Biochemical Changes in Urine with Urinary Tract Infection

BY

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Introduction

I. INTRODUCTION

Urinary tract infection is a common disorder at all ages and in both sexes. A healthy and normal Urinary tract is generally resistant to infection (Satoskar R.S., Bhandarkar S.D., 1988). Urinary tract infection is second in frequency to that of upper respiratory infection. (Chakraborty P., et. al., 1972). The incidence runs as high as 20 - 25% of the population (Engel. G, et.al., 1980).

The Urinary tracts of men differ from those of women. So it should be no surprise that urinary tract infection differ between both the sexes. Generally, men have fewer urinary tract infection despite having a less stringent criterion for diagnosis. Different organisms infect men, (Anthony J. Schaeffer, 1989).

Beginning with the onset of sexual activity, women have a 50 fold higher incidence of bacteriuria than men and this persists until they reach the geriatric age group when the prevalance is approximately the same among both the genders. Young, sexually active women between 18 and 35 years of age constitute the major risk group for urinary tract infection. (Stephan D.Fihnn, 1988).

Urinary tract infection is perhaps the most common complication of pregnancy resulting in high incidence of prematurity and abortions

(Yashodhara P. et.al., 1987). The prevalance of bacteriuria during pregnancy is 4 to 7% . Pregnancy itself does not appear to predispose to bacteriuria; this merely reflects the frequency of bacteriuria among young, sexually - active women (Patterson T.F., Andriole V.T., 1987).

Urinary tract infection is one of the major bacterial diseases of childhood. The risk of newborn girls falling ill during childhood with a symptomatic urinary tract infection is atleast 3 % ; for a boy about 1% (Jan Winberg, 1978). Several important characteristics distinguish urinary tract infection in infancy and childhood from the disease observed in the adult population. (Julia R. Spencer; Anthony J.Schaeffer.,1986).

Urinary tract infection is a common cause of morbidity and mortality in our community and is responsible for prolonging the days of hospitalization in surgical patients (Singh U.K., Bela Saigal, 1981). One to 3% of hospitalised urinary tract infections (Nocosomial urinary tract infection) result in secondary bacteremia and 10% of these infections are fatal (Erik H. Larsen et.al., 1986).

Urinary tract infection (UTI) is a common urologic condition and the diagnosis cannot be made without the bacteriological examination of urine (Engel et.al., 1980). So, UTI is defined as a condition characterized by the persistence of active multiplying bacteria in bladder urine. It occurs when 100,000 or more organisms are present in 1.0 ml of urine. When infection is present, WBC usually accompany with bacteria (Barar F.S.K., 1985).

Urinary tract infection may present itself in acute or chronic form. Acute infection localized to urethra and bladder causes increased frequency and urgency of micturition, dysuria and pain in the perineum. If the kidneys are involved (Pyelonephritis), the patients have loin pains, fever, chills and leucocytosis. Chronic Pyelonephritis may follow inadequately treated acute infections and is likely to develop in patients with a predisposing cause (Satoskar R.S., 1988).

The predisposing factors include instrumentation of Urinary tract, the presence of urethral catheters, dementia and concurrent infection such as pneumonia. (Anthony J. Schaeffer, 1986). U.T.I.in females is because of the anatomy of the genitourinary system and in older males because of enlarged prostrate (Doifode et al., 1982).

E. Coli and other coliforms account for the majority of naturally acquired U.T.I. Those acquired in the hospital following instrumentation are usually by other bacteria such as Pseudomonas and Proteus. K. Pneumoniae is a frequent cause of urinary infection. Infection may also be precipitated by urinary obstruction due to Calculi (Ananthanarayan R. Jayaram Paniker C.K., 1987).

In normal individuals, the pH value of urine is between 5.5 to 6.5 and is acidic. The quantity of Phosphorus excreted varies with dietary intake. Since Na and K are major cations of the diet,

they are also the major cations of normal urine. Chloride is the chief anion of urine and the amount excreted is approximately equal to the amount that has been ingested. The daily urinary excretion of calcium varies from 0.1 to 0.3g and it varies with the intake of diet. Normal urine contains traces of Protein (White, A. et al., 1978).

Tamm - Horsfall protein is a large glycoprotein of unknown function produced only by cells in the ascending limb of the loop of Henle. It forms the matrix of urinary casts and the slime found in urine left standing overnight. In patients with obstructive kidney disease or several vesicoureteral reflux, Tamm - Horsfall protein is deposited in renal interstitium and Bowman's space where it stimulates the formation of antibodies of Ig A, Ig G and IgM (Curtis A. Sheldon, Ricardo Gonzalez, 1984).

Recurrence of urinary infection is a common feature and chronic infection can lead to dangerous sequal like hypertension and renal failure. Besides the Clinically mainfest disease, "Silent" infections also occur. Their diagnosis is difficult because the voided urine is not sterile and mere presence of micro-organisms is no proof of U.T.I. (Kamet et al., 1980).

Reinfection denotes the occurrence of sequential infection caused by different organisms. It occurs promptly and predictably

after discontinuing antimicrobial therapy. Persistent infection denotes the occurrence of subsequential infection caused by the same organism. (Jackson E. Fowler, 1986).

Diagnosis of urinary tract infection correctly done at the earliest possible time not only saves the excessive modern therapy but also prevents the further damage of the urinary system. A wrong therapy may also lead the sensitive organisms to become resistant, thus endangering life. (Patnaik et al., 1983).

In the present investigation, the biochemical changes in urine with urinary tract infection have been evaluated so that it could help in the early detection of infection and saves the excessive modern therapy.

Review of Literature

REVIEW OF LITERATURE

The review of literature pertaining to the study "Biochemical changes in Urine with urinary tract infection" is discussed under the following headings:

I Introduction

II Classification

III Acute and Chronic urinary tract infection

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V Urinary tract Infection in males

VI Urinary tract Infection in females

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XII Biochemical changes in Urinary tract infection.

a) Immunopathalogical changes

b) Changes in protein

c) Bacteriuria.

d) Changes in Sodium, Potassium and Chloride

e) Changes in Phosphorus and Calcium.

XIII Treatment.

1. INTRODUCTION

Urinary tract infection (U.T.I.) is undoubtedly one of the common, medical as well as Surgical, gynaecological and obsterical problems in all the under developed, developing and developed countries of the world. Its general incidence in population is 4.4% (Jain et al., 1987).

Urinary tract infection results when the urinary tract of susceptible host is invaded by virulent bacteria usually E. Coli (Ronald. P., 1989) U.T.I. is of greatest frequency in the early years of life because it so commonly complicates infection elsewhere particularly in the respiratory and gastrointestinal tracts et al., 1972). U.T.I. is thus a disease that causes morbidity and inconvenience to many patients. U.T.I. is the denomination of several conditions that have one feature in common - Presence of significant amount of bacteria in urine. There is reason to believe that U.T.I. is not one disease entity but several diseases that differ in Pathogenesis (Harrison et al., 1978).

Urinary tract infection is synonymous with acute cystitis i.e., a bacterial infection limited to the urinary bladder. (Komaroff AL, 1984) U.T.I. sometimes leads to acute Pyelonephritis and bacteremia and is a major cause of morbidity (Stephan D. Fihn., 1988), U.T.I. is probably the most common complication of neurogenic bladder patients. Neurogenic bladder disturbances can lead to a tendency to infection caused by the presence of high residuals

of urine and stasis of the upper or lower urinary tract (Shlomo Raz and William E. Bradley., 1979).

U.T.I. are rare in patients with primary hypogammaglobulinaemia. This suggests that antibodies play little part in protecting against organisms such as E.Coli which commonly causes cystitis (Webster A.D.B. et. al., 1982). Vesico-urethral reflux and attempt of instrumentation of urinary tract may enhance spread of infection. (Doifode et.al., 1982).

2. CLASSIFICATION:

Urinary tract infection is used as a noncommittal term preferred when the localizattion of the infection has not been determined.

a) Classification with regard to Pathogenesis:

(i) Non obstructive UTI

(ii) Obstructive UTI

a) Neurogenic bladders.

b) "Screening bacteriuria"

b) Classification with regard to localization of infection:

(i) Urethritis - It remains to be proven that true bacterial Urethritis exists.

(ii) Cystitis and Pyelonephritis

(iii) Pyelitis - This is a term used especially in Europe to denote a febrile UTI. Bacteria reaching the renal pelvis may cause local inflammation with loin Pains.

(iv) Chronic Pyelonephritis - To describe a clinical condition characterized by continuous excretion of bacteria or by frequent recurrences of infection.

(v) Cystourethral Syndrome - This is often used for patients with the classic symptoms of "cystitis" but who are lacking demonstrable bacteriuria, Pyuria, suggesting inflammation.

Acute infection localized to urethra and bladder is known as cysto - urethritis.

(c) Classification with regard to management :

(i) First infection

(ii) Unresolved bacteriuria during therapy

(iii) Bacterial persistence "Recurrent"

(iv) Reinfections Urinary infections

The term "recurrent" obviously applies to patients with either bacterial persistence in a focus within the urinary tract or to reinfections from outside the urinary tract.

The term unresolved emphasize that the initial therapy has been inadequate (Jan Winberg, 1978).

