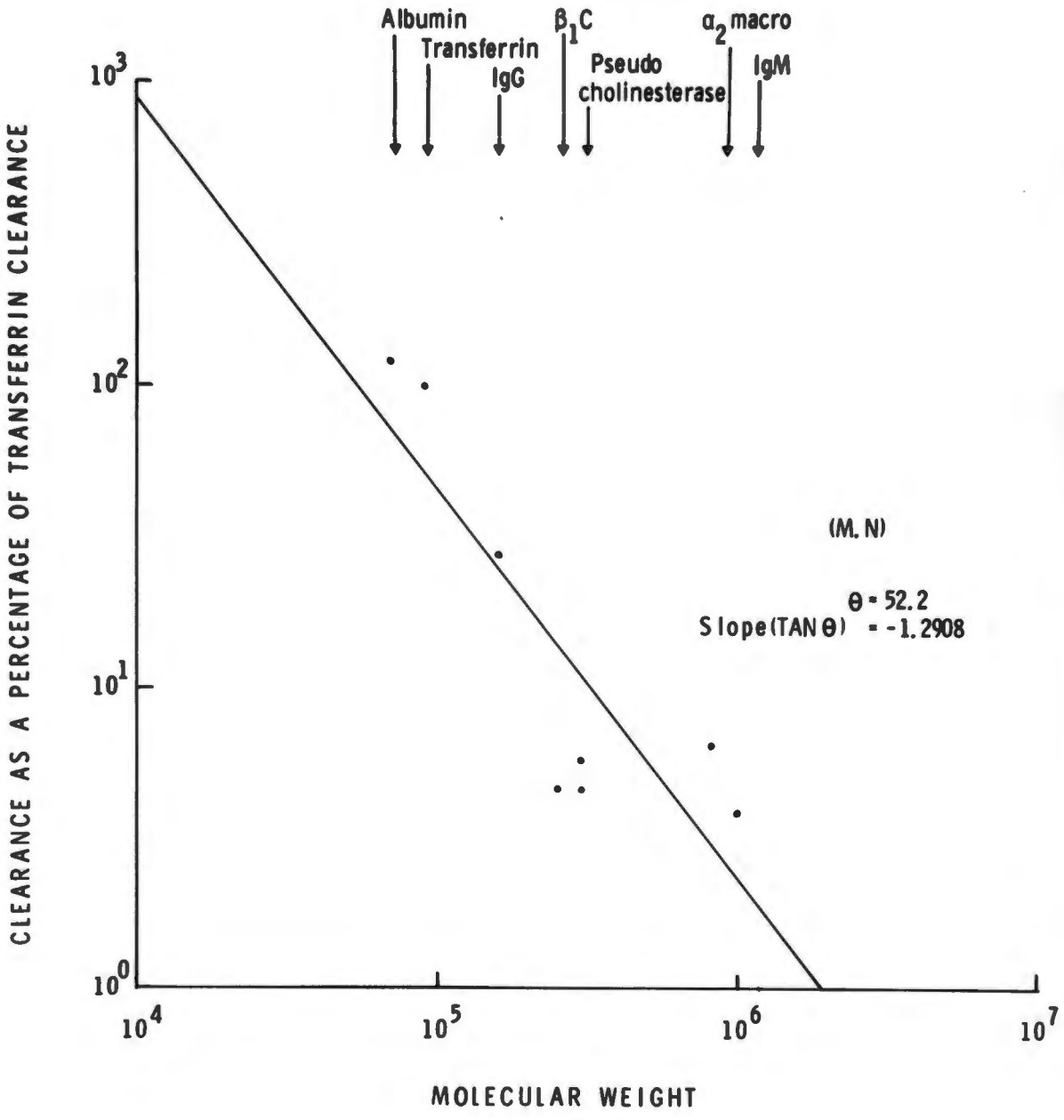
Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
α_2 macro
IgM
10^3
10^2
10^1
10^0
(M. N)
θ = 52.2
Slope(TAN θ) = -1.2908
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

Fig. iv

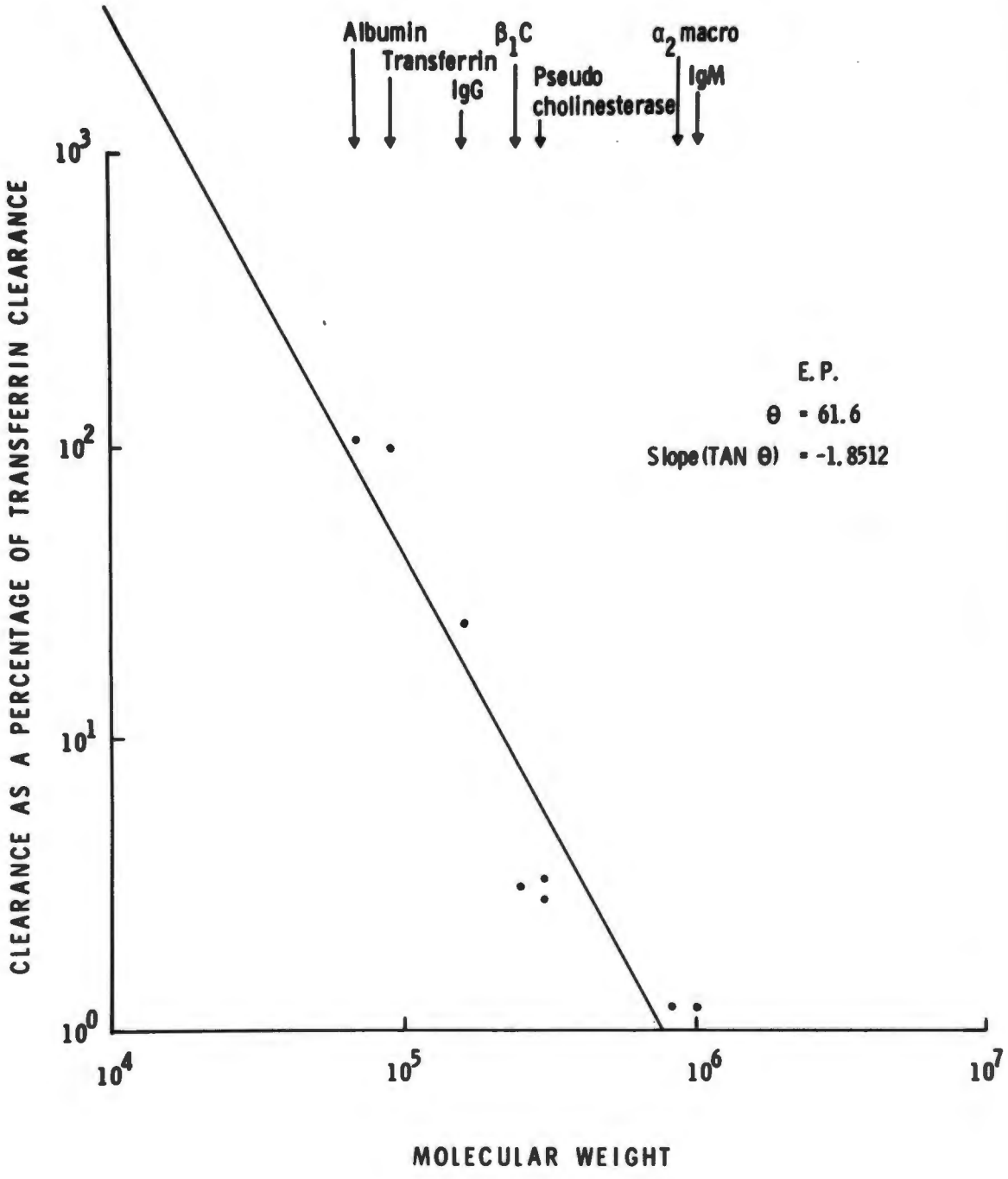
Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
α_2 macro
IgM
E.P.
θ = 61.6
Slope(TAN θ) = -1.8512
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

Fig. v

ranging from 18.4% to 24.4%. Ig G which is measured in unconcentrated urine carried an error in measurement of 12.5%. Thus in the derivation of a regression line based chiefly on these five protein clearances, the measurement carrying the greatest precision is apportioned equal value to each of four other measurements with far greater errors in measurement.

It has been suggested that a high Ig M clearance ratio relative to other proteins is indicative of urinary tract infection (Goddard and Hobbs, 1968). However, α-2-macroglobulin clearances serve as a check in this regard and these tended on the whole to follow the clearances of Ig M in the present patients where infection had been excluded (page 21). Higher macroglobulin clearance is particularly noticeable in patients with renal disease in pregnancy, (see Figure xi) but it is apparent in many of the other situations as well.

Ig G/transferrin clearance ratios have been shown to provide a satisfactory measurement in interpreting renal pathology in the nephrotic syndrome (Cameron and Blandford, 1966). A good correlation has been demonstrated between this measurement and the slope of the regression line of five to seven protein clearances on molecular weight (Simon et al., 1967). In the present investigation, the correlation between these two methods of arriving at a selectivity index is reasonable, provided "poor fit" regression lines are omitted. The Ig G/transferrin clearance ratio has the advantage both of

simplicity and probably of greater accuracy, since the error of measurement is smaller with these proteins. In addition, it is applicable to proteinurias of lesser severity, where the larger plasma proteins may be excreted in amounts not sufficient to permit clearance studies. For these reasons, the Ig G/transferrin and Ig G/albumin clearance ratios are mainly used in this study, as an expression of protein selectivity in pregnancy. For convenience, the mean ratio of Ig G/transferrin clearance and Ig G/albumin clearance is referred to in the text simply as the Ig G/transferrin ratio.

Protein selectivity in pregnancy

Table V shows the range of protein selectivities in 44 proteinuric pregnancies, obtained by both the above methods. Using the criteria of Cameron (1966) for the slope of the regression line and of Cameron and Blandford (1966) for the Ig G/transferrin clearance ratio, it will be seen that virtually all pregnancy proteinurias have a poorly selective pattern of protein excretion. The largest θ angle in the entire series is 67.0°, 3° less than Cameron's borderline value for selective proteinuria. The mean θ angle is 61.2° (S.D. 3.5°). The mean angle in Joachim's series of patients with established renal disease was 58.6° (S.D. 9.0°). Ig G/transferrin clearance ratios in Cameron and Blandford's series are compared in Figure vi with ratios found in pregnancy. Once again, protein clearance patterns are seen to compare with patterns found in established renal disease. None of the pregnant patients show the highly selective pattern (<0.1) seen

SELECTIVITY PATTERNS IN PROTEINURIA IN PREGNANCY COMPARED WITH NON PREGNANT NEPHROTICS: OF CAMERON & BLANDFORD (1966)

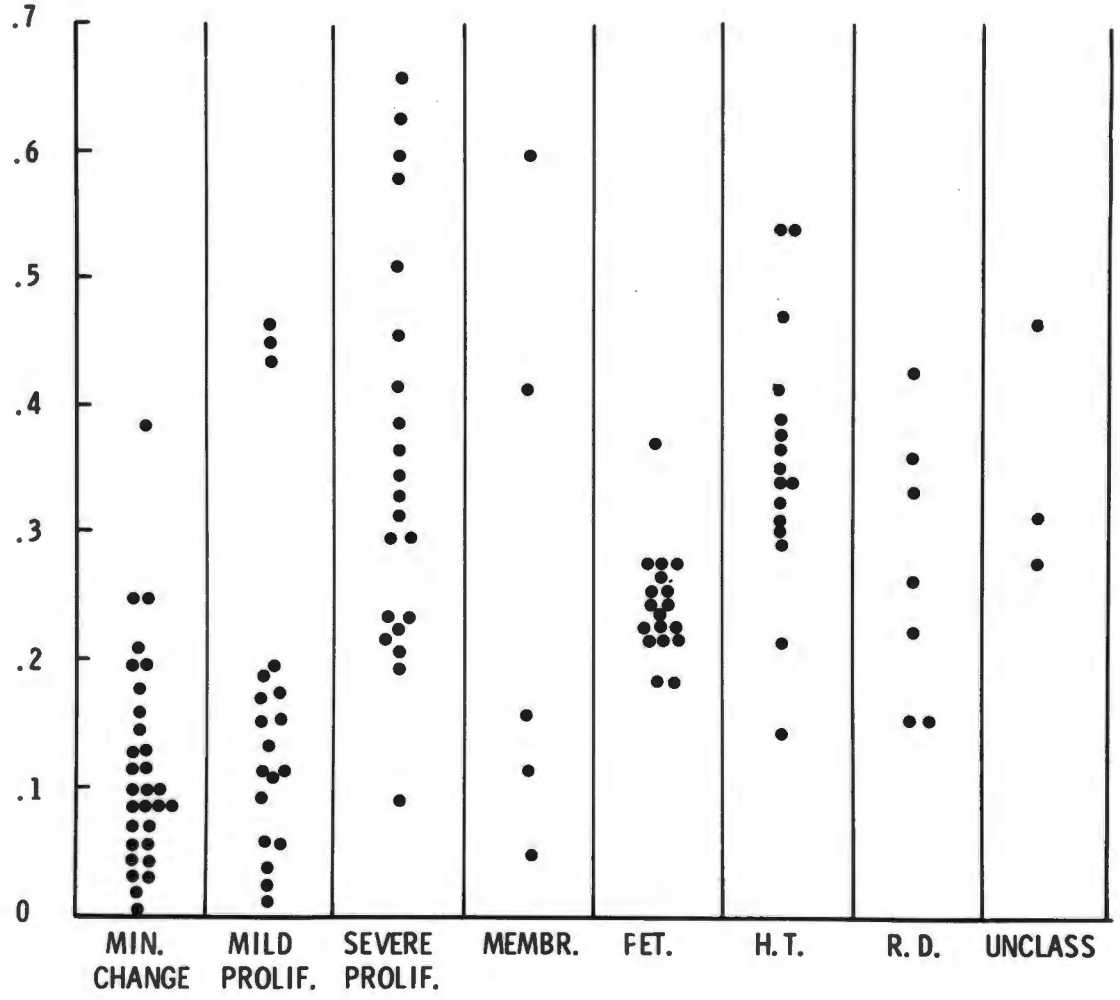

Fig. vi

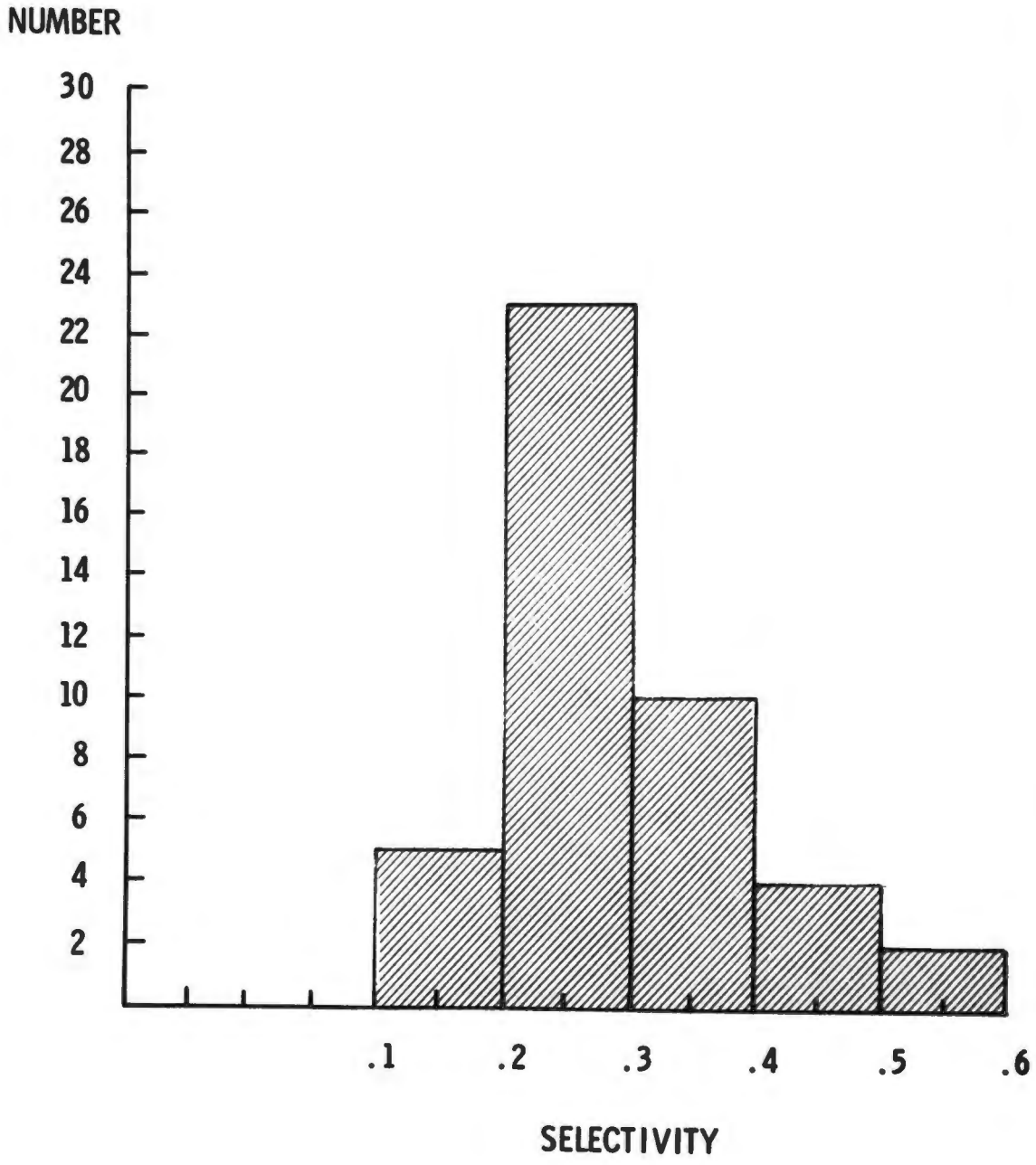

Fig. vii

in patients with "minimal change" glomerulonephritis. The mean Ig/transferrin clearance ratio in the series of pregnancy proteinurias is 0.29 (S.D. 0.09). The "spread" of the selectivity ratio in pregnancy is not as wide as in the nephrotic syndrome. Ig G clearance was found to be above 40% of the albumin and transferrin clearances in only six of the 44 cases. A histogram of the clearances found in the series is shown in Figure vii. This shows that 75% of the Ig G clearances fell between 20% and 40% of transferrin and albumin clearances. Further comparison between the different groups of pregnancy proteinurias are examined later.

Protein selectivity and level of proteinuria

At very low levels of proteinuria, selectivity ratios become increasingly more unreliable due to difficulties in interpretation of precipitin discs in the agar plates. All but six of the patients in the series had a protein excretion above two grams per day at the time of investigation. At some of these lower levels it was possible to study selectivity only on the basis of an Ig G/transferrin ratio, since the larger protein molecules were not present in the urine in amounts permitting quantitative measurement. Ig G globulin with a molecular weight of 160,000 was present in every case, as was transferrin (M.W. 90,000) and albumin (M.W. 69,000).

Figure viii relates protein selectivity (determined by Ig G/transferrin clearance) to quantity of protein excretion (determined by 24 hour collection of urine). It will be seen that on a cross-

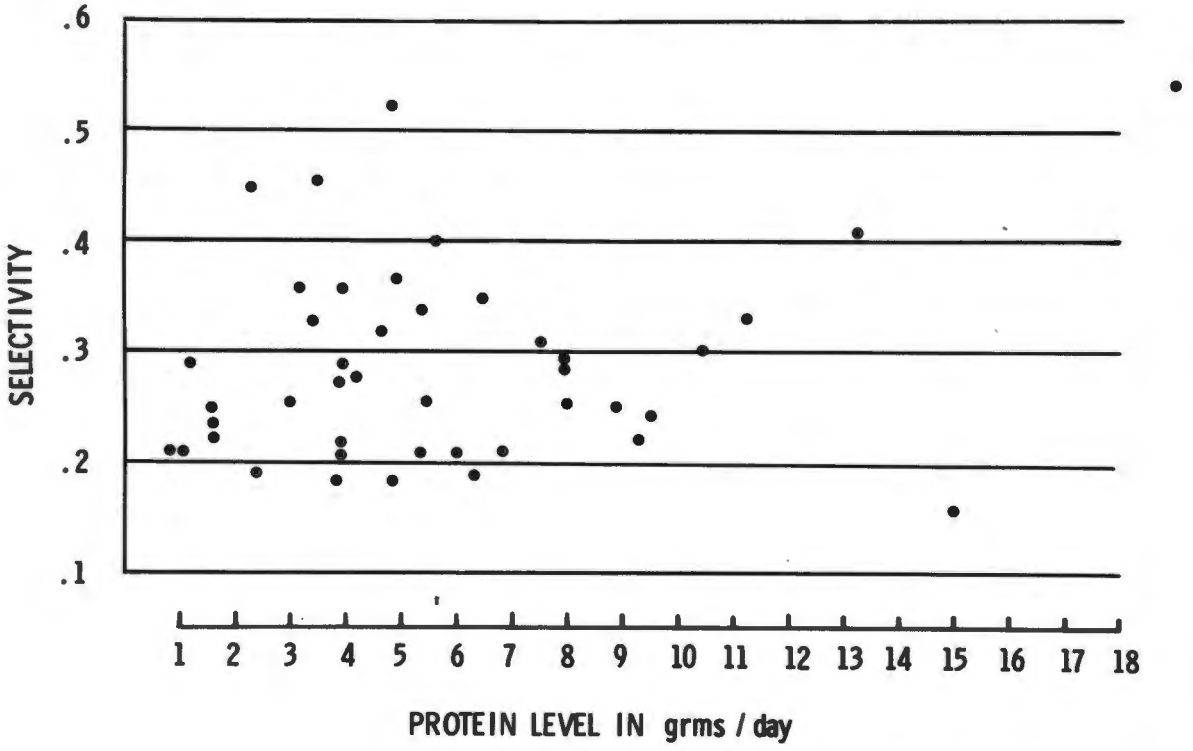
.6
.5
.4
.3
.2
.1
SELECTIVITY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PROTEIN LEVEL IN grms / day

Fig. viii

sectional basis where all patients' selectivities are compared with protein excretion at the time of study, there appears to be no correlation between selectivity and severity of protein loss. This was also observed by Joachim et al. (1964) in their study of nephrotic patients.

Further proof of this is provided by longitudinal studies of patients with pregnancy proteinuria. A group of patients in the present series was studied for varying periods of time from the onset of proteinuria through to term and into the puerperium and postnatal periods. Continuous studies in this group with respect to selectivity and level of proteinuria at the time of study are presented in Table VIII. It will be seen from this that whilst levels of protein excretion varied considerably, the selectivity ratios remained fairly constant throughout pregnancy. This consistency of selectivity in the presence of varying levels of proteinuria serves to support the thesis that tubular reabsorption of protein is non-selective.

Protein selectivity and the underlying pathology

In the presentation of renal selectivities in pregnancy, patients have been grouped according their presumed underlying pathology. For the purposes of comparison, only the hypertensive and pre-eclamptic groups are compared as there are too few in the group of patients with renal disease to draw any conclusions. The selectivities for all patients are listed in Table V.

TABLE VI

RENAL FUNCTION. A COMPARISON OF MEAN VALUES FOUND IN PREECLAMPSIA WITH THOSE FOUND IN ESSENTIAL HYPERTENSION (IN PREGNANCY)

	PRE-ECLAMPSIA	HYPERTENSION	STUDENT 't'
BLOOD UREA	29.4 mgs. per 100 ml. (± 10.3)	27.6 mgs. per 100 ml. (± 5.0)	P => 0.25
BLOOD URIC ACID	7.1 mgs. per 100 ml. (± 1.2)	6.7 mgs. per 100 ml. (± 0.87)	P => 0.1
CREATININE CLEARANCE	81.0 ml. per min. (± 25.1)	74.8 ml. per min. (± 21.3)	P => 0.25
SELECTIVITY Ig G Trans/Alb.	0.24 (± 0.04)	0.34 (± 0.1)	P =< 0.001
θ	63.3°	61.2°	P =< 0.001

The preeclamptic group of patients appeared to have a tighter range of selectivity than the hypertensive group. While practically all selectivities were in the poorly selective range (Ig/transferrin >0.20, $\theta < 70^{\circ}$), in the preeclamptic group only one patient (A.F.) had an Ig/transferrin ratio above 0.30 whereas in the hypertensive group eleven of the sixteen patients had ratios of 0.30 and above. This relationship was similarly noted in the slope of the regression lines. Again, patient A.F. was the only preeclamptic patient found to have a regression line the slope of which formed an angle (θ) of less than 61° with the horizontal. In the hypertensive group, seven of the sixteen patients had θ angles smaller than 61°. Thirteen of the sixteen hypertensive patients had θ angles of less than 63.1° (Joachim's lower limit for "intermediate" selectivity) whereas less than 50% (7 out of 15) preeclamptic patients had angles smaller than this.

Table VI compares three parameters of renal function between the preeclamptic and hypertensive groups. In addition, mean selectivity ratios of the two groups are compared and the significance of the difference between the two means examined. It will be seen that there is a highly significant difference between the mean selectivity ratio in the preeclamptic group and the mean in the hypertensive group. No other parameter of renal function shows any difference between the two groups.

Figures ix and x show diagrammatically the regression lines for protein clearance on molecular weight for the two different groups.

The mean slope of the regression line in the preeclamptic group is -1.9891 ($\theta = 63.3^{\circ}$). The mean slope of the line in the hypertensive group is -1.8221 ($\theta = 61.2^{\circ}$). The difference was found to be statistically significant ($P = <.001$).

The interpretation of these results is difficult. It would seem that protein excretion in patients with underlying hypertension is of a poorer selectivity than in patients with preeclampsia in late pregnancy. There is clearly a considerable degree of overlap. To what extent this overlap is dependent upon the differentiation of the groups remains uncertain.

Of paramount importance in this regard is the definition of hypertension. The figure, $^{140}/_{90}$ before the 20th week of gestation is generally taken as the dividing line between the normotensive and the hypertensive patient (Dieckmann, 1952, Reid and Teel, 1939, Cheatley and Annitto, 1947, Theobald, 1955), but many authorities would dispute this figure. Browne (1932) for a time adopted a standard of $^{130}/_{70}$, a standard that has received recent support (Vartan, 1958). Browne (1947) subsequently revised this figure to $^{120}/_{80}$ following the work of Robinson and Brucer (1939 and 1940). These authors found the normal range of blood pressure in women to lie between 90 and 120 systolic and 60 and 80 diastolic. They also made the important observation that even transient rises in blood pressure, including rises brought about by anxiety, suggest an underlying hypertensive tendency which will become overt at a later stage. Thus

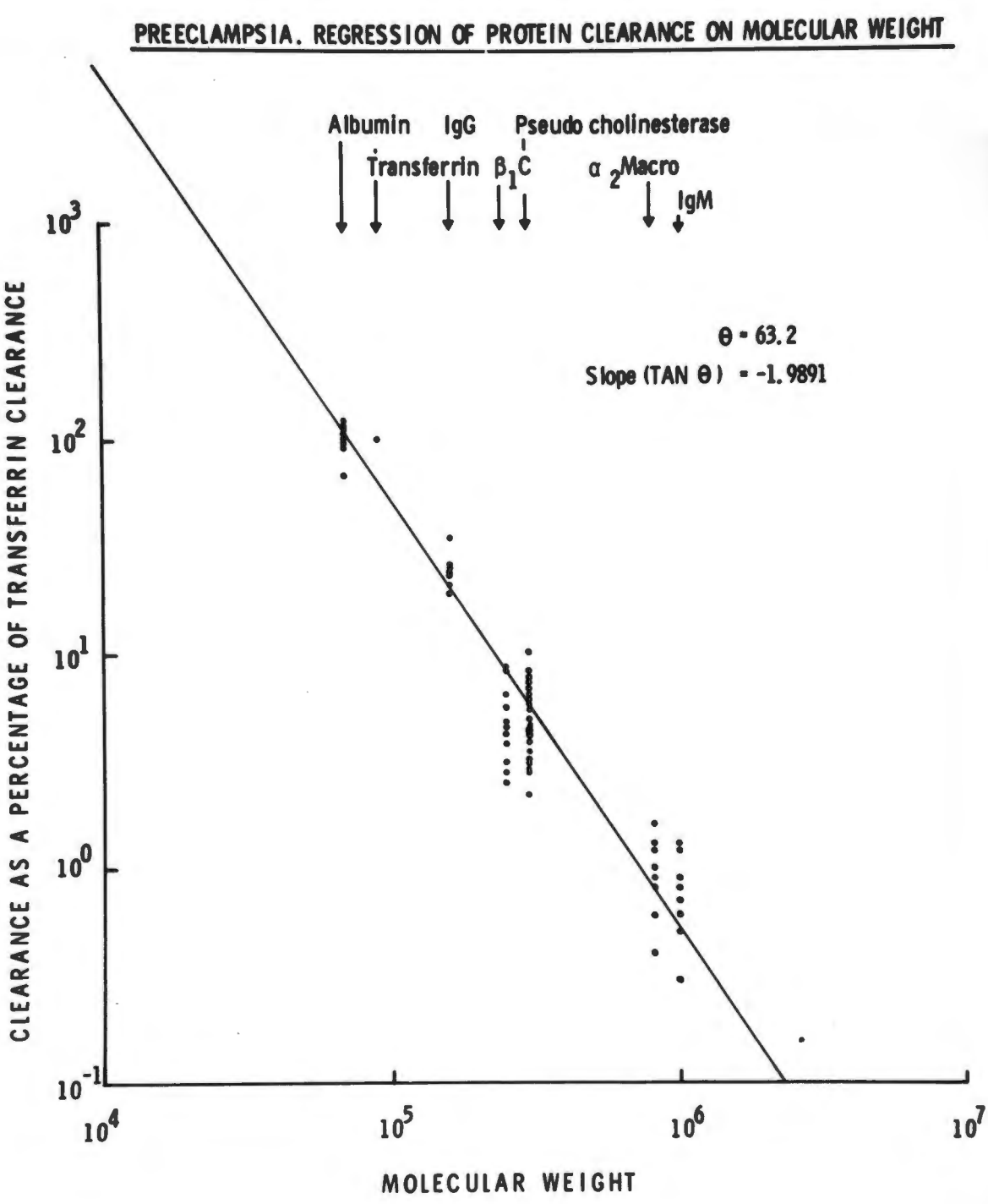
PREECLAMPSIA. REGRESSION OF PROTEIN CLEARANCE ON MOLECULAR WEIGHT
Albumin
IgG
Pseudo cholinesterase
Transferrin
β1C
α 2Macro
IgM
θ = 63.2
Slope (TAN θ) = -1.9891
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^-1
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

Fig. ix

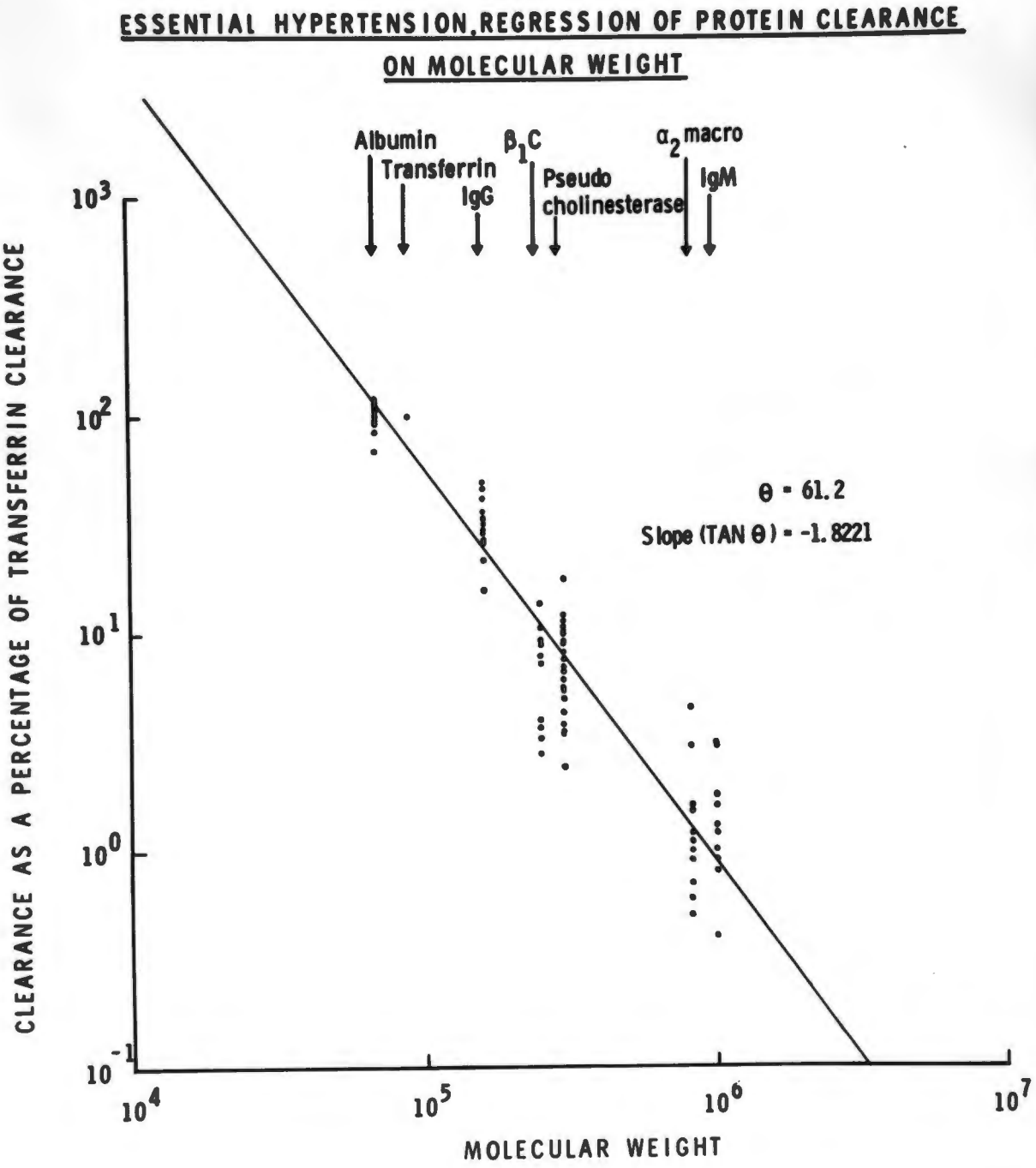
ESSENTIAL HYPERTENSION, REGRESSION OF PROTEIN CLEARANCE
ON MOLECULAR WEIGHT
Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
α_2 macro
IgM
θ = 61.2
Slope (TAN θ) = -1.8221
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^{-1}
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

Fig. x

we have two schools of thought, differing as to the importance of rest and reassurance prior to getting an assessment of a patient's blood pressure status. Kosmak (1931) recommended that several blood pressure readings should be taken, allowing the patient a period of rest prior to accepting a reading, but Browne and Dixon (1970) support Robinson and Brucer's impression that a rise in blood pressure due to anxiety is evidence of an underlying hypertensive tendency. Snodgrass (1968) found a significant fall in systolic and diastolic blood pressures after a short period of rest in both the normotensive and hypertensive groups. In a very large series of primiparous antenatal patients, MacGillivray (1961b) found the blood pressure around the 20th gestational week to lie between $^{130}/_{80}$ and $^{140}/_{90}$ in over 30% of cases. Less than 5% had blood pressures of $^{140}/_{90}$ and above. There was a definite increase in the incidence of albuminuria later in pregnancy in patients who had diastolic blood pressures over 80 mms. at the 20th week of gestation.

There are additional problems in attempting to define a normotensive and hypertensive group. Age is one, and this has been considered on page 62. The gestational age at which the patient is first seen is important in view of the frequently recorded finding of a "midtrimester fall", (Hare and Karn, 1924). A significant midtrimester fall occurred in 40% of Browne's series (1947). Chesley and Annitto (1947) found a midtrimester fall in 50% of their series of hypertensive pregnancies. Walters (1966), studying cardiac output in pregnancy,

found a small but significant fall in his series. On the other hand, Andros (1945) in a study of 300 normal private patients was unable to demonstrate a significant midtrimester fall in blood pressure. Snodgrass (1968), studying peripheral blood flow in pregnancy, found no change in blood pressure in her normotensive group, provided blood pressures were always taken after a rest period of ten minutes. Browne and Dixon (1970) would contend that this would be masking an underlying hypertension.

A useful approach is that of Browne (1958). He considered a patient with a blood pressure of $^{140}/_{90}$ and above before the 20th week of gestation as suffering from essential hypertension. Patients with pressures below $^{120}/_{70}$ were regarded as normotensive. All authorities would agree with these limits. There remained a "no-man's land" between 120-140 systolic and 70-90 diastolic which defied clear definition. Browne tended to feel that a lot of patients in this no-man's land were potentially hypertensive but sorting them out became extremely difficult.

It is with this approach in mind that the present clinical groups have been defined. There is little doubt that patients with blood pressures of $^{140}/_{90}$ and above prior to the 20th week of pregnancy are essential hypertensives, and it is primarily these that have been included in the "hypertensive" group. That some hypertensives might have crept into the preeclamptic group is possible, considering the problems inherent in the definition. However, it is unlikely

that the converse should have occurred, since preeclampsia rarely occurs before the end of the second trimester.

Poor selectivity ratios thus appear to be a feature of the hypertensive group, even allowing for the possible "escape" of some of its members into the preeclamptic group. The numbers, of course, are fairly small, but the high level of significance between the mean selectivities of the two groups would suggest that ratios above 0.3 indicate an underlying hypertension. The significance of this will be discussed further when foetal outcome and prognosis are considered.

Longitudinal studies

Protein clearance studies and determinations of selectivity were repeated at frequent intervals wherever proteinuria persisted for any length of time. In addition, every patient studied had a specimen of urine collected and examined for protein on the fourth day of the puerperium. Where significant amounts of protein were present, clearance studies were conducted. A further examination of the urine was performed six weeks after delivery and again clearances were studied where indicated.

The blood pressures and levels of proteinuria in the puerperium and at the postnatal attendances are shown in Table I. It will be seen that in the vast majority of preeclamptic patients and patients suffering from essential hypertension, proteinuria had completely disappeared by the fourth puerperal day (28 of the 34 cases). In none of these patients was protein present in the urine six weeks

after delivery. In the group with renal disease, proteinuria was consistently present in the puerperium, and had only disappeared from the urine at the six week postnatal attendance in two of the patients.