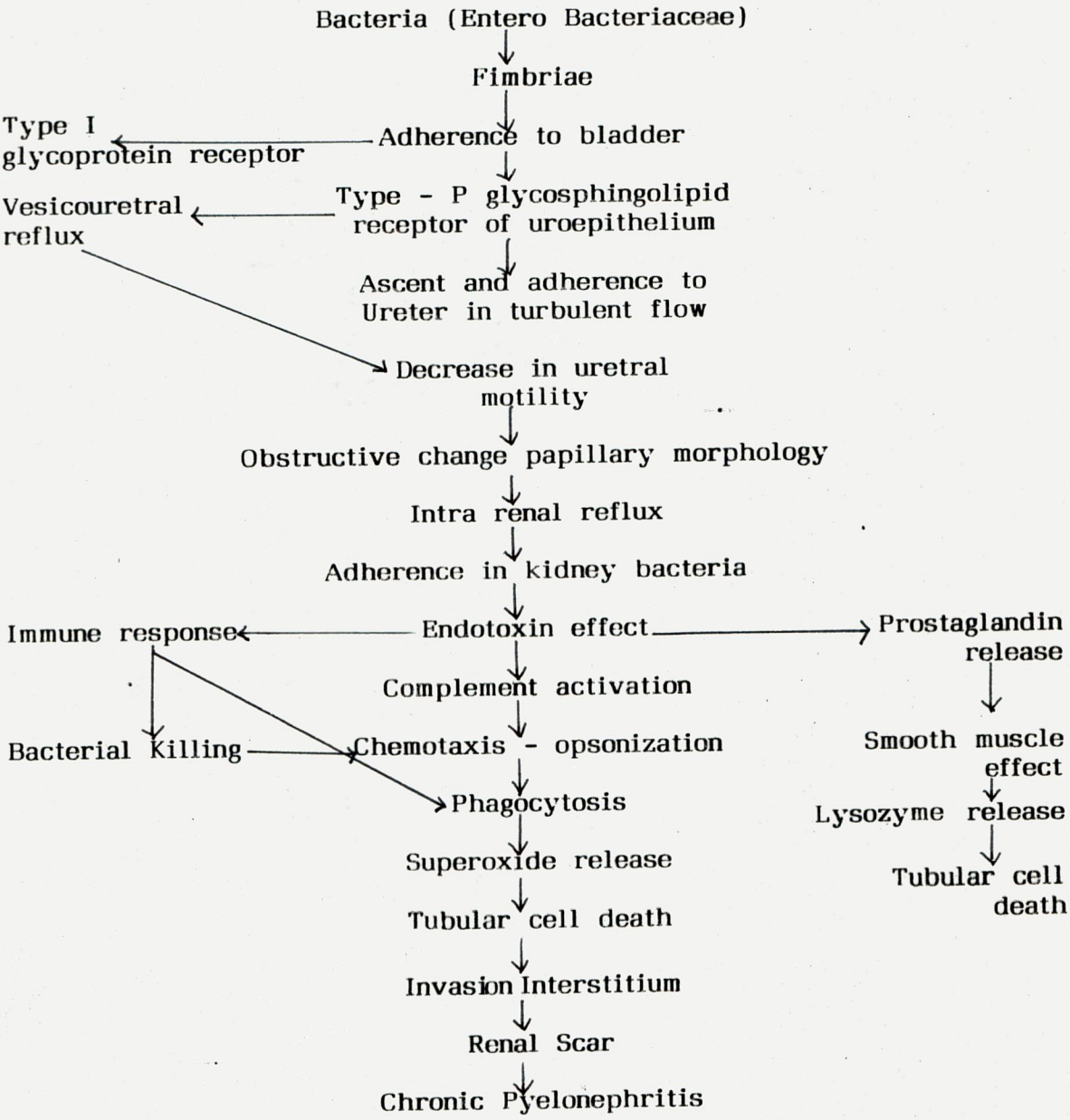
ACUTE AND CHRONIC URINARY TRACT INFECTION

Urinary tract infection may present itself in acute or chronic form.

Acute infection localized to the urethra and bladder (Cysto-urethritis) causes increased frequency and urgency of micturition, dysuria and pain in the perineum. Fever, chills and leucocytosis are generally absent. If the kidneys are involved (Pyelonephritis), the patient may have loin Pains, fever, chills and leucocytosis. Urine culture is positive and shows "significant bacteriuria". Urine is usually loaded with Pus cells in acute urinary infection. Chronic Pyelonephritis may follow inadequately treated acute infections.

Patients with chronic infection may have few urinary symptoms unless renal failure has supervene when Polyuria may be present. The urine may show a few pus cells. General loss of health and weight, anemia and hypertension are frequently present (Satoskar R.S. and Bhandarkar S.D. 1988).Pyelonephhritis due to proteus is toxic as the ammonia produced by bacillus interferes with complement and natural defence mechanism Ananthanarayan R and Jayaram Panicker C.K., 1987).

Pathogenesis of Pyelonephritis



(Roberts. T., 1983).

Bacteria usually reach the kidney to produce Pyelonephritis by ascending via the ureter. The organisms invade the interstitial tissue of the kidney. Patients who are said to have "acute complicated Pyelonephritis" have the more virulent forms of inflammatory disease and display the commonly described urographic abnormalities of Pyelonephritis. These include (a) Generalized renal enlargement (b) a diminished nephrogram (c) a delayed Pyelogram. In addition to the above abnormalities, calyceal and ureteral dilation have occasionally been reported. This has been commonly attributed to a decrease in ureteral peristalsis caused by bacterial endotoxin, particularly in association with E.Coli infection (Joseph N. Corriere and Carl M. Sandler, 1982).

4)BACTERIA IN THE URINE:

The normal urinary tract, except for the distal urethra, is sterile. A few bacteria may, however, be found even in properly collected urine of apparently healthy individuals. The most widely used method for identification of U.T.I. is the quantitative (colony count) culture of urine. Colony counts above 100,000 per ml. are designated as Significant bacteriuria and are considered diagnostic of infection while those below 10,000 may be regarded as due to contamination. A few apparently healthy individuals with normal urinary tracts are those on corticosteroid therapy, They excrete in their urine, bacteria in excess of 100,000 ml. Such individuals are said to have asymotomatic bacteriuria (Barar F.S.K., 1985), on the other hand, every patient with dysuria

due to urethritis may not have urinary tract infection i.e., Symptomatic abacteriuria (Sattoskar R.S. and Bhandarkar S.D., 1988).

5. urinary Tract Infection in Males :

U.T.I. in the male is uncommon in the absence of bladder outlet obstruction. The incidence is significant, but latent urodynamic abnormalities in patients presenting with unheralded single or recurrent attacks of infection in the absence of previous or concurrent symptoms suggestive of an underlying urodynamic disorder such as outlet obstruction (Booth C.M. et.al., 1981) Urinary Symptoms in men aged between 15 and 55 should prompt a through search for their cause.

(a) Pathogenesis and Bacteriology :

Cox and Hinman and Stamey and associates showed over longitudinal studies that colonization of the urethra with organisms that cause U.T.I. Preceded the onset of acute-cystitis in individuals who were prone to infection. Colonization seems to be an important first step for infection. Fowler and Stamey showed in several studies that bacteria that were more likely to cause infection and were more often associated with infection had a higher adherence rate to squamous epithelium (Lowell Parsons, C, 1986).

95% of the U.T.I. are due to Gram-negative bacili. E.Coli

is the commonest offender and next to it are Proteus, Klibsiella, Aerobacter and Pseudemonas aeruginosa (Pyocyanea). [Edmund Farrar.W, 1983.]

b) Symptoms :

Symptoms in adults are pyuria, urinary discomfort, fever, renal type of pain, burning on urination, hematuria, nocturia and neurogenic bladder dysfunction (Bhupendera M. Tolia et.al., 1981).

6. URINARY TRACT INFECTION IN FEMALES:

It is estimated that 10 to 20% of females will experience bacterial infection of the urinary tract sometimes during life. Although many infections are accompained by alarming discomfort and voiding symptomatology, the infecting organisms are generally confined to the bladder urine (Jackson E. Fowler, 1986).

a) Pathogenesis and Bacteriaology:

Pathogenesis of U.T.I. in the females has been postulated to involve 3 primary mechanism : hematogenous, lymphatic and retrograde extension of organisms directly from the rectum.

(i) Hematogenous dissemination is the principal route by which Staphylococcal organisms seed the kidney leading to Pyelonephritis and may possible be an important route for E.Coli in patients who do not have vesicc urteral reflux.

(ii) Lymphatic obstruction has been proposed as a potential mechanism by which organisms could proceed from the vaginal Vault to the perivesical lymphatics up the ureters to the kidney or even from the colony to the kidney.

(iii) Retrograde involvement of the urinary bladder in infection is the most widely accepted mechanism for infection (Lowell Parson C, 1985).

The vast majority of these infections in pregnant women are uncomplicated, easy to cure and pose little or no threat to renal function (Martin C. McHenry and Allan J. Weinsten, 1983).

Urinary tract infection in pregnancy may exist in symptomatic or asymptomatic forms. Many studies suggest that both anemia and under nutrition are associated with a higher incidence of asymptomatic bacteruia. The high incidence of U.T.I. in pregnancy is attributed to changes in urinary tract leading to stasis and increased excretion of nutrients like glucose and B-Complex Vitamins etc. which form a good medium for bacterial growth (Yashodhara. P et.al., 1987). So urinary tract infection is perhaps the most common complication of Pregnancy (Warre R Jones, 1979).

As in cystitis, 70% of cases of Pyelonephritis are caused by E.Coli (Stmm. WE et.al., 1987). The second important cause of urinary tract infection is Staphylococcus saprophyticus, a coagulase

negative, Gram (+) Coccus usually distinguished by resistance to novobicin (Reid. G. Sobel. JD, 1987). The microbiology of U.T.I. during gestation is not different from that in non pregnant woman except that Streptococcus agalactiae may be found more commonly (Patterson TF and Andriole VT, 1987). Klebsiella Species cause about 5% of infections, whereas Enterobacter species and Proteus species cause 2% of infection outside hospital (Allan R. Ronald, 1984).

b) Symptoms :

The dysuria syndrome, or the sensation of pain or discomfort when voiding urine occurs sometime during the life of most women. It is usually accompained by urgency and frequency and sometimes by suprapubic pain and gross hematuria. Patients with acute pyelonephritis present with fever, chills, an ache in the lumber flank and generalised symptoms of headache and musclar pain (Allan R. Ronald, 1984).

7)URINARY TRACT INFECTION IN PEDIATRICS :

In this age group, urinary infection occurs three times more often in boys than in girls and is commonly associated with bacteremia. It is assumed that in many infant boys bacteremia is the primary event, U.T.I. developing secondarily. In addition, several authors have shown that asymptomatic urinary infection is not uncommon in the new born. Female infants who develop

febrile urinary infection can be anticipated to have structural abnormalities responsible for pyelonephritis (Edmond T. Gonzales 1985). When normal development of detrusor and sphincter coordination fails to occur in early childhood, voiding abnormalities may result, which complicate urinary tract infection (Julia R. Spencer and Athony J. Schaeffer, 1986). Reflux is more commonly diagnosed in boys during infancy and in girls during childhood peaking at 3 to 7 years of age. Reflux usually mild in degree has been noted to occur clinically in association with acute urinary tract infection and to disappear when the urine becomes sterile. Conversely, severe reflux contributes to the occurance of urinary infection because it provides a reservior of residual urine (Lower R. King; Selwyn. B. Levitt, 1986)

(a) Pathogenesis and Bacteriology :

Bacteria are believed to enter the urinary tract mainly by two routes: the hematogenous and ascending. It is generally assumed but not proved that most infections are ascending and that those appearing during the new born period are blood borne (Jan Winberg, 1978).

The group B beta-hemolytic streptococcus is the major pathogen for the newborn (Edmond T. Gonzales, 1985).

b) Symptoms :

Symptomatic neonatal urinary tract infection is a manifestation of generalised septicemia. The symptoms are varied :

Symptom	(%)
Weight loss	76
Fever	49
Cyanosis or gray color	40
Distended abdomen	16
Jaundice	7
CNS Symptoms (Generalised convulsion, loss of consciousness, and respiratory inadequacy	23

Pain over the loins appears during childhood and enuresis appears more frequently with increasing age (Jan Winberg, 1978).

8. HOSPITAL ACQUIRED URINARY TRACT INFECTION :

The urinary tract is the most common site of hospital acquired bacterial infection. It is indicated that 2% of all patients admitted to a hospital in the U.S. will acquuire UTI during hospital admission. As many as 90% of nocosomial (Hospital - acquired) infections have been ascribed to urinary tract instru-

mentation is related to the placement and maintenance of indwelling urinary catheters. Approximately 75-80% of nocosomial bacteriuria follows Catheterization of the urinary tract, with an additional 5 to 10% of patients becoming infected from other forms of urologic manipulation. Other forms of nocosomial U.T.I. may be blood borne. Other predisposing factors include alcoholism, granulocytopenia, malnutrition, the use of corticosteroids and immunosuppressive agents for organ transplantation (Culley C. Carson III, 1988).

Transplant recipients are at increased risk for bacterial infection as well as opportunistic infections with viruses, fungi and protozoa. Urinary tract infections are increased above the high frequency which are noted in the nonpregnant transplant patients. Invasive procedures are placement of a foley cathetar, fetal monitoring with a scalp electrode and intrauterine pressure monitoring during oxytocin augmented labor (Susan Hou, 1989) A high proportion of patients with retention had urinary infection at some stage and in most of these the urine was infected at the time of operation (Higgins, P.M. et.al., 1981). Transurethral resection of bladder tumors is the treatment of choice for low grade bladder tumors. Few studies have reported urinary infection following transurethral resection of bladder tumor. The postoperative urinary infection after transurethral resection of bladder tumor is 19.4% (Goldwasser B et.al., 1983).

About 3 - quarters of nocosomial urinary infection are due to gram negative bacilli. Escherichia coli accounts for approximately 50% of these bacteriurias followed by Proteus, Pseudomonas, Klebsiella. Enterobacter and Serratia (Erik H.Larsen et.al., 1986). Serratia Marcescens is a water borne organism that is ubiquitous in the hospital environment (Krieger J.N. et.al., 1980). The remaining quarter is likely due to Gram positive organism such as Streptococcs and Staphylococcus (Erik H.Larsen et.al., 1986).

9) RECURRENT URINARY TRACT INFECTION :

In approaching a patient with recurrent infection, it is important to categorize the type of recurrence in order to adopt an appropriate management strategy. The most common type of recurrence is reinfection by a bacterium that is different from initially infecting strain. Although both infections may be caused by the same species, the organisms can be differentiated on the basis of colonial morphology or antimicrobial sensitivities. A far less common form of recurrence often termed as relapse is characterized by the reappearance of the original infecting strain in the urine within two weeks after infection therapy (Stamm.WE et.al., 1987).

Longitudinal studies of adult women with cystitis show that approximately 3/4th of those experiencing an initial episode will subsequently experience only sporadic recurrent infections at a rate of less than one infection per year (Maback CE, 1971)

The incidence of recurrent urinary tract infection in childern without reflux or in children in whom reflux has been surgically corrected does not greatly differ from that in children with reflux. The danger poses by Vesicoureteral reflux is its potential to convert lower urinary tract into pyelonephritis with consequent renal damage (Lowell R. King, Selwyn B. Levitt, 1986).

10. URINARY TRACT INFECTION AND RENAL CALCULL :

Branched renal calculi usually are composed of magnesium ammonium phosphate (Struvite) and carbonate apatite, and are caused by infection of the urine with bacteria that systhesize the enzyme urease. Ammonium is released by the breakdown of urea by urease. The urine becomes highly alkaline and struvite and carbonate apatite are crystallized (Fowler J.E., 1984).

Proteus infection was closely associated with stone recurrences following complete stone clearance at initial surgery. Prompt eradication of urinary infection in the immediate post-operative period is the most crucial factor in preventing new recurrances and failure to achieve this leads to a significantly higher recurrence rate (Androulakakis P.A. et.al., 1982).

11. DIAGNOSTIC BIOCHEMICAL PARAMETERS IN URINARY TRACT INFECTION

Symptoms are notoriously unreliable for the diagnosis of bacterial urinary tract infection. The characteristic laboratory findings is bacteriuria (10^5 Colonies/ml) or documentation of other

infectious agent. The other possible laboratory findings include pyuria, leukocyte cast, positive nitrite (Some organisms), positive urine leukocyte esterase (dipstick) (Norbert W. Tietz, 1987).

1. Urine Microscopy

Microscopic urinalysis remains of great value in evaluating a woman with symptoms of an acute urinary tract infection. Unfortunately as performed in the typical hospital or clinic laboratory, the urine analysis is of limited utility in assessing the presence of infection since specimens are given only cursory examination and results are nonquantitative. On the other hand, a brief, though careful inspection of urine, with or without gram staining can be extremely useful (Fihnn SD, Stamm. WE, 1983).

Another useful test that can be performed while the patient waits is examining voided urine for the presence of pyuria (defined as the presence of $>$ 10 - 20 leukocytes / ul) using a hemocytometer - counting chamber. Compared to examination of urinary sedi-ment, this method provides an index of pyuria that is much more highly correlated with the presence of infection and requires less time to perform (Stamm. WE, 1983). Pyuria is present nearly in all women with acute urinary tract infection. In clinical studies, the presence of Pyuria is 80 - 95% sensitive for the presence of U.T.I. (including those with bacterial counts 10^4) and 50 - 76% specfic when urine is examined for bacteria or leukocytes

it is valuable to ascertain whether RBC are present or not. (Johnsor JR, Stamm We, 1987).

(2) Urine Culture :

It is reasonable to initiate antibiotic treatment when urine microscopy reveals bacteriuria or pyuria. If these testt are negative, urinary tract infection is less likely and a quantitative urine culture should be performed. Traditionally, practicing physicians have considered a culture positive when growth of $\geq 10^5$ uropathogenic bacteria / ml is found (Fihnn. SD, Stamm. WE, 1983).

(3) The Fairley test :

The bladder washout (Fairley test) is used as a standard of accuracy by many investigators who find it atleast 90% sensitive in adults. It is less reliable in children especially if there is considerable reflux and it is not reliable in patients with neurogenic bladders.

An indwelling catheler is inserted and a control sample of urine is obtained for Grams staining and culture. A solution of 50ml of 0.9% saline containing 2 vials of fibrinolysin plus desoxyribonuclease (Elase) and an antimicrobial drug is instilled into the bladder and left for 45 minutes to detach organisms from

the bladder wall and inhibit their multiplication. The bladder is drained and rinsed with 1 to 2 litres of sterile water or saline in 50-100ml portions with the last portion being left in the bladder for a few minutes, during which 10mg of furosemide is given intravenously. A sample of this final rinsing portion is cultured (time zero). The foley catheter is then clamped, to be opened to obtain specimens for cultures at 10,20,30 and 60 minutes. The volume of urine obtained each time is also noted. To be considered diagnostic of upper urinary tract infection, the colony count at time zero must be 1% of that in control specimen, and there must be a 10% rise in the count in the subsequent samples.

(4) The stamey Test :

Culture of urine obtained by ureteral catheterization is widely accepted as "good standard" against which other methods should be measured and has the additional advantage of showing whether a renal infection is unilateral or bilateral. The bladder is irrigated throughly via the cystoscope to reduce the number of bacteria available for carriage up the ureters. Two ureteral catheters are then passed into bladder, and the residual fluid there is withdrawn through both catheters for culture (Curtis A.Sheldon, Ricardo Gonzalez 1984).

(5) Cytodiagnostic Urine Analysis :

Cytodiagnostic urine analysis (CUA) bypasses the need

for sophisticated microscopes by visualisation of cytocentrifuged and papni coalou - stained urine sediment. The study does not specifically address diagnosis of urinary tract or renal desease based, on Sediment abnormalities.

This technique provides important sensitivity in the identification of renal hematuria. They have observed that RBC casts show leaching of Hb pigment from cells as they age changing the cells from orange - staining to pink and finally to transparent "ghost". There have been a few cases with WBC cast numerous enough to suggest Pyelonephritis (Diane L. Eggensperger et.al., 1989).

12BIOCHEMICAL CHANGES IN URINE WITH URINARY TRACT INFECTION:

(a) Immunopathological changes:

Urine from normal human contains antibodies. Patients with repeated infections have an augmented excretion. The antibodies of normal urine are IgG and IgA classes and are probably locally synthesized (Jan Winberg, 1978). Specimens of urine from individuals with urinary tract infection were most frequently reactive with antisera to IgG (88%) and IgA (63%), The study by Brien P.O. (1980) shows clearly that specimens from patients with U.T.I. have detectable levels of IgG, and IgA.

(b) Proteins:

The Proteins present in normal urine in part are derived from the plasma proteins and in part from the urinary tract. IgA

and Tamm Horsfall protein are the urinary tract proteins of special interest. In renal disease or other diseases affecting renal function, excretion of protein is increased, as a result changes in glomeruli allowing increased passage of proteins. Proteinuria associated with infection is post renal (Varley et. al., 1980).

(c) Bacteriuria

Timely culture of significant bacteriuria (Colony counts above 100,000 per ml) is essential to the diagnosis and treatment of U.T.I. Routine urine analysis is time consuming. The rapid determination of bacteriuria not only could improve patient care and reduce the cost of treatment but also reduce the time and cost required to process negative urine specimen (Plastt. H.L. et. al., 1986).

(d) Sodium Potassium and Chloride:

If kidney function is damaged, the ability to conserve SodiuM, Potassium and chloride is frequently lost, kidneys may retain them rather than eliminate. The excess of these cause fluid retention resulting in oedema, severe disorders of sodium potassium chloride and water balance. It is necessary that high intake of sodium potassium and chloride in diet need to be restricted. Toxicity of Potassium occurs frequently in renal failure when the kidney is not capable of excreting excessive Potassium (Harpet et. al., 1981) Urinary losses of sodium, potassium arise during recovery from acute distruction. Renal tubular disease affects the excretion of potassium. This occurs in such cases of chronic pycLonephritis

and during recovery fromacute renal failure (Varley et. al., 1980).

(e) Phosphorus and Calcium:

Phosphate toxicity is rare except when acute or chronic kidney failure prevents normal phosphate excretion (Martin et. al., 1981). urinary tract infection associated with stone has increased phosphorus and calcium. Patnaick et. al., (1983), in his study or urinary tract infection found the increased excretion of phosphorus in urine.

13) TREATMENT

The urinary concentration of an antibiotic has been shown to be more important than serum concentration for treatment of UTI (Bagley D.H. et. al., 1982). In an acute infection due to an unidentified organism and with an acid urine it is reasonable to start with a sulphonamide or cotrimoxazole. If the infected urine is alkaline, co-trimoxazole or amplicillin should be tried first. For attacks following rapidly with the same organism may be relapses in treating a relapse it is wise to use a drug capable of achieving high tissue levels (e.g. Ampicillin) rather than one which does not (eg. Nitrofuranton) (Laurence D.R. Bennett P.N, 1980). The use of a single dose or a single day treatment for uncomplicated UTI in children has obvious attraction. These include improved compliance, more rapid resolution of symptoms, less risk of side effects and reduced cost. A single dose of trimethoprim effects a cure rate at 48 hrs, similar to that achieved with a conventional seven day course of cotrimoxazole, but this is accompanied by an unacceptably high

risk of local recurrence (Nolan et. al., 1989).