Twenty-two patients had proteinuria which was present for periods of from one to six weeks permitting repeated investigations during the antenatal period and further investigations following delivery.

Table VII in the Appendix shows the longitudinal clearance studies of seven proteins of varying molecular weights in these patients. Included in this table are patients who had only one set of clearances done antenatally, but whose protein levels in the puerperium were high enough to permit puerperal clearance studies. Total urinary protein levels at the time of study are also presented in the table.

The clinical follow up of selected patients is presented in the patient's summaries in the Appendix.

Serial protein selectivities for each of these patients are presented in Table VIII. Despite fluctuations in the level of proteinuria, selectivity remained remarkably constant for an individual patient from week to week. Where proteinuria persisted into the puerperium, the pattern of protein excretion seen prior to delivery was retained.

Proteinuria in patients with renal disease in pregnancy appeared to follow a different pattern. Selectivity ratios antenatally

TABLE VIII

LONGITUDINAL STUDIES OF PROTEIN SELECTIVITY IN PREGNANCY

PREECLAMPSIA

NAME	ANTENATAL STUDIES (Clearance = Ig G/Transferrin. Urinary protein level in brackets)				PUERPERIUM	POSTNATAL CLINIC
	1.	2.	3.	4.		
K.C.	0.22 (6.2 Grams)	0.22 (6.7 Grams)			0.20	-
E.S.	0.26 (8.0 Grams)	0.25 (2.6 Grams)			-	-
M.M.	0.25 (4.2 Grams)	0.19 (6.4 Grams)	0.22		-	-
P.F.	0.24 (7.9 Grams)	0.24 (8.0 Grams)			0.21	-
F.S-M.	0.20 (2.3 Grams)	0.19	0.17 (2.2 Grams)		-	-
L.S.	0.23 (6.2 Grams)	0.21 (3.0 Grams)	0.14	0.18	-	-
E.P.	0.23 (1.5 Grams)	0.26 (9.7 Grams)	0.26		-	-
J.T.	0.24 (4.5 Grams)	0.23 (9.2 Grams)			0.17	-
C.T.	0.24 (9.0 Grams)				0.26	-

TABLE VIII (Cont'd)

HYPERTENSION

NAME	ANTENATAL STUDIES (Clearance = Ig G/Transferrin. Urinary protein level in brackets)			PUERPERIUM	POSTNATAL CLINIC
	1.	2.	3.		
D.H.	0.30 (4.6 Grams)	0.28 (10.5 Grams)	0.25 (3.8 Grams)	0.22	-
P.P.	0.27	0.25 (8.0 Grams)	0.25 (6.6 Grams)	-	-
E.B.	0.29 (8.0 Grams)	0.28 (4.0 Grams)		-	-
M.K.	0.34 (4.1 Grams)	0.32 (6.5 Grams)	0.37	-	-
N.M.	0.39 (4.4 Grams)	0.43 (14.4 Grams)	0.43 (6.3 Grams)	-	-
S.K.	0.33 (3.2 Grams)	0.30	0.33 (11.2 Grams)	-	-
J.S.	0.32 (4.6 Grams)			0.34	-
H.D.	0.36 (2.9 Grams)	0.37 (4.8 Grams)	0.37	0.39	-
E.G.	0.50 (10.7 Grams)			0.51	-

TABLE VIII (Cont'd)

RENAL DISEASE

NAME	ANTENATAL STUDIES (Clearance = Ig G/Transferrin. Urinary protein level in brackets)					PUERPERIUM	POSTNATAL CLINIC
	1.	2.	3.	4.	5.		
M.N.	0.23 (4.3 Grams)	0.42 (3.9 Grams)	0.42	0.34 (7.5 Grams)	0.30	0.29 (2.9 Grams)	0.06 (1.8 Grams)
P.C.(1)	0.24 (3.8 Grams)	0.17	0.20 (2.4 Grams)	0.17		0.17	0.09 (2.2 Grams)
P.C.(2)	0.22 (2.4 Grams)	0.14 (3.2 Grams)	0.19	0.19 (3.4 Grams)		0.17	-
S.B.	0.26 (5.8 Grams)	0.30 (6.2 Grams)	0.25	0.23 (8.0 Grams)		0.21 (7.2 Grams)	0.18 (11.4 Grams)
R.F.	0.40 (3.2 Grams)					0.40	0.08 (2.4 Grams)
L.F.	0.22	0.23 (6.3 Grams)	0.22 (4.7 Grams)			0.25	-
F.R.	0.31 (1.6 Grams)	0.34 (5.5 Grams)	0.44 (2.6 Grams)			0.30	-

UNCLASSIFIED TOXAEMIA

M.P.	0.25	0.26 (3.0 Grams)				-	-

were indistinguishable from selectivity found in other forms of pregnancy proteinuria. However, following delivery, proteinuria persisted in these patients into the puerperium and was present six weeks after delivery in four of the seven patients. In three of the four patients with persistent proteinuria, a highly selective pattern of protein excretion was found at this stage, whereas antenatally the pattern had been poorly selective.

Discussion

Studies of urinary protein in pregnancy have been largely confined to conventional methods, using paper electrophoresis. This method, however, serves only to differentiate proteins according to their charge and mobility and has nothing to do with molecular weight. Thus the high globulin content relative to albumin in mild preeclampsia that surprised Parviainen et al. (1951) could well have been low molecular weight microglobulins passing through a sound glomerular membrane. In their more severe grades of preeclampsia, albumin was the chief protein present and there was an impressive deficiency of α2-globulins compared to their relative proportion in blood plasma! (α2-macroglobulin with a M.W. of 820,000 is by far the main component of the α2-globulin fraction.) Lorincz et al. (1961) found a general increase in urinary globulin in pregnancy proteinuria. They noted some differences between protein excretion patterns in essential hypertension, preeclampsia and chronic nephritis. An increase in urinary globulin relative to albumin has been reported from several sources (Dieckmann, 1952, Mack, 1955).

Similar results were obtained by Eastman (1931) who estimated urinary albumin/globulin ratios in preeclampsia using a quantitative chemical method. He was struck by the relatively large amounts of globulin in the urine and came to the conclusion that damage to the glomerular capillary tuft was considerable in the pre-eclamptic state, permitting the passage of the larger globulin molecules. He suggested a temporary asphyxia as the mechanism involved, which would account for the complete resolution of the proteinuria once delivery occurred and the stress was removed.

All these methods were imprecise and the variations in results of the investigations highlighted this fact. Thus, Glass et al. (1963) found a higher albumin/globulin ratio in the urine of preeclamptics than most other workers had found, and this ratio varied to a large extent depending on the severity of proteinuria. Eastman also found that very heavy proteinuria was necessary before his method achieved any degree of accuracy.

The introduction of more sophisticated immunochemical methods has permitted more precise determination of the nature of the proteinuria in pregnancy. We are no longer measuring a spectrum of plasma globulins, which though in general are larger than albumin, include a number that are in fact smaller than the albumin molecule and which serve to confuse the measurement. By selecting specific globulins with molecular weights in excess of albumin, we are in a position to compare clearance ratios across the glomerular basement membrane.

The concept of selectivity provides a relatively precise measure of what is happening in the individual case.

There is little doubt that the mechanism of protein excretion in pregnancy is similar to that of most proteinurias, namely, filtration of unaltered plasma proteins through an abnormally porous glomerulus. The proteins in the urine of pregnant patients has been shown to be immunochemically identical to the patients' own plasma proteins (reaction of identity, page 50). Furthermore, the characteristic linear excretion pattern of protein according to the molecular weight is similar to the patterns demonstrated for protein excretion in the nephrotic syndrome. Thus, when individual proteins are quantitated according to molecular weight, smaller proteins are found to be excreted much more readily than larger ones, the difference narrowing as the glomerular permeability increases.

The striking feature of pregnancy proteinuria in general is its poor selectivity. Selectivity ratios have been shown to approach those found in more advanced grades of renal disease (page 78). It is not surprising that a range of globulins of relatively large molecular weight are found in the urine in considerable quantities. Ig G gamma globulin with a molecular weight more than twice that of albumin has a clearance in pregnancy proteinuria of 20% to 50% of the albumin clearance. Proteins with molecular weights around 300,000 have clearance ratios ranging from 2% to 18% of the albumin clearance; and macromolecules with molecular weights approaching 1,000,000 are

frequently present in pregnancy proteinurias with clearances usually around 1% of the albumin clearance. Previous suggestions that the appearance of ∝-2-macroglobulin in the urine of preeclamptics might have some specific connotation (McEwan, 1969) are not borne out by this study. The presence of any protein in the urine is proportional to its relative clearance which in turn is related to its molecular weight. The presence of macroglobulins in the urine in pregnancy will be related to the general linear excretion pattern in each particular case. Since its clearance value is a percentage of albumin clearance, the heavier the proteinuria, the more likely is it to be detectable. There is, however, a deviation from strict linearity in the excretion of macroglobulins in pregnancy proteinuria (page 76). For this reason, selectivities are more satisfactorily expressed by the simpler Ig G/transferrin clearance ratio.

Several features of protein excretion patterns in pregnancy require explanation when considered in relation to patterns in the nephrotic syndrome. Some of these features serve to throw a new light on the nature and causation of the glomerular leak giving rise to proteinuria.

Protein has been interpreted in terms of pore size of the glomerulus (see introduction). In health, these "pores" will permit the passage of the very low molecular weight proteins only, proteins the size of albumin and above being in general retained. However, as in all porous membranes, pores vary in size according to a normal

distribution about a mean. At the one extreme of this distribution will be the pores large enough to permit the passage of small amounts of larger proteins. This accounts for the presence in normal urine of small quantities of albumin and Ig G globulin (Berggard, 1970, Rowe and Soothill, 1961, Grant, 1957). It also will account for the finding of high globulin concentration relative to albumin in mild pre-eclamptics (Parviaien et al., 1951). Albumin retention was presumably relatively efficient, with little increase in glomerular membrane permeability. Urinary protein would thus approximate the proportions found in normal urine, in which the ratio of low molecular weight globulin to albumin is considerably higher than in most proteinurias.

The concept of pore size is a purely theoretical one. No pores have ever been seen under the electron microscope but the linearity of protein excretion on molecular weight together with the experimental data using dextrans presented in the introduction would tend to support the idea. Brewer (1951), using low molecular weight dextrans, found that below M.W. 7,000 all were excreted, falling to 20% excretion at 25,000M.W. and 6% at 38,000. Arturson and Wallenius (1964) found that clearance values of dextrans of M.W. below 15,000 had a renal clearance approaching that of creatinine, crossing the glomerular barrier without any noticeable restriction. Clearances fell off rapidly as M.W. increased, approaching zero at a M.W. of 60,000.

On the basis of this concept, it is reasonable to accept that damage to the glomerular basement membrane could result in an increasing porosity and a consequent increase in the size of proteins able to pass through. This has been convincingly demonstrated in the nephrotic syndrome, a good correlation having been found between protein selectivity and the extent of glomerular membrane damage found on microscopic examination of renal biopsy material. In keeping with the concept of glomerular pore size, highly selective protein clearance patterns are a feature of renal disease in which minimal change is found on microscopic examination of the glomeruli. Conversely, where glomerular membrane changes are extensive, they are accompanied by a characteristically poor selectivity (Blainey et al., 1960, Joachim et al., 1964, Cameron, 1966, Cameron and Blandford, 1966).

The morphological changes in the kidney in preeclampsia, however, could at the most be described as minimal. Characteristically, there is swelling of the endothelial cells of the capillary tuft, with resultant narrowing of the capillary lumina (Altchek, 1964, Pirani et al., 1961). There is no basement membrane change detectable even under electronmicroscopy, and in some cases of clinical preeclampsia, there are no detectable renal changes at all (MacCartney, 1968).

How then are we to account for a protein excretion pattern in pregnancy which, on the basis of the mechanisms discussed, suggests extensive glomerular membrane involvement? Furthermore, despite its

poorly selective quality, proteinuria virtually always clears immediately after delivery so that it would appear that the pregnant state is largely responsible for this hyperporosity of the renal glomerular membrane. This is borne out by the fact that in three patients with proteinuria due to renal disease in pregnancy, poorly selective patterns changed to highly selective patterns by the six week postnatal visit. Previous reports on protein selectivity in renal disease suggest that selectivity is a feature of a particular patient's disease and that this does not change over a considerable period of time, even in the presence of a remission (Blainey et al., 1960, Cameron and Blandford, 1966, Vere and Walduck, 1966). Although selectivity ratios were not available prior to the three above patients falling pregnant, it would seem that the protein excretion pattern was probably altered by the pregnant state and that pre-pregnant patterns were re-established once pregnancy was completed.

Kenney (1950) pointed out that the contradictory findings in renal efficiency tests in pregnancy suggest an unstable circulatory equilibrium in the kidney. There is histological evidence that the glomerular capillaries are markedly ischaemic in preeclampsia even though this is not obvious on every biopsy. The efferent arteriole which leaves the glomerular tuft is responsible for oxygenation of the renal cortex (Rhodin, 1967) and there can be little doubt that the narrowed capillary lumina described in preeclampsia will produce some degree of cortical ischaemia. Altchek (1964) has shown that the

glomerular lesion in preeclampsia appears after the development of hypertension, but precedes the appearance of proteinuria. It is possible that the ischaemia thus produced may be an important factor in bringing about a temporary increase in glomerular permeability. A vascular explanation would further explain the rapidity of the disappearance of protein from the urine following delivery. It would also serve to explain the decrease in renal plasma flow and glomerular filtration found in preeclampsia and hypertension and more particularly, the rapid return to normal of these renal function tests following delivery.

Cheeley et al., (1939) reviewed the evidence suggesting that vasoconstriction and tissue anoxia could result in transient proteinuria. This group used the cold pressor test of Hines and Brown (1933) to demonstrate that vasospasm produced by immersion of the hand in iced water was followed shortly afterwards by clinical proteinuria. De Alvarez and Richards (1954) were impressed with the intermittent character of pregnancy proteinuria and felt this could be best explained on a vascular basis. Once there is permanent glomerular damage as in established renal disease, proteinuria tends to be more permanent. Lamport (1942) has shown mathematically that decreased renal plasma flow in preeclampsia is the result of increased afferent glomerular arteriolar resistance and Assali et al. (1953) reported similar findings. This would serve further to embarrass the cortical blood supply derived from the efferent arteriole leaving a constricted glomerular capillary bed.

Poorly selective proteinurias have been found in acute nephritis (Blainey et al., 1960) and in the orthostatic and exercise proteinurias (Rowe and Soothill, 1961). In these conditions, once again poorly selective patterns of protein excretion are found where glomerular membrane damage is minimal or entirely absent. Orthostatic proteinurias are totally reversible as is the proteinuria of most pregnancy proteinurias. Acute nephritis is reversible in most cases. The glomerular capillaries tend to be constricted by a proliferation of the mesangial and endothelial cells with a consequent reduction in blood flow through the glomeruli. The mechanism operating in orthostatic proteinuria is as yet not clear but again haemodynamic factors have been postulated. The reduction of blood flow resulting from the upright posture is thought to be a primary factor in causation of this phenomenon, although clearly there are additional unknown factors (Robinson et al., 1963).

The poorly selective pattern of protein excretion found in these conditions in the presence of an apparently healthy kidney suggests a probable alternative mechanism to permanent glomerular membrane damage as a cause of increased glomerular membrane porosity. Temporary ischaemia resulting in reversible glomerular membrane damage is propounded as the possible alternative mechanism.

PROTEINURIA AS A FEATURE OF RENAL DISEASE IN PREGNANCY

Six patients in the series were either known to have, or presumed to have, renal disease complicated by pregnancy. One of these patients had a second pregnancy during the period of the study and has been included twice. Summaries of their case histories, together with criteria for the diagnosis of chronic renal disease and foetal outcome are shown in Table I. Two of the patients (R.F. and F.R.) had an established nephrotic syndrome prior to their falling pregnant. One of the patients (M.N.) had a renal biopsy done in view of her persistent heavy proteinuria and a diagnosis made of "minimal change" glomerulonephritis. The remaining three patients were presumed to have renal disease because of gross proteinuria in early pregnancy in the absence of hypertension. In two of these three cases, blood pressure remained normal throughout pregnancy. Proteinuria persisted in the puerperium in all seven pregnancies and was present in considerable quantities at the postnatal attendance six weeks after delivery in four of the seven.

Renal function and protein selectivity

In none of the seven pregnancies was renal function noticeably impaired. The highest recorded blood urea was 30 mg. per 100 ml. and the lowest recorded creatinine clearance was 86 ml. per minute.

The range of protein clearances as a percentage of the transferrin clearance is shown in Table V.

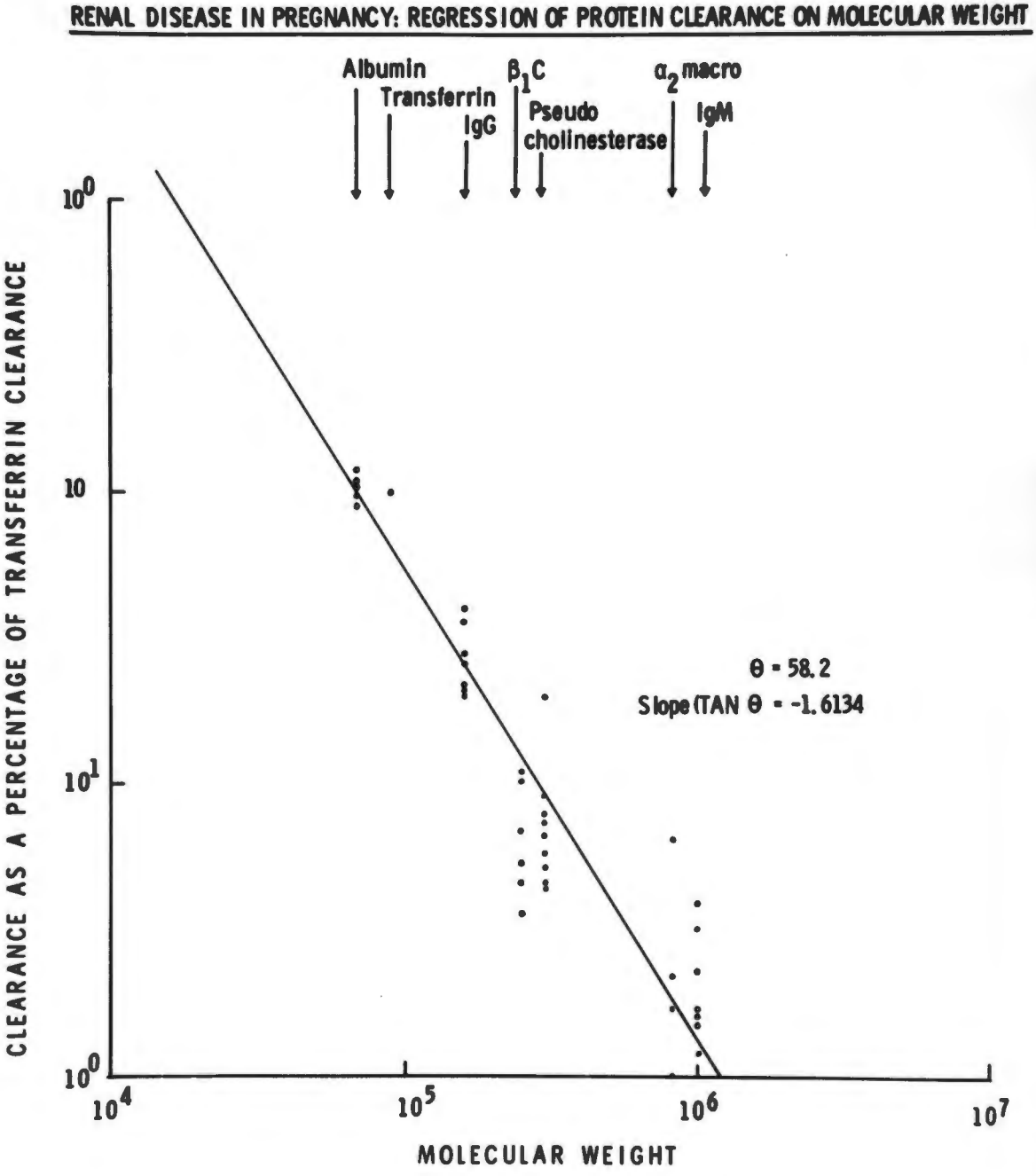
RENAL DISEASE IN PREGNANCY: REGRESSION OF PROTEIN CLEARANCE ON MOLECULAR WEIGHT
Albumin
Transferrin
IgG
β1C
Pseudo cholinesterase
α2 macro
IgM
θ = 58.2
Slope (TAN θ = -1.6134
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
MOLECULAR WEIGHT
10^0
10
10^1
10^0
10^4
10^5
10^6
10^7

Fig. xi

Graphic representation of the selectivity expressed as the mean slope of clearance on molecular weight for the group of six patients is shown in Figure xi.

There are not enough cases in this series to draw any definite conclusions regarding protein excretion patterns in patients who develop proteinuria in pregnancy on the basis of an underlying renal lesion. What appears to be most striking in this small group is the relative loss of linearity of protein excretion. This is illustrated by the marked difference in protein selectivity expressed by the two methods; both in many of the individual cases and in the group as a whole. The mean regression slope for the group of seven pregnancies was -1.6134 ($\theta = 58.2^{\circ}$). This is less than the slope for preeclampsia and for hypertension. On the other hand, mean selectivity expressed as Ig/transferrin, was 0.27 (S.D. 0.08), a higher selectivity than that found in the hypertensive group. Further opportunity for comparing selectivities by the two methods is afforded in Table V.

Proteinuria due to underlying renal disease differs primarily from that due to hypertension and preeclampsia in its persistence after delivery. In all seven pregnancies, heavy proteinuria persisted into the puerperium, whereas it had cleared by the fourth day in 28 of the 34 hypertensive and preeclamptic patients. Furthermore, proteinuria was still present in four of the seven pregnancies six weeks after delivery. The striking reversion to a highly selective pattern of protein excretion in three of these four patients has been previously commented upon (page 87).

Progress and outcome of the pregnancies

This is summarised in Table I.

All seven pregnancies were normotensive (B.P. $\frac{140}{90}$) when first seen in the first trimester.

Hypertension supervened in five of the pregnancies and in this group one pregnancy had to be terminated at 25 weeks because of uncontrollable hypertension and symptoms of imminent eclampsia. One of the hypertensive group was terminated at 34 weeks with the successful delivery of live twins. The remaining three patients were delivered after the 36th week of gestation, all resulting in live births of infants whose birth weights were below the tenth percentile for the stage of gestation (see page 104).

The two patients who remained normotensive throughout pregnancy were delivered of live infants with birth weights well above the tenth percentile.

These results are summarised in the table.

TABLE IX

	Number	Foetal Loss	Birth Weight Below 10th percentile
No hypertension	2	0	0
Hypertension	5	1 (20%)	4 (80%)

Discussion

An extensive review of renal disease in pregnancy would be unwarranted here, particularly in view of the small number of patients in the series. However, this small series does serve to illustrate some of the more recent concepts of renal disease in pregnancy and these will be discussed.

The extreme pessimism attendant upon the association between renal disease and pregnancy has been replaced by a more rational approach to the problem, following the gradual realisation that with careful antenatal care, the outcome for mother and baby is considerably better than was once thought.

Gibberd (1931) expressed the pessimism of the time when he reviewed 224 patients with proteinuria in pregnancy. Quoting a 60% foetal mortality in those with chronic nephritis, he goes on to say "considering the total foetal death rate, we see at once that when pregnancy occurs in a woman already suffering from a chronic renal lesion, the chance of a foetal catastrophe is very great, so that in allowing pregnancy to proceed in such a patient we are allowing the mother to take a considerable risk for the sake of a very problematical infant". This view is reflected more recently by Dieckmann et al. (1958) who felt the risk was too high in these cases to permit pregnancy to continue, and by Browne and Browne (1960) who advised extreme caution in allowing any to continue.

Recent reviews have been considerably more optimistic. Felding (1969) found no increase in the risk of prematurity and perinatal loss in patients with chronic normotensive nephropathy. In patients who commenced pregnancy with renal disease and a raised blood pressure, however, foetal loss was 50%. Mackay (1963) found a 10% foetal loss in patients with proteinuria only and a 30% loss when preeclampsia supervened. He too found a high perinatal loss (60%) where hypertension was present at the commencement of the pregnancy. He stressed the importance of a rising blood urea as a bad prognostic sign. Wilson (1958) found a foetal loss of 5.7% in patients with renal disease presenting with albuminuria alone. He grouped the remaining patients together finding a foetal loss of 34% in those patients with renal disease plus hypertension or nitrogen retention (or both). He found that a rise in blood pressure before the 30th week carried a bad prognosis and seven of the pregnancies in the series were terminated.

Wilson further stressed the apparent benign effect pregnancy had on renal function in these patients. Only nine of the 53 patients showed any deterioration in renal function at all, and then not enough to raise the blood urea in most cases. In his follow up, 17 of the 24 patients in whom the late prognosis was known were unaffected by their pregnancies. He concluded that apart from a patient with severe hypertensive complications during pregnancy, pregnancy appeared to have no adverse effect on the condition.

The findings in the small series described in the present investigation would appear to agree with recent reviews on the subject, both with respect to renal function and foetal prognosis. Thus in the two patients where proteinuria was the only symptom, the pregnancies proceeded normally with the birth of healthy babies of satisfactory birth weight. Four of the five pregnancies in which hypertension supervened, however, were associated with low birth weight infants and foetal loss (through termination) occurred in one of the two patients where blood pressure levels rose above $^{160}/_{110}$.

FOETAL LOSS AND GESTATIONAL AGE

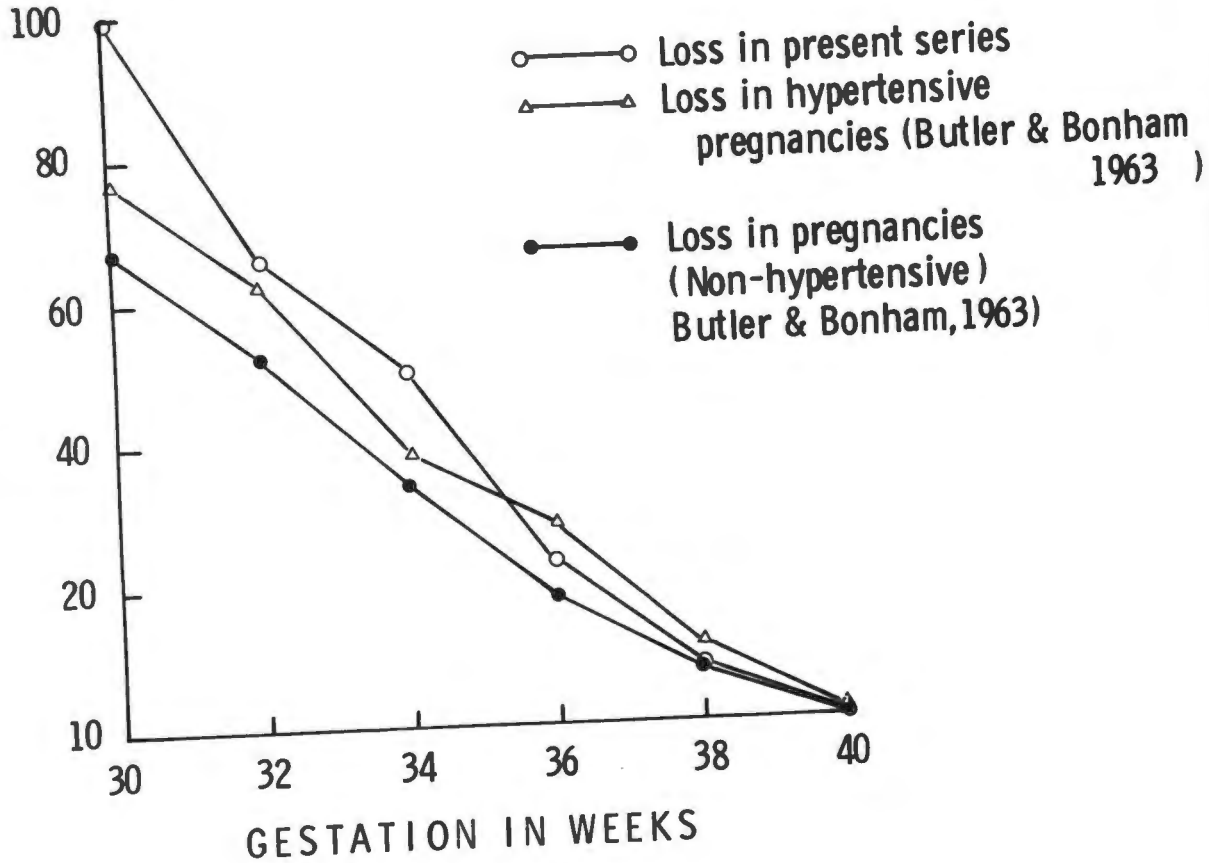

Fig. xii

PROTEINURIA IN PREGNANCY AND FOETAL PROGNOSIS

The foetal prognosis in patients with renal disease and proteinuria has been discussed. On the whole, in the absence of hypertension, proteinuria alone has a favourable prognosis, and even in those patients where hypertension develops in the latter weeks, foetal survival remains of the order of 70%. A different situation arises where blood pressure is raised at the outset of pregnancy or develops prior to the 30th week of gestation. In these cases, foetal loss appears to be of the order of 50% to 60%.

The present chapter deals with foetal prognosis in the entire series of 44 pregnancies. The relationship between protein selectivity and foetal outcome is dealt with and some conclusions are drawn as to the value of protein selectivity studies in the evaluation of pregnancy proteinuria and the assessment of foetal prognosis. In particular, comparisons are drawn between patients thought to have preeclampsia and those with essential hypertension.

Foetal survival in relation to maturity

The total foetal loss in the series of 44 pregnancies was 11 (250 per 1,000). There was one case of hydatidiform mole.

Nine infants were delivered before the commencement of the 34th week of gestation because of anxiety regarding their intrauterine environment. Figure xii shows the distribution of foetal loss according to the gestational age. This confirms the high risk of premature delivery of these infants and the necessity of being

able to decide which infants can be permitted a few more weeks of intra-uterine life. Six of the eleven foetal deaths occurred amongst nine patients delivered before the 34th week of gestation (66%). Foetal loss above 34 weeks gestation was 11%. Of the six deaths prior to 34 weeks, three were hypertensive patients and the birth weights of these three infants were all below the tenth percentile for their gestational age. Three of the deaths in this premature group occurred in preeclamptics, and of these, two had birth weights above the tenth percentile for the gestational age. With the exception of N.M. who had an intra-uterine death, all of these six patients were delivered by Caesarean section as a planned procedure, and all six infants were born alive.

Figure xii is a graphic comparison of foetal loss at varying gestational ages in this series compared with the findings in 16,994 pregnancies amongst which 5,998 were "toxaemic" (Butler and Bonham, 1963). The patterns are basically the same, although the percentage loss is considerably higher in this series than the overall loss in Butler's "hypertensive" pregnancies. This can be explained in part by the small numbers involved in this series. In addition, Butler's percentages included all pregnancies which were not normotensive, whereas the present series deals with more severe grades of preeclampsia and hypertension. Butler's overall foetal loss in all preeclamptics was 42 per 1,000 whereas when protein was present in a catheter specimen of urine, this rose to 93 per 1,000.

Foetal outcome and proteinuria

In a series as small as this, it is difficult to compare foetal outcome between two groups according to foetal loss alone. An attempt has thus been made to examine the foetal outcome according to the degree of intra-uterine growth retardation caused by the two syndromes. This is achieved by comparing foetal weight at delivery with the expected weight at the particular stage of gestation.

Several studies of foetal growth have been undertaken, in an attempt to determine a mean birth weight for each gestational age. Two of these studies are represented graphically in Figure xiii showing a close correlation between two entirely different sets of figures. The 90th and 10th percentiles are plotted for each gestational age. Infants whose birth weights fall below the 10th percentile for their gestational age are regarded in general terms to be "small for dates" babies.

Interpretation of the results according to these criteria must be guarded. In the first instance, the series is small. In addition, many of the infants were born very prematurely, at a time when the foeto-maternal mechanism has a fair amount of reserve to compensate for adverse factors and may thus sustain intra-uterine growth satisfactorily in a situation where it may be unable to do so at a later stage of gestation (Gruenwald, 1966). Furthermore, the 10th percentile is a fairly arbitrary dividing line, and the birth weights of several of the infants in the series fall very close to

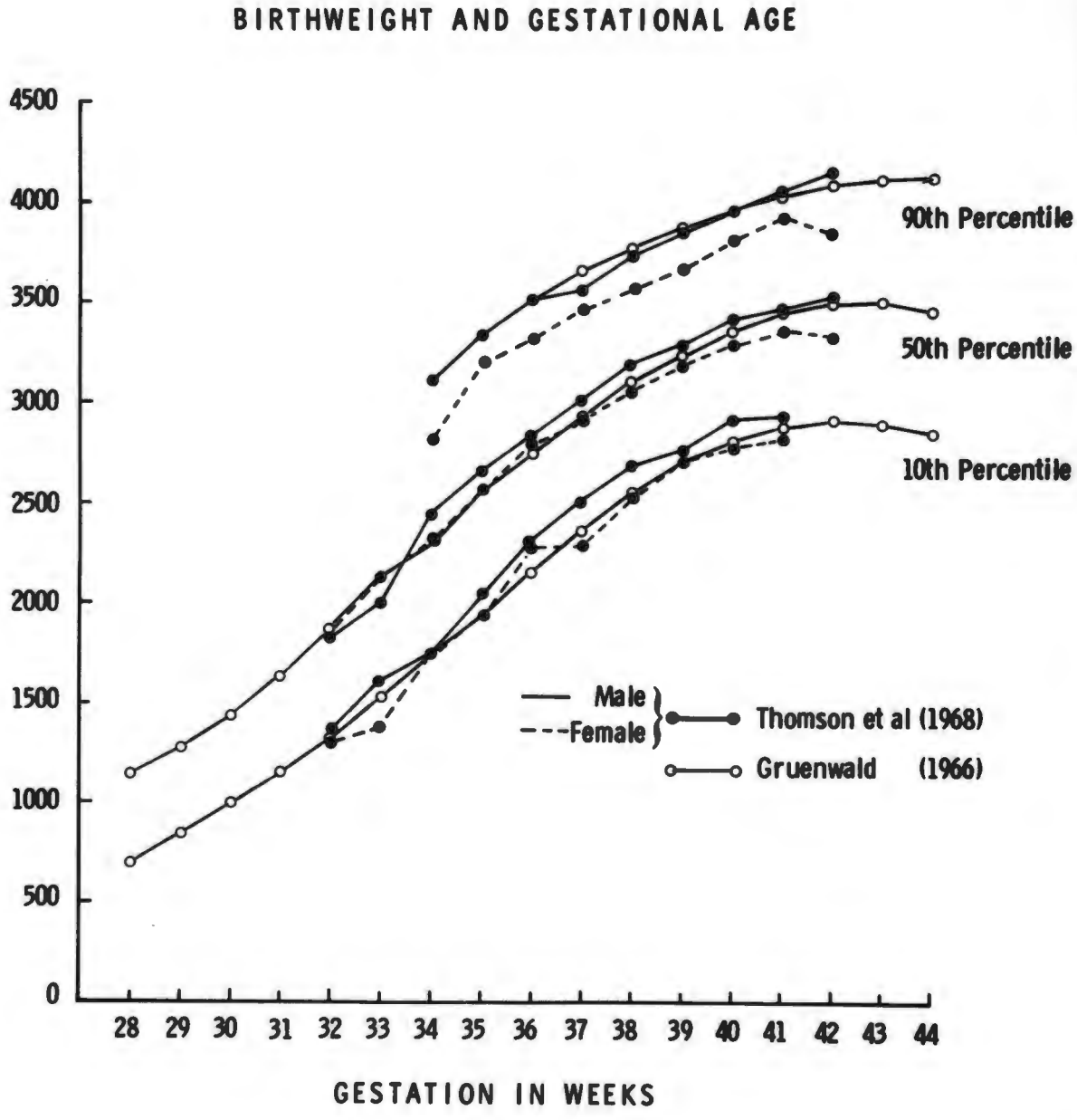
BIRTHWEIGHT AND GESTATIONAL AGE
4500
4000
3500
3000
2500
2000
1500
1000
500
0
90th Percentile
50th Percentile
10th Percentile
Male
Female
Thomson et al (1968)
Gruenwald (1966)
28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
GESTATION IN WEEKS

Fig. xiii

this figure. It is however a workable figure which has found general acceptance, and for this reason it is employed here.

Table X compares the relationship between proteinuria, protein selectivity and foetal outcome in the two defined groups, preeclampsia and hypertension.

It will be seen that in the preeclamptic group, only one patient of the nine who had proteinuria of less than two weeks duration gave birth to a "small for dates" infant (below the 10th percentile). Two neonatal deaths in this group resulted from very premature deliveries of babies whose birth weights were above the 10th percentile for their period of gestation (C.T. and E.S.). Ig G/ transferrin selectivity ratios were between 0.2 and 0.3, as they were in the majority of the preeclamptic cases.

Seven preeclamptic patients had proteinuria of two weeks or more duration, and of these five gave birth to "small for dates" babies. All selectivities were in the 0.2 to 0.3 range. One of the four died in the neonatal period, from extreme prematurity. A further infant was lost during delivery (E.P.). Intrapartum asphyxia occurred after a 36 hour labour with the birth of a 2,605 gram infant which was stillborn. Birth weight was above the tenth percentile.