Experimental Procedure

III. EXPERIMENTAL PROCEDURES

The experimental procedures relating to the study 'Biochemical changes in urine with urinary tract Infection' are presented in the following sequence:

A. Selection of subjects.
B. Collection of Urine.
C. Determination of IgG.
D. Determination of IgA and IgM.
E. Estimation of Total Proteins.
F. Culture of the Urine Specimen.
G. Estimation of Sodium and Potassium.
H. Estimation of Chloride.
I. Estimation of Calcium.
J. Estimation of Phosphorus.

A. Selection of Patients:

Twenty five patients with urinary tract infection were selected from the Department of Urology - Coimbatore Medical College Hospital. Three of them were in-patients while the rest were out-patients. Patients were of both sexes. In in-patients, 2 of them were males and one female. In Out-patients 15 of them were males, 6 were females and one was a child. 25 individuals of the matching age sex and apparently free from diseases were selected as controls.

B. Collection of Urine:

For bacteriological examination, random samples of urine were collected in a sterile container, Urine specimen should be cultured preferably within 1 hour of collection. For routine examination, the urine (random sample) should be collected in a sterile container. The best preservative for general purpose is Toluene. 1.0 ml. of Toluene is placed in the receptacle before urine is added (K.M. Samuel, 1986).

C. Determination of the Immunoglobulin IgG:

The level of IgG in urine was determined by the method of Radial immunodiffusion assay. The details are presented in Appendix - I (Marcini, G. et. al., 1965).

D. Determination of the Immunoglobulin IgA and IgM:

The level of IgA and IgM in urine wasdetermined by the Method of Radial immunodiffusion assay. The procedure is given in Appendix - II (Marcini G. et. al., 1965).

E. Estimation of Total Proteins:

Urinary proteiN level was estimated by "Technique of Richterrich" (Varley et. al., 1980), the details of which are given in Appendix - III.

F. Microbiological Examination:

The urine samples were collected ina a sterile container and the micro-organisms were identified by culture study as given in Appendix - IV.

G. Estimation of Sodium and Potassium:

The level of sodiuM and Potassium in urine was estimated by 'Flame Photometer' (Varley et. al., 1980). The procedure for estimation is given in Appendix - V.

H. Estimation of Chloride:

Urinary Chloride level was estimated by 'Method of van slyke' (Varley et. al., 1980). The procedure is given in Appendix - VI.

I. Estimation of Calcium:

The level of Calcium in Urine was determined by 'Method of Clark and Collip' (Varley et. al., 1980). The procedure for which is given in Appendix - VII.

J. Estimation of Phosphorus:

The amount of phosphorus excreted in urine is determined by the method of Gomorri (K.M. Samuel, 1986). The details are presented in Appendix - VIII.

Results and Discussion

IV RESULTS AND DISCUSSION

The findings pertaining to the present study are discussed under the following headings:

A. Distribution of the urinary tract infected patients.

B. Biochemical analysis of urine.

   1. Proteins and immunoglobulins.
   2. Sodium and Potassium
   3. Calcium, Chloride and Phosphorus
   4. Microbiological examination.

C. Comparative study of the patients infected with E. Coli and other microbes.

   1. Distribution of patients .
   2. Proteins and immunoglobulins.
   3. Sodium and Potassium.
   4. Calcium, Chloride and Phosphorus.

A. DISTRIBUTION OF URINARY TRACT INFECTED PATIENTS

Among the 150 patients who attended the out patient ward of the urology section of Coimbatore Medical College Hospital, 25 were found to suffer from urinary tract infection and they were selected for the study.

Table - I indicates the distribution of controls and patients according to age and sex.

TABLE - I

DISTRIBUTION OF SELECTED CONTROLS AND PATIENTS ACCORDING TO AGE AND SEX

Age group in years	NUMBER OF SAMPLES			
	CONTROLS		PATIENTS	
	MALES	FEMALES	MALES	FEMALES
5-15	--	1	--	1
16-25	--	2	--	2
26-35	4	1	4	1
36-45	2	1	2	1
46-55	5	1	5	1
56-65	1	1	1	1
66-75	5	1	5	1
TOTAL	17	8	17	8

Out of the 25 patients, 17 were males, 7 were females and one patient was a 5 year old child.

B. BIOCHEMICAL ANALYSIS OF URINE:

The urine samples were collected as discussed under Chapter III. All the samples were collected before the patients underwent treatment.

For statistical analysis the urine parameters were considered irrespective of age and sex.

(1) PROTEIN AND IMMUNOGLOBULINS:

Table - II shows the mean urinary protein and immunoglobulin values of controls and patients.

TABLE - II

MEAN URINARY PROTEIN VALUES OF CONTROLS AND PATIENTS

Parameters	Mean ± S.D.		't' value
	Controls	Patients	
Proteins (mg/dl)	12.44 ± 3.98	134.07 ± 93.13	6.522**
Immunoglobulins (mg/dl)			
IgG	--	--	
IgA	--	--	
IgM	--	--	

** Significant at 1% level.

The urinary protein was found to be in the range of 6 to 20 mg per dl with a mean value of 12.44± 3.98 mg per dl in controls. In controls, the protein excreted was in agreement with the normal range of 8 to 20 mg per dl urine (Oser, 1973). Whereas in patients the urinary protein ranges from 29 to 380 mg with a mean value of 134.07 ± 93.13 mg per dl. The mean urinary protein levels were increased several fold in patients and the increase was statistically significant at 1% level. The increased excretion of proteins in urine might be due to changes in glomeruli that occur during some degree of kidney damage in urinary infection (White A et.al., 1978.

Fig.I indicates the levels of urinary protein in controls and patients.

In this study, it was noticed that there was no excretion of immunoglobulins (IgG, IgA and IgM) in urine of both controls and patients. But the study by Brien P.O. (1980) showed that specimens of urine from patients with urinary tract infection have detectable levels of IgG and IgA.

Immunodiffusion plates-1,2 and 3 shows that the immunoglobulins were not excreted in urine.

Sodium and Potassium:

Table - III depicts the urinary sodium and Potassium level in controls and patients.

TABLE - III

MEAN URINARY SODIUM AND POTASSIUM LEVELS IN CONTROLS AND PATIENTS

Parameter	MEAN ± S.D.		't' Value
	Controls	Patients	
Sodium (mEq/1)	127.48 ± 13.73	165.64±58.44	3.174 **
Potassium (mEq/1)	40.28±13.73	31.48±11.24	2.478 *

** Significant at 1% level.

* Significant at 5% level.

The mean value of urinary excretion of sodium in controls was found to be 127.48 ± 13.73 milliequivalents per litre with a range of 107 to 148 milliequivalents per litre where as in patients the mean value of urinary excretion of sodium was 165.64 ± 58.44 milliequivalents per litre. The urinary excretion of sodium in controls was within normal range 100 to 150 milliequivalents per litre (Varley, 1980). In patients the urinary excretion of sodium was increased over the control value and this increase was found to be statistically significant at 1% level.

The urinary excretion of Potassium in controls ranged between 34 to 48 milliequivalents per litre with a mean value of 40.28 ± 13.73 millieqivalents per litre. The mean value of urinary excretion of Potassium in pa tients was found to be 31.48 ± 11.24 milliequivalents per litre with a range of 19 to 52 milliequivalents per litre. The values of controls were within the normal range, 38 to 45 milliequivalents per litre of urine (Varley, 1980). The mean value of urinary Potassium was decreased than the control and this decrease was statistically significant at 5% level.

Due to damage in Kidney function following urinary infection the ability to conserve sodium and potassium is lost.

Hence there will be a decrease in the excretion of sodium and potassium (David.W.Martin et.al., 1985.) In this study, there was a decrease in the excretion of Potassium but not sodium, this might be due to increased intake of salt by the selected subjects.

Fig.II depicts the range of urinary sodium, potassium and chloride levels.

(3) Calcium, Chloride and phosphorus:

Table - IV shows the mean values of Calcium, Chloride and Phosphorus in urine of controls and patients.

TABLE IV

MEAN URINARY CALCIUM, CHLORIDE AND PHOSPHORUS LEVELS IN CONTROLS AND PATIENTS

Parameters	Mean ± S.D.		't' value
	Controls	Patients	
Calcium (mg/dl)	10.54±3.27	10.46±4.95	0.067 NS
Chloride (mEq/1)	117.64±23.40	121.64±29.92	0.103 NS
Phosphorus (mg/dl)	58.30±7.13	87.79±17.86	7.667 **

** Significant at 1% level.

NS:Not significant

The mean value of urinary calcium in controls was found to be 10.54 ± 3.27 mg/dl with a range of 5 to 14 mg per dl whereas in patients it ranged from 4 to 21 mg per dl with a mean value of 10.46 ± 4.95 mg per dl. The mean value of urinary calcium in controls and patients were within the normal range, 7.5 to 13.0 mg per dl as reported by Harper (1985).

The mean urinary calcium level is increased only in infection associated with renal calculi (Martin et.al., 1981).
In this study, only one patient was found to be having infection associated with calculi. The urinary calcium level of the same patient was also found to be increased above the normal range (21mg/dl).

The mean value of urinary chloride in controls was found to be 117.64 ± 23.40 mEq/1 with a range of 68 to 148 mEq/1 whereas in patients it ranged from 48 to 150 mEq/1 with a mean value of 121.64 ± 29.92 mEq/1. The mean value of urinary chloride in controls and patients were within the normal range, 96 - 156 mEq/1 (Varley, 1980).

But according to the reports given earlier by Harper (1985), there is retention of chloride when there is renal damage following urinary infection and hence there is decreased excretion of chloride.

The mean values of urinary phosphorus in patients was found to be 87.79 ± 17.86 mg per dl with a range of 62 to 115 mg per dl whereas in controls it ranged from 42 to 67 mg per dl with a mean value of 58.30 ± 7.13 mg per dl. The mean value of urinary phosphorus in controls were within the normal range, 45 to 70 mg per dl (Harper, 1985).

The level of phosphorus in urine of patients was found

to be increased and this increase was statistically significant at 1% level. This was in agreement with the reports given by Patnaik (1983).

Fig. III represents the levels of urinary calcium and phosphorus.

4. Microbiological Examination:

There was growth of microbes in the sample when culture of the specimen was carried out. The microbes identified were E.Coli, Pseudomonas Pyocyanae, Pseudomonas aeroginosa, Proteus and Klebsiella.

There was no growth of microbes when the urine samples of the control subjects were cultured.

The patients were grouped according to the type of the microorganisms identified as per the culture studies and the statistical study between the groups were also done.

C. COMPARATIVE STUDY OF THE PATIENTS INFECTED WITH E.COLI AND OTHER MICROBES

1. Distribution of Patients:

Table - V gives the classification of patients according to the type of organisms that have caused infection.