In the hypertensive group, there were eight patients who had proteinuria lasting less than two weeks. Six of the eight had "small for dates" babies, with two neonatal deaths. One stillbirth occurred

TABLE X

URINARY PROTEIN LEVELS, DURATION OF PROTEINURIA, SELECTIVITY AND FOETAL RESULT

PREECLAMPSIA

NAME	BLOOD PRESSURE		PROTEINURIA		SELECTIVITY	FOETUS	
	LEVEL	DURATION in weeks	LEVEL in grams per 24 hrs	DURATION in weeks	(Ig/trans.)	RESULT	PERCENTILE
K.C.	155/100	4	6-7	2	0.21	A	10 +
A.W.	150/100	2	1	1	0.21	A	10 +
F.S-M.	170/120	5	2-3	3	0.19	A	10 -
B.M.	160/125	1	1-2	1	0.24	A	10 -
E.P.	150/110	7	9-10	5	0.24	SB	10 +
M.G.	170/105	< 1	4-5	< 1	0.18	A	10 +
A.F.	220/140	4	4-5	< 1	0.35	A/A	10 +
M.M.	180/110	4	4-5	4	0.21	A	10 -
J.G.	170/110	< 1	4-5	< 1	0.20	A	10 +
B.T.	150/100	1	1-2	1	0.26	A	10 +
E.S.	175/110	1	8-9	1	0.26	NND	10 +
C.T.	170/120	4	9-10	1	0.25	NND	10 +
J.T.	165/110	4	9-10	2	0.23	A	10 -
S.D.	160/120	1	6-7	< 1	0.21	A	10 +
M.W.	160/90	2	1-2	1	0.23	A	10 +
L.S.	170/110	5	6-7	5	0.19	NND	10 -
M.P.	160/120	4	5-6	4	0.26	A	10 -

TABLE X (Cont'd)

ESSENTIAL HYPERTENSION

NAME	BLOOD PRESSURE		PROTEINURIA		SELECTIVITY	FOETUS	
	LEVEL	DURATION in weeks	LEVEL in grams per 24 hrs	DURATION in weeks	(Ig/trans.)	RESULT	PERCENTILE
P.P.	190/115	-	8-9	3	0.28	A	10 -
J.S.	150/120	-	4-5	1	0.32	A	10 -
B.M.	170/120	-	3-4	< 1	0.46	A	10 -
N.M.	170/110	-	4-5	4	0.40	IUD	10 -
U.K.	180/115	-	15-16	1	0.16	NND	10 -
E.B.	180/140	-	4-5	2	0.29	A	10 +
J.R.	220/125	-	1	1	0.21	A	10 +
M.M.	160/95	-	3-4	2	0.32	A	10 -
H.D.	160/100	-	4-5	5	0.37	A	10 -
S.K.	170/110	-	11-12	3	0.33	A	10 +
M.K.	170/110	-	6-7	5	0.35	A	10 +
N.R.	220/120	-	3-4	≤ 1	0.36	NND	10 -
D.S.	180/110	-	5-6	≤ 1	0.55	A	10 -
W.A.	170/120	-	4-5	1	0.33	A	10 -
E.G.	210/120	-	24	1	0.53	SB	10 +
D.H.	160/105	-	10-11	3	0.30	NND	10 -

TABLE X - ABBREVIATIONS

(See abbreviations for Table I)

due to intrapartum asphyxia. This infant was normal weight for dates. Selectivities were generally above 0.3, but two of the eight had selectivities less than 0.3, and patient U.K. had the lowest selectivity ratio of the whole series. Of the eight patients with proteinuria of more than two weeks duration, five infants were "small for dates" at birth. There was one neonatal death and one intrauterine death. All selectivities were above 0.3 with the exception of P.P. (selectivity 0.28) and E.B. (selectivity 0.29).

Discussion

A rise in perinatal foetal loss attendant upon hypertensive pregnancies is well recognised. Difficulties arise, however, when it comes to comparing figures reported by different workers. The variation in criteria used to define the different pathological groups is one of the main difficulties, but added to this problem is the inconsistency of methods of comparison. Thus, whilst recognising preeclampsia and essential hypertension in pregnancy as two separate entities, albeit with awkwardly merging borders, many authors proceed to discuss them as one group when considering foetal loss. FitzGerald and Clift (1958) made the important observation that foetal loss resulted from two different situations, one in which the infant is normal weight for its period of gestation and one in which growth is clearly retarded. However, they failed to differentiate between patients suffering from preeclampsia and those suffering from essential hypertension in pregnancy. Thus they studied the influence of

proteinuria on the whole group of "toxaemias", coming to the conclusion that albuminuria was no reliable guide to those pregnancies which would result in low birth weight infants. In general terms, the present investigation would bear this out since clear evidence of intra-uterine growth retardation was present in only sixteen of the 34 cases of "toxaemia" with proteinuria. If, however, we consider only the hypertensive group in the present study, evidence of retarded growth is found to be present in eleven out of sixteen cases. The three cases described by FitzGerald and Clift showing striking evidence of intra-uterine growth retardation were probably all cases of essential hypertension rather than preeclampsia.

Carey and Liley (1959) analysed the factors responsible for intra-uterine death in preeclampsia. They too failed to differentiate between preeclampsia and hypertension in their analysis although they made the observation that the duration of pregnancy at the onset of the condition was of paramount importance in the causation of intra-uterine death. Although there was a further association between intra-uterine death and the severity of both hypertension and proteinuria, this association was not a strong one. They felt that this was deceptive in that foetal loss takes no account of infants that "nearly" died. This suggests that an additional yardstick is required for assessing the effect of hypertensive conditions on the progress of pregnancy.

TABLE XI

FOETAL LOSS (PER 1,000) IN HYPERTENSIVE DISEASE IN PREGNANCY

	PREECLAMPSIA				HYPERTENSION			
	MILD	MODERATE	SEVERE	WITH PROTEINURIA	MILD	MODERATE	SEVERE	WITH PROTEINURIA
Taylor et al. (1954) (4,432 cases)	66 all groups (including those with proteinuria)			93	157 all groups (excludes terminations which were probably more in those with proteinuria)			230
Wellen (1953) (1,400 cases)	31	-	158	-	66	-	165	-
Rogers et al. (293 cases)	-	-	77	-	-	-	167	-
Butler & Bonham (1963) (2,487 cases)	24	36	49	98	36	32	49	125
Browne & Dodds (1940) (589 cases)	130 all groups				330 all groups			
Tillman & Watson (1935) (1,036 cases)	241 all groups				30	36	300	333
Friedberg (1966) (806 cases)	77		162		108		245	
Claireaux (1961) (3,281 cases)	84		187		147 all groups			
Browne (1961)	24		85					

Baird et al. (1957) analysed 7,168 primiparous pregnancies over a seven year period. They were able to demonstrate retarded foetal growth in patients with severe preeclampsia. In mild pre-eclampsia and essential hypertension, foetal birth weight did not differ significantly from the normal range, at similar gestational ages. Hypertensive patients who developed proteinuria showed an identical pattern of intra-uterine growth retardation as did pre-eclamptic patients. Duration of the condition appeared to have only a slight bearing on foetal weight.

In the present study, duration of proteinuria would appear to be of considerable importance in the preeclamptic group, though less so in the hypertensive group. In the latter group, the presence of proteinuria was associated with a high incidence of low birth weight infants, even when proteinuria was of short duration. This association has been previously noted, where foetal weight in the same week of gestation decreased in proportion to the duration of proteinuria (Jarvinnen et al., 1958).

The different methods of comparing groups is exemplified in Table XI where an attempt has been made to present a group of reported series in such a way as to permit comparison with the results of the present investigation. Thus Browne and Dodds (1940) compared foetal loss in preeclampsia with loss in hypertension without considering difference in severity. Their criteria for preeclampsia was a blood pressure below $^{130}/_{70}$ until the 20th week of gestation, rising above

this in later pregnancy. In Tilman and Watson's paper (1935) on the other hand, blood pressures below $^{130}/_{90}$ were accepted as normal and levels between $^{130}/_{90}$ and $^{150}/_{110}$ were considered mild hypertension. Their foetal loss of 241 per 1,000 in their preeclamptic group is probably due to the fact that proteinuria appeared to be a prerequisite for inclusion in the group. Wellen (1953) compared foetal loss in preeclampsia and hypertension in 1,400 pregnancies retrospectively. Foetal loss was higher in the hypertensive group, but no comparison was drawn between patients with and without proteinuria. Friedberg (1966) found a higher perinatal loss in hypertensive patients than in preeclamptics and in patients with proteinuria and those without. He failed, however, to compare preeclamptic patients who developed proteinuria with hypertensive patients who did so.

Taylor et al. (1954) conducted one of the more comprehensive examinations of perinatal loss in hypertensive pregnancies. They made an attempt to study the effect of different symptoms of "toxaemia" in a series of clearly demarkated groups. In a study of 4,432 patients they demonstrated quite convincingly the higher perinatal loss in patients with essential hypertension compared with those with preeclampsia, particularly where this was associated with proteinuria. Butler and Bonham (1963) have done likewise in a nationwide survey. Their foetal loss in the absence of proteinuria was similar in the two groups. Proteinuria raised the mortality in the essential hypertension group more than it did in the preeclamptic group.

Although these series are not exactly comparable, there is little doubt that essential hypertension carries a considerably greater foetal risk than does preeclampsia of an equivalent severity. Proteinuria increases the risk in both situations, but more particularly again in the essential hypertensive group. An analysis of the results in the present study suggests that not only is proteinuria a greater foetal hazard in essential hypertension but that it is also less selective in this condition. For this reason, protein selectivity studies may be of assistance in detecting the foetus at greater risk. Unfortunately, this is not an absolute indicator as there is a considerable overlap between groups. However, in general, poorer selectivity ratios (Ig G/transferrin above 0.3 or a regression slope of less than 63^{o}) are suggestive of essential hypertension in pregnancy, with the greater foetal hazard as its inevitable accompaniment.

SUMMARY

SUMMARY

1. Radial immuno-diffusion has been used to investigate renal protein selectivity in proteinuria of pregnancy.
2. Proteinuria was attributed to preeclampsia in eighteen patients, to essential hypertension in sixteen patients and to underlying renal disease in six patients, by careful longitudinal study and the exclusion of infection. The pathology in a further three patients was not defined.
3. Seven proteins with molecular weights ranging from 69,000 (albumin) to 1,000,000 (19S Ig M gamma globulin) were measured in serum and urine taken on the same day from the proteinuric patients. Proteins were measured quantitatively by radial immuno-diffusion and a urine/plasma ratio obtained for each protein. Each urine/plasma ratio was expressed as a percentage of the urine/plasma ratio for transferrin and albumin of the same patient. The slope of the regression line of protein clearance (as a percentage of transferrin clearance) on molecular weight is an expression of protein selectivity in a particular patient.
4. The clearance of Ig G gamma globulin as a percentage of the transferrin and albumin clearances was found to be a useful and simpler method of expressing selectivity. This obviates the necessity of concentrating the urine which is essential if the larger protein molecules are to be measured.

5. With the exception of 7S Ig G gamma globulin, two proteins of similar molecular weights were measured at each molecular weight range in order to validate the clearance measurements. Proteins of similar molecular weights were found to have closely related clearance.
6. Pseudocholinesterase with a molecular weight of 300,000 was included to confirm the clearance pattern of β_1^A/C globulin (molecular weight about 250,000). Pseudocholinesterase was measured both chemically and immunochemically, the two methods providing a closely related clearance pattern in most cases.
7. Establishing a satisfactory recovery of 19S Ig M globulin and duplicating studies by including α-2-macroglobulin of similar molecular weight, it was shown that in pregnancy, clearances do not behave entirely linearly for molecular weight. This is further justification for the use of the simpler Ig G/transferrin clearance ratio.

Results

1. A poorly selective pattern of protein excretion was found in practically all cases of proteinuria of pregnancy, irrespective of the underlying pathology. Thus none of the regression slopes approached the highly selective slopes described in patients with minimal change renal lesions in the nephrotic syndrome. Ig G/transferrin clearance ratios were poorly selective in all but five of the 44 pregnancies and even these five would at most be classified as moderately selective.

Pregnancy proteinuria, though in general totally reversible, had a pattern of excretion similar to that found in advanced renal disease.

2. The range of selectivities in preeclampsia was much tighter than in patients with essential hypertension. Ig/transferrin ratios were found to lie between 0.18 and 0.30 in all but one of the patients studied, whereas eleven of the sixteen hypertensive patients had selectivity ratios above 0.30. Poorer selectivity was a feature of essential hypertension according to both methods of expressing selectivity and the difference was statistically significant in each case. There was no statistical difference between any of the other parameters of renal function in preeclampsia and hypertension.
3. Protein selectivities in patients with renal disease were similar to those in preeclampsia when expressed as the Ig/transferrin ratio. Departure from linearity was considerable however in the macroglobulin range so that selectivities expressed by the two methods correlated poorly in this condition.
4. Longitudinal follow up studies of renal protein selectivity showed a remarkably consistent pattern for the same patient from week to week. Where proteinuria persisted into the puerperium, selectivity was found to be very similar to the pattern found antenatally.
5. In three patients with renal disease where proteinuria was found to be present six weeks after delivery, a highly selective pattern of protein excretion was found, whereas during pregnancy, a poorly selective pattern was consistently found. Proteinuria in renal disease persisted into the puerperium in all of the seven pregnancies.

6. There was no correlation between the severity of proteinuria and the protein selectivity. This was shown both in cross-sectional study (where different patients had varying levels of proteinuria) and in longitudinal study (where urinary protein levels fluctuated but selectivity remained constant in the same patient).
7. In patients with renal disease, the presence of heavy proteinuria in the absence of hypertension carried a favourable foetal prognosis. Where hypertension supervened, evidence of retarded foetal growth was present in four out of five cases. One pregnancy was terminated.
8. Foetal growth retardation was present in eleven of the sixteen patients with essential hypertension, irrespective of duration of proteinuria. In the preeclamptic patients, foetal growth retardation was a feature only of those patients in whom proteinuria had been present for more than two weeks.
9. Ig G/transferrin ratios above 0.30 tend to be associated with essential hypertension. This may serve as an aid to differentiating preeclampsia from essential hypertension associated with pregnancy. Since hypertension carries a graver foetal prognosis when associated with proteinuria than does preeclampsia, protein selectivity studies may serve as a guide to the foetal prognosis in these situations.

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APPENDIX

APPENDIX I

EXPERIMENT To assess the percentage recovery of protein after concentration through a Sartorius collodion bag membrane filter using ^{125}I albumin and $^{125}I\alpha$-2-macroglobulin

Two identical experiments were performed, one using ^{125}I albumin and the other using $^{125}I\alpha$-2-macroglobulin.

The protein solutions were each dialysed twice to get rid of any free ^{125}I.

Thereafter, each protein solution was passed through three separate concentration procedures and the percentage recovery estimated on each occasion.

The large amount of free ^{125}I after dialysis of $^{125}I\alpha$-2-macroglobulin indicates a poorly labelled sample, but there was sufficient labelled protein to proceed with the experiment.

^{125}I Albumin

Dialysis 1

	Gamma counts over 30 seconds
Before concentration	950,700
After concentration	797,200
Dialysate (mainly free ^{125}I)	125,441
Residue in collodion bag	8,479

Approximate percentage labelling 87%

Dialysis 2

	Gamma counts over 30 seconds
Before concentration	797,200
After concentration	715,547
Dialysate	70,974
Residue in collodion bag	9,479

Dialysis 3

Before concentration	715,200
After concentration	660,845
Dialysate	45,719
Residue in collodion bag	8,624

Percentage recovery 92.4%

Dialysis 4

Before concentration	660,845
After concentration	621,855
Dialysate	30,949
Residue in collodion bag	8,002

Percentage recovery 94.1%

Dialysis 5

Before concentration	621,855
After concentration	567,132
Dialysate	45,902
Residue in collodion bag	7,050

Percentage recovery 91.2%

Mean percentage recovery of albumin = 92.6%.

$^{125}I\alpha_2$-macroglobulin

Dialysis 1

	Gamma counts over 30 seconds
Before concentration	1,409,044
After concentration	502,842
Dialysate (mainly free ^{125}I)	897,202
Residue in collodion bag	8,642

Approximate percentage labelling 46%

Dialysis 2

Before concentration	502,842
After concentration	441,998
Dialysate	48,947
Residue in collodion bag	10,004

Dialysis 3

Before concentration	441,998
After concentration	423,876
Dialysate	9,704
Residue in collodion bag	6,998

Percentage recovery 95.9%

Dialysis 4

Before concentration	423,876
After concentration	408,616
Dialysate	7,211
Residue in collodion bag	7,822

Percentage recovery 96.4%

Dialysis 5

	Gamma counts over 30 seconds
Before concentration	408,616
After concentration	388,185
Dialysate	8,909
Residue in collodion bag	8,995

Percentage recovery 95.0%

Mean percentage recovery of α-2-macroglobulin = 95.8%

APPENDIX II

Immunisation schedule for preparation of antisera against human albumin, transferrin, Ig G globulin and Ig M globulin

New Zealand White rabbits were immunised over a period of four weeks with each antigen, and bleeding of the rabbit was performed a week later.

Antigen

Pure human protein antigens prepared by fractionation and separation through a Sephadex column.

An emulsion is prepared from equal parts of emulsion and Freund's complete adjuvant.

Immunisation schedule

Day 0 1 ml. emulsion given intramuscularly

Day 14 1 ml. emulsion given intramuscularly

Day 21 1 ml. emulsion given intramuscularly

Day 22 0.5 ml. emulsion given intraperitoneally

Day 23 0.5 ml. emulsion given intraperitoneally

Day 24 0.5 ml. emulsion given intraperitoneally

Day 31 Rabbit bled

Blood is centrifuged and serum taken off and stored at $-20^{o}C$ with sodium azide preservative.

At a convenient time, immuno-electrophoresis of human serum is run against the antiserum raised, and impurities in the antiserum in the form of contaminating antibodies are noted. These are then adsorbed out using appropriate antigens to produce a pure monospecific antiserum.

APPENDIX III

Precision and reproducibility of the method were tested by making twelve separate estimations of individual protein values in serum and urine from the same patient. The means, standard deviation and coefficient of variation were estimated from adjoining serum and urine wells in each instance, to reduce the error caused by position of a well in the plate.

Albumin

	Serum	Urine	Clearance
	2,900	140	.048
	3,200	180	.056
	2,800	160	.057
	2,900	150	.052
	3,200	160	.05
	2,850	140	.049
	2,900	150	.05
	2,800	150	.054
	3,100	150	.048
	3,200	160	.05
	3,050	160	.052
	2,750	150	.055
Mean	2,970	154	.052
Variance	28,842	1,291	.000025
Standard deviation (S.D.)	170	40	.005
Coefficient of variation $\left(\frac{S.D.}{mean} \times 100\right)$	5.8%	23.3%	9.6%

Transferrin

	Serum	Urine	Clearance
	450	20	.044
	390	17	.043
	400	22	.055
	380	20	.053
	390	20	.052
	430	25	.058
	380	18	.047
	380	24	.063
	430	22	.051
	350	18	.051
	350	20	.057
	350	18	.051
Mean	390	20	.052
Variance	1,096	6.2	.000033
Standard deviation	33	2.5	.0058
Coefficient of variation	8.5%	12.3%	11.1%

Ig G Globulin

	Serum	Urine	Clearance
	1,200	15	.012
	1,100	15	.014
	1,100	15	.014
	970	10	.01
	990	14	.014
	1,200	15	.012
	1,000	17	.017
	990	15	.015
	1,200	17	.014
	950	15	.016
	1,100	16	.015
	1,150	17	.015
Mean	1,079	15	.014
Variance	9,172	3.5	.00004
Standard deviation	96	1.9	.002
Coefficient of variation	8.8%	12.5%	14.3%

	β_1^A/C Globulin	Ig M Globulin	α-2-macroglobulin
Serum values in 12 samples	100	140	290
	100	160	310
	110	150	280
	95	145	280
	85	165	290
	90	145	295
	120	145	300
	80	150	320
	95	155	280
	100	145	290
	105	135	290
	115	150	300
Mean	100	149	294
Variance	138	229	151
Standard Deviation	12	15	12
Coefficient of variation	11.7%	10.1%	4.2%

Protein values in concentrated urines

Three separate concentrates of the same urine were prepared according to the method described on page 35. The concentrations were x 25, x 50, and x 100. β_1^A/C globulin, Ig M globulin and α-2-macroglobulin values were estimated in each of the three concentrates, and converted into values for the unconcentrated urine by appropriate multiplication. Each concentrate was estimated four times for each protein, making a total of twelve estimations.

The total protein content in the urine under examination was 195 mg. per 100 ml., measured by the Biuret method described previously.

β_1^A/C Globulin

	Actual Value	Value for unconcentrated urine
Concentrated x 25	6	0.24
	7	0.28
	6	0.24
	8	0.32
Concentrated x 50	12	0.24
	16	0.32
	10	0.2
	9	0.18
Concentrated x 100	20	0.2
	30	0.3
	24	0.24
	25	0.25

Mean	0.25
Variance	0.0021
Standard deviation	0.046
Coefficient of variation	18.4%

α -2-macroglobulin was measurable after concentrating urine x 50 and x 100. Ig M globulin was only measurable after concentrating urine x 100.

α -2-macroglobulin

	Actual Value	Value for unconcentrated urine
Concentrated x 50	4	0.08
	5	0.1
	5	0.1
	5	0.1
Concentrated x 100	8	0.08
	10	0.1
	12	0.12
	16	0.16
Mean		0.105
Variance		0.00065
Standard deviation		0.0256
Coefficient of variation		24.4%

Ig M Globulin

Concentrated x 100	2	0.02
	4	0.04
	0.5	0.05
	1	0.01

APPENDIX IV

Measurement of serum pseudocholinesterase activity by a spectrophotometric method

Twenty separate readings on the same specimen.

Serum values in units (1 unit = O.D. difference of 0.001 at 1 minute)	44, 45, 45, 46, 45, 44, 45, 44, 42, 44, 45, 45, 44, 44, 46, 43, 45, 46, 44, 44.
Mean	44.5
Variance	1
Standard deviation	1
Coefficient of variation	2.24%

APPENDIX V

Statistical Data

ABBREVIATIONS

"t" = Student "t" test
P = Probability
S.D. = Standard deviation
Var. = Variance
d.f. = Degress of freedom

1. Tests of significance for differences in mean values between all proteinuric pregnancies and a matched control group of normal pregnancies at similar gestational ages. (Patients with renal disease have been excluded.)

Blood Cholesterol	Normal Pregnancy	Proteinuric Pregnancy
Number of cases	20	34
Mean value	240.25	231.5
Variance	1190.7	1627
S.D.	34.5	40.3

$t_{(d.f.52)} = 0.67$ $P => .2$

Blood Urea

Number of cases	20	36
Mean value	20.8	28.4
Variance	14.27	63.1
S.D.	3.8	7.9

$t_{(d.f.54)} = 4.01$ $P = <.0005$

Blood Uric Acid

Number of cases	20	36
Mean value	5.8	6.9
Variance	1.5	1.2
S.D.	1.2	1.1

$t_{(d.f.54)} = 3.48$ $P = <.001$

Creatinine Clearance	Normal Pregnancy	Proteinuric Pregnancy
Number of cases	20	29
Mean value	105	78.5
Variance	616.7	526.3
S.D.	24.8	22.9

$t_{(d.f.47)} = 4.27$ $P = <.0005$

2. Tests of significance for differences in mean values between patients with preeclampsia and those with hypertension.

Age	Preeclampsia	Hypertension
Number of cases	18	16
Mean age	25.4	29.3
Variance	31.36	36
S.D.	5.6	6

$t_{(d.f.32)} = 1.95$ $P = <.05$

Blood Cholesterol		
Number of cases	18	16
Mean value	226	224

Blood Uric Acid		
Number of cases	18	16
Mean value	7.1	6.7
Variance	1.5	0.75
S.D.	1.2	0.87

$t_{(d.f.32)} = 1.077$ $P => 0.1$

Blood Urea		
Number of cases	18	16
Mean value	29.4	27.6
Variance	105.8	24.6
S.D.	10.3	5.0

$t_{(d.f.32)} = 0.58$ $P => 0.25$

Creatinine Clearance	Preeclampsia	Hypertension
Number of cases	16	12
Mean value	81	748
Variance	630.1	452.8
S.D.	25.1	21.3

$t_{(d.f.26)} = 0.68$ $\quad P => .25$

Selectivity Ratios

Number of cases	18	16
Mean value	0.24	0.34
Variance	0.0016	0.01
S.D.	0.04	0.1

$t_{(d.f.32)} = 3.865$ $\quad P =< .0005$

3. Regression of log. protein clearance (y) on log. molecular weight (x).

Regression slopes were determined and all regression lines drawn by computer analysis.

Test of significance between regression slopes for preeclampsia and essential hypertension.

Preeclampsia $\quad y = 10.877911 - 1.822115x$
S.D. of regression coefficient = 0.052255

Essential hypertension $\quad y = 11.661302 - 1.989136x$
S.D. of regression coefficient = 0.046265

To test the difference

$$\frac{(b_1 - b_2) - 0}{\text{Var. } b_1 + \text{Var. } b_2}$$

$$= \frac{0.783391}{0.069793}$$

$$= 11.224492$$

$P =< .0005$

where b_1 = slope of regression for preeclamptic group
b_2 = slope of regression for hypertensive group.

SELECTED CASE HISTORIES

PRE-ECLAMPSIA. TWIN PREGNANCY.

A.F. Age 22 Parity 0 Booked patient West Indian

L.M.P. 27. 10. 69. E.D.D. 3. 8. 70.

Date when first seen: 13. 3. 70. Gestation: 19 weeks.

No past medical history.

Family history. Had a twin sister. No family history of hypertension.

Examination at first attendance.

B.P. 130/70. Urine clear. No oedema. Weight 139 lbs. Uterine size larger than period of gestation.

Summary of ante-natal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
24	26	125/75	Nil	Nil	147 lbs.
28	32	130/80	Nil	Nil	147 lbs.
30	34	120/70	Nil	Nil	151 lbs.

Twin pregnancy diagnosed at this stage and patient admitted for rest.

Further progress.

Hypertension developed at rest in hospital. At 31 weeks, blood pressure was 130/90 after which it remained raised. Proteinuria appeared for the first time at 35 weeks. Blood pressure rose to 220/140 with 4 grams of protein in the urine in 24 hours. Limited improvement on heavy sedations (Morphine 15 mgs.).

Labour. Surgical induction of labour at 35 weeks. Vaginal delivery of twin female infants weighing 2,040 grams each.

Puerperium. Blood pressure settled to 130/90. Protein cleared within four days.

Post-natal clinic (six weeks post-delivery).

B.P. 110/80. Urine clear.

A.F.

Laboratory investigations.

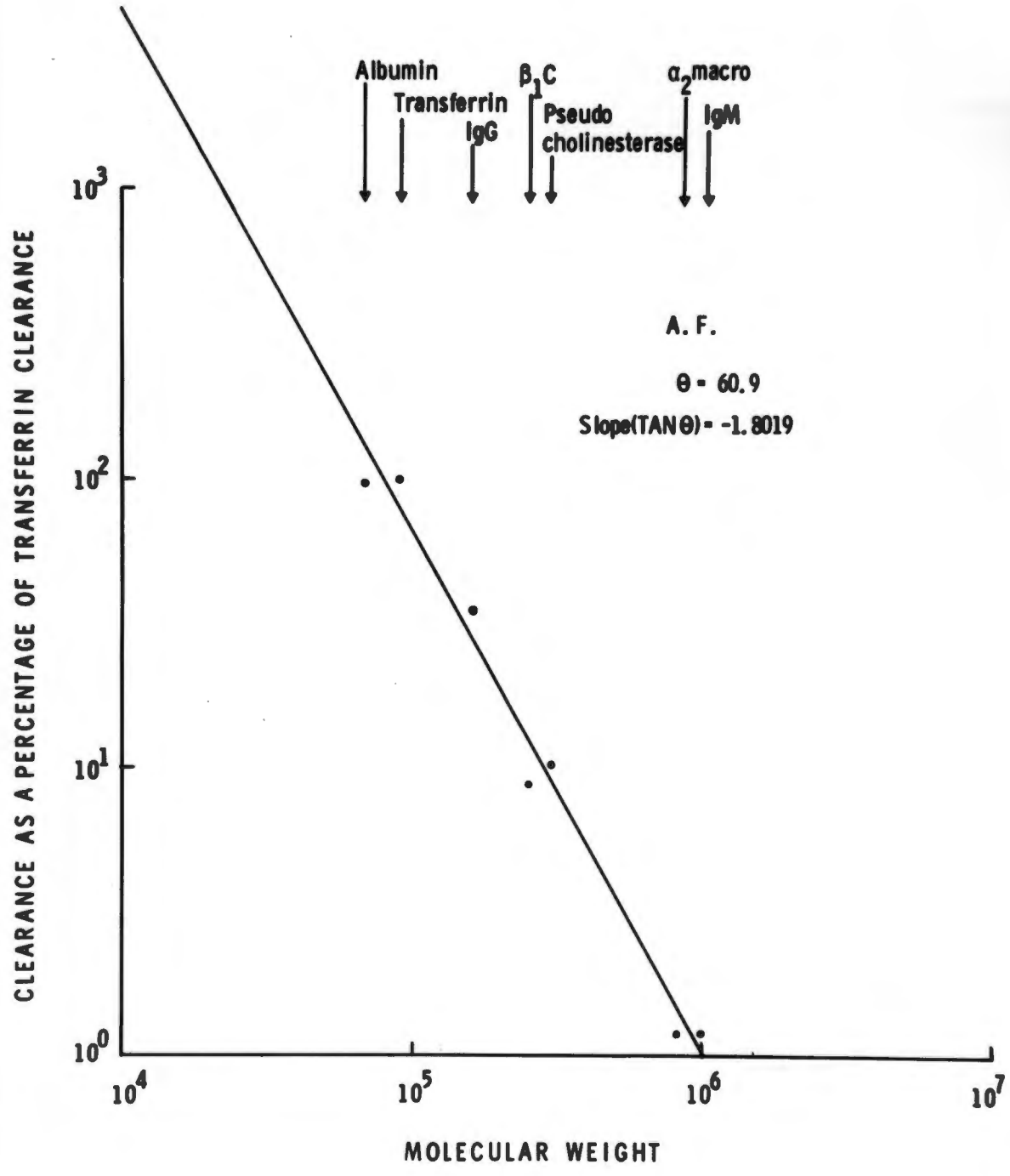
Haemoglobin: 11.8 grams per 100 ml. Blood urea: 64 mgs. per 100 ml. Blood uric acid 9.7 mgs. per 100 ml. Blood cholesterol 185 mgs. per 100 ml. Serum albumin/globulin ratio: 2.2/2.3. Creatinine clearance 63 ml. per minute. Total urinary protein 4.0 grams in 24 hours.

Microscopy of urine.

No casts. Few leucocytes and epithelial cells.

Protein clearances (Urine/serum ratios).

Albumin	0.10
Transferrin	0.103
IgG.	0.036
β 1 A/C	0.009
Pseudocholinesterase (immunological)	-
Pseudocholinesterase (chemical)	0.011
α2 macroglobulin	0.0012
IgM	0.0012
IgG/Transferrin	0.35
IgG/Albumin	0.36


Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
$\alpha_2$macro
IgM
A. F.
Θ = 60.9
Slope(TANΘ) = -1.8019
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

PRE-ECLAMPSIA. POSTPARTUM ECLAMPSIA.

F.S.-M. Age 32 Parity 0 Booked patient English

L.M.P. 2. 1. 69. E.D.D. 9.10.69.

Date when first seen: 1. 4. 69. Gestation: 13 weeks

No past medical history.

No significant family history.

Examination at first attendance.

B.P. 140/80. Urine clear. Weight 160 lbs. Uterine size 13 weeks.

Summary of ante-natal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
17	17	110/70	Nil	Nil	162 lbs.
21	21	100/60	Nil	Nil	166 lbs.
25	25	110/70	Nil	Nil	170 lbs.
28	28	110/60	Nil	Nil	177 lbs.
32	32	130/85	Nil	Nil	183 lbs.
33	33	145/90	Nil	Nil	183 lbs.

Admitted to hospital.

Further progress.

Blood pressure settled initially on bed rest. However, after eight days it began to rise and proteinuria appeared for the first time. Hypertension and proteinuria persisted throughout the remainder of the pregnancy. Serial estimations of 24 hour urinary oestriol excretion were satisfactory for the period of gestation.

F.S.-M.

Labour. Surgical induction of labour at 37 weeks. Spontaneous vaginal delivery of a male infant weighing 2,330 grams. This was followed two hours later by two eclamptic fits. These were controlled using rectal Avertin.

Infant. Satisfactory progress.

Puerperium. Rapid improvement. Protein cleared completely from the urine by the 4th puerperal day. B.P. 110/70 on the 10th day.

Post-natal attendance (six weeks after delivery).

B.P. 110/70. Urine clear.

Laboratory investigations.

Haemoglobin 12.5 grams per 100 ml. Blood urea 27 mgs. per 100 ml. Blood uric acid 6.0 mgs. per 100 ml. Blood cholesterol 180 mgs. per 100 ml. Serum albumin / globulin ratio 2.3/ 3.5. Creatinine clearance 87 ml. per minute. Total urinary protein 2.3 grams in 24 hours.

Microscopy of urine. No casts. A few epithelial cells and leucocytes.

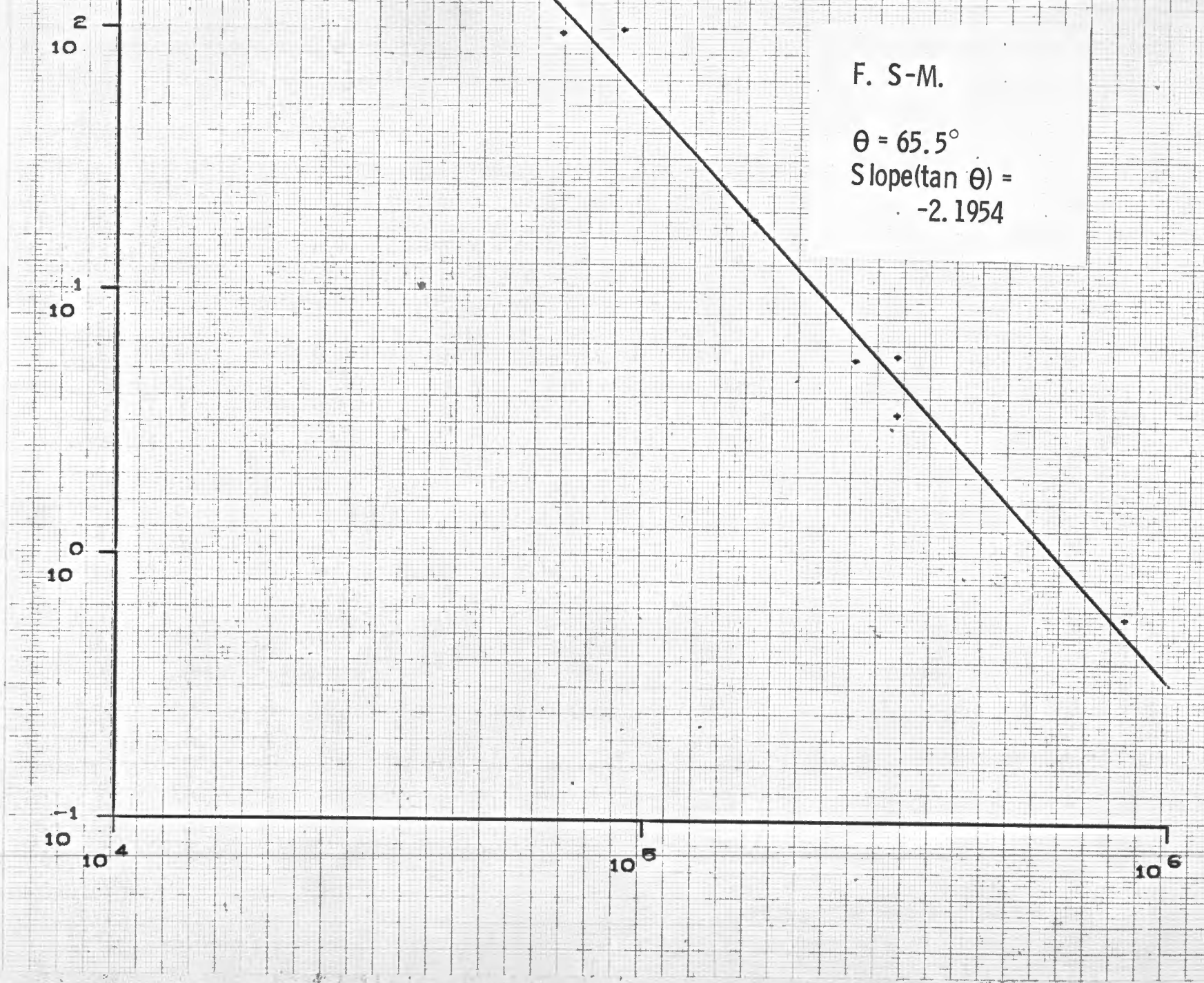
Protein clearances (urine/serum ratios).

	34 weeks	35 weeks	36 weeks
Albumin	0.075	0.055	0.06
Transferrin	0.070	0.059	0.067
IgG.	0.01	0.011	0.011
β1 A/C globulin	0.004	0.0035	0.0035
Pseudocholinesterase (immunological)	0.0031	0.0032	0.0038

F.S.-M.

Protein clearances (urine/serum ratios) (continued)

	34 weeks	35 weeks	36 weeks
Pseudocholinesterase (chemical)	0.0041	0.0015	0.0025
α_2 macroglobulin	0.0003	0.0005	0.0005
IgM.	-	-	-
IgG/Transferrin	0.20	0.19	0.17
IgG/Albumin	0.19	0.19	0.18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PRE-ECLAMPSIA

E.S. Age 19 Parity 0 Booked patient English

L.M.P. 23. 7. 69. E.D.D. 30. 4. 70.

Date when first seen: 29. 10. 69. Gestation: 12 weeks

No past medical history.

No significant family history.

Examination at first attendance.

B.P. 130/70. Urine clear. Weight 122 lbs. Uterine size: 12 weeks.

Summary of ante-natal care (shared with General Practitioner).

Uncomplicated. Referred back to hospital ante-natal clinic at 28 weeks.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
28	28	130/70	Nil	Nil	131 lbs.
30	28	150/90	50 mgs.	+	

Admitted to hospital.

Further progress.

Progressive deterioration in condition despite bed rest. B.P. rose to 175/110. Proteinuria increased to 8 grams daily. Caesarean section performed at 31 weeks in view of deteriorating condition.

Infant. Female 1,190 grams. Condition poor at birth. Neonatal death.

Puerperium. B.P. 135/75. Protein cleared.

Post-natal attendance (six weeks after delivery).

B.P. 120/70. Urine clear.

E.S.

Laboratory investigations (ante-natal).