TABLE - V

DISTRIBUTION OF THE PATIENTS ACCORDING TO THE TYPE OF ORGANISMS THAT HAVE CAUSED INFECTION

Organisms causing infection	Number of Patients affected	% of infection
E.Coli	14	56
Pseudomonas pyocyanae	1	4
Klebsiella	7	28
Proteus	2	8
Pseudomonas aeroginosa	1	4
TOTAL	25	100

Out of the twenty-five patients suffering from urinary tract infection, fourteen were infected by E.Coli., seven by Klebsiella, one by Pseudomonas pyocyanae , one by Pseudomonas aeroginosa and two by Proteus. It was found that infection by E.Coli was more predominant. Fifty six per cent of the patients had E.Coli infection.

2. Proteins and Immunoglobulins:

Table - VI gives urinary protein and Immunoglobulin levels in patients infected with E.Coli and in patients infected with other microbes.

TABLE - VI

URINARY PROTEINS AND IMMUNOGLOBULIN LEVELS IN PATIENTS INFECTED WITH E.COLI AND OTHER MICROBES

Infective organisms	Number of patients	Urinary Proteins Mean ± S.D. mg/dl	Urinary Ig. Mean ± S.D. mg/dl
E.Coli	14	123.85±79.89	-
Other Microbes	11	137.98±113.29	-
't' value		0.364 NS	-

NS: Not Significant.

The mean value of urinary proteins in patients infected with E.Coli was found to be 123.85 ± 79.89 mg per dl with a range of 43.96 to 203.74 mg per dl, whereas in the case of infection with other microbes, the same ranged from 24.69 to 251.27 mg per dl with a mean value of 137.98 ± 113.29 mg per dl. There was an increase in the excretion of protein in the case of infection by other microbes, but this increase was not statistically significant. There was no excretion of immunoglobulins in the case of infection with E.Coli as well as by other microbes.

3. Sodium and Potassium:

Urinary sodium and Potassium levels in patients infected

with E.Coli and other microbes are shown in Table - VII.

TABLE - VII

URINARY SODIUM AND POTASSIUM LEVELS IN PATIENTS INFECTED WITH E.COLI AND OTHER MICROBES.

Infective Organisms	Number of patients	Urinary Sodium	Urinary Potassium
		mEq/1 Mean ± S.D.	mEq/1 Mean ± S.D.
E.Coli	14	148.64±65.88	31 ± 14.02
Other Microbes	11	141.81±88.03	28.45 ± 13.74
't' VALUE		0.220^{NS}	0.453^{NS}

NS: Not Significant

The urinary sodium levels ranged from 82.76 to 214.52 mEq/1 with a mean of 148.64 ± 65.88 mEq/1 in patients infected with E.Coli and in patients infected with other microbes, the urinary sodium levels ranged from 53.78 to 229.84 milliequivalents per litre with a mean of 141.18 ± 88.03 mEq/1. The increase in urinary sodium level in case of E.Coli infection over the infection by other microbes was not statistically significant.

The urinary potassium levels ranged from 16.98 to 45.02 milliequivalents per litre with a mean value of 31±14.02 milliequivalents per litre in case of patients infected with E.Coli whereas

in case of patients infected with other microbes it ranged from 14.71 to 42.19 milliequivalents per litre with a mean value of 28.45 ± 13.74 milliequivalents per litre. There was no significant difference in the excretion of potassium in the case of patients infected with E.Coli and other microbes.

4. Calcium, Chloride and Phosphorus :

Table VIII depicts the urinary level of calcium, chloride and phosphorus in patients infected with E.Coli and other microbes

TABLE VIII

Infective Organisms	Number patients	Urinary calcium	Urinary chloride	Urinary Phosphorus
		Mean±S.D. mg/dl	Mean±S.D. mEq/l	Mean±S.D. mg/dl
E.Coli	14	10.78±5.72	98.21±39.55	81.88±25.03
Other Microbes	11	11.04±4.81	133.27±17.41	88.13±19.18
't' value		0.121 NS	2.728 **	0.692 NS

NS: Not Significant

**: Significant at 1% level

The urinary calcium level ranges from 5.06 to 16.50 mg per dl with a mean value of 10.78 ± 5.72 mg per dl in case

of E.Coli infection whereas in the case of infection with other microbes, it ranges from 6.23 to 15.85 mg per dl with a mean value of 11.04 ± 4.81 mg per dl. There was no significant difference in urinary calcium level in the case of patients infected with E.Coli and other microbes.

The urinary levels of chloride ranges from 58.66 to 137.76 mg per dl with a mean value of 98.21±39.55 mg per dl in case of E.Coli infection whereas in the case of infection with other microbes it ranged from 115.86 to 150.68 mg per dl with a mean value of 133.27±17.41 mg per dl. The excretion of chloride in urine in the case of infection with other microbes was significant at 1% level.

The urinary phosphorus level in case of E.Coli infection ranged from 56.85 to 106.91 mg per dl with a mean value of 81.88± 25.03 mg per dl where as in the case of infection with other microbes it ranged from 68.95 to 107.31 mg per dl with a mean value of 88.13 ± 19.18 mg per dl. There was no significant difference in urinary phosphorus level in case of patients infected with E.Coli and other microbes.

FIG. I

MEAN URINARY TOTAL PROTEIN LEVEL IN CONTROLS AND PATIENTS

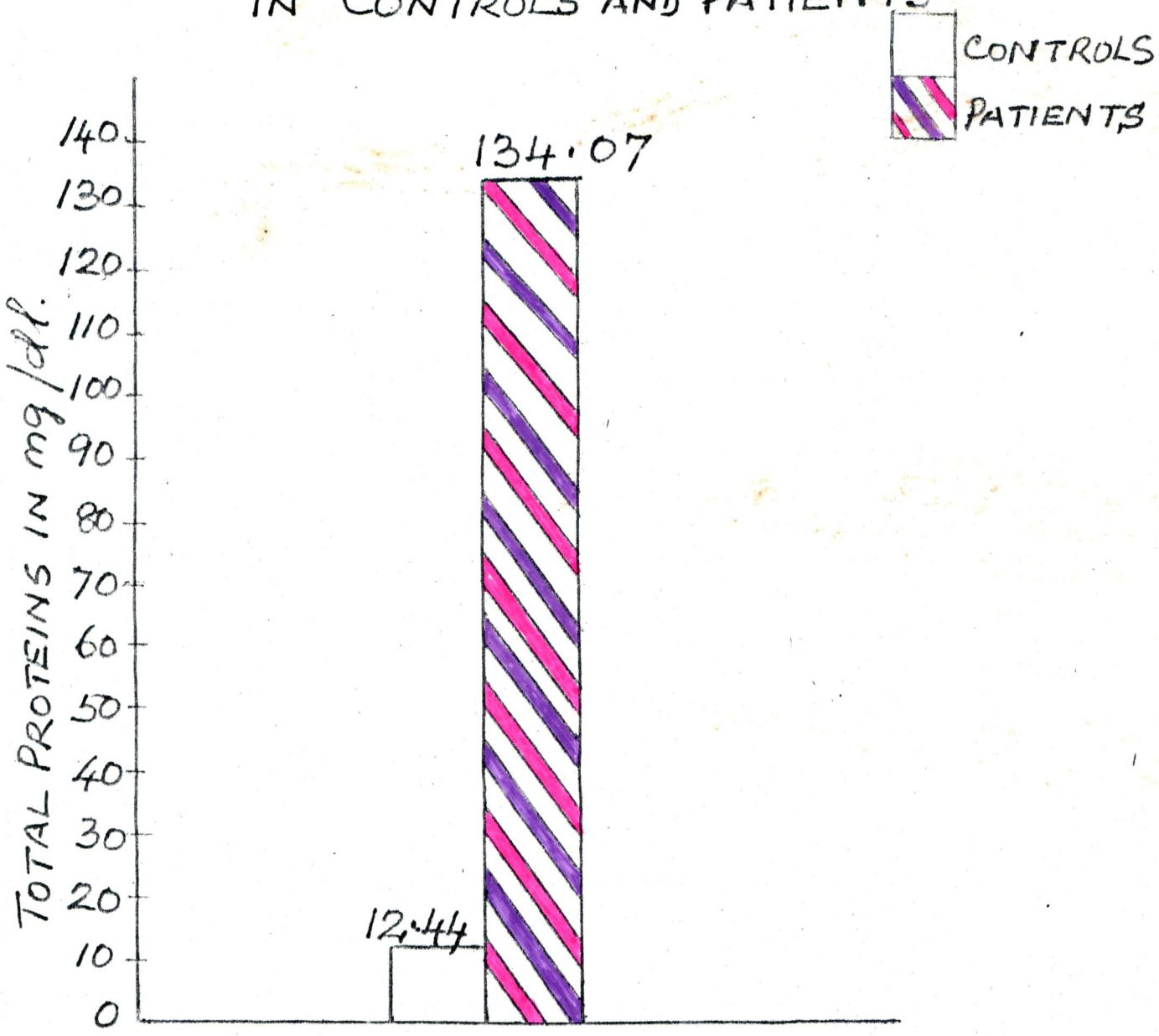


FIG. II

MEAN. URINARY SODIUM, POTASSIUM AND CHLORIDE LEVELS IN CONTROLS AND PATIENTS.