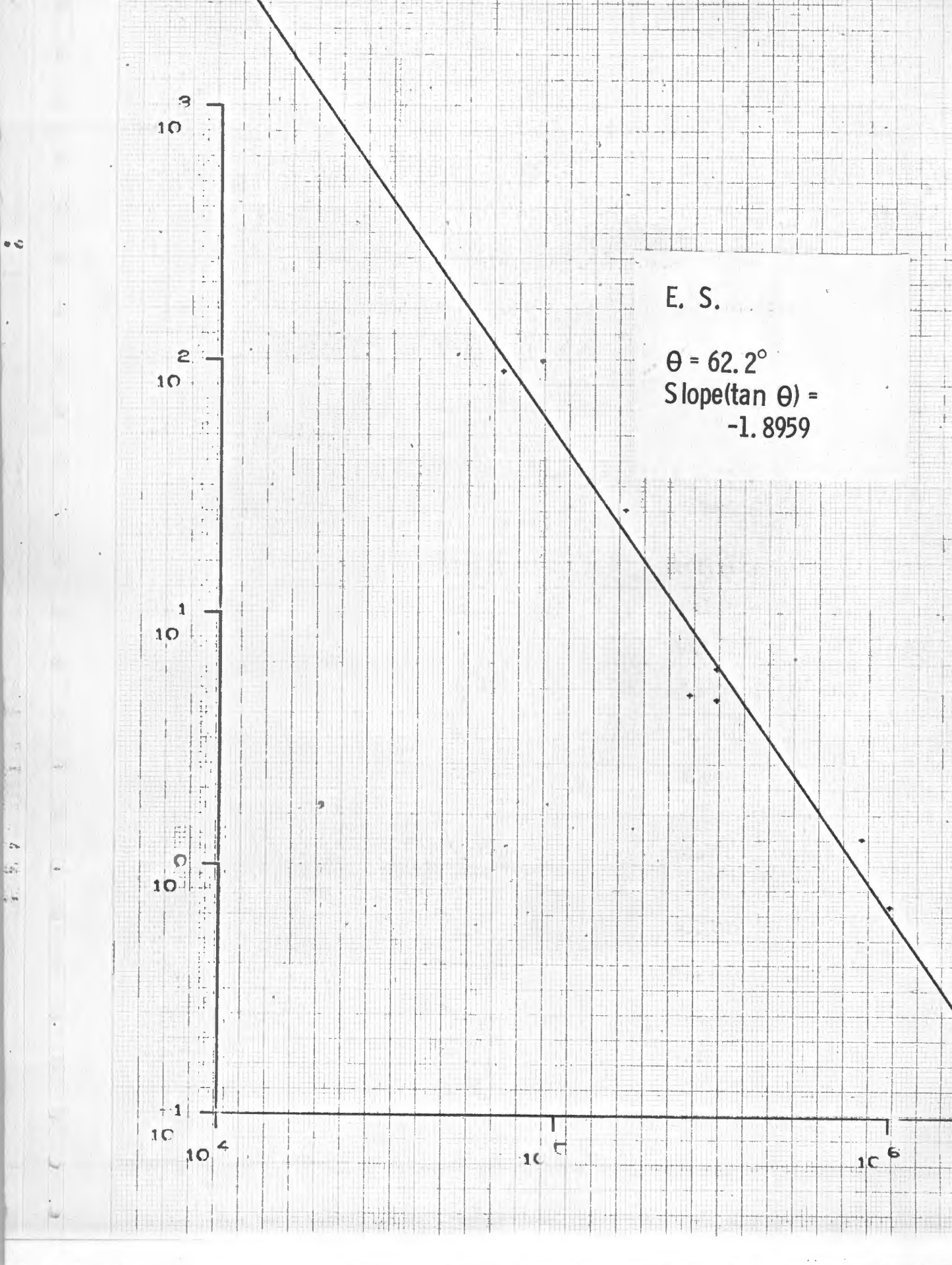
Haemoglobin: 11.9 grams per 100 ml. Blood urea: 25 mgs. per 100 ml. Blood uric acid: 7.2 mgs. per ml. Blood cholesterol: 175 mgs. per 100 ml. Creatinine clearance: 64 ml. per minute. Total protein excretion: 8.0 grams in 24 hours.

Microscopy of urine:

No casts. Occasional leucocytes and epithelial cells.

Protein clearances (urine/serum ratios)

	30 weeks	31 weeks
Albumin	0.049	0.10
Transferrin	0.056	0.106
IgG.	0.014	0.028
β1A/C globulin	0.0028	0.005
Pseudocholinesterase (immunological)	0.003	0.006
Pseudocholinesterase (chemical)	0.0025	0.005
α 2 macroglobulin	0.0007	0.0014
IgM.	0.0003	0.001
IgG/Transferrin	0.26	0.25
IgG/Albumin	0.28	0.26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PRE-ECLAMPSIA. HYDATIDIFORM MOLE.

P.F. Age 24 Parity 0 Emergency admission British

L.M.P. 12. 4. 69. E.D.D. 19. 1. 70.

Date when first seen: 10. 8. 69. Gestation: 17 weeks.

No past medical history.

No significant family history.

Past obstetric history. 1st trimester miscarriage: November 1968.

B.P. recorded at the time: 130/75.

Present history. Admitted as an emergency at 17 weeks' gestation with:

1. Hypertension. B.P. 210/130.
2. Oedema.
3. Proteinuria 8.0 grams in 24 hours.

Clinical examination.

Marked oedema. B.P. 210/130. Uterine size 18 weeks.

Foetal heart not heard.

Laboratory investigations.

Haemoglobin: 11.5 grams per 100 ml. Blood urea: 34, 33 mgs. per 100 ml. Blood uric acid: 7.7 mgs. per 100 ml. Serum albumin/globulin ratio: 2.6/2.2, 1.7/2.9. Blood cholesterol: 195 mgs. per 100 ml. Total urinary protein: 8.0, 1.3, 4.3 grams per 24 hours. Creatinine clearance: 64 ml. per minute.

Microscopy of urine.

Large numbers of hyaline and granular casts. Occasional red cells and leucocytes.

Further progress.

Abdominal hysterotomy and delivery of hydatidiform mole.

P.F.

Puerperium. Rapid improvement. B.P. 180/110 on fourth day. 130/90 on eighth day. Proteinuria 500 mgs. per 100 ml. on fourth day. Trace only on discharge.

Laboratory investigations in the puerperium.

Haemoglobin: 10.8 grams per 100 ml. Blood urea: 23 mgs. per 100 ml. Blood uric acid: 6.0 mgs. per 100 ml. Creatinine clearance: 123 ml. per minute.

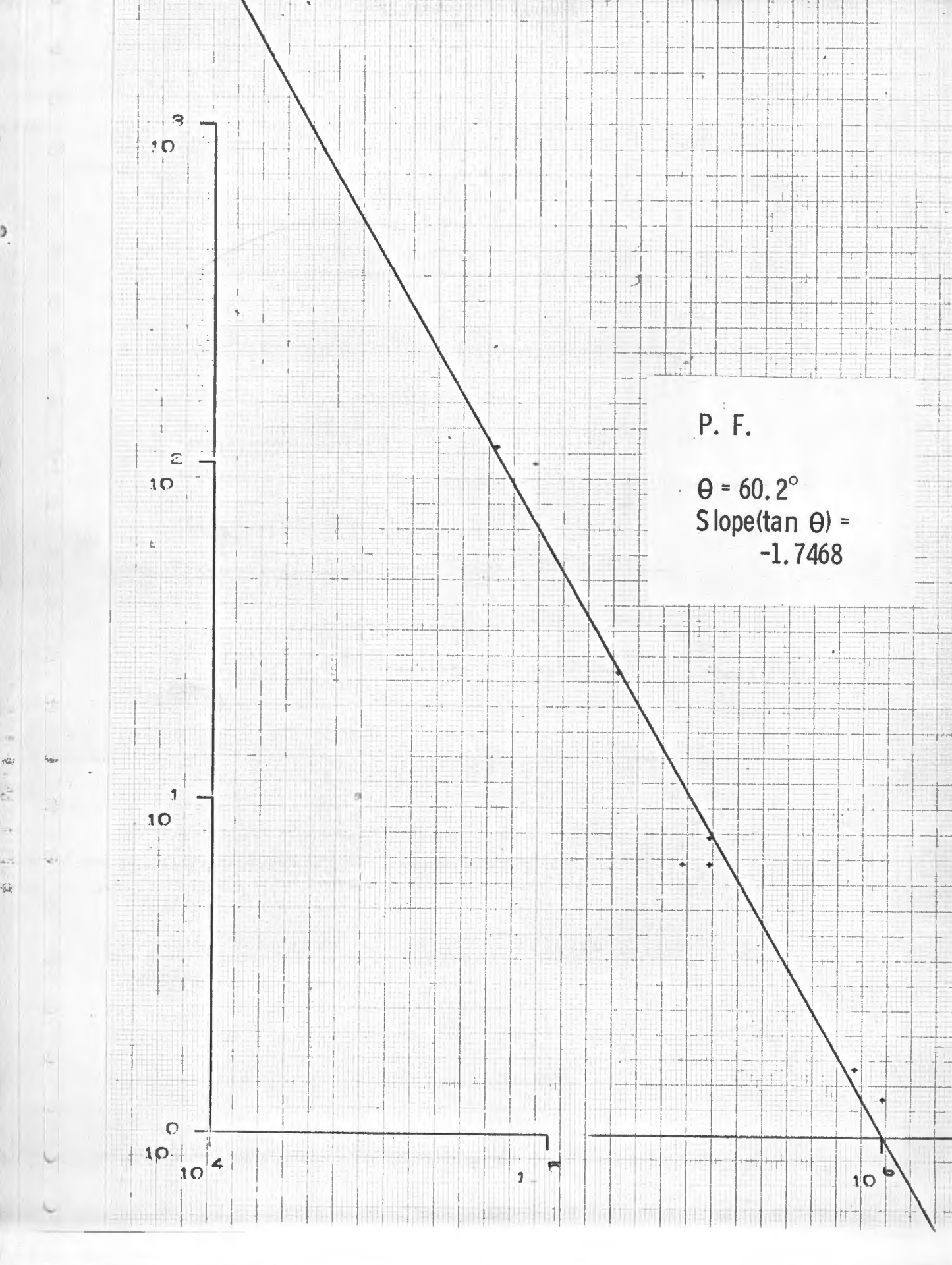
Microscopy of urine.

No granular casts. No red cells. Occasional leucocytes and epithelial cells.

Post-natal attendance. B.P. 120/70. Urine clear.

Protein clearances (urine/serum ratios)

	17 weeks	18 weeks	Puerperium
Albumin	0.171	0.186	0.175
Transferrin	0.157	0.167	0.140
IgG.	0.037	0.040	0.031
β1 A/C globulin	0.0096	0.0114	-
Pseudocholinesterase (immunological)	0.0127	0.012	-
Pseudocholinesterase (chemical)	0.008	0.014	-
α-2 macroglobulin	0.0015	0.0036	-
IgM.	-	0.0029	-
IgG/Transferrin	0.24	0.24	0.21
IgG/Albumin	0.22	0.21	0.17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PRE-ECLAMPSIA

E.P. Age 28 Parity 0 Booked patient West Indian

L.M.P. 13. 9. 69. E.D.D. 20. 6. 70.

Date when first seen: 16. 1. 70. Gestation: 18 weeks

No past medical history.

No significant family history.

Examination at first attendance.

B.P. 120/70. Urine clear. No oedema. Weight 108 lbs.

Uterine size 18 weeks.

Summary of ante-natal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
22	22	130/80	Nil	Nil	112
26	26	105/65	Nil	Nil	117
29	29	130/100	Nil	Nil	121

Admitted to hospital.

Further progress.

Blood pressure levels fluctuated between 120/80 and 140/100. Proteinuria appeared for the first time at 31 weeks, increasing considerably in amount and associated with oedema. Urinary oestriol excretion remained satisfactory. B.P. at 36 weeks rose to 150/110 and induction of labour was performed.

Labour. Surgical induction. Desultory labour lasting 36 hours. Foetal distress at full dilatation. Forceps delivery of stillborn male infant, weighing 2,605 grams.

Puerperium. Blood pressure returned rapidly to normal levels (130/80) and proteinuria disappeared by the fourth puerperal day.

Postnatal attendance. (six weeks postdelivery).

B.P. 130/70. Urine clear.

E.P.

Laboratory investigations. (ante-natal)

Haemoglobin: 12.4 grams per 100 ml. Blood urea: 24, 14 mgs. per 100 ml. Blood uric acid: 5.2, 5.1 mgs. per 100 ml. Blood cholesterol: 175, 190 mgs. per 100 ml. Creatinine clearance: 62, 82 ml. per minute. 24 hour protein excretion: 9.7 grams.

Microscopy of urine.

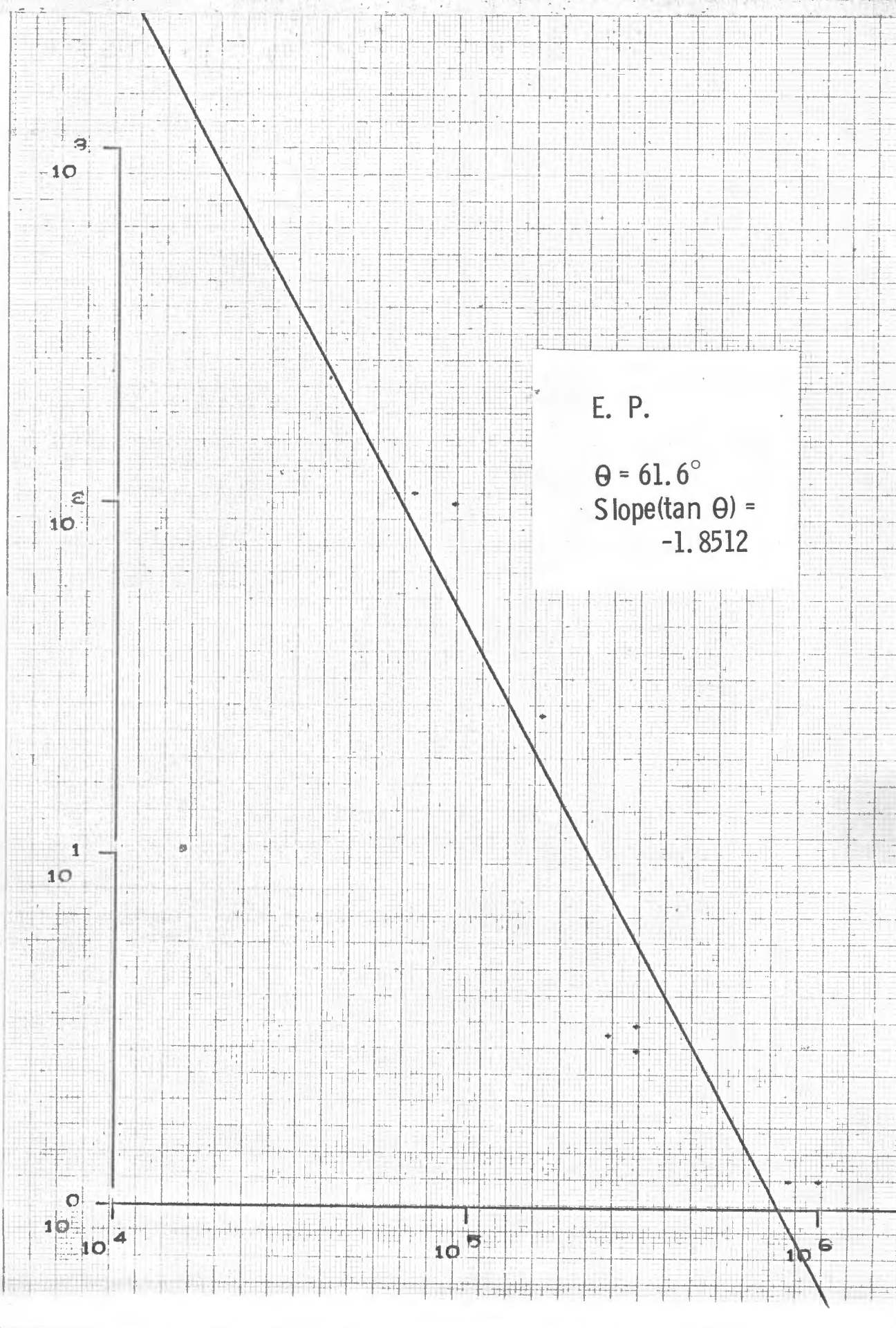
Large numbers of granular casts. A few leucocytes and epithelial cells. No red cells.

Laboratory investigations (post-natal).

Urine microscopy. No casts seen.

Protein clearances (urine/serum ratios)

	34 weeks	35 weeks	36 weeks
Albumin	0.036	0.57	0.33
Transferrin	0.03	0.58	0.33
IgG.	0.007	0.16	0.095
β1 A/C globulin	0.0013	0.022	0.055
Pseudocholinesterase (immunological)	-	0.018	0.0114
Pseudocholinesterase (chemical)	-	0.02	0.0067
α2 macroglobulin	0.0003	0.0068	0.0036
IgM.	-	0.0057	0.0043
IgG/Transferrin	0.23	0.26	0.26
IgG/Albumin	0.19	0.26	0.26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PREECLAMPSIA

K.C. Age 26. Parity 1. Booked patient. Indian.

L.M.P. 8.9.69 E.D.D. 15.6.70.

Date when first seen: 5.11.69. Gestation: 9 weeks.

No past medical history.

No significant family history.

Past obstetric history.

1964: Spontaneous abortion at 10 weeks gestation.

1965: Spontaneous abortion at 14 weeks gestation.

Examination at first attendance.

B.P. 110/70. Urine clear. Weight 123 lbs.

Summary of antenatal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
12	12	120/60	Nil	Nil	124
20	20	115/55	Nil	Nil	131
24	24	140/75	Nil	Nil	136
28	28	155/100	Nil	+	144

Admitted to hospital.

Further progress.

Mild hypertension persisted. Proteinuria appeared at 30 weeks gestation, becoming progressively heavier and at 32 weeks protein level was 6.7 grams per day with marked generalised oedema. Delivery decided upon in view of deteriorating condition.

Labour.

Caesarean section at 32 weeks. Female infant weighing 1310 grams.

Infant.

Satisfactory progress in intensive care unit.

M.C.

Puerperium.

B.P. 130/90. Protein 100 mgs. on the 4th day.

Postnatal attendance (6 weeks after delivery).

B.P. 110/70. Urine clear.

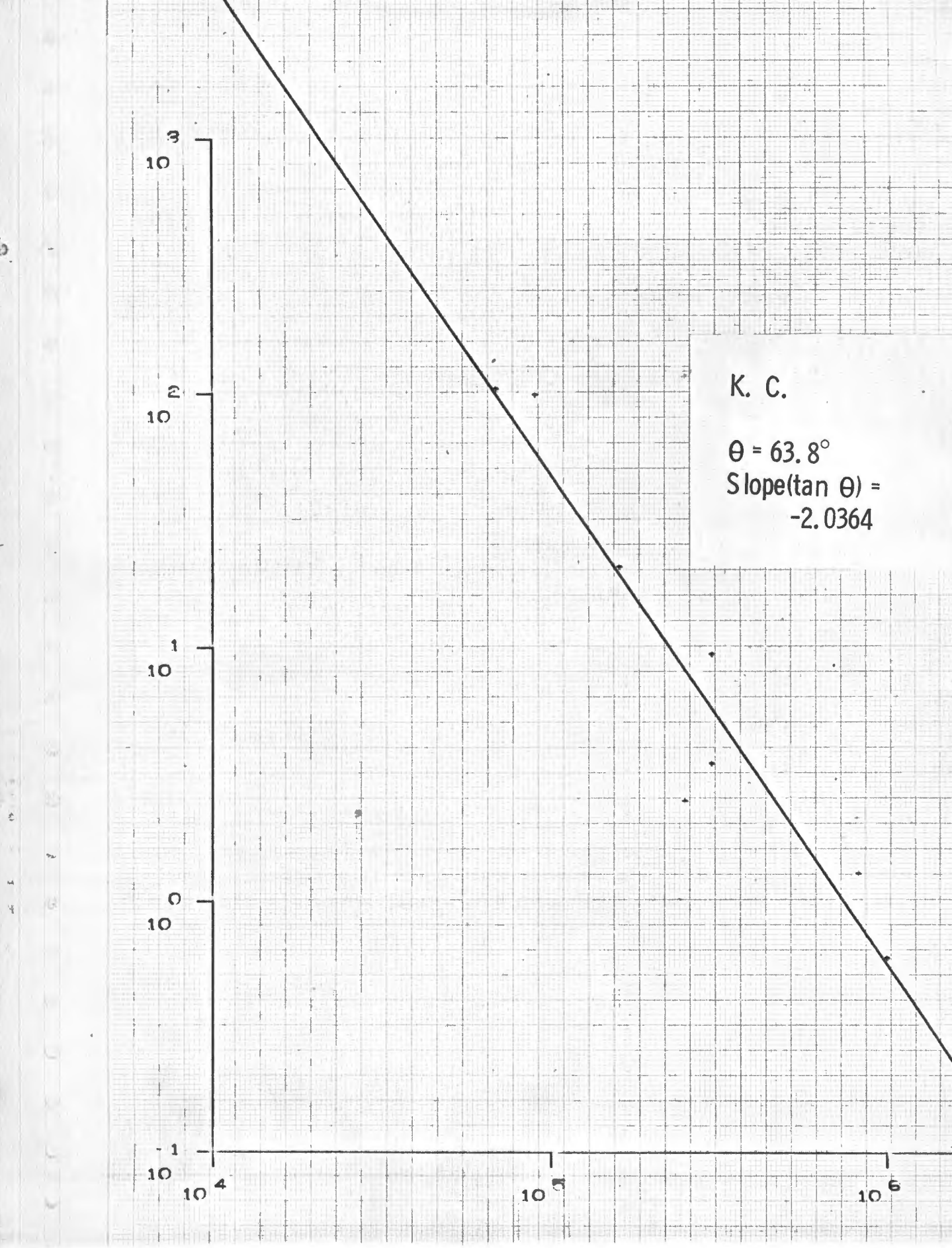
Laboratory investigations (antenatal).

Haemoglobin 12.6 grams per 100 ml. Blood urea 26 and 30 mgs. per 100 ml. Blood uric acid 9.2 mgs. per 100 ml. Blood cholesterol 238 mgs. per 100 ml. Serum albumin/globulin ratio 2.7/2.9. Creatinine clearance 83 ml. per minute. Total urinary protein 6.7 grams and 6.2 grams in 24 hours.

Urine microscopy: No casts. A few leucocytes and epithelial cells.

Protein clearances (urine/serum ratios).

	31 weeks	32 weeks	Puerperium
Albumin	0.10	0.138	0.024
Transferrin	0.088	0.138	0.025
IgG	0.020	0.03	0.005
βI A/C	0.0025	0.003	-
Pseudocholinesterase (immunological)	0.0057	0.007	-
Pseudocholinesterase (chemical)	0.003	0.005	-
α 2 macroglobulin	0.0013	0.0018	-
IgM	0.0006	0.0007	-
IgG/Transferrin	0.22	0.22	0.20
IgG/Albumin	0.20	0.22	0.21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ESSENTIAL HYPERTENSION

N.M. Age 26. Parity 0. Booked patient. Irish.

L.M.P. 11.7.69. E.D.D. 18.8.70.

Date when first seen: 24.10.69. Gestation: 14 weeks.

No past medical history.

No significant family history.

Examination at first attendance.

B.P. 140/90. Urine clear. No oedema. Weight 178 lbs.

Uterine size: 14 weeks.

Summary of antenatal care.

Gestation	B.P.	Urine	Weight
25	130/80	N.A.D.	178
26	140/95	N.A.D.	176
27	145/100	N.A.D.	178
28	150/105	Protein +	180.

Admitted to hospital.

Further progress.

Blood pressure levels remained above 140/100 on complete bed rest and proteinuria persisted, rising to levels as high as 14.4 grams in 24 hours. Foetal heart was no longer heard after 31 weeks and spontaneous labour commenced at 32 weeks with delivery of a macerated stillbirth weighing 1200 grams.

Puerperium.

Blood pressure remained up. B.P. 170/110 on discharge.

Urine: no protein.

Laboratory investigations.

Haemoglobin 12.2 grams per 100 ml. at 30 weeks gestation. Blood urea 34 mgs per 100 ml. Blood uric acid 6.1 mgs per 100 ml. Blood

N.M.

cholesterol 230 mgs. per 100 ml. Serum albumin/globulin ratio 2.3/3.5. Total urinary protein 14.4 grams in 24 hours. Creatinine clearance 78 ml. per minute.

Urine microscopy.

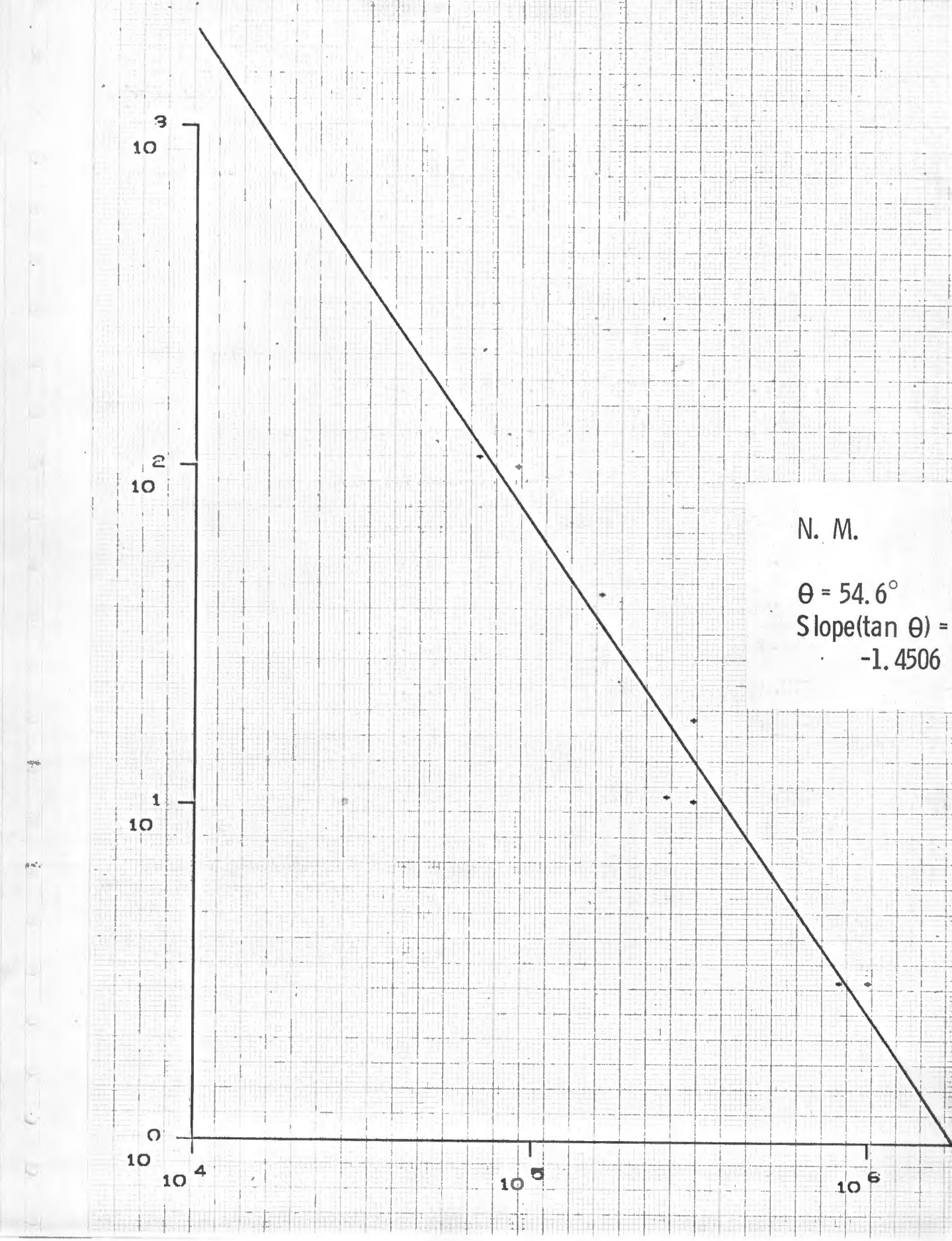
Large numbers of epithelial cells and granular casts.

Puerperium.

Blood urea 25 mgs. per 100 ml. Blood uric acid 6.8 mgs. per 100 ml. Urine: no protein. Microscopy negative.

Protein clearances (urine/serum ratios)

	29 weeks	30 weeks	31 weeks
Albumin	0.10	0.30	0.12
Transferrin	0.092	0.30	0.107
IgG	0.036	0.130	0.046
β 1 A/C	0.113	0.253	0.009
Pseudocholinesterase (immunological)	0.013	0.025	0.012
Pseudocholinesterase (chemical)	0.02	0.054	0.017
α 2 macroglobulin	0.004	0.009	0.004
IgM	0.003	0.009	0.003
IgG/Transferrin	0.39	0.43	0.43
IgG/Albumin	0.36	0.43	0.38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ESSENTIAL HYPERTENSION.

J.S. Age 26. Parity 0. Booked patient. Indian.

L.M.P. 5.10.69 E.D.D. 12.7.70.

Date when first seen: 19.1.70. Gestation: 16 weeks.

No past medical history.

No family history available.

Examination at first attendance.

B.P. 130/95. Urine clear. No oedema. Weight 134 lbs.

Uterine size: 16 weeks.

Summary of antenatal care (shared between hospital and general practitioner).

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
20	20	130/80	Nil	Nil	137
28	28	130/85	Nil	Nil	146
30	30	150/120	++++	+	154

Admitted to hospital.

Further progress.

Blood pressure settled to 140/100 on bed rest. Oedema partly resolved. Proteinuria reduced in amount but remained present.

Labour.

Spontaneous onset of labour 6 days after admission. Normal delivery of male infant weighing 906 grams.

Infant.

Satisfactory progress in intensive care unit.

Puerperium.

B.P. settled to 130/90. Proteinuria was present on the4th day but absent on the 8th day.

Postnatal attendance (six weeks after delivery).

B.P. 125/95 Urine clear.

J.S.

Laboratory investigations.

On admission.

Haemoglobin 12.5 grams per 100 ml. Blood urea: 35 mgs. per 100 ml. Blood uric acid 7.4 mgs. per 100 ml. Blood cholesterol 225 mgs. per 100 ml. Serum albumin/globulin ratio 2.6/3.1. Creatinine clearance 58 ml. per minute. Total urinary protein 4.6 grams in 24 hours.

Urine microscopy: Granular casts. No red cells. Occasional white cells and epithelial cells.

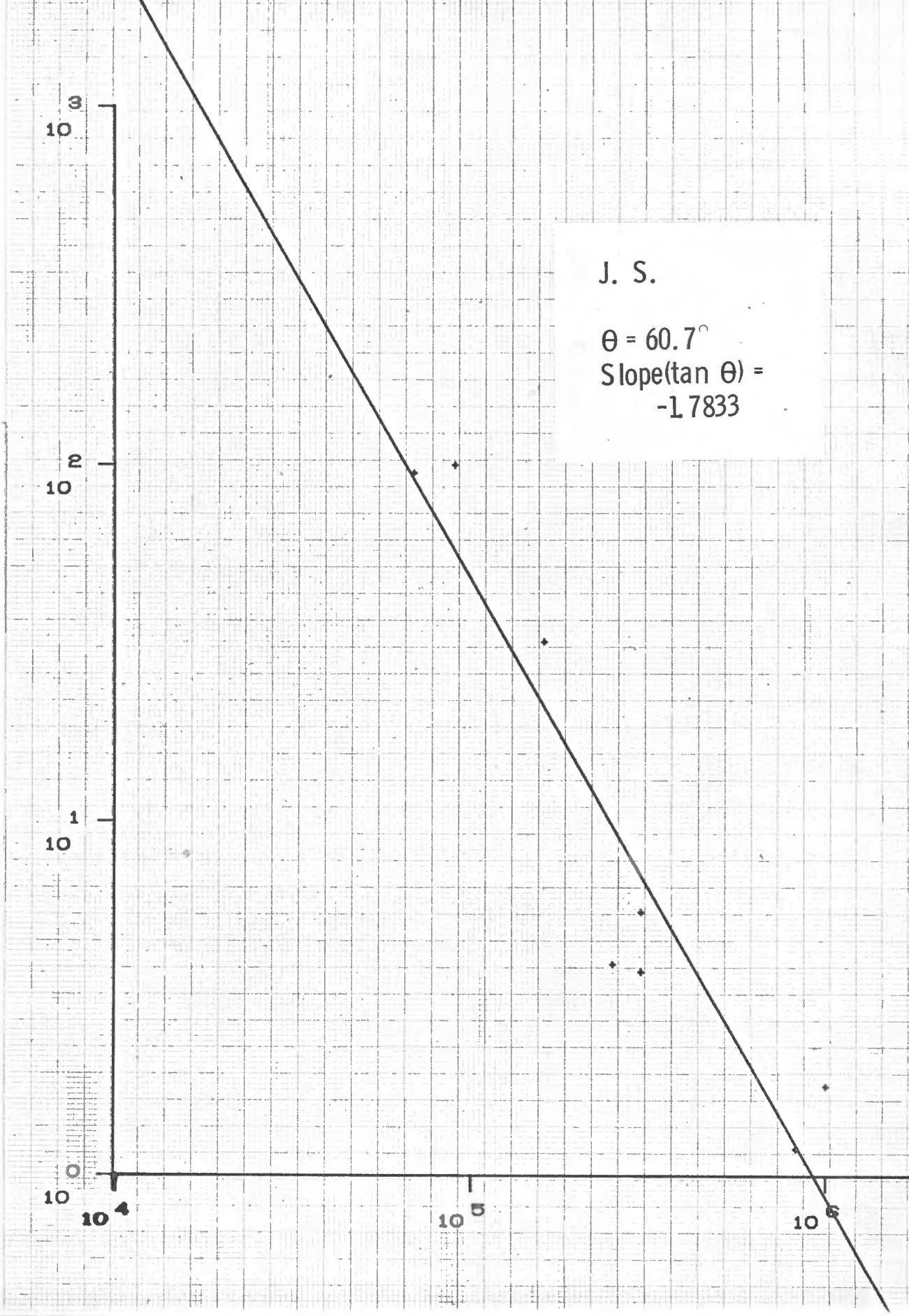
Puerperium.

Blood urea 33 mgs. per 100 ml. Blood uric acid 6.8 mgs. per 100 ml. Serum albumin/globulin ratio 3.0/4.2. Urinary protein 100 mgs. per 100 ml.

Urine microscopy: Red cells. No casts.

Protein clearances (urine/serum ratios).

	Antenatal	Puerperium
Albumin	0.184	0.047
Transferrin	0.193	0.042
IgG	0.062	0.014
β I A/C	0.008	-
Pseudocholinesterase (immunological)	0.011	-
Pseudocholinesterase (chemical)	0.075	-
α 2 macroglobulin	0.0025	-
IgM	0.0035	-
IgG/Transferrin	0.32	0.34
IgG/Albumin	0.34	0.30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ESSENTIAL HYPERTENSION.

U.K. Age 29. Parity 1. Booked patient. English.

L.M.P. 24.2.69. E.D.D. 3.12.69.

Date when first seen 3.6.69. Gestation: 14 weeks.

No past medical history.

No significant family history.

Past obstetric history.

1964: Severe "pre-eclampsia". Accidental antepartum haemorrhage at 34 weeks gestation.

3 lb. 11 oz. stillborn infant delivered spontaneously.

1966: Spontaneous first trimester abortion at 12 weeks.

Examination at first attendance.

B.P. 120/70. Urine clear. No oedema. Weight 160 lbs.

Uterine size 14 weeks.

Summary of antenatal care.

Gestation	B.P.	Urine	Weight
20	130/90	N.A.D.	162
24	125/80	N.A.D.	163
26	130/90	N.A.D.	165
28	130/100	Trace of protein.	166

Admitted to hospital.

Further progress.

Blood pressure rose to 180/130 soon after admission, subsequently fluctuating between this level and 140/100. Heavy proteinuria began to appear from 29 weeks and blood pressure remained uncontrollable. Caesarean section was performed at 30 weeks gestation because of uncontrollable hypertension and heavy proteinuria.

U.K.

Infant.

Male, 850 grams at birth, which died shortly after delivery.

Puerperium.

Blood pressure 140/90. Urine: no proteinuria.

Postnatal attendance (6 weeks after delivery).

B.P. 180/120. Urine: no proteinuria.

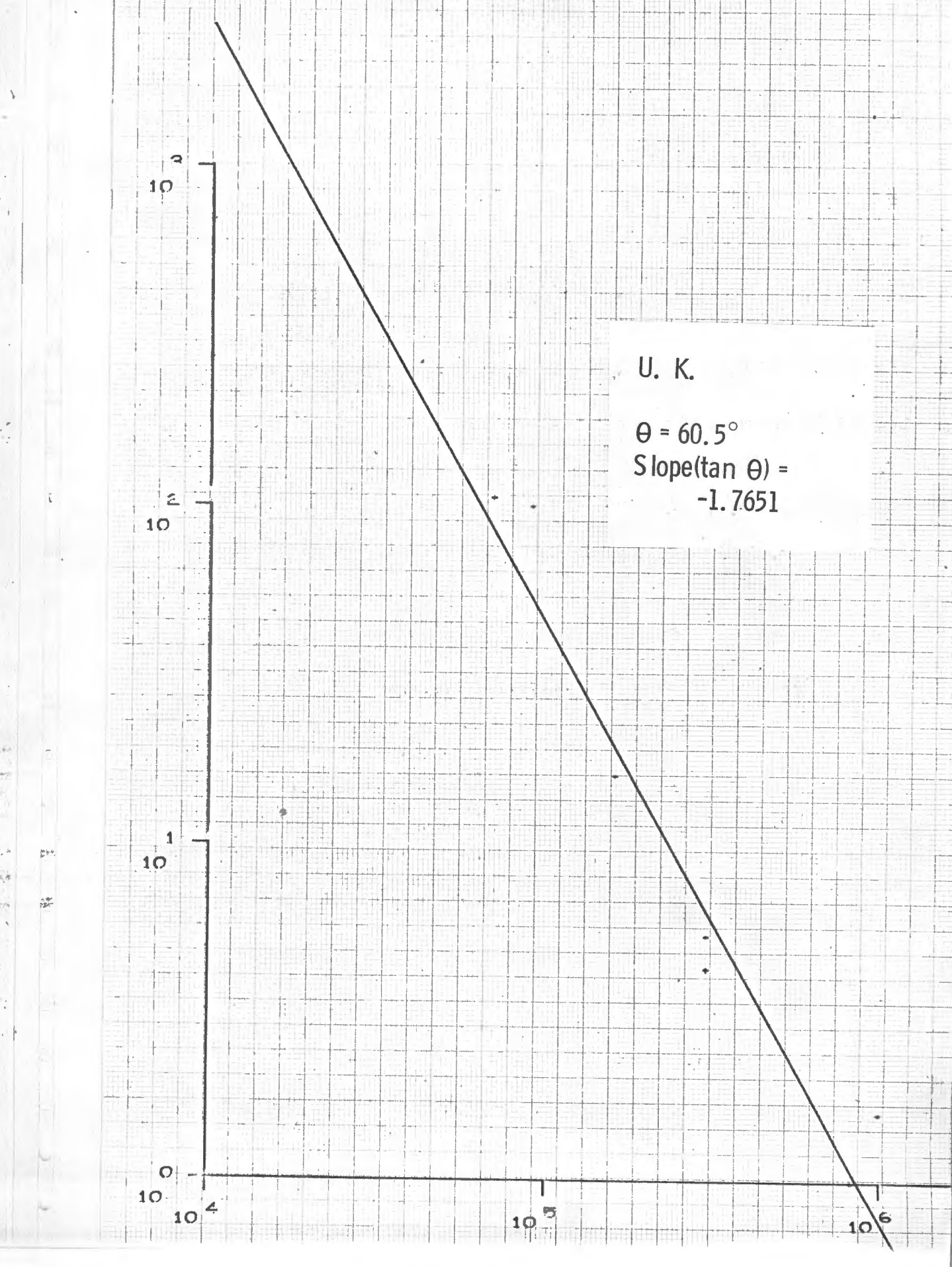
Laboratory investigations (on admission).