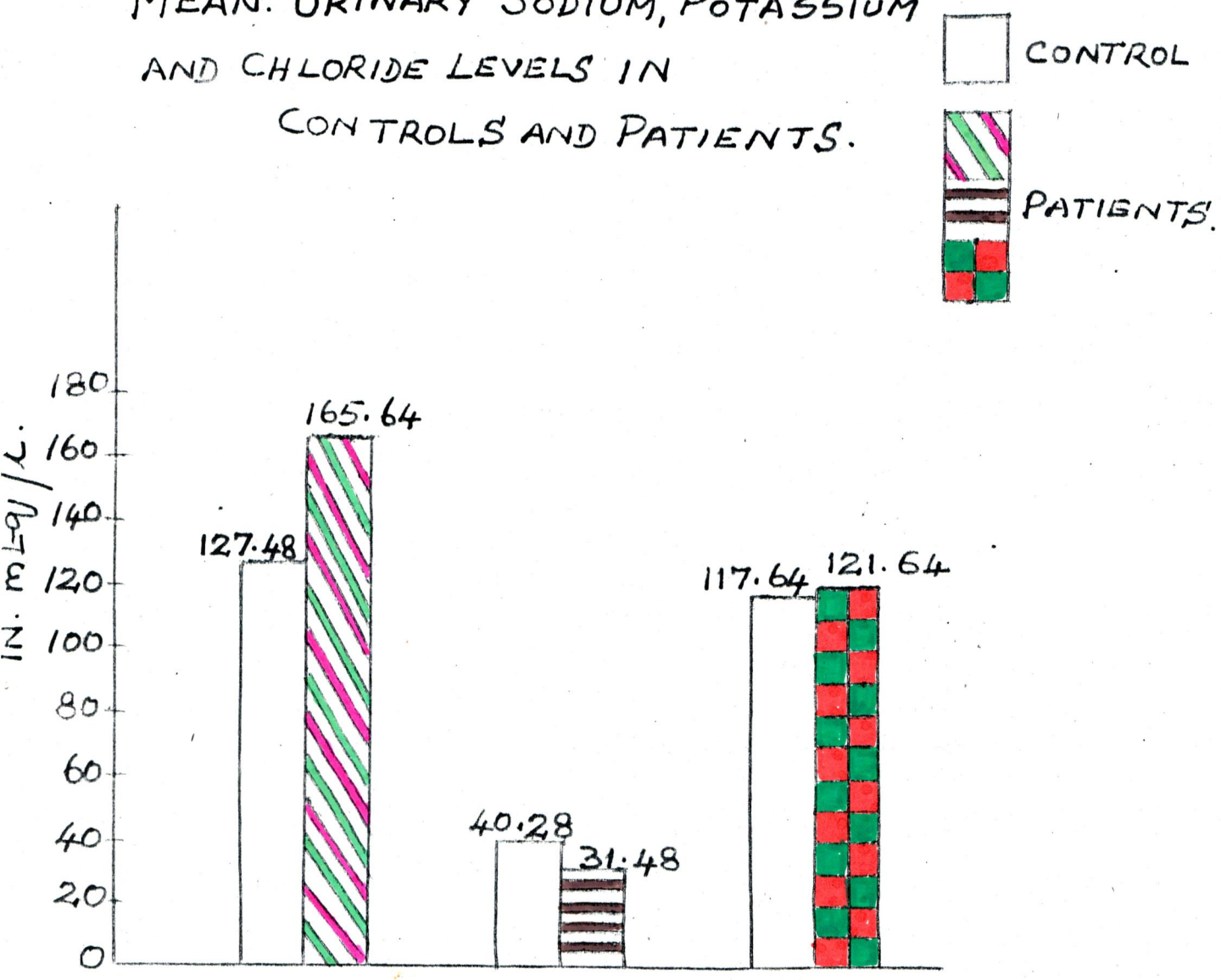
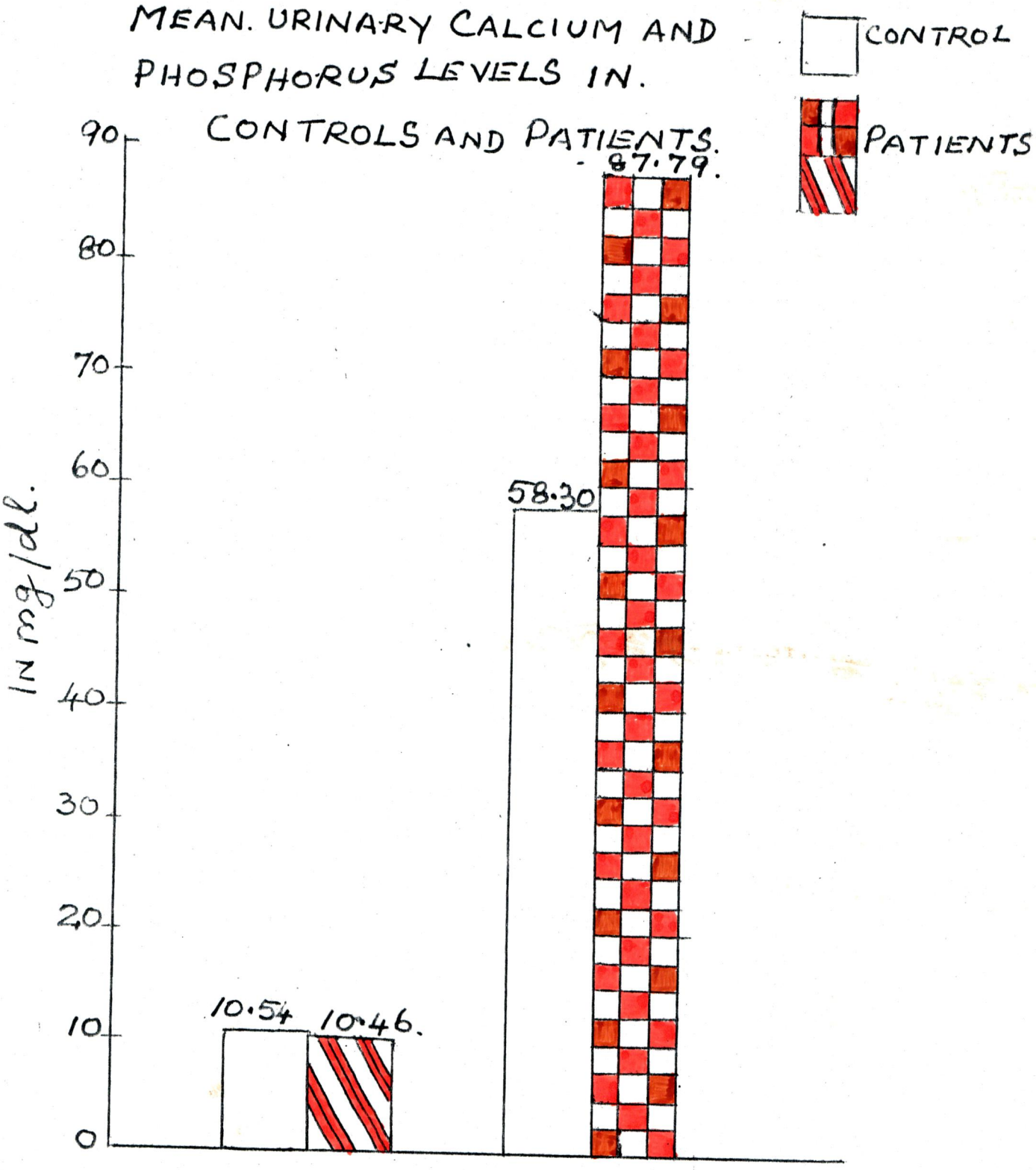


FIG. III.


MEAN. URINARY CALCIUM AND PHOSPHORUS LEVELS IN. CONTROLS AND PATIENTS.
CONTROL
PATIENTS
IN mg/dl.
90
80
70
60
50
40
30
20
10
0
10.54
10.46.
58.30
87.79.

IMMUNO DIFFUSION PLATES

PLATE I

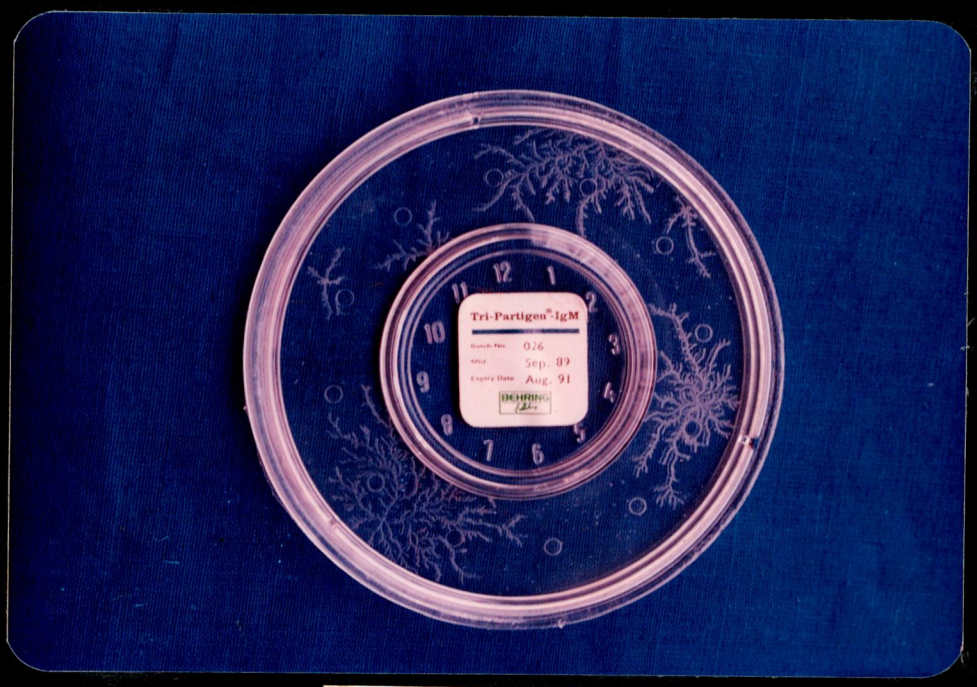


PLATE II


HC-Partigen®-IgG
Batch No. 014
Mfd. Sep. 89
Expiry Date Aug. 91
BEHRING

PLATE III

Summary and Conclusion

V. SUMMARY AND CONCLUSION

This study was undertaken to find out the biochemical changes in urinary tract infection. Among the 150 patients who attended the out-patient ward of the urology section of Coimbatore Medical College Hospital, 25 were found to suffer from urinary tract infection and they were selected for this study. The age of the patients ranged from 5 to 74 years. Out of the 25 patients, 17 were males and the rest were females. An equal number of healthy controls of matching age, sex and apparently free from diseases were selected

The urine samples were collected from all the patients before they underwent treatment. The urine samples were also collected from the controls. The protein, immunoglobulins, sodium, potassium, calcium, chloride and phosphorus content of the urine samples were determined. In addition to this, microbiological examination of the urine was also done.

The mean value of urinary protein was found to be 12.44 ± 3.98 mg per dl in controls, whereas the same was found to be between 134.07 ± 93.13 mg perdl in patients. The mean urinary protein levels were found to be increased in patients and the increase was statistically significant at 1% level. The increased excretion of protein in urine might be due to changes in glomeruli that occurs during some degree of kidney damage in urinary infection. In this study it was noticed that there was no excretion of immunoglobulin in urine of both patients and controls.

The mean value of urinary excretion of sodium in controls was found to be 127.48 ± 13.73 milliequivalents whereas in patients the mean level of urinary excretion of sodium was 165.64 ± 58.43 milliequivalents per litre. In patients the urinary excretion of sodiuM was found to be increased over the control values and this increase was statistically significant at 1% level.

The mean urinary excretion of PotassiuM was found to be 40.28 ± 13.73 milliequivalents per litre in controls, whereas the same was found to be 31.48 ± 11.24 milliequivalents per litre in patients. The mean value of urinary ptassium was found to be decreased than the control and this decrease was statistcally significant at 5% level.

Due to damage in kidney function following urinary infection, the ability to conserve sodiuM and Potassium is lost, hence there will be decrease in the excretion of sodium and potassium. But in this study there was a decrease in the excretion of Potassium but not sodium, this might be due to increased intake of salt by the selected subjects.

The mean value of urinary calcium in controls was found to be 10.54 ± 3.27 mg perdl whereas in patients, the mean value was 10.46 ± 4.95 mg per dl. The mean values of urinary calcium in controls and patients were within the normal range (7.5 to 13.0 mg%). It is found to be increased only in infection associated with renal calculi. In this study only one patient was found to be having

infection associated with calculi and the urinarycalcium level of the same patient was found to be above the normal range (21 mg/dl).