Haemoglobin 11.5 grams per 100 ml. Blood urea 35 mgs. per 100 ml. Blood uric acid 7.2 mgs. per 100 ml. Blood cholesterol 218 mgs. per 100 ml. Serum albumin/globulin ratio 3.1/2.8. Creatinine clearance 84 ml. per minute. Total urinary protein 15.0 grams in 24 hours.

Urine microscopy: Granular casts. A few red cells and white cells.

Protein clearances (urine/serum ratios).

Albumin	0.59
Transferrin	0.558
IgG	0.098
β I A/C	0.022
Pseudocholinesterase (immunological)	0.03
Pseudocholinesterase (chemical)	0.024
α 2 macroglobulin	0.006
IgM	0.007
IgG/Transferrin	0.16
IgG/Albumin	0.15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ESSENTIAL HYPERTENSION

W.A. Age 29. Parity 2. Booked patient. West Indian.

L.M.P. 24.11.69 E.D.D. 31.8.70.

Date when first seen: 28.1.70. Gestation: 9 weeks.

Past medical history.

Essential hypertension diagnosed in 1968. B.P. 200/110.

No cause found after full examination. Commenced treatment on Methyldopa 250 mgs. three times daily.

No family history available.

Past Obstetric history.

1960: Normal delivery 7 lb. 2 oz. male infant.

1965: Normal delivery 7 lb. 6 oz. male infant.

Mild hypertension (140/90) was noted at term in the pregnancy.

Examination at first attendance.

B.P. 150/90. Urine clear. No oedema. Weight 142 lbs.

Uterine size: rather large for dates, 10-12 week gestation.

On Methyl Dopa 250 mgs. thrice daily.

Summary of antenatal care.

Gestation.	B.P.	Urine.	Weight.
12	170/120	N.A.D.	141
14	160/110	N.A.D.	141
16	150/105	N.A.D.	143
18	150/100	N.A.D.	144
No significant change until:			
32	150/105	Protein 90 mgs.	154

Admitted to hospital.

W.A.

Further progress.

Hypertension persisted but proteinuria disappeared on bed rest. Proteinuria reappeared and persisted after 36 weeks gestation. Labour was induced at 38 weeks gestation.

Labour.

Surgical induction of labour at 38 weeks gestation. Spontaneous delivery of a female infant weighing 2340 grams.

Infant.

Satisfactory progress.

Puerperium.

B.P. 140/110 on the 4th day after delivery. Urine clear.

Postnatal attendance (6 weeks after delivery).

B.P. 140/100. Urine clear.

Laboratory investigations (37 weeks).

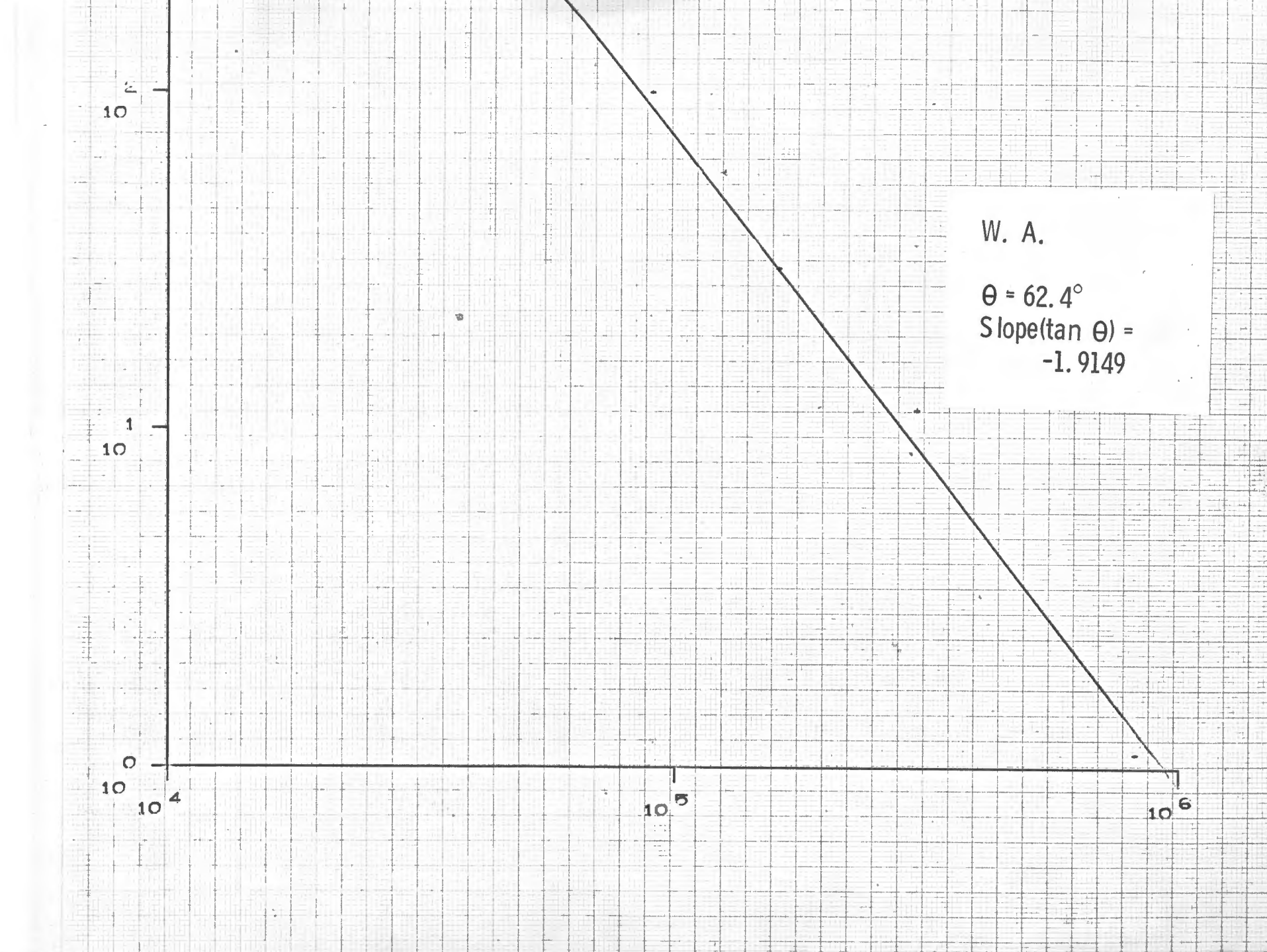
Haemoglobin 12.2 grams per 100 ml. Blood urea 38 mgs. per 100 ml. (at 36 weeks) and 32 mgs. per 100 ml. (at 37 weeks). Blood uric acid 8.2 mgs. per 100 ml. Serum albumin/globulin ratio 2.9/2.7. Blood cholesterol 240 mgs. per 100 ml. Creatinine clearance 75 ml. per minute. Total daily protein 5 grams per day.

Protein clearances (urine/serum ratios).

Albumin	0.060
Transferrin	0.050
IgG	0.015
α_1 I A/C	-
Pseudocholinesterase (immunological)	-

W.A.

Pseudocholinesterase (chemical)	0.006
α 2 macroglobulin	0.005
IgM	-
IgG/Transferrin	0.30
IgG/Albumin	0.25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ESSENTIAL HYPERTENSION.

H.D. Age 30. Parity 1. Booked patient. English.

L.M.P. 7.1.69 E.D.D. 14.10.69

Date when first seen: 24.2.69. Gestation: 7 weeks.

No past medical history.

Family history.

Mother suffers from essential hypertension. On antihypertensives.

Past obstetric history.

1968: Intra-uterine death at 32 weeks gestation.

Severe "pre-eclampsia."

Examination at first attendance.

B.P. 145/90. Urine clear. Weight 145 lbs.

Uterine size: 8 weeks.

Summary of ante-natal care.

Blood pressure fluctuated between 140/80 and 140/100.

Proteinuria appeared for thefirst time at 30 weeks gestation.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
30	30	160/100	Trace	Nil	165
31	31	160/100	320 mgs./ 100 ml.	Nil	166

Admitted to hospital.

Further progress.

B.P. fluctuated on bed rest, never falling below 130/90. Proteinuria lessened but did not disappear.

Caesarean section performed at 34 weeks gestation. This was prompted by falling 24-hour urinary oestriol excretion.

Infant.

Male 1840 grams. Satisfactory progress.

H.D.

Puerperium.

B.P. 140/100. Proteinuria cleared by the 10th day.

Postnatal attendance. (six weeks after delivery).

B.P. 140/90. Urine clear.

Laboratory investigations (on admission).

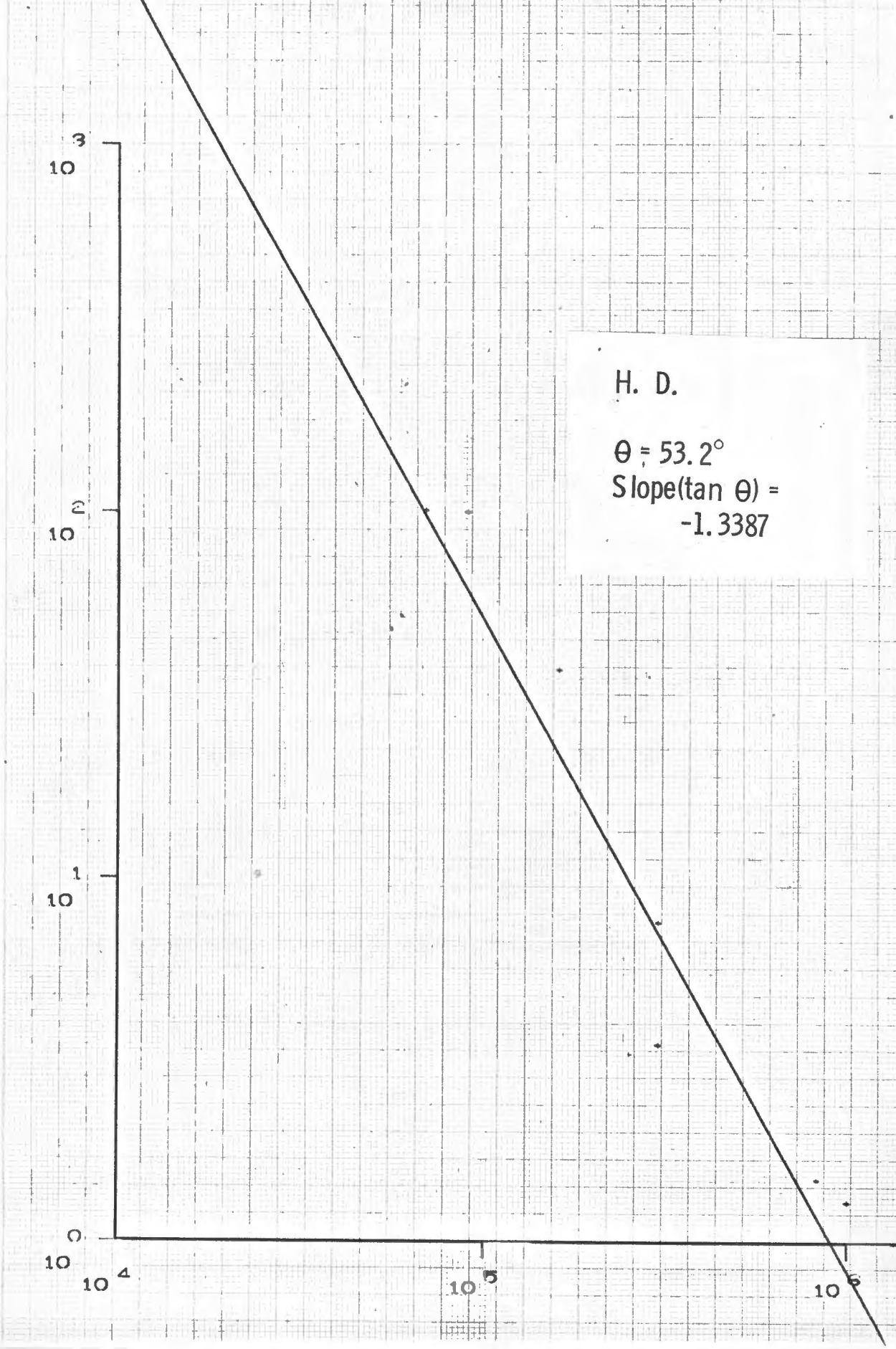
Haemoglobin 11.8 grams per 100 ml. Blood urea 25 mgs per 100 ml. Blood uric acid 7.2 mgs. per 100 ml. Blood cholesterol 170 mgs per 100 ml. Total urinary protein 4.8 grams in 24 hours.

Urine microscopy.

Granular casts. A few leucocytes and epithelial cells.

Protein clearances (urine/serum ratios)

	33 weeks	34 weeks	35 weeks	Puerperium
Albumin	0.083	0.158	0.067	0.168
Transferrin	0.078	0.157	0.069	0.167
IgG	0.028	0.059	0.025	0.065
β I A/C	-	0.006	0.002	0.0056
Pseudocholinesterase (immunological)	-	0.0015	0.002	0.006
Pseudocholinesterase (chemical)	-	0.0125	0.005	0.015
α2 macroglobulin	-	0.0024	-	0.0038
IgM	-	0.0021	-	0.0028
IgG/Transferrin	0.36	0.37	0.37	0.39
IgG/Albumin	0.34	0.38	0.38	0.38


H. D.
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10^3
10^2
10^1
10^0
10^4
10^5
10^6

RENAL DISEASE

R.F. Age 38. Parity 6. Booked patient. West Indian.

L.M.P. 1.1.70. E.D.D. 8.10.70.

Date when first seen: 22.4.70. Gestation: 16 weeks.

Past medical history.

Proteinuria of nephrotic proportions discovered after her previous pregnancy in 1966. No renal functional impairment.

Renal biopsy not performed.

Persistent heavy proteinuria.

No significant family history.

Past obstetric history.

6 normal full-term deliveries.

Proteinuria in last pregnancy but not hypertensive.

Examination at first attendance.

B.P. 110/75. Urine: Protein +++. Oedema +. Weight 171 lbs.

Uterine size: 16 weeks.

Summary of antenatal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
20	20	120/60	++	+	174
22	22	120/70	+++	+	174

Admitted for investigation.

Further progress.

Suddendevelopment at 24 weeks of severe hypertension with associated headache. B.P. 210/110. Unable to control hypertension. Therefore termination of pregnancy by abdominal hysterotomy was decided upon.

Puerperium.

B.P. 160/90. Protein 250 mgs. per 100 ml.

R.F.

Postnatal attendance (6 weeks post-delivery).

Blood pressure 130/90. Proteinuria 200 mgs. per 100 ml.

Laboratory investigations (antenatal).

Haemoglobin 12.2 gms. per 100 ml. Blood urea 21 mgs. per 100 ml. Uric acid 4.5 mgs. per 100 ml. Blood cholesterol 165 mgs. per 100 ml. Serum albumin/globulin ratio 2.6/3.8. Creatinine clearance 86 ml. per minute. Total urinary protein 13.2 grams in 24 hours.

Urine microscopy.

Granular and waxy casts. A few red cellsand leucocytes.

Postnatal attendance.

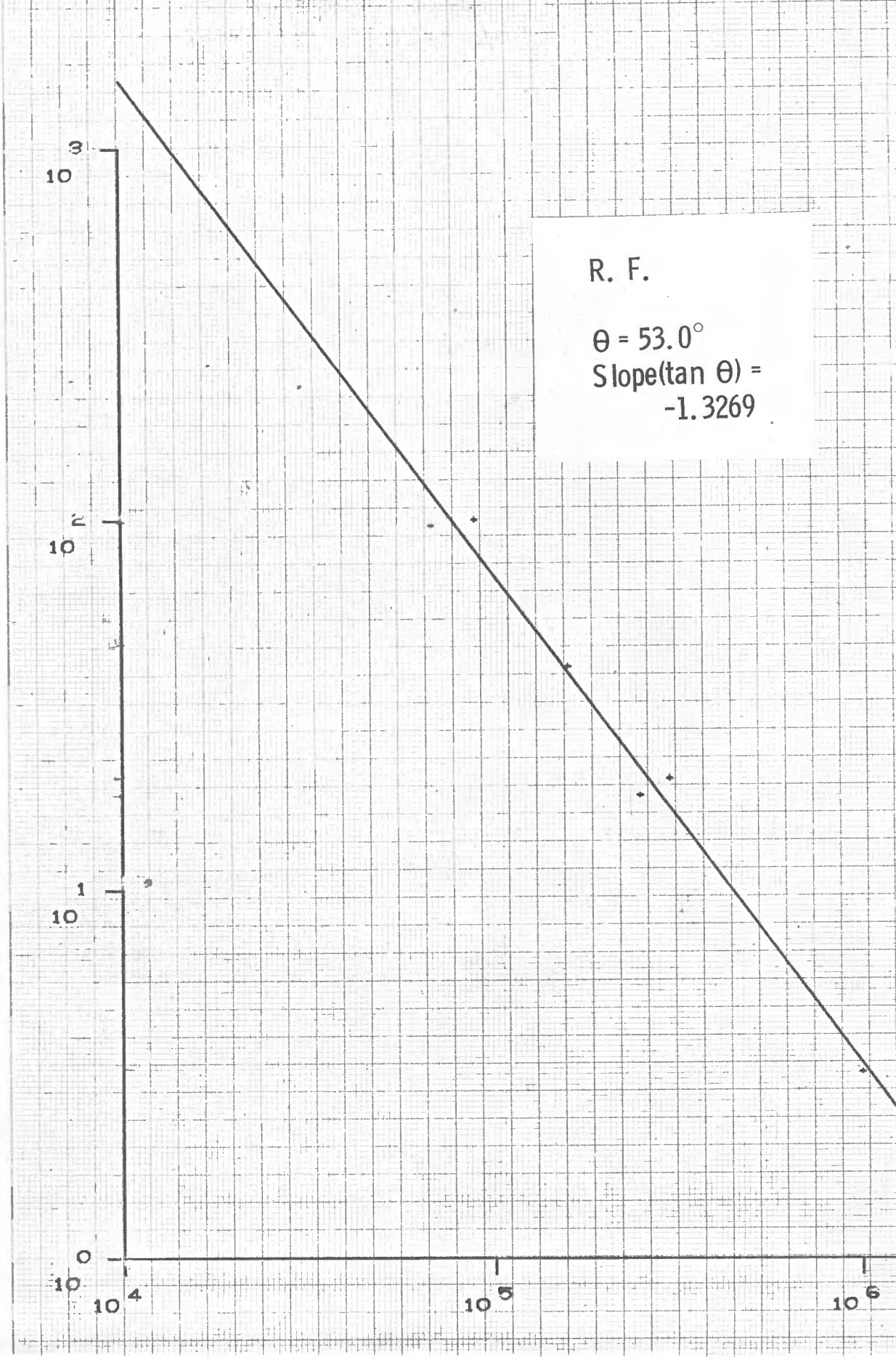
Blood urea 23 mgs. per 100 ml. Creatinine clearance 112 ml. per minute.

Urine microscopy.

Granular casts. Occasional leucocytes and epithelial cells.

Protein clearances (urine/serum ratios).

	24 weeks.	Puerperium.	Postnatal clinic.
Albumin	0.192	0.1	0.037
Transferrin	0.20	0.1	0.039
IgG	0.08	0.04	0.003
β 1 A/C	0.0364	0.0167	-
Pseudocholinesterase (immunological)		0.0143	
Pseudocholinesterase (chemical)	0.04	0.0167	
α 2 macroglobulin	0.0064	0.0029	
IgM		0.0026	
IgG/Transferrin	0.40	0.40	0.08
IgG/Albumin	0.43	0.40	0.09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3
10
2
10
1
10
0
10
10^4
10^5
10^6

RENAL DISEASE

S.B. Age 22. Parity 0. Booked patient. English.

L.M.P. 5.12.69. E.D.D. 11.9.70.

Date when first seen: 12.4.70. Gestation: 20 weeks.

Past medical history.

Hospitalised with acute nephritis at age 8.

No significant family history.

Examination at first attendance.

B.P. 120/70. Urine: Protein +++. No oedema. Weight 121 lbs.

Uterine size: 20 weeks.

Summary of antenatal care.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
24	24	120/70	++	Nil	123
28	28	120/80	+++	++	129

Admitted to hospital.

Further progress.

Oedema resolved on bed rest. Proteinuria persisted but blood pressure remained normal throughout pregnancy.

Labour.

Surgical induction of labour at 37 weeks gestation.

Normal vaginal delivery.

Infant.

Male, 2690 grams. Satisfactory progress.

Puerperium.

Proteinuria persisted. Blood pressure 120/70.

Postnatal attendance (6 weeks after delivery).

B.P. 140/85. Proteinuria: 500 mgs. per 100 ml.

S.B.

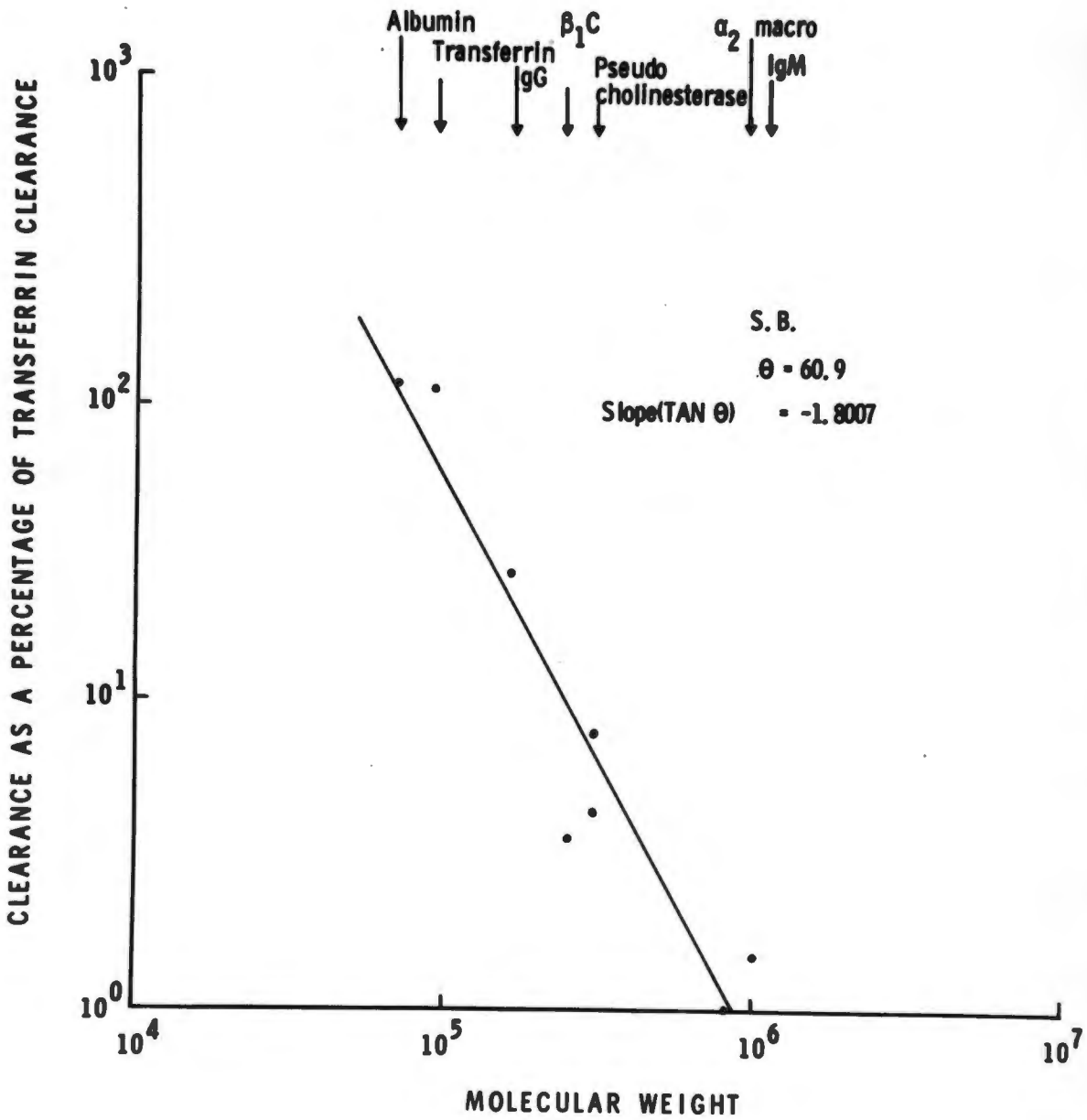
Laboratory investigations (antenatal).

Haemoglobin 12.8 grams per 100 ml. Blood urea 17 mgs. and 25 mgs. per 100 ml. Blood uric acid 3.8 mgs. per 100 ml. Serum albumin/globulin ratio 2.9/2.4. Creatinine clearance 101 and 84 ml. per minute. Total urinary protein 8.0 grams in 24 hours.

Urine microscopy: Granular casts and a few leucocytes. Occasional red cells.

Protein clearances (urine/serum ratios).

Gestation (in weeks)	28	30	34	38	Puerperium	P.N.C.
Albumin	0.093	0.1	0.09	0.15	0.12	0.171
Transferrin	0.095	0.1	0.081	0.141	0.145	0.152
IgG	0.025	0.03	0.02	0.032	0.03	0.0278
β I A/C	0.003	0.003		0.006	0.005	0.006
Pseudocholinesterase (immunological)	0.005	0.003		0.008		0.008
Pseudocholinesterase (chemical)	0.008	0.0067		0.0125		0.0125
α 2 macroglobulin	0.0016	0.001		0.0025	0.0029	0.0038
IgM		0.001			0.004	0.0004
IgG/Transferrin	0.26	0.30	0.25	0.23	0.21	0.18
IgG/Albumin	0.27	0.30	0.22	0.21	0.25	0.16


Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
α_2 macro
IgM
S. B.
Θ = 60.9
Slope(TAN Θ) = -1.8007
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

RENAL DISEASE

M.N. Age 34. Parity 2. Booked patient. English.

L.M.P. 11.7.68. E.D.D. 18.4.69.

Date when first seen: 26.9.68. Gestation: 10 weeks.

No past medical history.

No significant family history.

Past obstetric history.

1960: Full-term normal delivery of a male infant weighing 5 lbs. 2½ ozs.

1963: Full-term normal delivery of a female infant weighing 6 lbs. 10½ ozs.

Examination at first attendance.

B.P. 110/70. Urine: 200 mgs. protein. No oedema.

Weight 125 lbs. Uterine size: 10 weeks.

Summary of antenatal care.

Patient remained normotensive until 34 weeks gestation.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
17	17	120/70	300 mgs. per 100 ml.	Nil	130
24	24	110/70	200 mgs. per 100 ml.	Nil	134
30	30	120/60	280 mgs. per 100 ml.	+	136
34	34	150/90	440 mgs. per 100 ml.	+	142
36	36	135/70	160 mgs. per 100 ml.	+	144
38	38	140/90	400 mgs. per 100 ml.	+	146

Admitted to hospital.

Further progress.

Spontaneous onset of labour at 39 weeks.

M.N.

Labour.

Normal vaginal delivery.

Infant.

Female 2150 grams. Satisfactory progress.

Puerperium.

Blood pressure 130/90. Proteinuria 250 mgs. per 100 ml.

Postnatal attendance (6 weeks after delivery).

B.P. 120/90. Protein 400 mgs. per 100 ml.

Investigations (antenatal).

These were monitored throughout the pregnancy.

	22 weeks	32 weeks	38 weeks	Puerperium	Postnatal
Blood urea (in mgs.per 100 ml.)	20	25	22	36	33
Blood uric acid	4.7	-	5.3	4.4	4.1
Cholesterol	420	-	-	-	-
Albumin/globulin ratio	2.4/3.0		1.9/2.4		
Creatinine clearance			148 ml/min.		117 ml/min.
24 hour urinary protein			7.5 grams		10.3 grams.
Microscopy of urine			Granular casts. No red cells		Granular casts. No red cells.

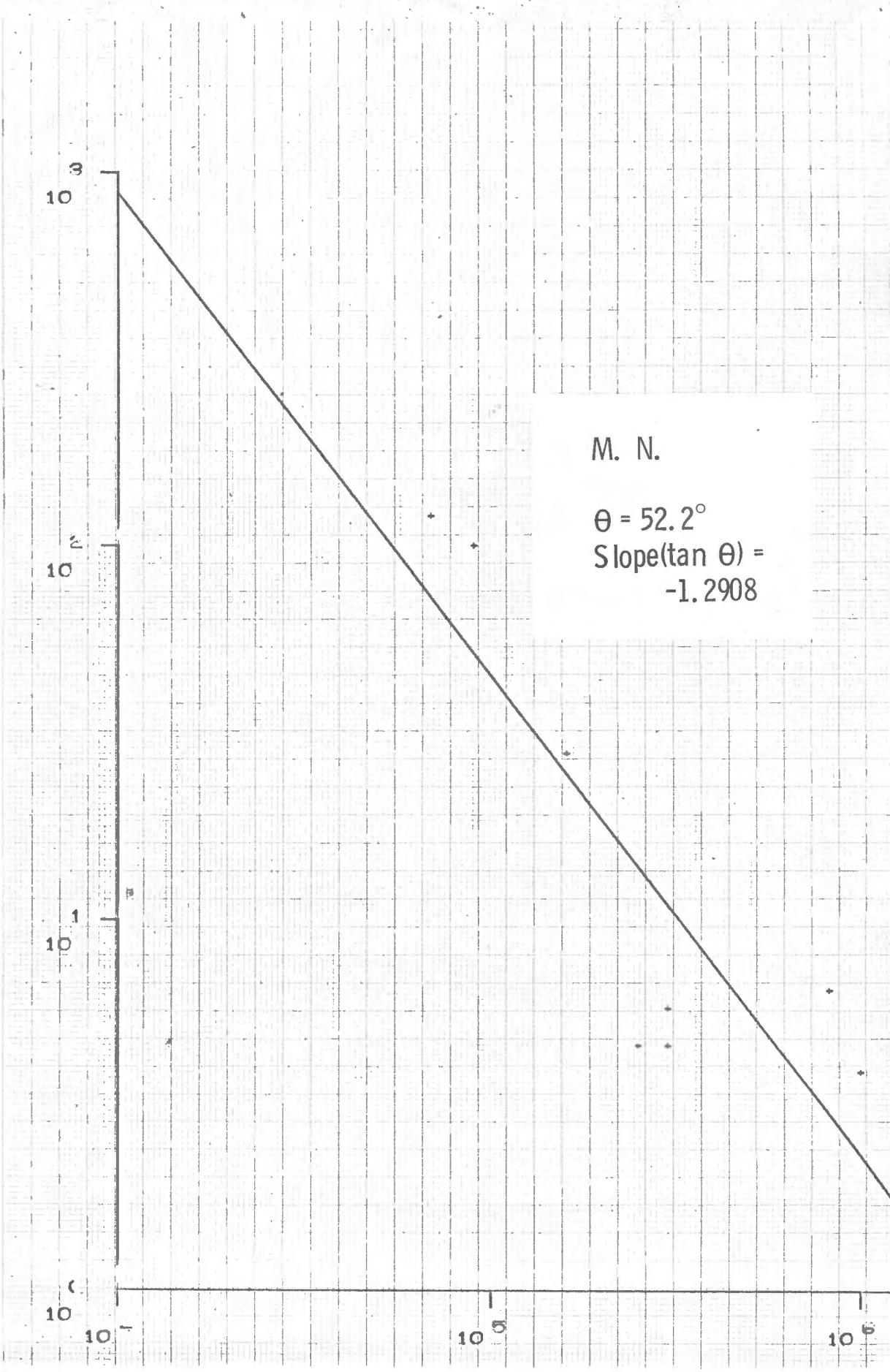
Renal biopsy.

This was performed following delivery and showed a "minimal drainage" renal lesion.

M.N.

Protein clearances (urine/serum ratios)

Gestation (weeks)	27	29	33	35	38	Puerperium	P.N.C.
Albumin	0.108	.086	.170	.216	.299	.268	.01
Transferrin	0.092	.076	.170	.134	.277	.285	.017
IgG	0.021	.033	.071	.052	.083	.059	.001
β I A/C	0.0059	-	.013	-	.011	-	-
Pseudocholinesterase (immunological)	0.003	-	-	-	.016	-	-
Pseudocholinesterase (chemical)	0.0061	-	.0105	-	.0126	-	-
α 2 macroglobulin	0.0036	-	.0061	-	.0121	-	-
IgM	0.0048	-	.012	-	.02	-	-
IgG/Transferrin	0.23	0.42	.42	.34	.30	.21	.06
IgG/Albumin	0.19	0.38	.42	.34	.28	.22	.06


M. N.
θ = 52.2°
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10 2
10 1
10 0
10 1
10 5
10 6

UNCLASSIFIED TOXAEMIA

M.P. Age 40. Parity 0. Booked patient. British.

L.M.P. 30.10.69. E.D.D. 6.8.70.

Date when first seen: 24.2.70. Gestation: 16 weeks.

No past medical history.

Family history.

Mother thought to have essential hypertension.

Examination at first attendance.

B.P. 120/65. Urine clear. No oedema. Weight 137 lbs.

Uterine size: 16 weeks.

Summary of antenatal care (shared between hospital and general practitioner).

Referred to G.P. after booking. Seen again at 32 weeks.

Gestation	Size	B.P.	Urine (protein)	Oedema	Weight
32	32	160/80	Nil	Nil	154
34	34	160/115	++	+	158

Admitted to hospital.

Further progress.

Raised blood pressure and proteinuria persisted after admission.

An elective Caesarean section was performed at 35 weeks gestation with the birth of a male infant weighing 2350 grams.

Infant.

Satisfactory progress.

Puerperium.

B.P. settled to 130/90. Urine clear.

Postnatal attendance (6 weeks after delivery).

B.P. 150/90. Urine clear.

M.P.

Laboratory investigations (on admission).

Haemoglobin 12.4 grams per 100 ml. Blood urea 25 mgs. per 100 ml. and 23 mgs. per 100 ml. Uric acid 6.2 mgs. per 100 ml. and 4.5 mgs. per 100 ml. Albumin/globulin ratio 3.0/2.7 and 3.3/3.2. Creatinine clearance 85 ml. per minute. Total urinary protein 3.0 grams in 24 hours.

Urine microscopy: Granular casts. Few white cells and epithelial cells.

Protein clearances (urine/serum ratios).