The mean value of urinary choride in controls was found to be 117.64 ± 23.40 mEq/1, whereas in patients the same was found to be 121.64 ± 29.92 mEq/1. The mean values of urinary chloride in controls and patients were within the normal range (96 - 156 mEq/1).

The mean value of urinary phosphorus in controls was found to be 58.30 ± 17.8mg per dl, whereas in patients the mean value was found to be 87.79 ± 17.86 mg per dl. The increased excretion of urinary Phosphorus in patients was statistically significant at 1% level.

Also the organisms that have caused the infection were identified by the culture studies, and were found to be E. Coli. Pseudomonas Pyocyanae. Pseudomonas aeroginosa., Proteus and Klebsiella.

The patients were grouped according to the type of micro organism identified and statistical study between the groups was also done.

The mean value of urinary proteins in patients infected with E. Coli. was found to be 123.85 ± 79.89 mg perdl, whereas in the case of patients infected with other microbes the same was

found to 137.98 ± 113.29 mg. per dl. There was no significant difference in the excretion of proteins in urine in the case of patients infected with E. Coli and other microbes.

There was no excretion of immunoglobulins in the case of infection with E. Coli as well as by other microbes.

The mean urinary sodiuM level in patients with E. Coli infection was found to be 148.64 ± 65.88 m Eq/1, where as the same was found to be 141.81 ± 88.03 m Eq/1 in the case of patients infected with other microbes. There was no significant difference in the urinary sodium level in the case of patients infected with E. Coli and other microbes.

The patients infected with E. Coli was found to have a mean urinary potassium value of 31 ± 14.02 m Eq/1, where as the same in the case of patients infected with other microbes was found to be 28.45 ± 13.74 m Eq/1. There was no significant difference in the urinary potassium levels in the case of patients infected with E. Coli and other microbes.

The mean value of urinary calcium in patients infected with E. Coli was found to be 10.78 ± 5.72 mg per dl where as in the case of patients infected with other microbes if was found to be 11.04 ± 4.81 mg dl. There was no siginificant difference in urinary calcium level in the case of patients infected with E. Coli and other microbes.

The urinary level of chloride in patients infected with E. Coli was found to be 98.21 ± 39.55 m Eq/1, where as in patients infected with other microbes it was significant at 1% level. The reason for the increased excretion of chloride was not known.

The mean urinary phosphorus level in case of patients with E. Coli infection was found to be 81.88 ± 25.03 mg per dl whereas in the case of infection with other microbes the same was found to be 88.13 ± 19.18 mg per dl. There was no significant difference in urinary phosphorus level in case of patients infected with E. Coli and other microbes.

In summary, the urinary levels of protein, sodiuM and phosphorus were found to be increased in the urinary tract infected patients when compared with subjects, whereas the urinary level of potassium was found to be decreased in the infected patients when compared with normal healthy individuals.

The urinary calcium and chloride levels were found to be within the normal range. None of the patients excreted immunoglobulins.

Recommendations for future studies:

The serum levels of different components need to be assessed to understand better the biochemical changes in urinary tract infection.

In thus study, the sodium excretion in urine was found to be higher than in controls, whereas the Literature have shown a decreased excretion of the same element. Hence a study can be done by taking into account the amount of salt consumed by the subjects.

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Appendix

APPENDIX - I

QUANTITATIVE DETERMINATION OF THE IMMUNOGLOBULIN IgG.

(Marcini,G. et.al.,1965)

Composition:

HC-Partigen IgG. Immunodiffusion plate contains a prepared agar gel in which H-chain specific antiserum to Human IgG is incorporated. The antiserum is produced by immunization of sheep and goats.

Preservatives:

Sodium azide (1 mg/ml).

Sodium p-ethyl-mercury-mercapto-benzene-Sulfonate (atmost 0.1 mg/ml).

Method:

HC - Partigen IgG is suitable for quantitative IgG determinations (using reference values supplied with each plate).

(1) Open the plate and leave the plate opened to stand for about 5 minutes at room temperature to allow any condensation water that may have accumlated in the wells to evaporate.

(2) Procedure:

Well 1 is filled with 5ml of control urine, well 2-12 are each filled with undiluted 5 ml of the respective urine under test.

(3) Close the plate tightly and leave it to stand at room temperature. Evaluation may be made after a minimum diffusion time of 50 hours.

(4) At the end of the given diffusion time, the diameter D of the precipitin rings should be measured accurately to 0.1 mm using a suitable calibrated instrument.

(5) Evaluation:

The immunoglobulin concentrations related to the measured diameters are read directly from the table of refrence values. The results are reliable only when the value found for control urine applied to well 1 lies within the confidence range taken from the table of values enclosed with each of the control urine. With Hoechest Behring control urine, the confidence range is 15% of the immunoglobulin concentration given with each pack.

If the protein concentrations of the urine samples diverge considerably from the normal value, this means that the resulting precipitin ring diameters will fall outside the assay range of the plate. In this case, the examination must be repeated, using higher or lower dilutions of the urine sample with isotonic saline.

APPENDIX - II

QUANTITATIVE DETERMINATION OF THE IMMUNOGLOBULINS IgA and IgM.

Composition:

Tri- Partigen Immunodiffusion plates contained a prepared agar gel in which H-chain specific antiserum to the respective immunoglobulin is incorporated. The antiserum is produced by immunization of sheep and goats.

Preservatives:

Sodium azide (1mg/ml)

Sodium P-ethyl-mercury-mercapto-benzene-Sulfonate (atmost 0.1 mg/ml)

Method:

Tri-Partigen is suitable for quantitative immunoglobulin determinations (using reference values supplied with each plate).

(1) Open the plate and leave the opened plate to a stand for about 5 minutes at room temperature to allow any condensation water that may have accumulated in the wells to evaporate.

(2) IgA and IgM are determined using undiluted urine

(3) Procedure:

Well 1 is filled with 5 ml of control urine. Wells 2-12 are

each filled with 5 ml of the respective urine under test.

(4) Close the plate tightly and leave it to stand at room temperature Evaluation may be made after a minimum diffusion time of 50 hours (IgA) and 80 hours (IgM).

(5) At the end of the given diffusion time, the diameter D of the preciptin rings should be measured accurately to 0.1 mm using a suitable calibrated instrument.

(6) Evaluation:

The immunoglobulin concentrations related to the measured diameters are read directly from the table of reference values. The results are reliable only when the value found for the control urine applied to well 1 lies within the confidence range taken from the table of values enclosed with each pack of the control urine. With Hoechst Behring control urine, the confidence range 15% of the immunoglobulin concentration is given with each pack.

If the protein concentrations of the urine samples diverage considerably from the normal value, this means that the resulting precipitin ring diameters will fall outside the assay range of the plate. In this case, the examination must be repeated, using higher or lower dilutions of the urine sample.

APPENDIX - III

Determination of Urinary Protein (Technique of Richterich)

(Varley et.al., 1980)

Reagents:

(1) Perchloric acid :

Dilute 5.7 ml of concentrated perchloric acid (specific gravity 1.70) to 100ml with distilled water.

(2) Biuret Reagent (Stock) :

Dissolve 45g of Rochelle Salt in about 400 ml of 0.2N Sodium hydroxide and 15g of Copper-sulphate ($CuSo_4$ $5H_2O$) stirring continuously until the solution was complete. Add 5.0g of Po tassium-io dide and made upto a litre with 0.2N Sodium hydroxide.

(3) Dilute Biuret Reagent :

Dilute 200ml. of stock reagent to a litre with 0.2N sodium hydroxide which contains 5g of potassium-iodide per litre.

(4) Albumin standard :

Weigh 400mg of Albumin and dissolve in o.9% saline solution and make upto 100ml, with the same so that 1.0ml of this solution contains 4mg of protein.

Procedure:

Test for Total Protein: To 2.5ml of urine add 2.5ml of ice cold perchloric acid. Allow to stand for 10 minutes. Centrifuge vigorously. Discard the Supernatant.

Standards: Into a series of test-tubes pipette out 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the standard protein solution. Made upto 2.5 ml with distilled water.

Blank: Distilled water 2.5 ml.

Add 2.5 ml of Biuret reagent to each tube, mix throughly and let it stand at room temperature for 30 minutes. After cooling compare the colours in a colorimeter using a green filter.

A standard graph is drawn by plotting concentration of protein on X-axis and colorimeter reading on Y-axis. The amount of protein present in urine is then calculated from the standard graph.

APPENDIX - IV

CULTURE OF THE URINE SPECIMAN

(SAMUEL K.M. 1986).

(a) Collection of material for bacterial culture :

The urine sample for culture must be collected in a sterile container by observing strictest possible aseptic precautions necessary at all stages starting from the collection till the final stage of reading the test.

(b) Inoculation - Streak Plate Method :

A sterile loop is taken in the right hand (the loop is heated red-hot). It is dipped into the urine specimen in the culture tubes and the loop is removed without touching the sides of the tube. The charged loop is then be inoculated into a fresh culture observing all the above procedure.

In streak plate method, the culture material (Urine Specimen) is spread on the surface of the medium in the petri dish with name and date and immediately put in the incubator for 24 hrs.

CULTURE MEDIA :

Nutrient agar :

It is a general purpose medium for cultivattion of

Micro-organism a base for enriched or special purpose media

Beef extract, Bacto	3g.
Peptone, Bacto	5g.
Sodium Chloride	5g
Distilled water	1000 ml.
Agar	25g.

Mix them and steam for about 2 hours, adjust the reaction to PH 6.8. clear the fluid with egg albumin. Filter and bottle. Autoclave at 5 lbs. Pressure for 30 minutes for steaming for 30 minutes each day on three successive days.

After 24 hours incubation, if there is growth of the organism in the culture, prepare a smear of the colony from the media. They are first emulsfied in a drop of normal saline on a clean slide and the smear is fixed by gentle heat over the flame. Then they arestained for the identification of the organism.

Gram's staining Method (Modified, Jensen) :

(i) Methyl violet 6B aqeous solution

(ii) Lugol's Iodine :

Iodine	1g
Potassium Iodide	2g
Distilled water	100ml

(iii) Counter Stain :

Carbol Fuchsin (1:15) - diluted with distilled water.

(iv) Absolute alcohol or acetone for decolourisation

PROCEDURE :

Prepare the smear, cover the film with Methyl violet solution and allow the stain and wash off with Lugol's iodine. Allow for a minute. Wash off the iodine with spirit or actone until the colour ceases to come out of the smear. This is easily seen by holding the slide against a white back ground. Wash with water, apply counter stain for 30 to 60 sedonds. Wash again with water and dry in air. If the diluted carbol fuschin in used as a counter stain it should be allowed to act only 20 to 30 seconds. After drying, observed, under the microscope.