	34 weeks	35 weeks
Albumin	0.045	0.044
Transferrin	0.069	0.065
IgG	0.017	0.017
β I A/C	-	-
Pseudocholinesterase (immunological)	-	-
Pseudocholinesterase (chemical)	-	0.0027
α 2 macroglobulin	-	-
IgM	-	0.0008
IgG/Transferrin	0.25	0.26
IgG/Albumin	0.32	0.32

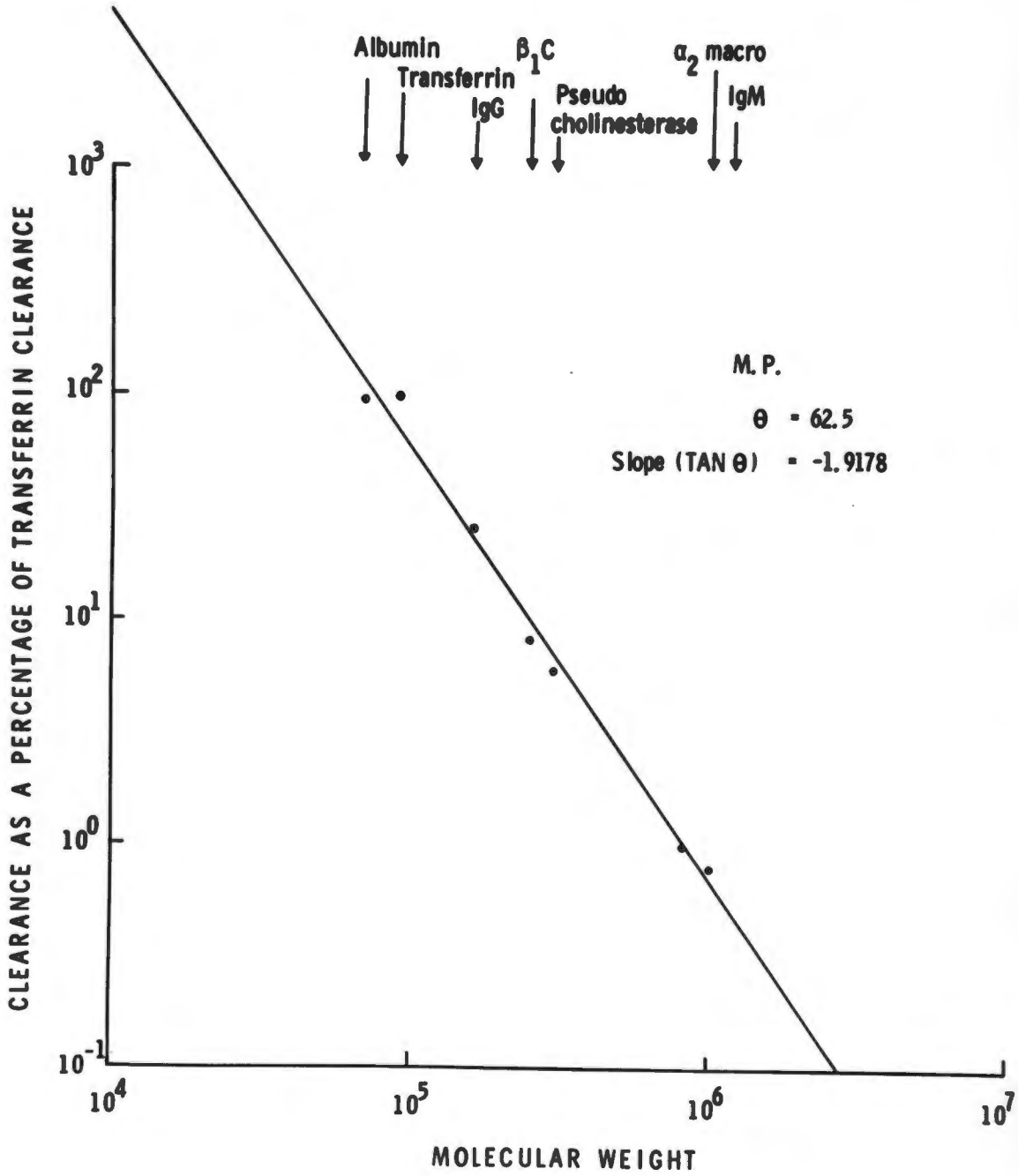

Albumin
Transferrin
IgG
β_1C
Pseudo cholinesterase
α_2 macro
IgM
M. P.
θ = 62.5
Slope (TAN θ) = -1.9178
CLEARANCE AS A PERCENTAGE OF TRANSFERRIN CLEARANCE
10^3
10^2
10^1
10^0
10^{-1}
10^4
10^5
10^6
10^7
MOLECULAR WEIGHT

TABLE VII

LONGITUDINAL FOLLOW UP OF PROTEIN CLEARANCES EXPRESSED AS A PERCENTAGE OF TRANSFERRIN CLEARANCE

PREECLAMPSIA

NAME		ALB.	Ig G	β_1C	PSEUDOCHOLIN.		α-2-M	Ig M
					IMM.	CHEM.		
K.C.	31 weeks	114	22	2.8	5.7	3.3	1.3	0.6
	32 weeks	100	22	2.2	5.2	3.6	1.3	0.5
	Puerperium	100	20	-	-	-	-	-
E.S.	30 weeks	94	26	4.7	6.0	4.7	1.3	0.9
	31 weeks	88	25	5.0	6.1	4.4	1.3	0.5
M.M.	32 weeks	113	25	3.0	-	2.1	0.8	0.8
	33 weeks	100	19	3.0	-	2.0	1.6	1.0
	34 weeks	137	22	2.4	-	2.4	0.4	0.5
P.F.	16 weeks	113	24	6.2	8.4	4.8	0.9	0.9
	17 weeks	111	24	6.8	7.2	8.4	2.2	1.7
	Puerperium	125	21	-	-	-	-	-
F.S-M.	34 weeks	107	20	5.7	4.4	5.9	0.4	-
	35 weeks	93	19	5.9	2.5	5.8	0.8	-
	36 weeks	90	17	5.2	3.7	5.7	0.7	-
L.S.	27 weeks	98	23	4.2	5.0	3.4	0.6	0.3
	28 weeks	110	21	-	-	4.4	-	-
	29 weeks	72	14	-	-	-	-	-
	30 weeks	100	18	-	-	-	-	-
E.P.	34 weeks	120	23	4.0	-	-	-	-
	35 weeks	100	26	3.8	3.2	3.5	1.2	1.0
	36 weeks	100	26	1.6	3.5	2.0	1.1	1.3
J.T.	32 weeks	100	24	5.2	4.9	6.9	0.9	-
	33 weeks	110	23	7.9	11.8	7.8	2.0	2.2
	Puerperium	78	17	-	-	-	-	-
C.T.	31 weeks	93	24	4.5	4.4	6.9	1.3	0.9
	Puerperium	104	26	3.3	3.8	6.8	-	-

TABLE VII (Cont'd)

HYPERTENSION

NAME		ALB.	Ig G	β_1C	PSEUDOCHOLIN. IMM.	PSEUDOCHOLIN. CHEM.	α-2-M	Ig M
P.P.	28 weeks	98	27	-	-	-	0.7	0.3
	29 weeks	106	25	-	-	-	0.7	0.4
	30 weeks	88	25	-	-	-	0.6	0.4
E.B.	34 weeks	90	29	3.3	2.5	3.2	0.8	1.2
	35 weeks	93	28	2.3	2.2	3.6	0.4	-
M.K.	31 weeks	95	34	8.6	7.3	9.7	1.0	-
	32 weeks	88	32	6.1	5.1	7.4	1.1	1.0
	33 weeks	100	37	8.7	7.5	7.5	1.0	-
N.M.	29 weeks	101	39	12.5	13.5	20.0	4.5	3.5
	30 weeks	100	43	8.2	8.4	18.1	2.9	2.9
	31 weeks	112	43	11.1	9.0	15.6	3.4	2.7
S.K.	33 weeks	97	33	3.8	6.7	8.3	1.0	0.9
	34 weeks	81	30	-	-	-	-	-
	35 weeks	100	33	3.5	5.5	10.0	0.9	0.5
J.S.	30 weeks	95	32	4.0	5.6	3.8	1.2	1.8
	Puerperium	112	34	-	-	-	-	-
H.D.	32 weeks	106	36	-	3.8	-	-	-
	34 weeks	100	37	3.8	2.9	8.0	1.5	1.3
	35 weeks	97	37	2.8	3.7	7.2	-	-
	Puerperium	100	39	3.3	3.7	9.4	2.3	1.7
E.G.	34 weeks	85	50	8.8	12.1	12.1	4.5	3.1
	Puerperium	100	51	-	-	-	-	-
D.H.	31 weeks	118	30	12.1	7.6	7.2	0.9	0.9
	33 weeks	83	28	7.4	5.9	7.4	0.7	0.7
	34 weeks	91	25	8.1	6.7	6.5	1.2	1.3
	Puerperium	97	22	8.5	6.3	7.4	1.5	0.9

TABLE VII (Cont'd)

RENAL DISEASE

NAME		ALB.	Ig G	β_1C	PSEUDOCHOLIN. IMM.	PSEUDOCHOLIN. CHEM.	α-2-M	Ig M
F.R.	32 weeks	94	31	9.8	8.4	8.8	1.4	-
	33 weeks	93	34	7.4	5.8	8.7	1.8	1.8
	34 weeks	137	44	13.4	6.3	9.9	1.7	1.6
	Puerperium	104	30	-	9.8	-	1.5	-
L.F.	33 weeks	92	22	-	-	-	-	-
	34 weeks	106	23	12.5	-	9.2	2.6	2.7
	35 weeks	97	22	9.3	6.7	6.7	2.0	1.6
	Puerperium	100	25	-	-	7.0	-	-
M.N.	27 weeks	117	23	3.3	3.3	6.6	3.9	5.2
	29 weeks	113	42	-	-	-	-	-
	33 weeks	100	42	-	-	6.2	3.6	7.0
	35 weeks	161	34	-	-	-	-	-
	39 weeks	108	30	5.8	5.8	4.6	4.3	7.2
	Puerperium	94	29	-	-	-	-	-
	P.N.C.	88	6	-	-	-	-	-
P.C.(1)	32 weeks	142	24	-	-	-	-	-
	34 weeks	147	17	14.3	5.6	8.1	4.5	-
	36 weeks	93	20	6.9	6.6	7.4	1.7	-
	38 weeks	92	17	-	-	-	-	-
	Puerperium	103	17	-	-	-	-	-
	P.N.C.	115	9	-	-	-	-	-
P.C.(2)	30 weeks	88	22	-	-	-	-	-
	31 weeks	145	14	-	-	-	-	-
	33 weeks	155	19	-	-	4.5	-	-
	36 weeks	116	17	-	-	7.8	1.2	1.2
	Puerperium	90	21	5.4	-	5.2	1.2	1.2

TABLE VII (Cont'd)

RENAL DISEASE (Cont'd)

NAME		ALB.	Ig G	β_1C	PSEUDOCHOLIN. IMM.	PSEUDOCHOLIN. CHEM.	α-2-M	Ig M
S.B.	28 weeks	98	26	-	5.0	8.5	1.7	-
	30 weeks	100	30	3.0	3.0	6.7	1.0	1.0
	34 weeks	111	25	-	-	-	-	-
	38 weeks	106	23	4.3	5.6	8.8	1.8	1.2
	Puerperium	83	21	3.5	-	-	2.0	2.8
	P.N.C.	113	18	3.9	5.2	8.2	2.5	2.6
R.F.	24 weeks	96	40	18.0	-	20.0	3.2	-
	Puerperium	100	40	18.0	14.0	17.0	2.9	2.6
	P.N.C.	98	8	-	-	-	-	-

UNCLASSIFIED TOXAEMIA

M.P.	34 weeks	65	25	-	-	-	-	-
	35 weeks	68	26	-	-	4.2	1.2	-

PROTEIN CLEARANCES (URINE/PLASMA RATIOS) FOR ALL PATIENTS NOT INCLUDED IN CASE SUMMARIES

NAME		ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLINESTERASE		α-2-M	Ig M
						IMMUN.	CHEM.		
A.W.		0.017	0.016	0.003	-	-	-	-	-
S.D.		0.101	0.102	0.021	0.008	0.006	0.004	0.0017	0.0011
B.M.		0.044	0.048	0.011	-	-	0.0015	0.0002	-
M.G.		0.096	0.141	0.33	0.004	0.0045	0.004	0.0011	0.001
A.F.		0.10	0.103	0.036	0.009	-	0.0105	0.0012	0.0012
J.G.		0.071	0.058	0.018	0.0015	-	0.0018	0.0003	0.0006
B.T.		0.030	0.031	0.008	-	-	-	-	-
M.W.		0.030	0.029	0.007	-	-	-	-	-
M.P.		0.072	0.075	0.020	0.006	-	0.0045	0.0006	0.0007
L.S.	27 weeks	0.115	0.117	0.027	0.0048	0.0058	0.004	0.0007	0.0004
	28 weeks	0.063	0.057	0.012	-	-	0.0025	0.0004	-
	29 weeks	0.021	0.029	0.004	-	-	-	-	-
	30 weeks	0.023	0.023	0.004	-	-	-	-	-
M.M.	32 weeks	0.069	0.061	0.015	0.0018	-	0.0013	0.0005	0.0005
	33 weeks	0.10	0.10	0.019	0.003	-	0.0020	0.0017	0.001
	34 weeks	0.082	0.06	0.013	0.002	-	0.0014	0.0002	0.0003
C.T.	31 weeks	0.085	0.091	0.022	0.0041	0.004	0.0063	0.0012	0.0008
	Puerperium	0.054	0.052	0.014	0.017	0.002	0.0036	-	-
J.T.	32 weeks	0.090	0.097	0.023	0.005	0.0049	0.0067	0.0009	-
	33 weeks	0.14	0.127	0.0298	0.01	0.015	0.01	0.0025	0.0028
	Puerperium	0.050	0.064	0.0083	-	-	-	-	-
M.M.		0.064	0.055	0.016	-	-	0.005	0.0006	-
N.R.		0.050	0.055	0.020	-	-	0.0033	0.0003	-
E.S.		0.115	0.164	0.076	0.0118	0.013	0.0178	0.0024	0.0015
B.M.		0.121	0.145	0.067	0.0192	0.0125	0.0139	0.0024	0.0017
J.R.		0.022	0.020	0.0043	-	-	0.001	-	-

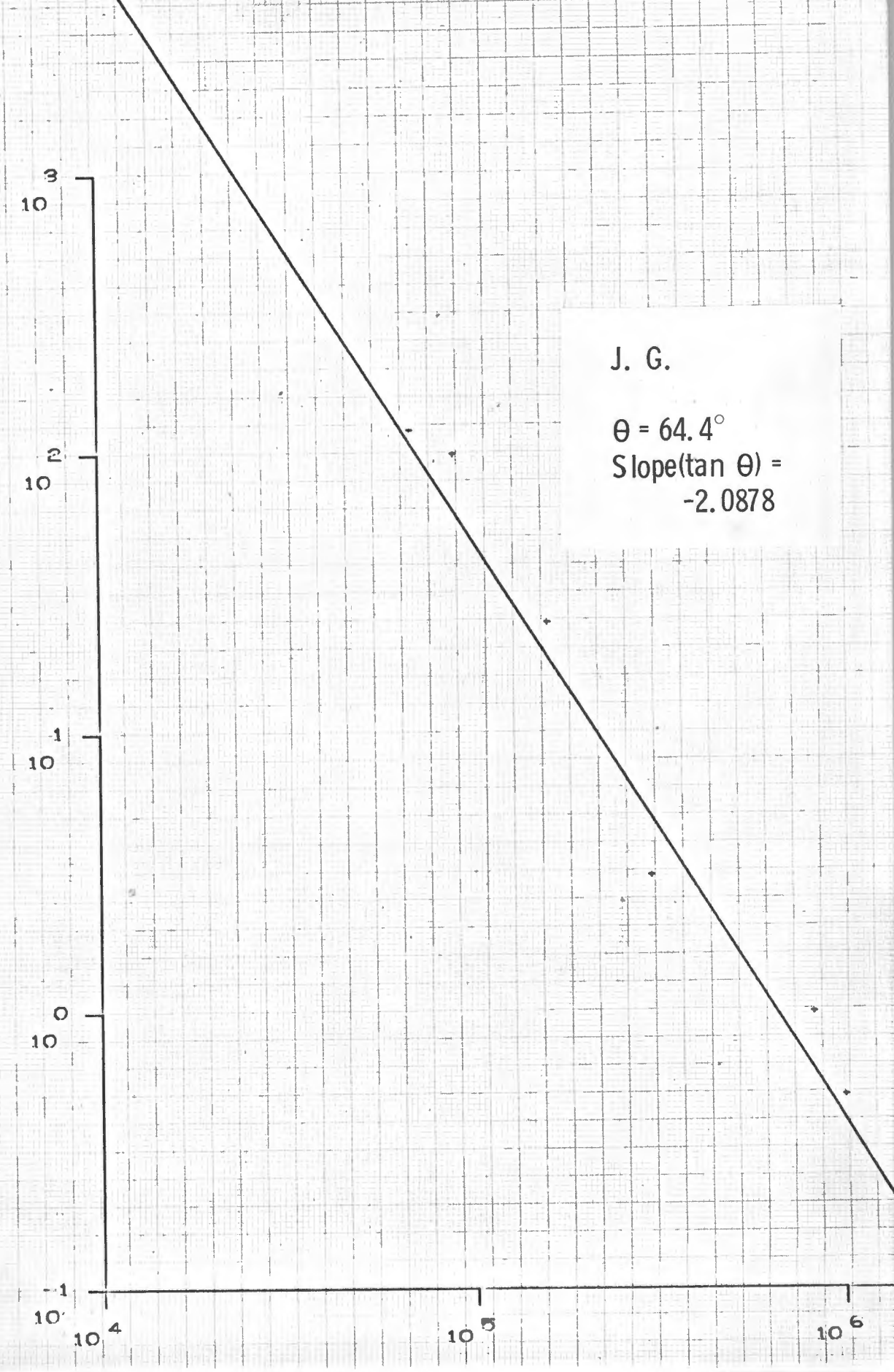
PROTEIN CLEARANCES (URINE/PLASMA RATIOS) FOR ALL PATIENTS NOT INCLUDED IN CASE SUMMARIES (Cont'd)

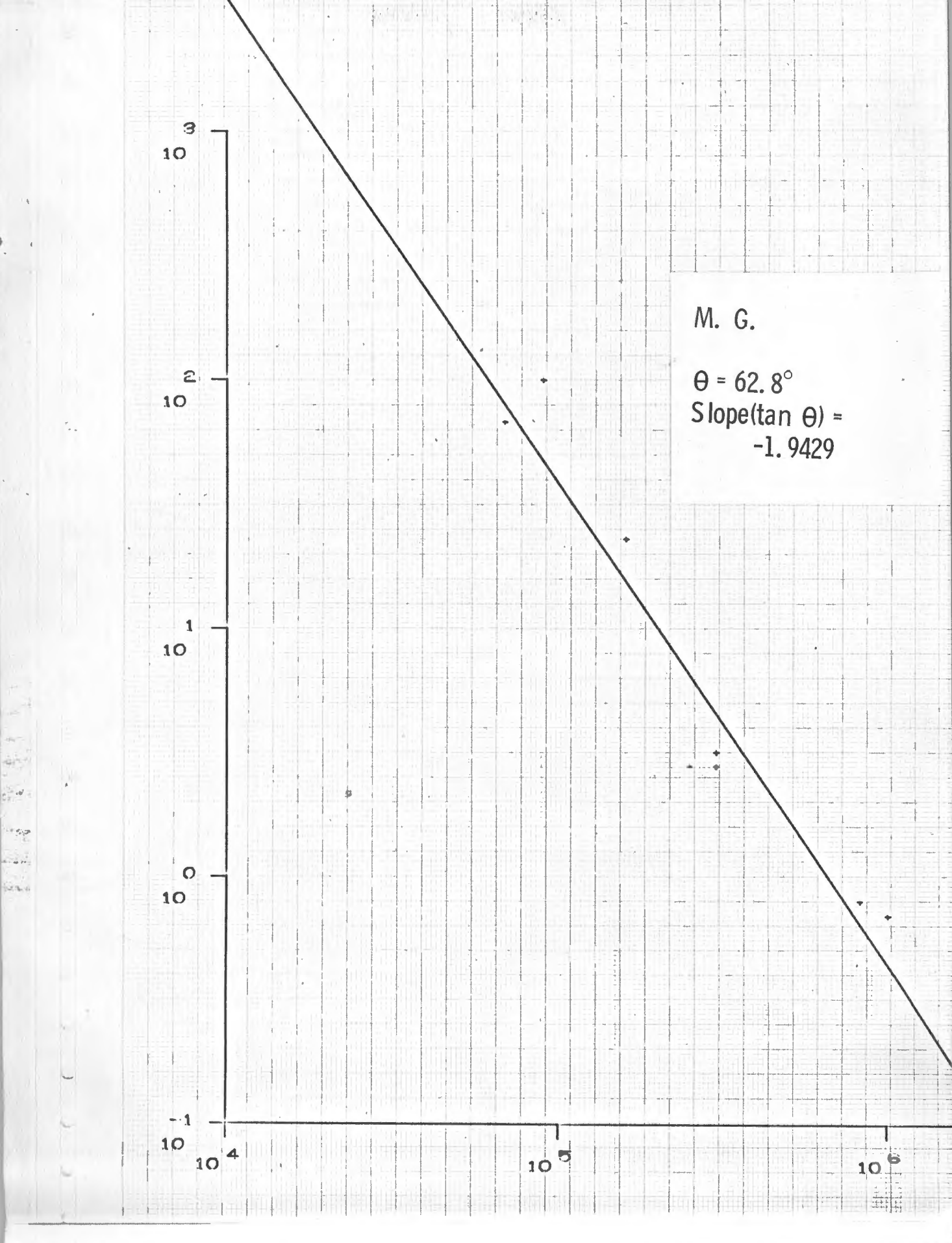
NAME		ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLINESTERASE		α-2-M	Ig M
						IMMUN.	CHEM.		
P.P.	28 weeks	0.1	0.102	0.028	-	-	-	0.0007	0.0004
	29 weeks	0.112	0.106	0.027	-	-	-	0.0006	0.0002
	30 weeks	0.10	0.114	0.028	-	-	-	0.0007	0.0004
E.B.	34 weeks	0.18	0.20	0.057	0.0067	0.005	0.0063	0.0015	0.0023
	35 weeks	0.052	0.056	0.016	0.0014	0.0013	0.002	0.0002	-
M.K.	31 weeks	0.059	0.062	0.021	0.0053	0.0045	0.006	0.0006	-
	32 weeks	0.083	0.094	0.027	0.0057	0.0048	0.007	0.0009	0.001
	33 weeks	0.067	0.067	0.025	0.0058	0.005	0.005	0.0007	-
S.K.	33 weeks	0.058	0.060	0.02	0.0023	0.004	0.005	-	0.0006
	34 weeks	0.044	0.054	0.016	-	-	-	-	-
	35 weeks	0.203	0.20	0.057	0.007	0.011	0.02	0.001	0.0017
E.G.	34 weeks	0.175	0.206	0.103	0.0182	0.025	0.025	0.0093	0.0063
	Puerperium	0.013	0.013	0.007	-	-	-	-	-
D.H.	31 weeks	0.065	0.055	0.0168	0.0067	0.0042	0.004	0.0005	0.0005
	33 weeks	0.14	0.168	0.048	0.0125	0.010	0.0125	0.0011	0.0013
	34 weeks	0.08	0.088	0.022	0.007	0.0059	0.0057	0.0011	0.0012
	Puerperium	0.066	0.068	0.0151	0.0056	0.0043	0.005	0.001	0.0006
P.C.(1)	32 weeks	0.054	0.038	0.009	-	-	-	-	-
	34 weeks	0.033	0.023	0.004	-	-	-	-	-
	36 weeks	0.041	0.044	0.017	0.0063	0.0024	0.0036	0.002	-
	38 weeks	0.033	0.036	0.006	0.0025	0.0025	0.0027	0.0017	-
	Puerperium	0.033	0.032	0.003	-	-	-	-	-
	P.N.C.	0.038	0.033	0.003	-	-	-	-	-
P.C.(2)	30 weeks	0.046	0.052	0.011	-	-	-	-	-
	31 weeks	0.080	0.055	0.008	-	-	-	-	-
	32 weeks	-	0.053	0.011	-	-	-	-	-
	33 weeks	0.068	0.044	0.008	-	-	0.002	0.0006	-
	34 weeks	-	0.048	0.009	-	-	-	-	-
	35 weeks	0.05	0.043	0.007	-	-	0.003	0.0005	0.0005
	36 weeks	0.075	0.083	0.018	0.0044	-	0.004	0.001	0.0015

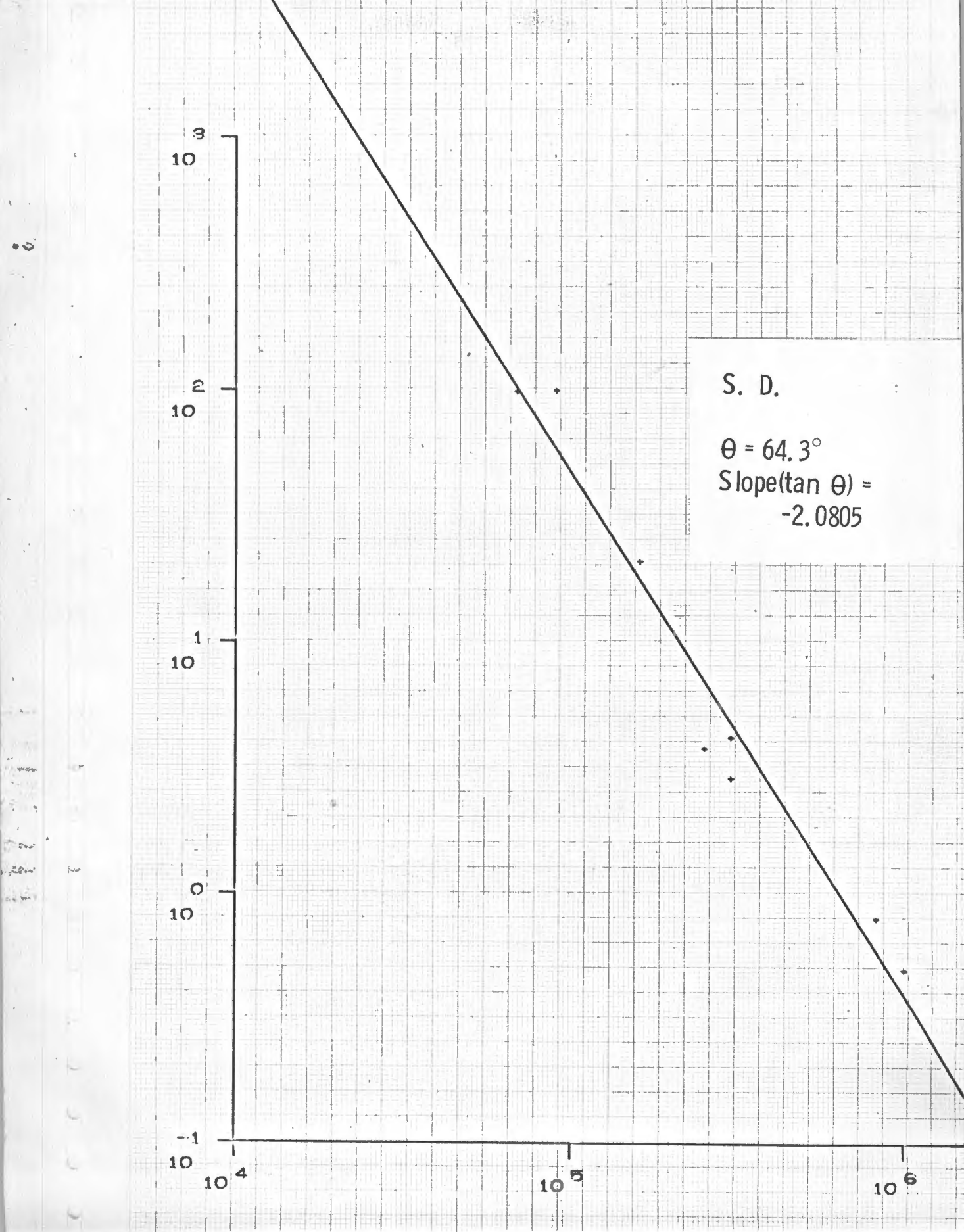
PROTEIN CLEARANCES (URINE/PLASMA RATIOS) FOR ALL PATIENTS NOT INCLUDED IN CASE SUMMARIES (Cont'd)

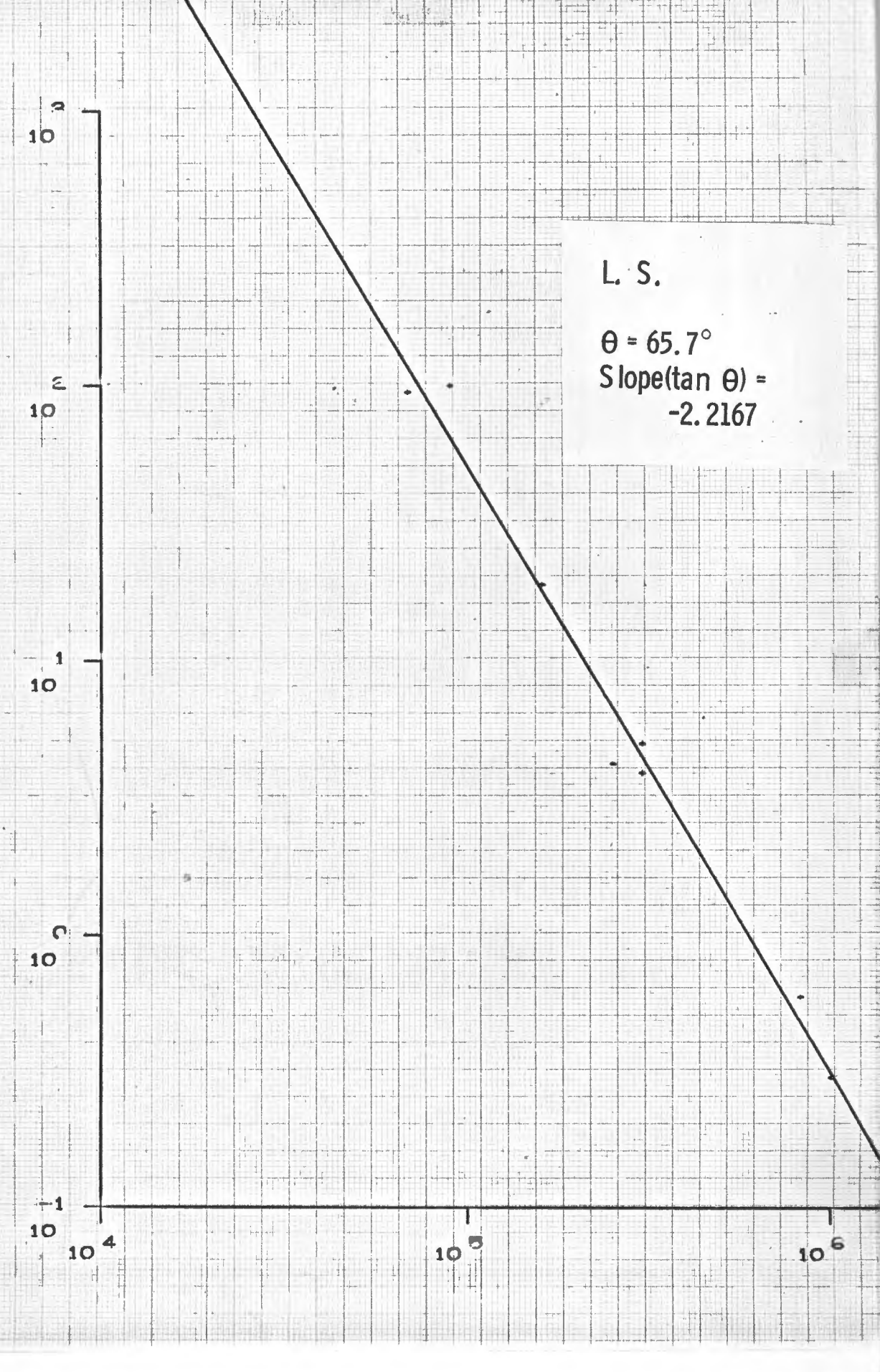
NAME		ALB.	TRANS.	Ig G	β_1C	PSEUDOCHOLINESTERASE		α-2-M	Ig M
						IMMUN.	CHEM.		
F.R.	30 weeks	0.08	0.085	0.026	0.0083	0.0071	0.0075	0.0012	-
	33 weeks	0.40	0.43	0.148	0.0318	0.025	0.0375	0.0077	0.0079
	34 weeks	0.158	0.115	0.05	0.0154	0.0073	0.0114	0.0019	0.0018
	Puerperium	0.10	0.088	0.026	-	-	0.0086	0.0013	-
L.F.	33 weeks	0.108	0.118	0.026	-	-	-	-	-
	34 weeks	0.15	0.141	0.032	0.0188	-	0.013	0.0038	0.0036
	35 weeks	0.15	0.155	0.035	0.014	0.01	0.01	0.0024	0.003
	Puerperium	0.10	0.10	0.025	-	-	0.007	-	-
M.G.		0.045	0.046	0.013	-	0.0037	0.004	0.001	0.0007
C.B.		0.026	0.028	0.012	-	-	-	-	-

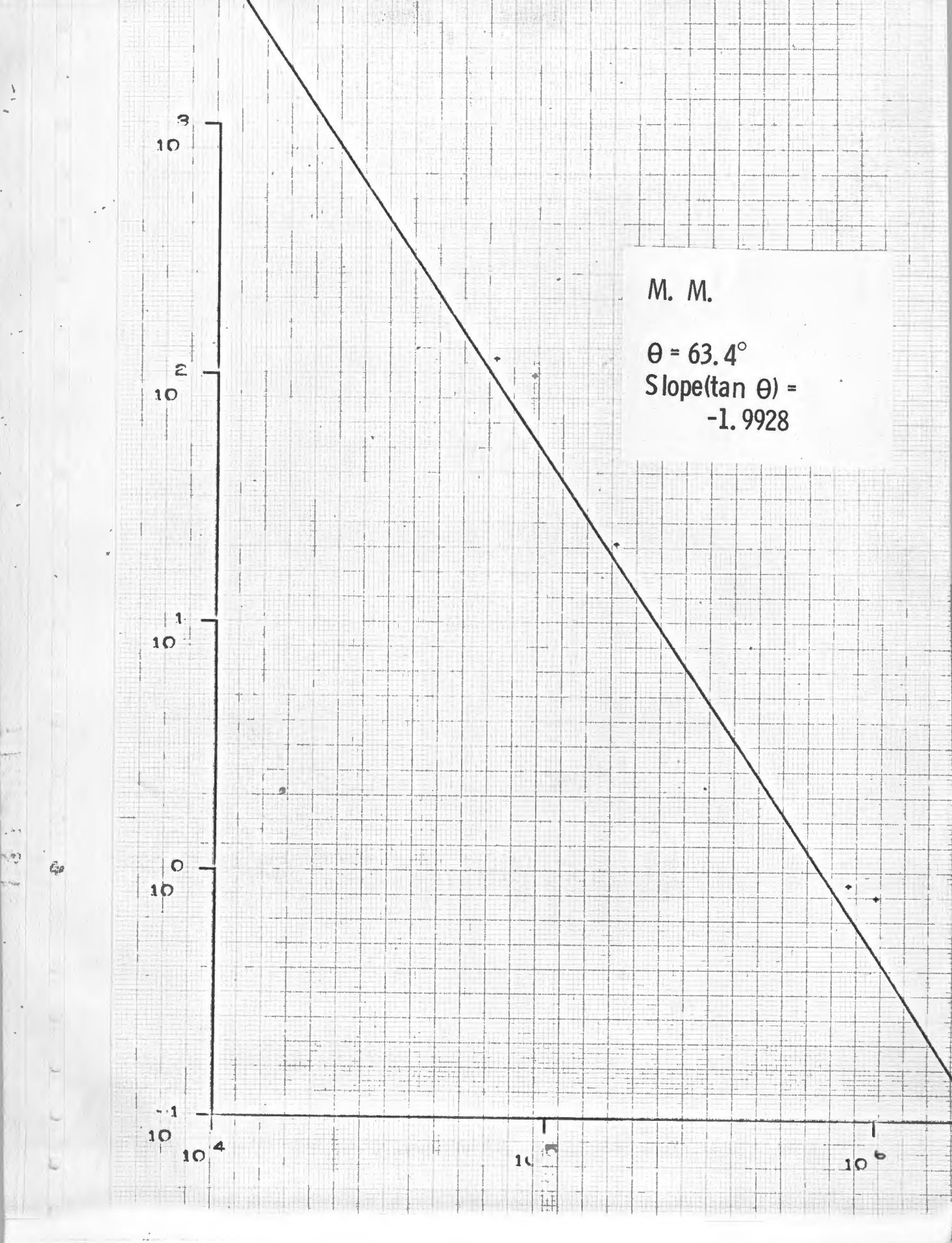
COMPUTER DRAWN REGRESSION LINES OF
PROTEIN CLEARANCE ON MOLECULAR WEIGHT
FOR PATIENTS NOT INCLUDED IN THE
CLINICAL CASE SUMMARIES

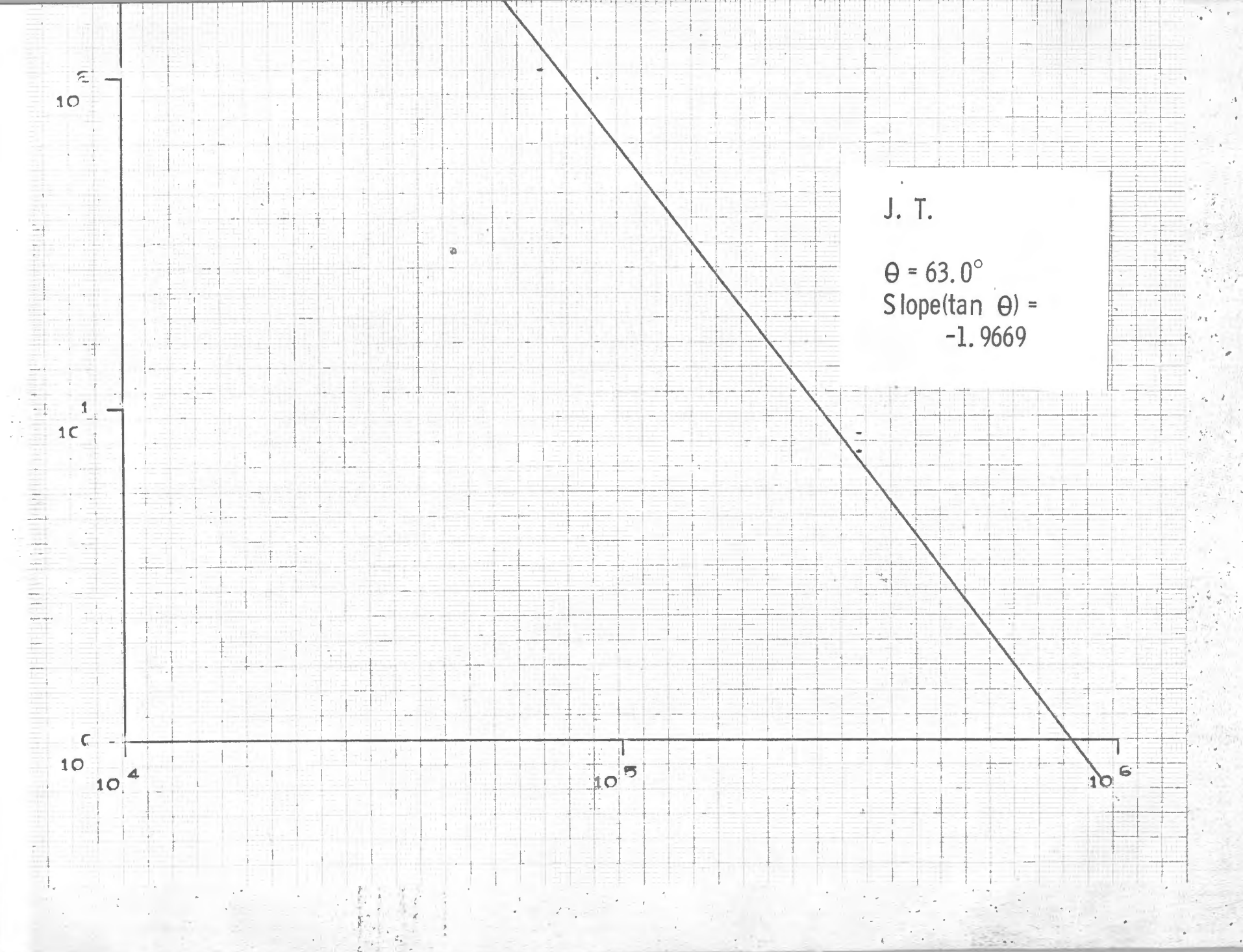

J. G.
θ = 64.4°
Slope(tan θ) =
-2.0878
3
10
2
10
1
10
0
10
-1
10
10^4
10^5
10^6

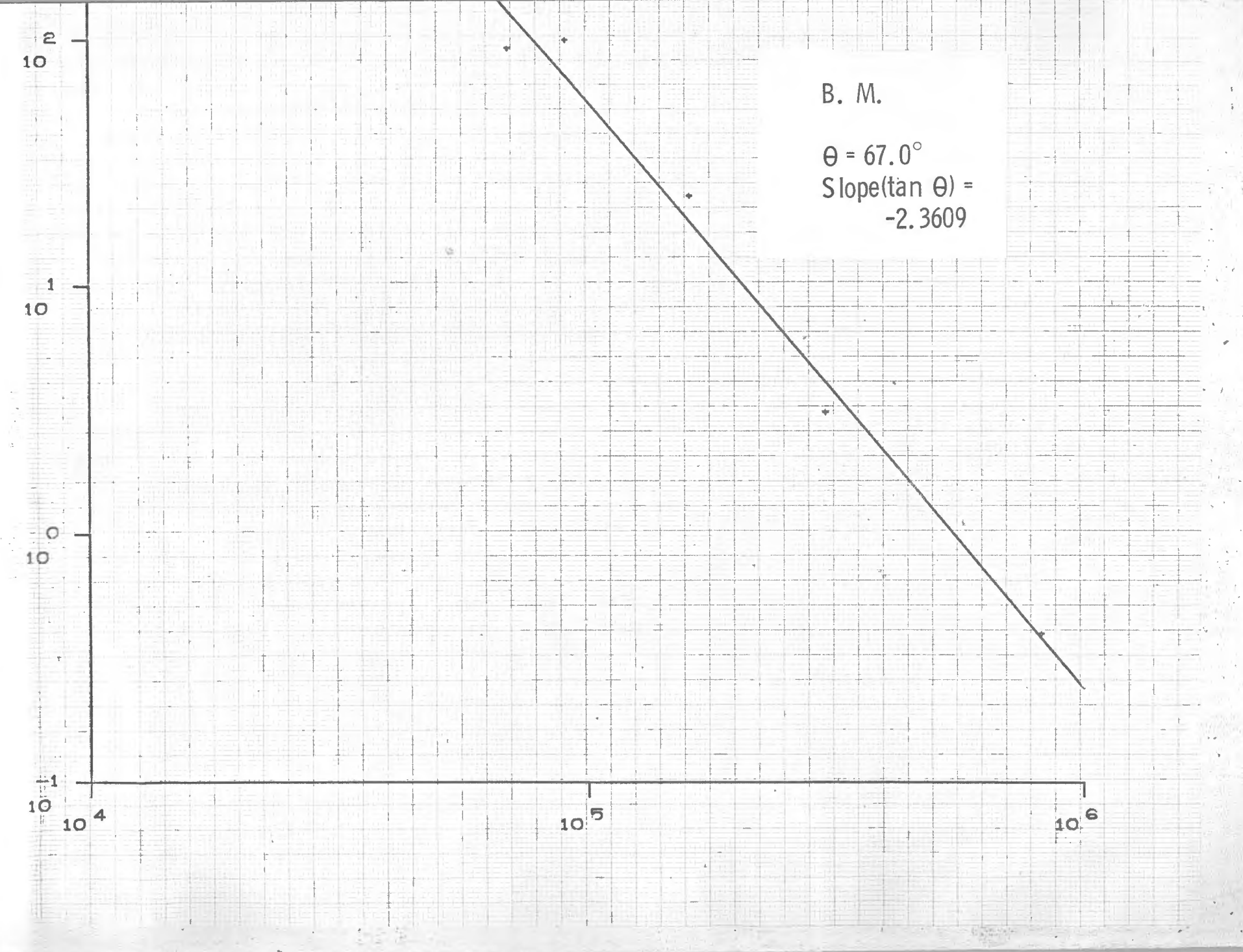

M. G.
θ = 62.8°
Slope(tan θ) =
-1.9429
3
10
2
10
1
10
0
10
-1
10
10^4
10^5
10^6

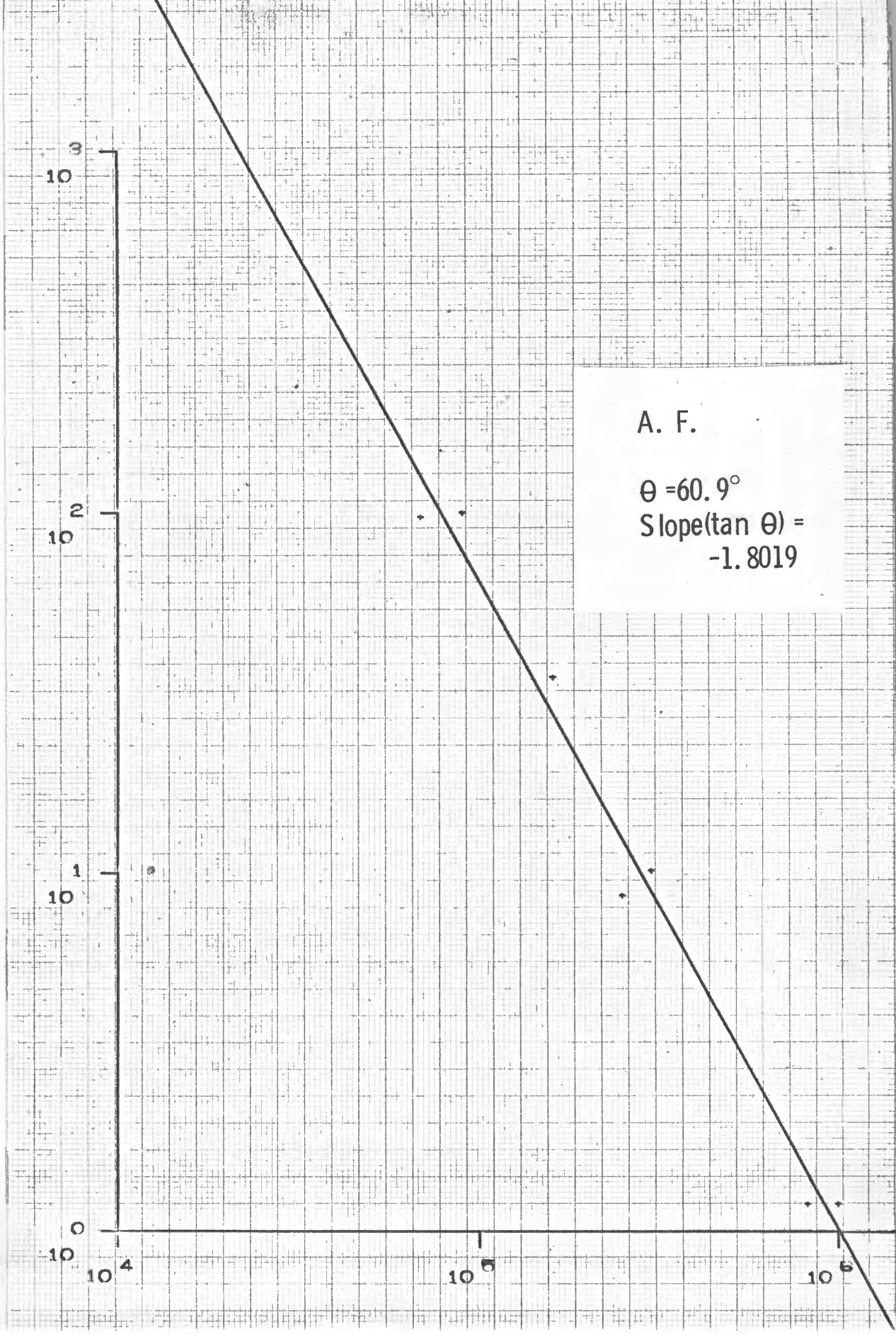

S. D.
θ = 64.3°
Slope(tan θ) =
-2.0805
10^3
10^2
10^1
10^0
10^-1
10^4
10^5
10^6

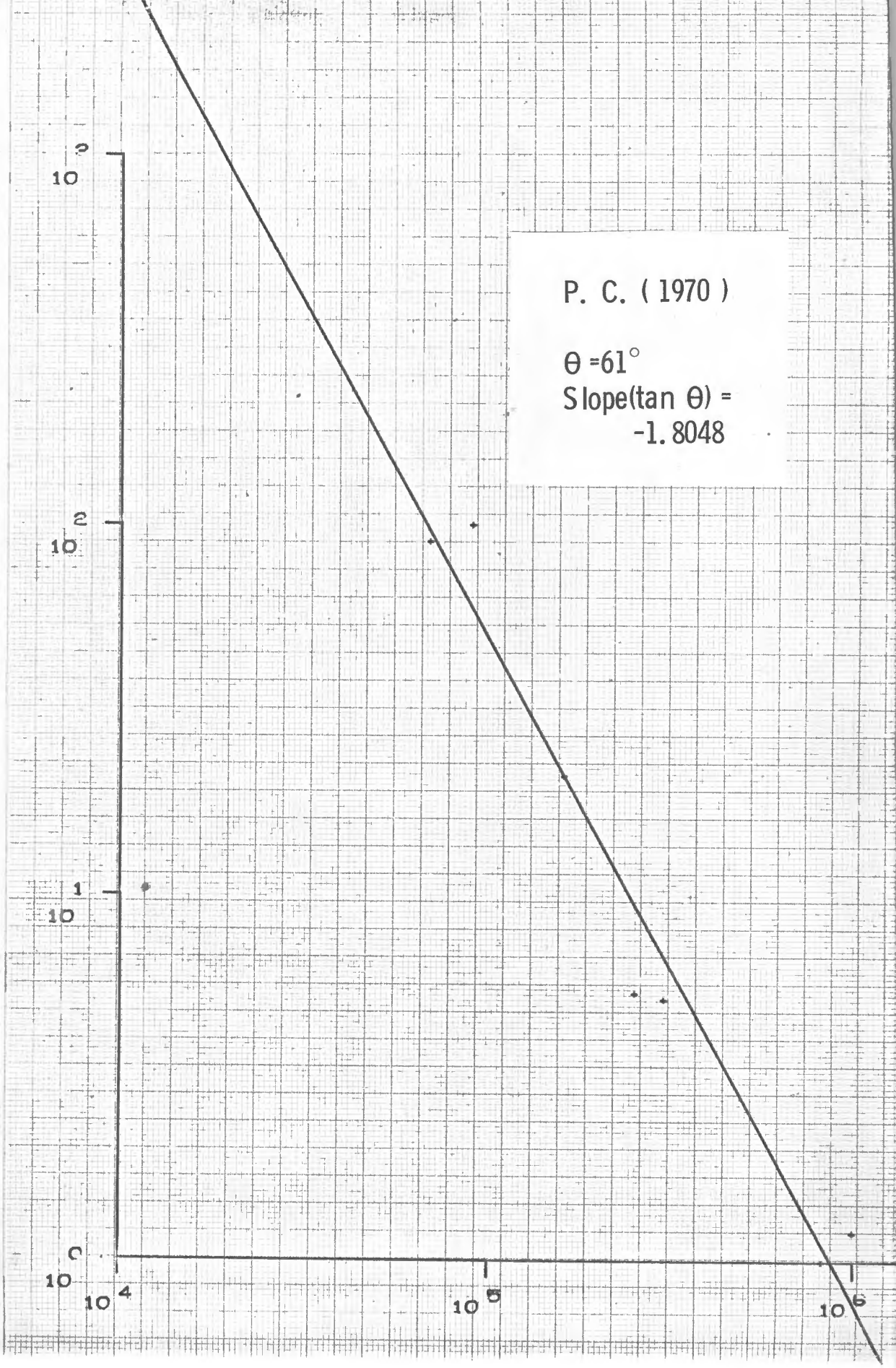

L. S.
θ = 65.7°
Slope(tan θ) =
-2.2167
10^3
10^2
10^1
10^0
10^-1
10^4
10^5
10^6

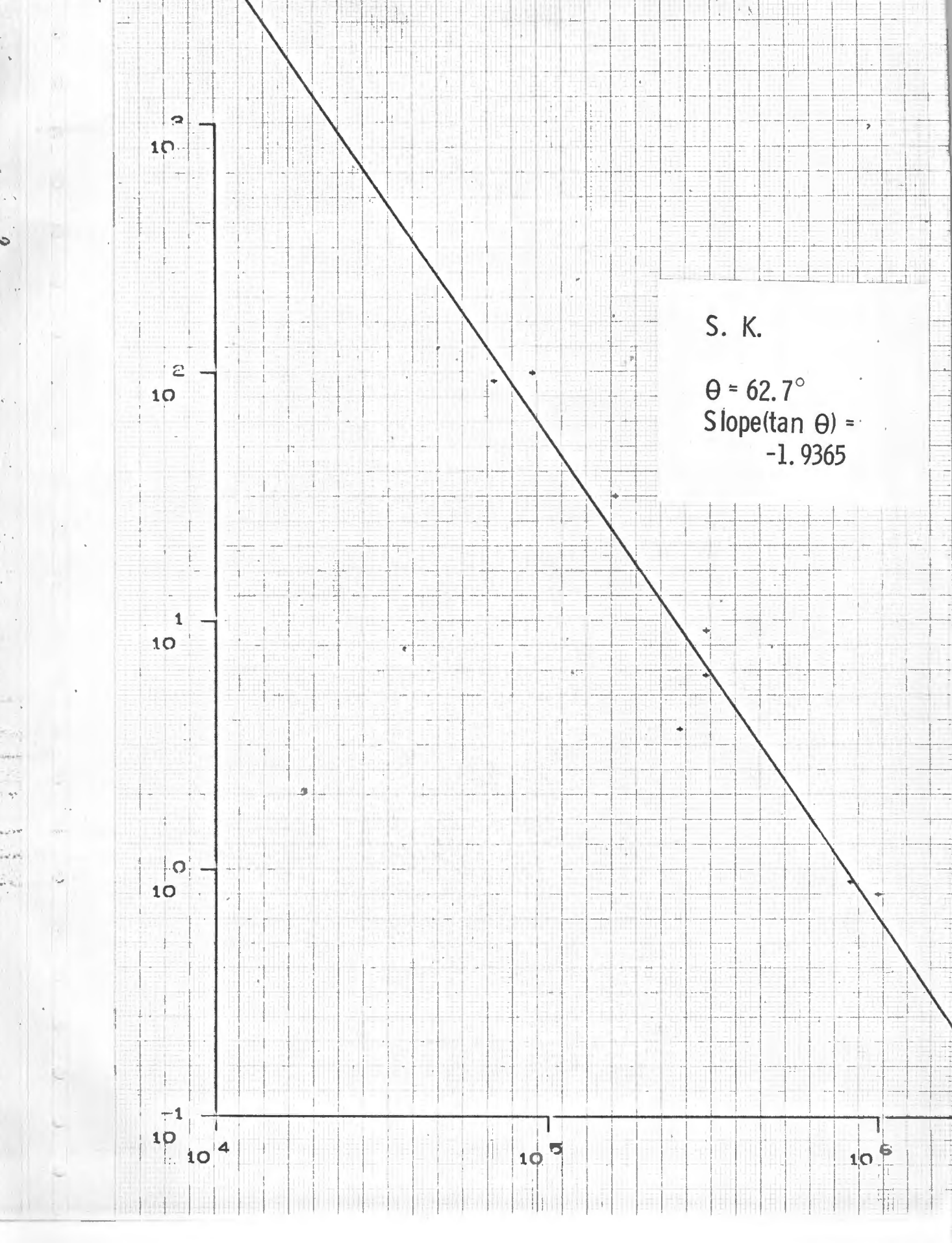

M. M.
θ = 63.4°
Slope(tan θ) =
-1.9928
10^3
10^2
10^1
10^0
10^-1
10^4
10^6

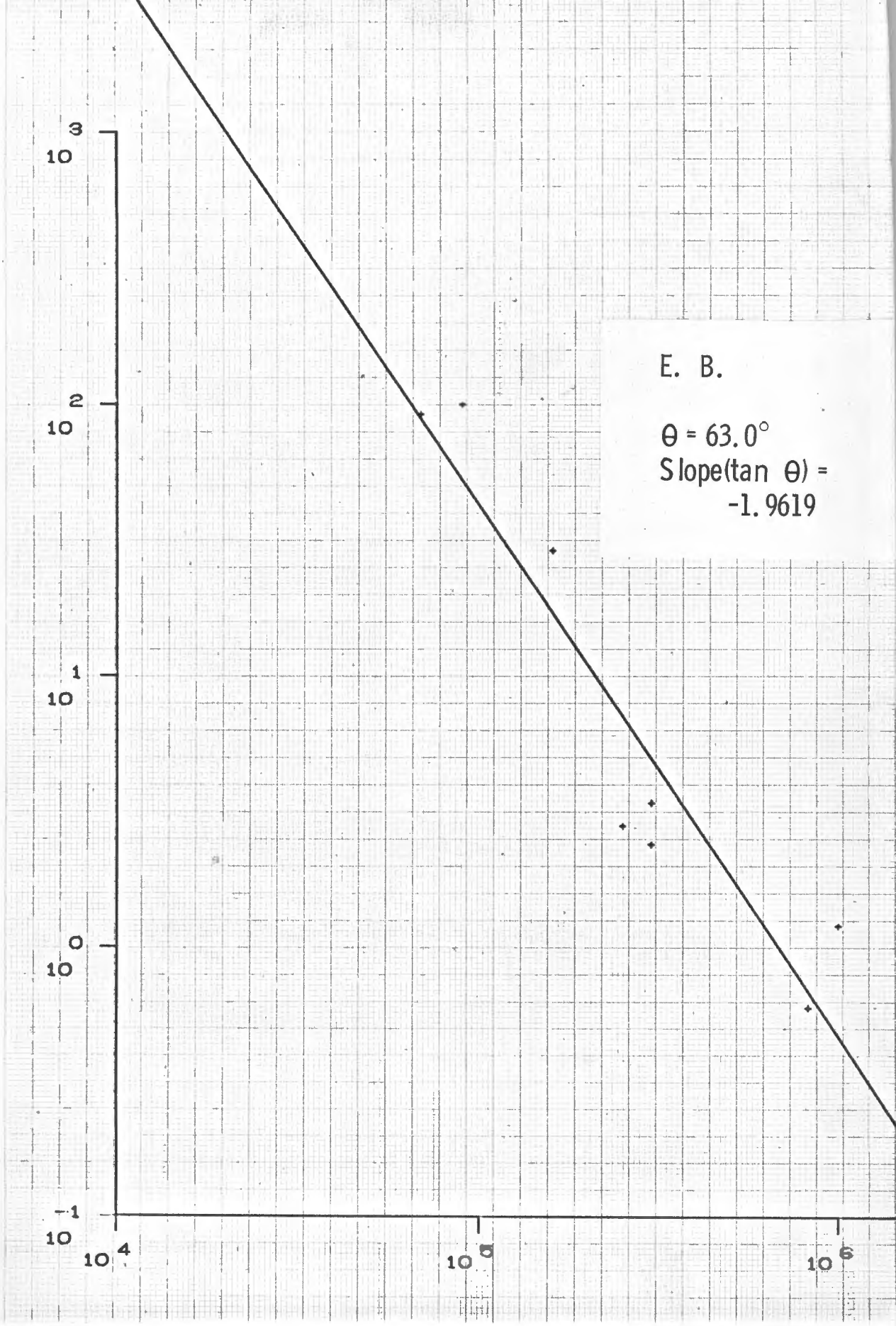

J. T.
θ = 63.0°
Slope(tan θ) =
-1.9669
10
1
10
10^4
10^5
10^6

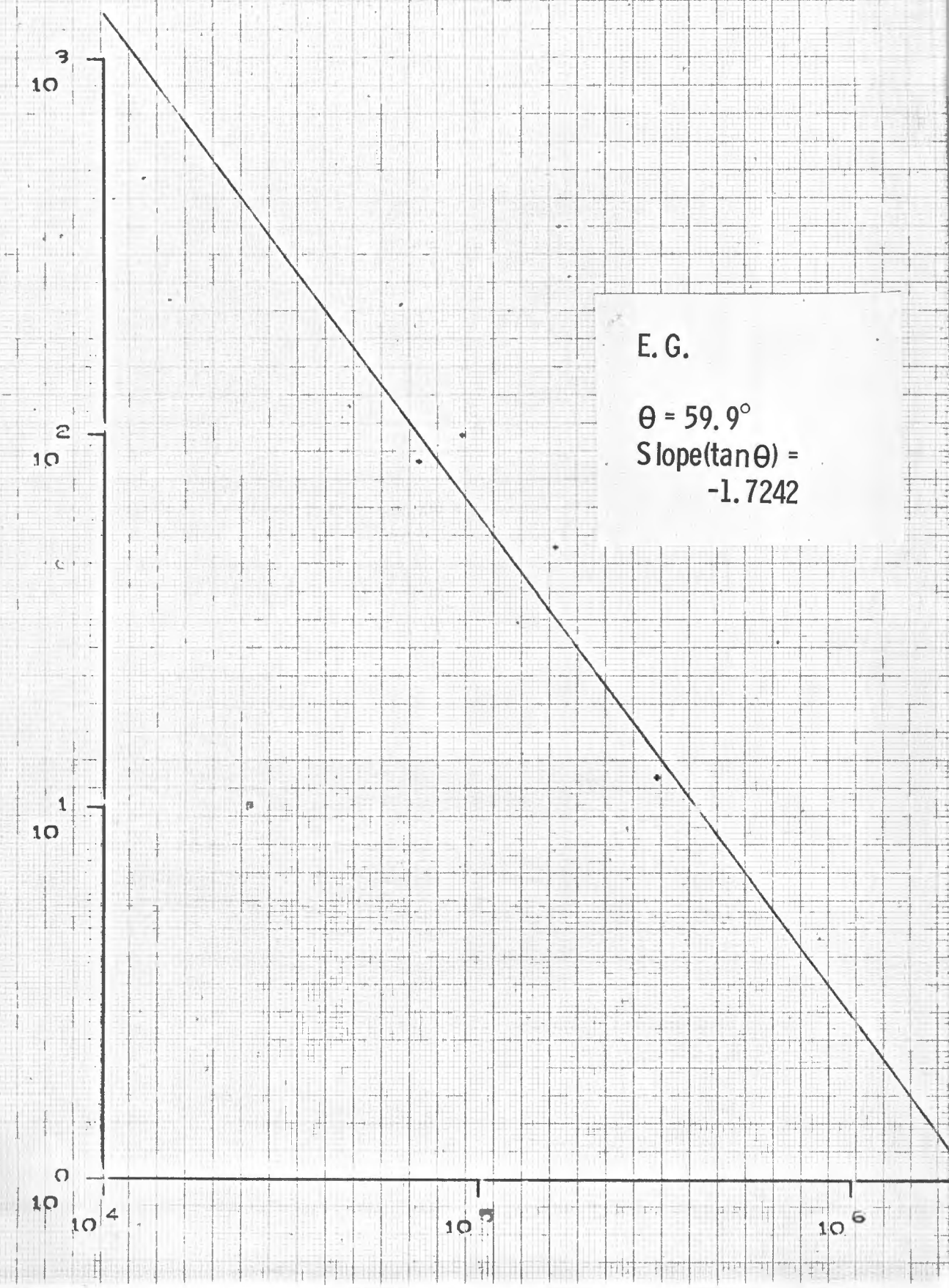

B. M.
θ = 67.0°
Slope(tan θ) =
-2.3609
2
10
1
10
0
10
-1
10
10^4
10^5
10^6

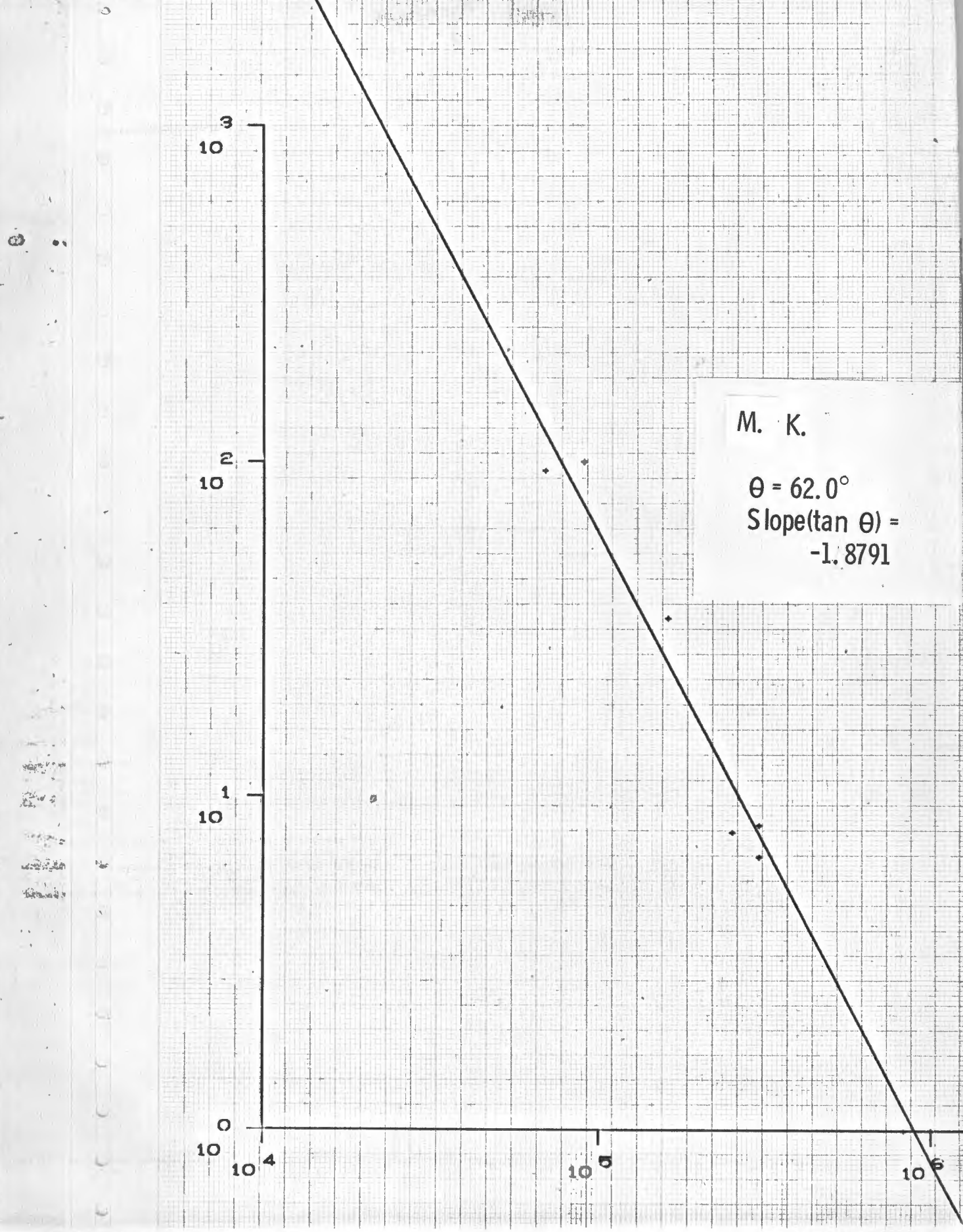

A. F.
θ =60.9°
Slope(tan θ) =
-1.8019
3
10
2
10
1
10
0
10
10 4
10 5
10 6

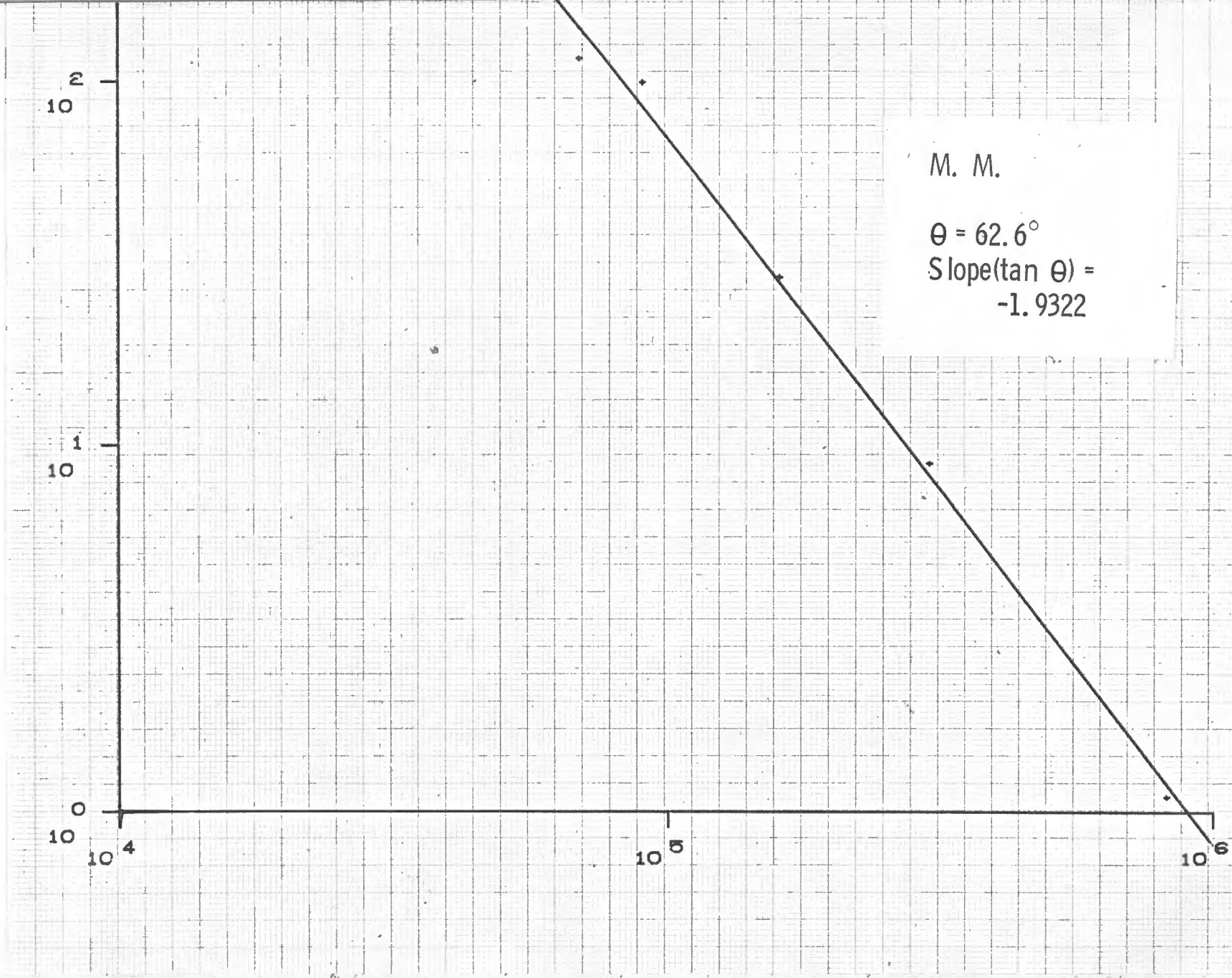

P. C. (1970)
θ =61°
Slope(tan θ) =
-1.8048
10^2
10^2
10^1
10^0
10^4
10^5
10^6

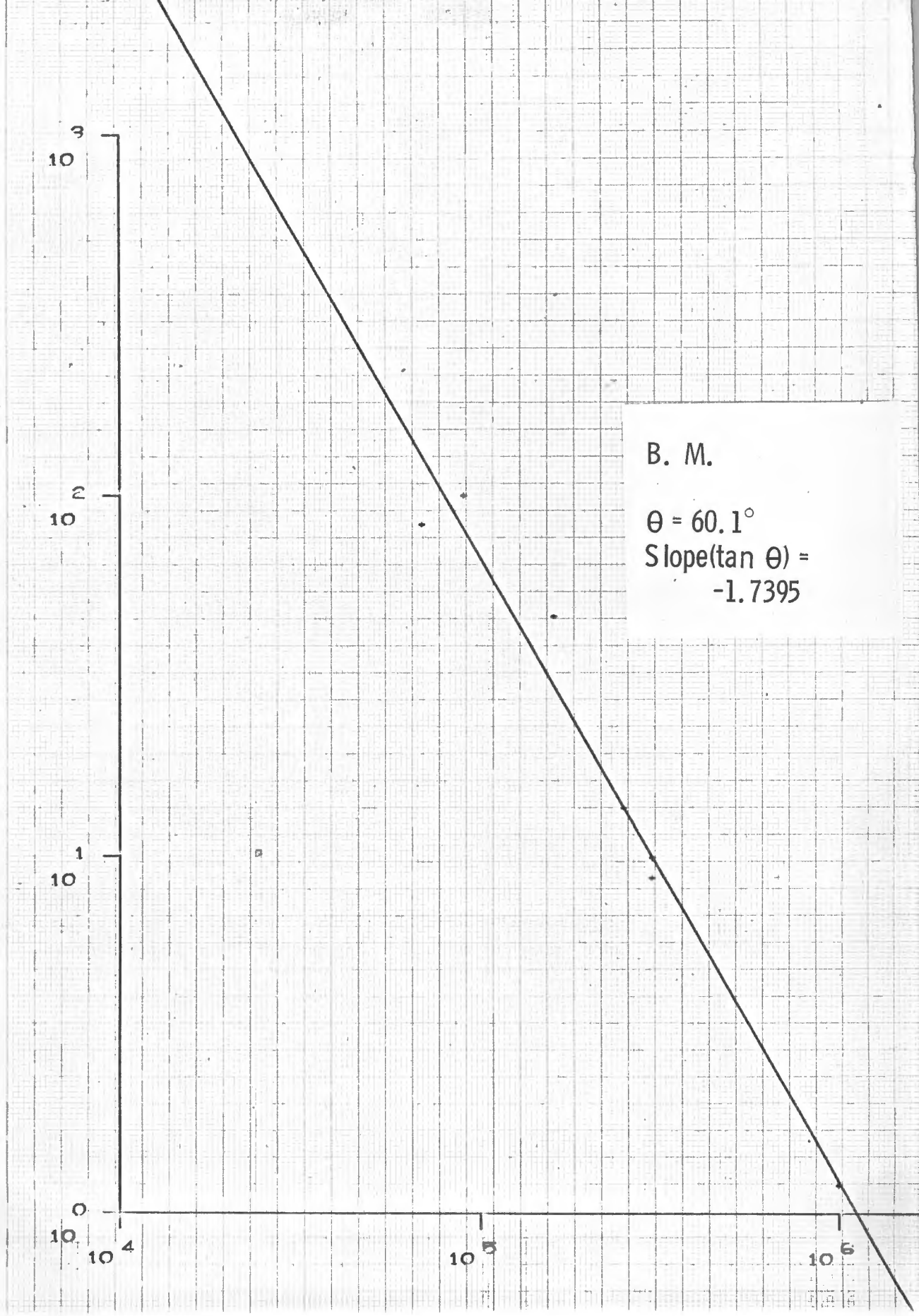

S. K.
θ = 62.7°
Slope(tan θ) =
-1.9365
10^{2}
10^{1}
10^{0}
10^{-1}
10^{4}
10^{5}
10^{6}

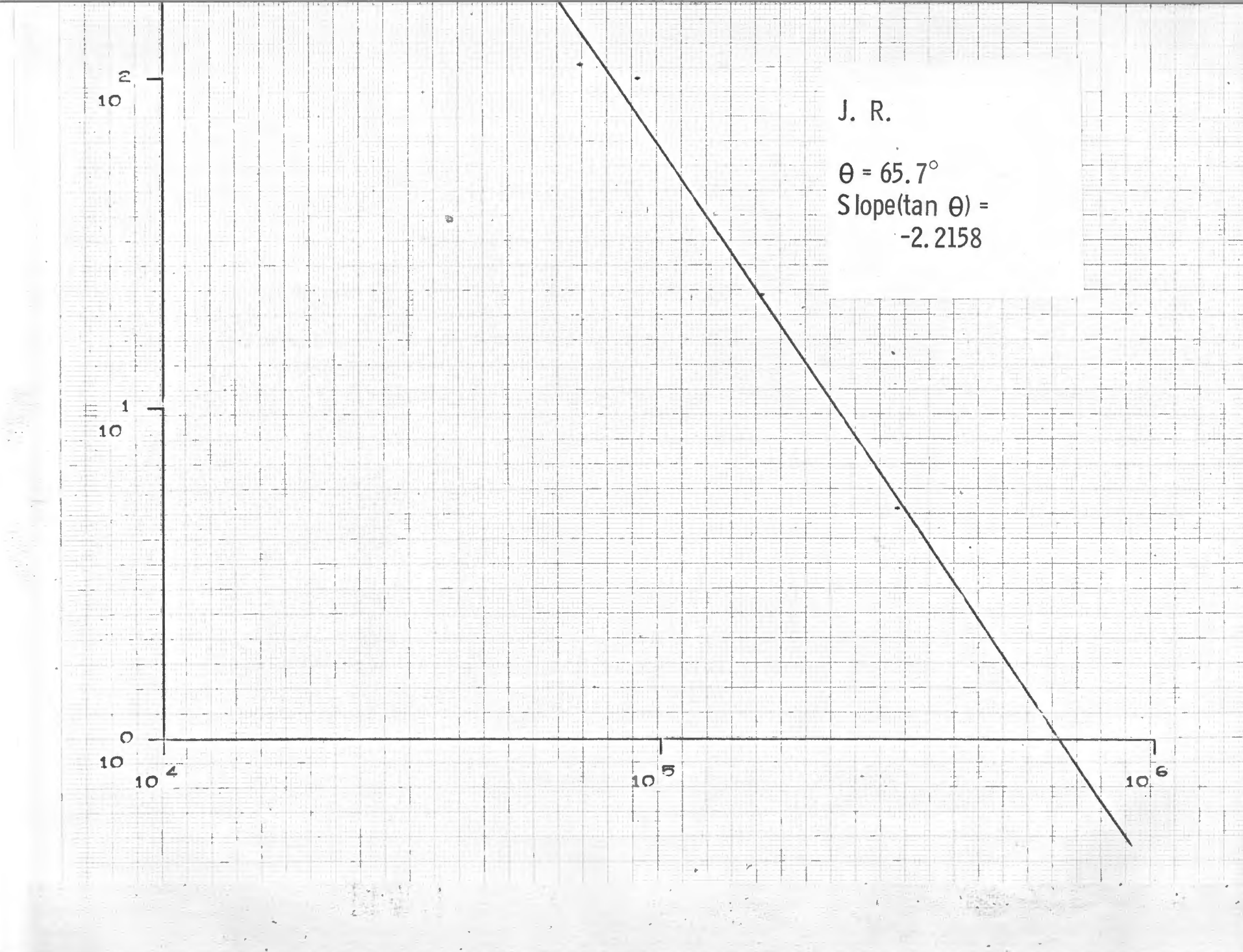

E. B.
θ = 63.0°
Slope(tan θ) =
-1.9619
10^3
10^2
10^1
10^0
10^-1
10^4
10^5
10^6

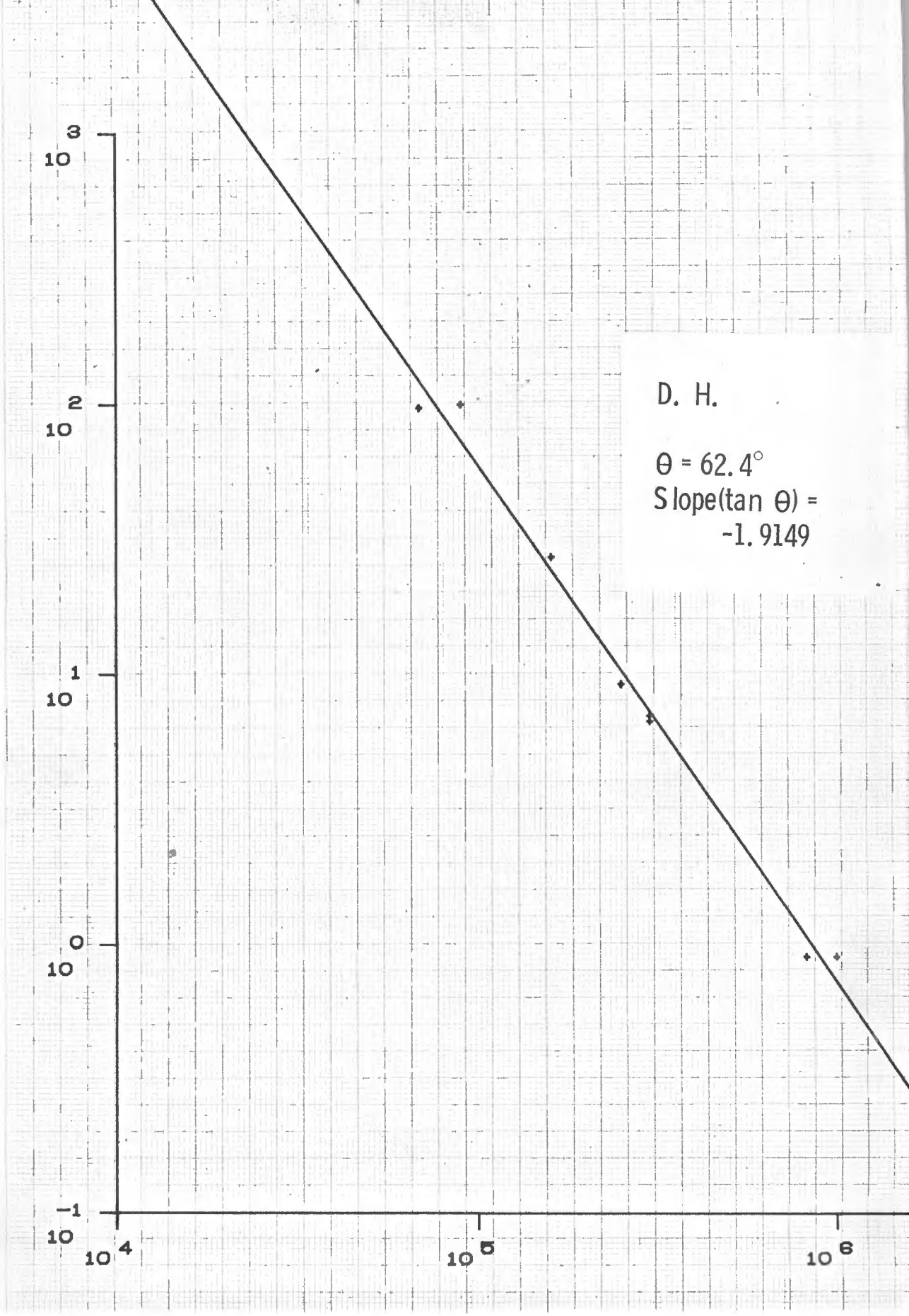

E. G.
θ = 59. 9°
Slope(tan θ) =
-1. 7242
10^3
10^2
10^1
10^0
10^-4
10^-3
10^-6

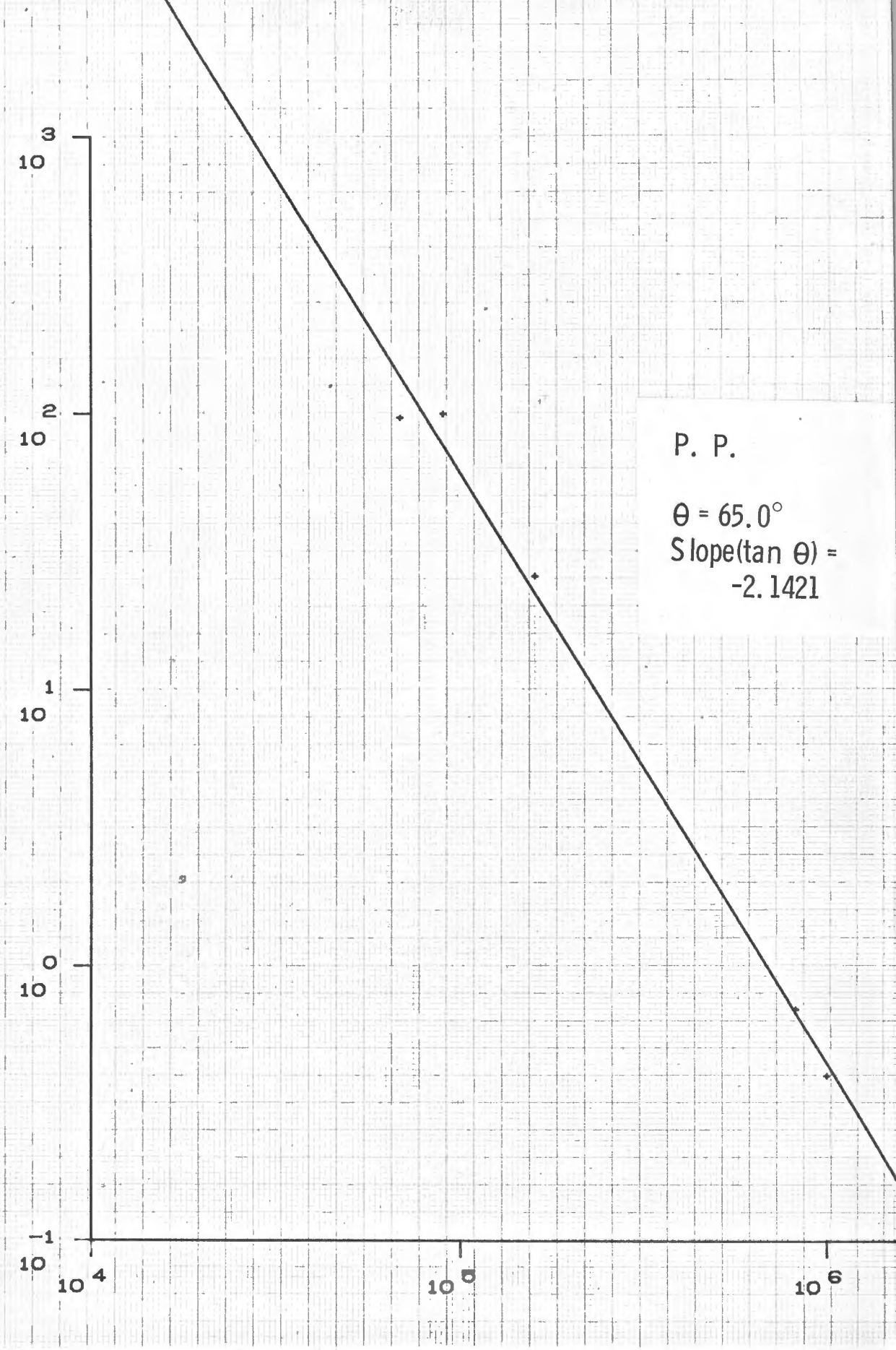

M. K.
θ = 62.0°
Slope(tan θ) =
-1.8791
10^3
10^2
10^1
10^0
10^4
10^5
10^6

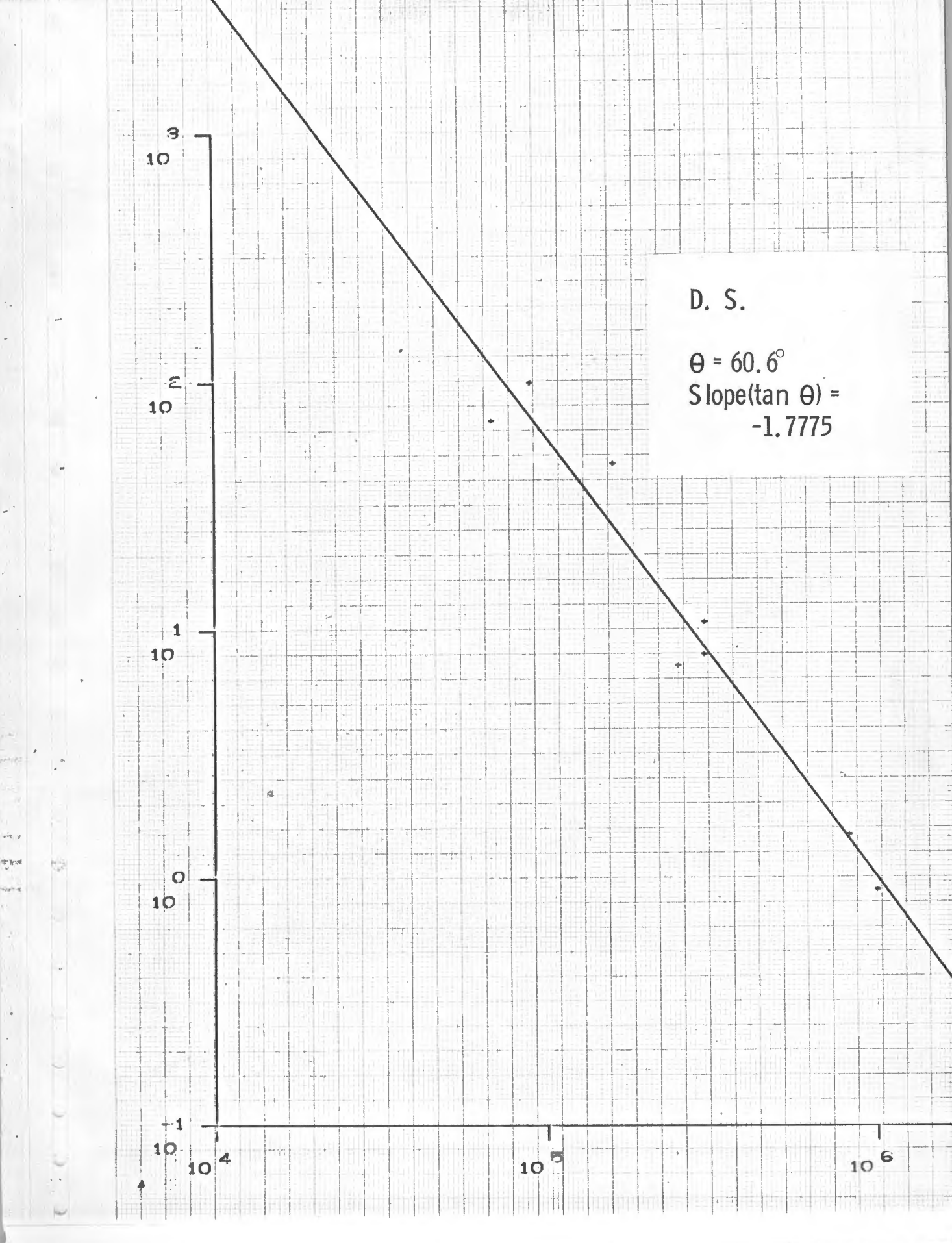

M. M.
θ = 62.6°
Slope(tan θ) =
-1.9322
10^2
10^1
10^0
10^4
10^5
10^6

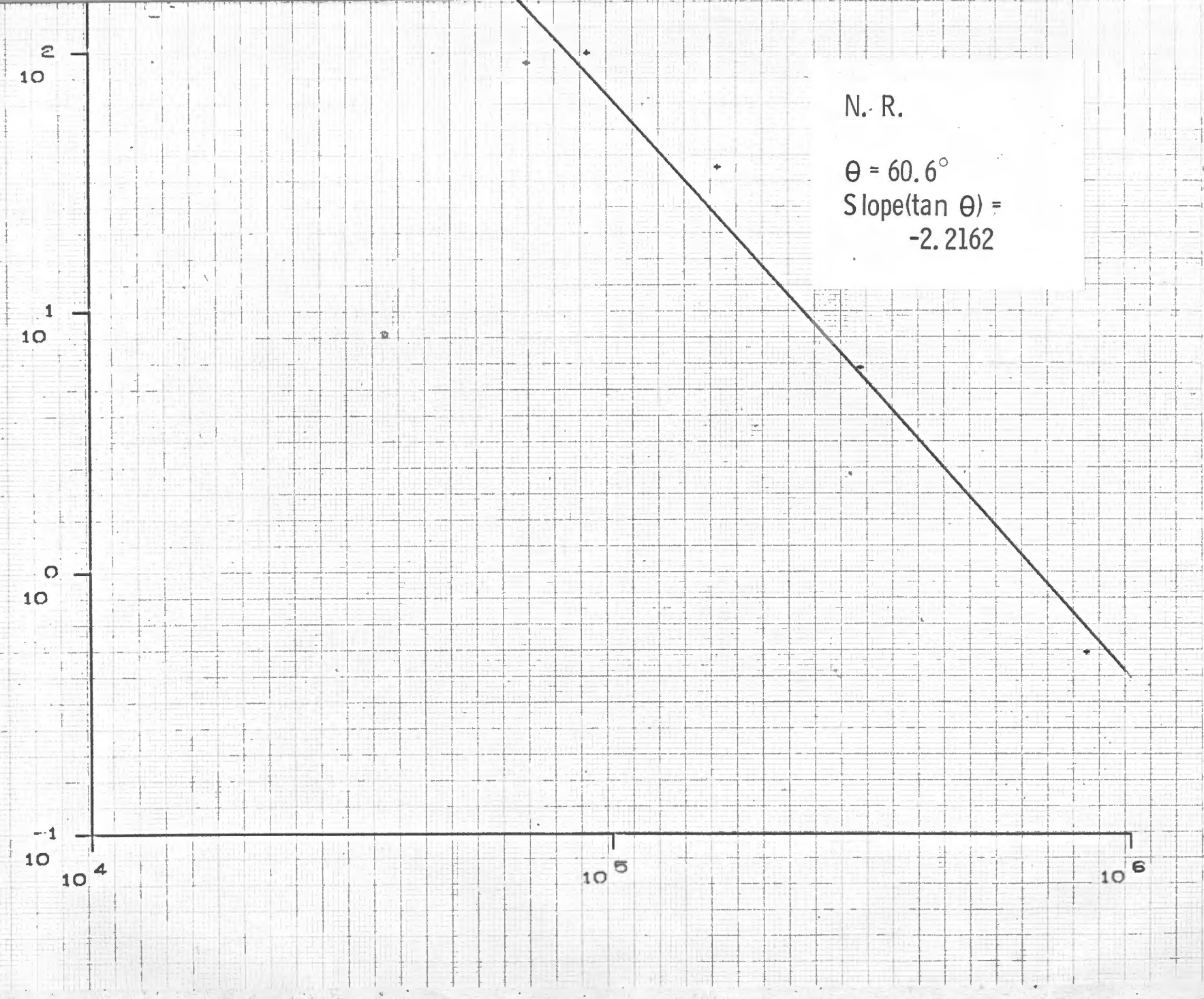

B. M.
θ = 60.1°
Slope(tan θ) =
-1.7395
3
10
2
10
1
10
0
10
10 4
10 5
10 6

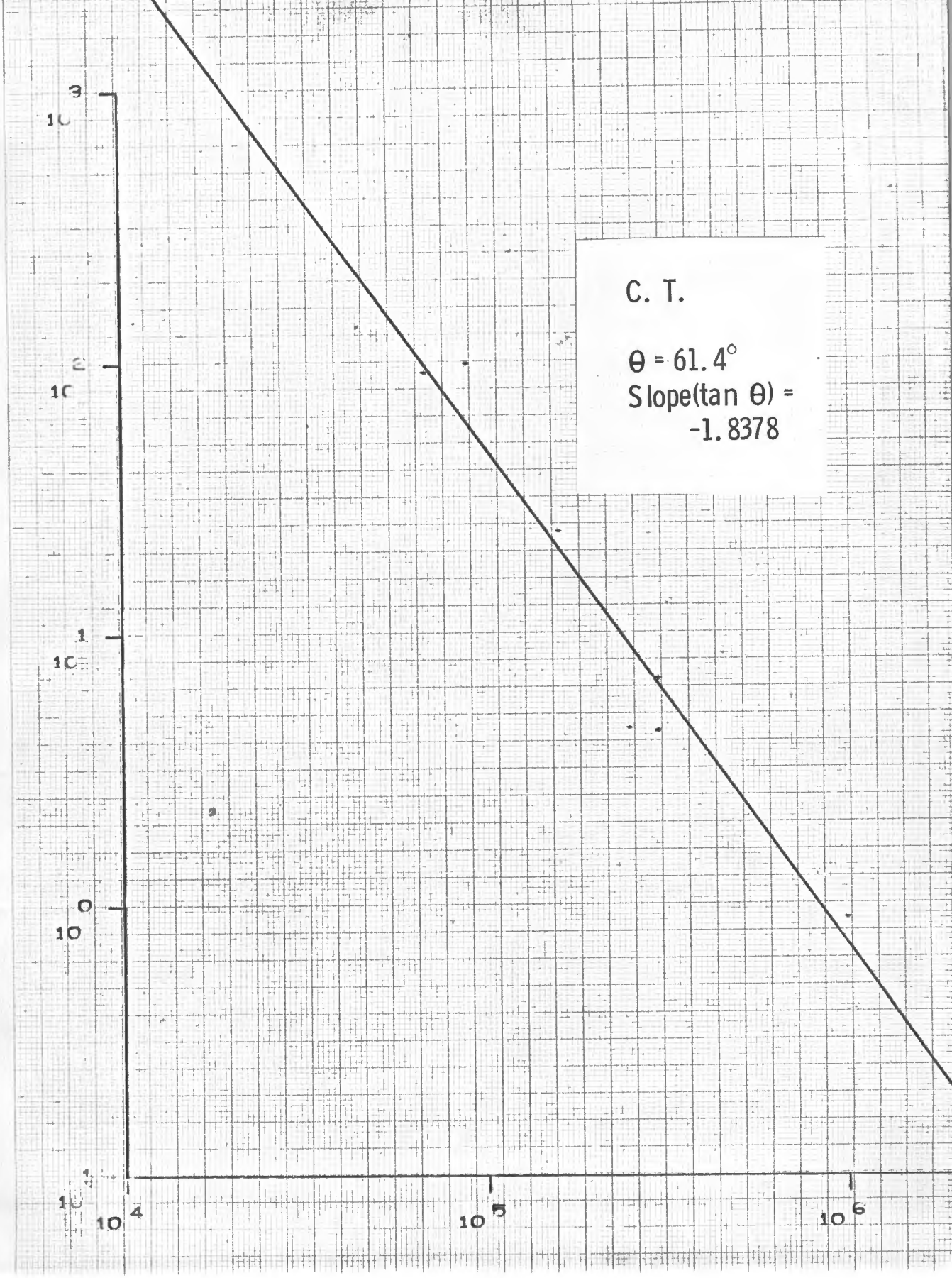

J. R.
θ = 65.7°
Slope(tan θ) =
-2.2158
10^2
10^1
10^0
10^4
10^5
10^6

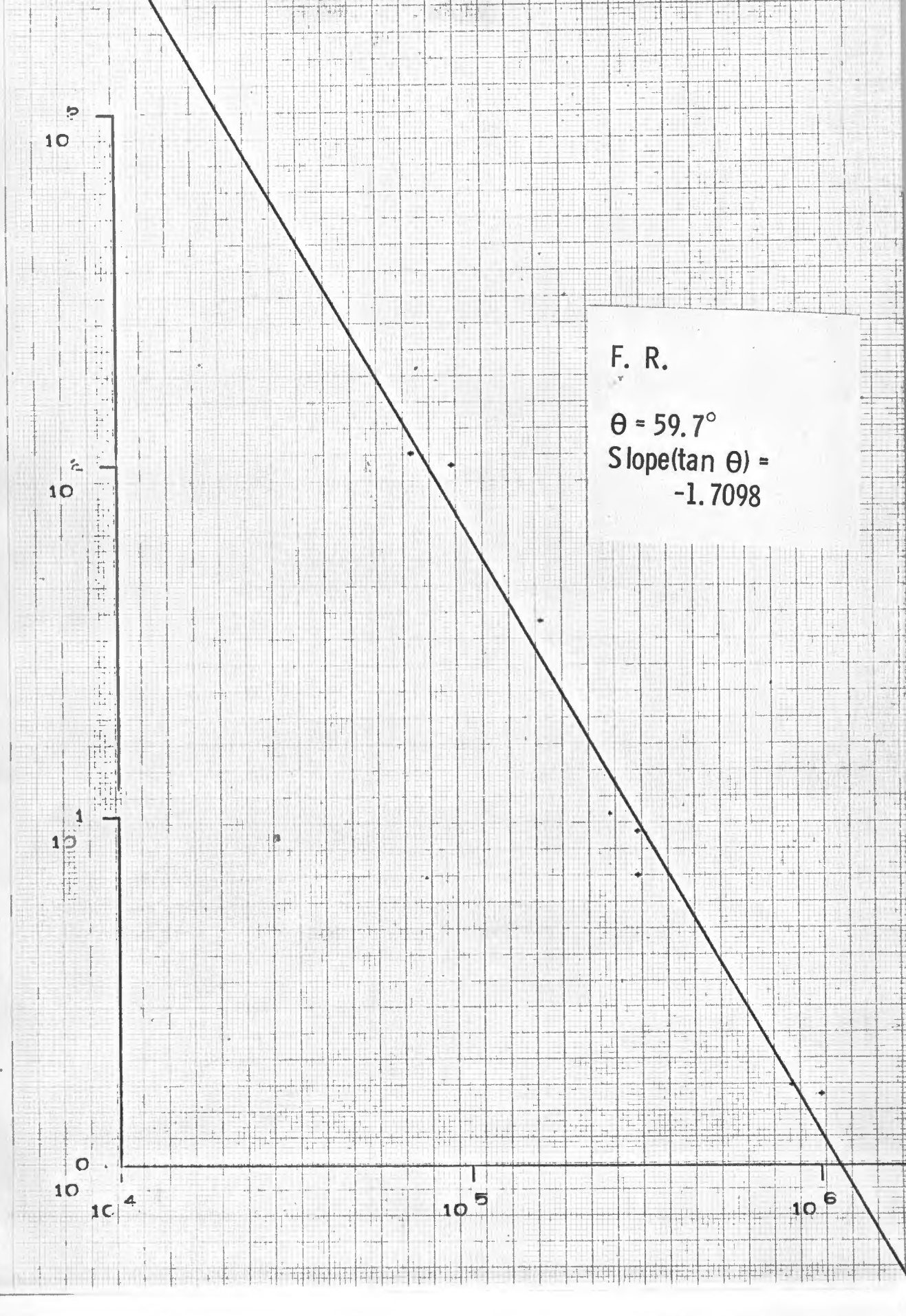

3
10
2
10
1
10
0
10
-1
10
10^4
10^5
10^6
D. H.
θ = 62.4°
Slope(tan θ) =
-1.9149

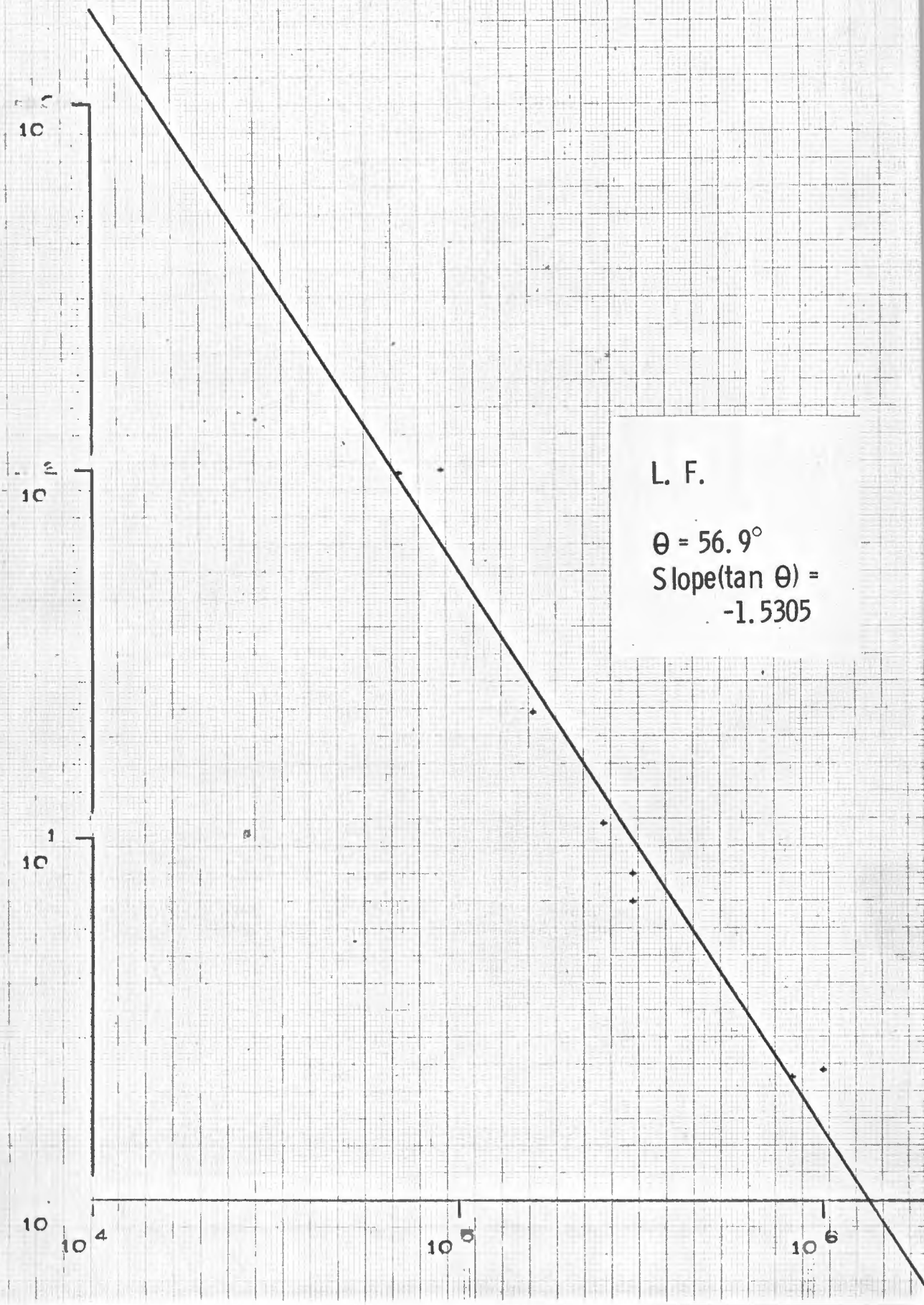

P. P.
θ = 65.0°
Slope(tan θ) =
-2.1421
3
10
2
10
1
10
0
10
-1
10
10 4
10 5
10 6

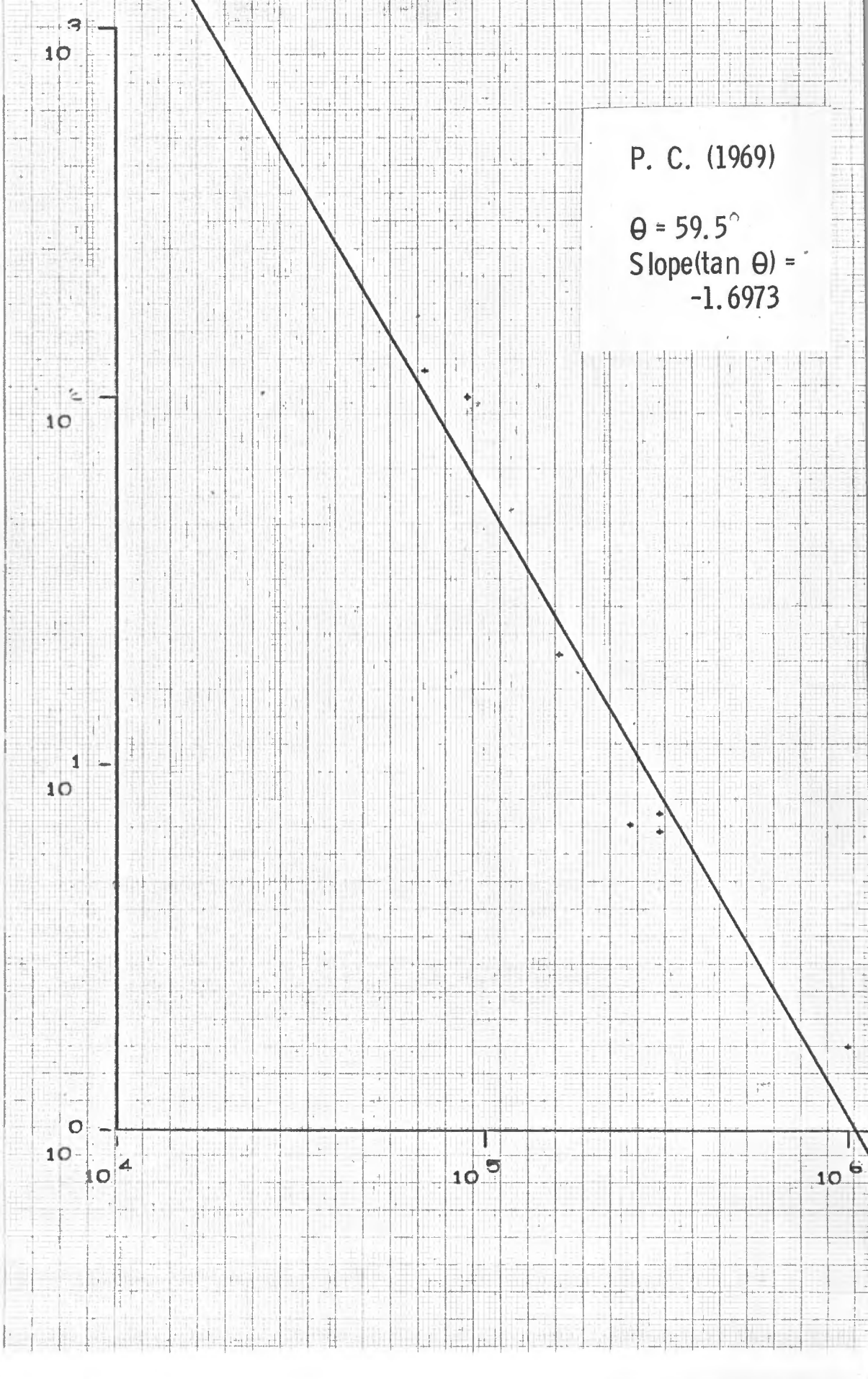

D. S.
θ = 60.6°
Slope(tan θ) =
-1.7775
10^3
10^2
10^1
10^0
10^-1