RESULT :

The bacteria are seen stained either violet (Gram-positive) or pink (Gram - negative) by the counter stain.

(a) Bacillus Pyocyaneus (Pseudomonas aeruginosa):

Morphology: Straight rods, 1.5 to 3 by 0.5 microns, motile with one of three terminal flagella; non-sporing.

Staining : Gram negative.

(b) Proteus

Morphology : Straight rods about the same size of B.Pyocyaneus; Pleomorphic; motile with Peritrichate flagella; non-sporing.

Staining : Gram negative.

(c) E.Coli.

Morphology : E.Coli is a gram negative straight rod, measuring 1-3 X 0.4 - 0.7 arranged single or in pairs. It is motile by Peritrichate flagella, though some strains may be non-motile.

Straining : Gram - negative.

(d) Klebsiella

: The genus Klebsiella consist of nonmotile, capsulated rod that grow well on ordinary media forming large, dome shapped, mucoid colonies of varying degrees of stickiness. Strains formerly labelled as nonmotile Aerobacter aerogenes (K.aerogenes) are now considered to be K.Pneumoniae

Staining : Gram negative.

APPENDIX - V

ESTIMATION OF SODIUM AND POTASSIUM

(Varley et.al., 1980)

The emission flame photometry is used in the estimation of sodium, and potassium.

The solution under analysis is sprayed as a fine mist into nonluminous flame which becomes coloured according to the characteristic emission of the metal. A very narrow band of wavelength corresponding to the element being analysed in selected by a light filter and allowed to fall on a photodetector. The output of the photodetector which is a measure of the concentration of the element is connected to a measuring system ie, (a) galvanometer, (b) measuring unit (c) digital unit (shuntbox)

Once the galvanometer or measuring unit is standarised with a solution of known concentration, the system will be ready for analysing the unknown sample.

Components of Flame Photometry :

The total system consists of 3 units - that is,

1. The compressor unit.
2. Burner chamber
3. A measuring unit (shunt box)

Working mechanism of Flame Photometry :

Compressed air from the compressor unit approximately with air pressure between 0.4 and 0.6 kg/cm and a gas from a suitable source ie. indane gas or oxyacetylene gas.

At the mixing chamber it consists of the atomizer assembly at controlled rates. The draught of the air the point of entry is used to draw the sample solution in the chamber through a fine atmomizer jet. Liquid will be drawn from the sampling beaker by suction and the excess liquid is condensed in droplets and the condensed liquid will emerge to the drain pipe. The mixture of the gas and atomised sample is passed into the burner and ignited, distilled water should be used throughout the entire ignition process.

The emitted light from the flame is collected by a lens and passed through the appropriate filter brought in front of it.

Filter : Transparent coloured glass plates.

The filter used here is to select the wavelengths.

Wave-lengths for sodium - 589 nm

Potassium - 766 nm

The filtered light is then passed on to energies a photodetector. The output is indicated in the measuring unit either a galvanometer or digital unit.

Preparation of standard electrolytes :

Standards should be put through each time when determinations are done. Standard stock Na and K :

A) Stock Na : 1000 mEq/l - 58.5g of NaC1/l of distilled water.

B) Stock K : 100 mEq/l - 7.46 Kcl/l of distilled water

A series of stock standard solutions can be prepared from these as follows:

For routine use the stock standard can be prepared as follows :

SODIUM	mEq/1	ml. of stock A	ml.of stock B
	140	70	25

Made upto 500ml with distilled water.

POTASSIUM	mEq/1	ml.of stock A	ml. of stock B
	5	70	25

Made upto 500ml with distilled water

These solutions should be then diluted in the same way as the urine to give standard for use in the instrument. ie, usually 1 in 100 dilution.

APPENDIX - VI

ESTIMATION OF CHLORIDE (METHOD OF VAN SLYKE)

(Varley et. al., 1980)

Principle:

Chloride is precipitated as Silver Chloride by the addition of Silver nitrate solution. Excess of Silver nitrate is titrated against standard thiocyanate solution in the presence of ferric alum as indicator.

Reagents:

(1) 0.05N Silver nitrate solution:

Dissolve 9.495 g of silver nitrate in water and made upto a litre with distilled water. The solution which should be standarised against sodium chloride was kept indefinitely in a brown bottle.

(2) 0.02 N Potassium thiocyanate:

Prepare at intervals by diluting a stock 0.1N solution.

(3) Concentrated nitric acid.

(4) 5% solution of Ferric alum.

(5) Potassium dichromate solution (Indicator).

Procedure:

To 1.0 ml of urine add 3.0 ml of Silver nitrate solution and 2.0ml of concentrated nitric acid. Heated over a flame till a pale yellow colour was obtained. Cool and then add 6.0 ml of ferric alum. Then titrate with 0.02N thiocyanate (Standardised against silvernitrate of known strength using Potassium dichlromate as an indicator) until a reddish brown colour persisting for 10-15 seconds was obtained.

To determine the standard 2.0ml of concentrated nitric acid, 3.0ml of silver nitrate and 6.0ml of ferric alum were taken and titrated against 0.02N thicyanate to the same end point as above.

Calculations:

The difference between the two titre values gives a measure of the amount of chloride in 1.0ml of urine in terms of 0.02N solution.

$$\text{M.Eq of Chloride/litre} = (\text{Titre value of Standard} - \text{Titre value of unknown}) \times 20$$

APPENDIX - VII

ESTIMATION OF CALCIUM

(Method of clark and Collip)

(Varley et. al., 1980)

Aim:

To estimate the amount of Calcium in the given sample of urine.

Principle:

Calcium is precipitated from urine as the oxalate. Magnesium is not coprecipitated as the conditions are selected to increase the solubility in acid and the oxalate ion is determined titrimetrically by titration with potassium permanganate.

Reagents:

(1) Ammonium oxalate 4% solution.

(2) Ammonia 2% (V/V) Solution.
Dilute 2.0ml of ammonia (Specific gravity 0.88) to 100ml with water.

(3) Potassium permanganate 0.01N
Prepared freshly before use by diluting stock 0.1N solution.

(4) Approximately normal sulphuric acid.

Procedure:

To 2.0ml of urine add 2.0ml of distilled water and 1.0ml of 4% ammonium oxalate solution. Let it stand overnight at room temperature. Centrifuge and remove the supernatant fluid without disturbing the precipitate. Add 3.0ml of 2% ammonia down the inside of the tube.

Mix the precipitate. Centrifuge and pour off the supernatent. This is repeated until the precipitate was washed completely. Repeat this till the supernatant gives no precipitate with calcium chloride solution. Add 2.0ml of approximately normal sulfuric acid. Mix and dissolve the precipitate with acid. Warm by placing in a beaker containing almost boiling water to complete the solution of oxalate. Remove and titrate with 0.01N Potassium permanganate keeping the mixture at 70 - 75°C to a faint pink colour which persisted for about a minute.

As a blank titrate 2.0ml of N/10 sulphuric acid to the same end point. The difference between the two titrations gives the volume of 0.01N potassium permanganate required to titrate the calcium oxalate.

Calculation:

$$\text{Amount of Calcium in 100 ml of urine} = \frac{(\text{Titre value of urine} - \text{Titre value of blank}) \times 0.2 \times 100}{2}$$

APPENDIX - VIII

ESTIMATION OF URINARY PHOSPHORUS

(According to Gomorri)

(K.M. Samuel; 1986)

Metol is used as reducing agent instead of amionnapthol sulphonic acid. This reagent is easier to handle & is less affected by variations in the amount of acid present. Only one molybdate reagent is required.

Reagents:

(1) Trichloroacetic acid 10% W/V in distilled water.

(2) Ammonium molybdate solution:

Dissolve 7.5g in about 200 ml. distilled water, add 100ml of 10N sulphuric acid and make upto 400ml of distilled water.

(3) Metol Reagent (P-methylaminophenol Sulphate):

1g. in 100ml. of 3% SodiuM bisulphi te solution.

(4) Standard phosphate solution:

Dissolve 0.2197g of potassium dihydrogen phosphate in distilled water, make upto a litre and add few drops of chloroform.

Technique:

Add 0.8ml of urine to 7.2ml of 10% Trichloracetic acid, mix well and filter or centrifuge. Set up three tubes containing respectively 5.0 ml. (0.5ml of urine) of the filtrate (the unknown); 0.5ml of standard (=0.025 mg. phosphorus) plus 4.5 ml of 10% Trichloracetic acid (the standard); and 5 ml. of the trichloracetic acid (the blank). To each tube add 1.0 ml of each Ammonim molybdate and 1.0ml of metol solutions. Allow to stand for 30 minutes, and read at wave length 680 mu. or using Red filter against the blank set at zero.

Calculation:

Mg. Inorganic phosphorus per 100ml urine.

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.025 \times \frac{100}{0.5}$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 5$$

S.No.	Infecting Organism	Total Proteins mg/dl	Sodium mEq/l	Potassium mEq/l	Calcium mg/dl	Chloride mEq/l	Phosphorus mg/dl
1.	E.Coli	112.5	148	30	13.0	118	103.85
2.	E.Coli	226.0	85	17	3.0	62	105.85
3.	E.Coli	133.2	288	35	9.0	138	69.33
4.	Pseudomonas pyocyanae	63.68	159	36	21.0	140	95.86
5.	E.Coli	30.3	123	52	12.0	90	64.91
6.	E.Coli	230.47	123	19	4.0	48	62.90
7.	E.Coli	29.75	78	21	6.0	138	104.80
8.	Klebsiella	29.45	272	39	12.0	136	80.80
9.	Klebsiella aeruginosa	80.66	136	51	7.0	110	109.80
10.	Klebsiella aeruginosa	25.12	158	38	5.0	118	115.80
11.	Proteus	230.0	180	25	9.0	132	45.93
12.	Proteus	382.11	184	22	14.0	98	83.88
13.	Klebsiella aeruginosa	279.23	280	23	11.0	140	69.90

S.No.	Infecting Organism	Total Proteins mg/dl	Sodium mEq/1	Potassium mEq/1	Calcium mg/dl	Chloride mEq/1	Phosphorus mg/dl
14.	E.Coli	160.0	185	31	7.0	140	94.87
15.	E.Coli	60.0	148	28	6.0	82	79.89
16.	E.Coli	228.0	172	38	16.0	148	94.87
17.	E.Coli	176.0	236	48	13.0	68	81.88
18.	E.Coli	136.0	125	20	4.0	136	111.85
19.	E.Coli	220.0	140	50	19.0	134	105.80
20.	E.Coli	39.50	190	41	18.0	127	98.29
21.	Klebsiella aeruginosa	68.0	80	17	6.0	148	65.00
22.	Klebsiella aeruginosa	132.0	120	22	12.0	146	80.00
23.	Pseudomonas aeruginosa	135.0	160	39	11.5	150	93.00
24.	E.Coli	52.21	140	24	8.0	144	90.00
25.	Klebsiella	92.54	231	21	15.0	150	85.71