10^4
10^5
10^6

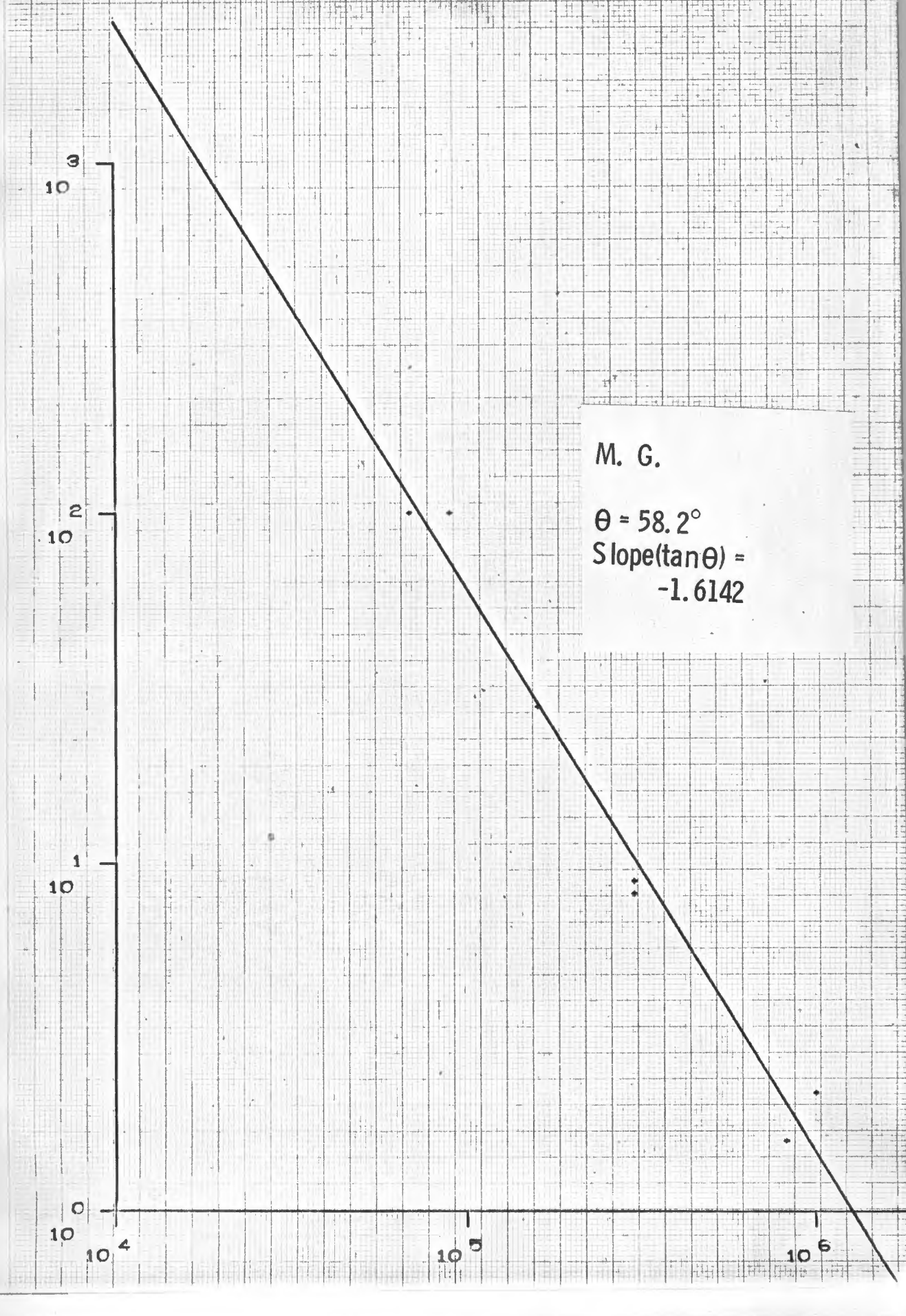

N. R.
θ = 60.6°
Slope(tan θ) =
-2.2162
10^2
10^1
10^0
10^{-1}
10^4
10^5
10^6


C. T.
θ = 61.4°
Slope(tan θ) =
-1.8378
3
10
2
10
1
10
0
10
10^4
10^5
10^6


F. R.
θ = 59.7°
Slope(tan θ) =
-1.7098
10
10
10
10
10
10
10
10
10


L. F.
θ = 56.9°
Slope(tan θ) =
-1.5305
10
10
1
10
10
10 4
10 5
10 6


P. C. (1969)
θ = 59.5°
Slope(tan θ) =
-1.6973
3
10
2
10
1
10
0
10
10^4
10^5
10^6


3
10
2
10
1
10
0
10
10^4
10^5
10^6
M. G.
θ = 58.2°
Slope(tanθ) =
-1.6142

A D D E N D U M

In order to confirm the hypothesis that differences in protein excretion patterns exist between patients with pre-eclampsia and those with hypertension, the influence of several other variables on renal protein selectivity was tested in the patients studied.

Influence of parity on renal selectivity

There were 28 primiparous patients and 15 multiparous patients studied. Mean selectivity for the primiparous group was 0.279 (S.D. 0.075). Mean selectivity for the multiparous group was 0.28 (S.D. 0.09). There is no significant difference between these means. (t on 41 degrees of freedom = 0.208 P = 0.836)

Influence of age on renal protein selectivity

There was no relationship between age and protein selectivity. The calculation of the linear regression curve of selectivity on age gave a correlation coefficient R = 0.049 t on 41 degrees of freedom = 0.314 P = 0.38

Influence of the stage of onset of proteinuria on selectivity

Selectivity rates were tested against the gestational age at which proteinuria first appeared. No relationship was found to exist between these two variables. R = 0.08968 t on 40 degrees of freedom = 0.56947. P = 0.29.

Influence of renal function on selectivity

The extent to which selectivity was influenced by renal function (as determined by creatinine clearance) was examined. No relationship was found to exist between creatinine clearance and protein selectivity. R = - .00518. t on 34 degrees of freedom = - .03022 P = 0.49

Influence of blood pressure levels on selectivity

The relationship between selectivity and the magnitude of diastolic blood pressure was examined. No relationship was found between these two variables. R = 0.14998. t on 41 degrees of freedom = 0.97130 P = 0.17.