

**GRANULOCYTE FUNCTION IN STABLE  
CRF AND END-STAGE RENAL DISEASE  
PATIENTS ON HAEMODIALYSIS AT  
KENYATTA NATIONAL HOSPITAL**

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fulfilment for the degree of  
MASTER OF MEDICINE (MEDICINE)**

**of the**

**UNIVERSITY OF NAIROBI**

**By**

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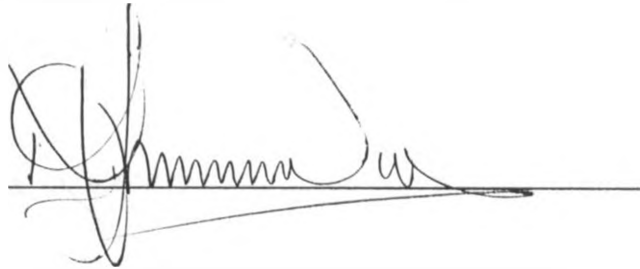
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## DECLARATION

This dissertation is my original work and has not been presented for a degree work in any other University.

A handwritten signature in black ink, appearing to read 'Joseph Ntarindwa', written over a horizontal line.

JOSEPH NTARINDWA M.B.CH.B ( MUK )

This dissertation has been submitted for examination with my approval as a University Supervisor.

A handwritten signature in black ink, appearing to read 'Leander S. Otieno', written over a horizontal line.

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DEDICATED

*To Fred and those with him who gave their lives for the benefit of others.*

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## SUMMARY

Phagocytosis and the ability to kill Candida albicans in-vitro (candidacidal activity) in three groups of subjects were compared: Group 1 - patients with end-stage renal disease (ESRD) on maintenance haemodialysis (H.D) Group 2 - patients with stable chronic renal failure (CRF) who were not on dialysis. Group 3 - normal healthy controls.

Group 1 were 20; 13 males and 7 females, group 2 were also 20; 14 males and 6 females and group 3 were 21; 14 males and 7 females.

The study showed that the phagocytic activity in autologous plasma was lower in group 2 (uraemic patients who were not on haemodialysis) than group 1 (patients who were on haemodialysis) ( $P < 0.02$ ). The phagocytic activity was also lower in group 2 patients than group 3 (controls) ( $P < 0.02$ ). When the neutrophils from group 1, 2 and 3 subjects were suspended in AB rhesus negative (AB Rh -ve) plasma, the phagocytic activity was lower in group 2 than group 1 patients ( $P < 0.002$ ). The phagocytic activity in AB Rh -ve plasma for group 2 patients was also lower than for group 3 (controls) ( $P < 0.002$ ). There was no significant difference in neutrophils from group 2 and 3 subjects both in autologous and AB Rh -ve plasma.

The candidacidal activity of group 3 was the highest of the three groups but the difference was statistically significant only when comparing groups 2 and 3 ( $P < 0.02$ ). Immunoglobulin and complement profiles in the three groups were also compared and no significant differences were found.

## INTRODUCTION AND LITERATURE REVIEW

Vulnerability to infection by uraemic patients was reported by among others Masson and associates as early as 1949 (1). Balch during the Korean war in 1955 observed that soldiers with battle injuries tended to have more serious infections when they developed acute renal failure (2). Merrill found renal failure in Boston to be a major cause of death in renal patients with or without dialysis(3). Montogomerle and Kalmanson noted that renal failure patients had high incidences of infection often with fatal outcome(4).

Various hypotheses have been suggested to explain the above findings. Skin abnormalities have been found in uremic patients pre- and post-dialysis. These include pruritus, ichthyosis, excoriation ecchymoses, uraemic frost and exfoliation (5-7). Histochemical changes have also been described, namely atrophy of skin epidermis (5), increase in elastin fibres in papillary layer and perifollicular region (8,9), increase in urea nitrogen in stratum corneum (9), atrophied eccrine sweat glands and increase in nitrogenous compounds in uraemic breath (10) and saliva (11). These changes may be responsible for delayed wound healing and contribute to increased nasal and cutaneous carriage of staphylococcus aureus reported by Goldblum and co-workers (12) and Klman et al (13).

Uraemic patients and those on maintenance haemodialysis have been reported to have very high incidences of respiratory tract infections (4,14). In addition to uraemic lung disease (15,16), a number of other changes in the respiratory tract probably help to increase the incidence of respiratory tract infections. These include coating of the tracheo-bronchial tree with thick tenacious mucus, thickened alveolar septa covered with hyaline appearing membrane and intra alveolar fibrinous exudate. In 1966 Goldstein and Green demonstrated impaired clearance of coagulase positive Streptococci in nephrectomised mice and they suggested that this may be due to impaired lung macrophage and mucocilliary action caused by uraemia(17).

The urinary tract has also been reported to be commonly infected during uraemic state (4,14). The infection in this situation may be related to catheterisation a common procedure employed on these patients. Oliguria also causes relative stasis and encourages proliferation of urinary pathogens (18). Impaired urine concentration in CRF causes passage of dilute urine without any antibacterial activity (19).



Other observations have been made in patients on maintenance H.D. Leonard and associates reported five adult patients who developed osteomyelitis while on maintenance H.D (20). Three of these patients had renal osteodystrophy and two were on corticosteroid therapy. However Lameirie and colleagues reported one case of an adult male who developed spontaneous osteomyelitis and was neither on corticosteroids nor had renal osteodystrophy(21). Haemodialysis patients have been found to be more prone to tuberculosis (TB) than the general population. Andrew found that 10 out of 172 patients who were on H.D between July 1967 and July 1976 in San Francisco had developed TB(22). He found that H.D patients were 12 times more prone to TB infection than the general Californian population. Four of the ten patients had pulmonary TB alone. Other sites affected by TB were meninges, pleura, kidneys, liver and mediastinal lymph nodes. Apart from Mycobacterium tuberculosis (MTB), other mycobacteria have been found to cause disease in renal patients (23). These include M. avium intracellulare and M. fortium. Almost similar findings were made by Sasaki and Akiba in Japan (24).

Patients with CRF have also been found to be affected by dermatophyte infections. Onorato and colleagues reported two cases of thrombosed fistulae which were infected with Cephalosporidium species along with Staph. aureus(25). Infective endocarditis in association with H.D has also been reported by a number of investigators (26-30). Other studies have shown that patients on H.D tend to become chronic carriers of hepatitis B surface antigen (HBsAg) when they become infected with hepatitis B virus. A report by Mc'Ligeyo and Otieno (31) has shown that 3 out of 71 patients who were initially HBsAg negative at the on-set of H.D, had seroconverted to positive and this required a period of only 3 months. Other studies in Berlin showed that more than 50% of H.D patients who were HBsAg positive continued to test positive beyond 6 months period(32). This reflects the inability of H.D patients to clear the hepatitis B virus from their systems.

The single most common source of infection in patients with H.D is thought to be the gastrointestinal tract (GIT) (14). Montgomerie and colleagues demonstrated Klebsiella pneumonia infection in faecal flora of 3 out of 5 patients on H.D who had Klebsiella pneumonia infections of the skin, chest and maxillary sinuses, and these organisms were further found to be identical serotypes(33).

The tendency for intestinal bacteria to cause extra intestinal infections may be related to the GIT mucosal defects associated with uraemia and H.D. A study done at K.N.H. by Joshi and his group, showed that only 33% of CRF patients had normal upper GIT(34). The abnormalities found included gastritis, duodenitis and oesophagitis with abnormal mucosal folds in the antrum. Similar study by Zukerman et al gave similar results(35). Further more, Gordon and Johnson reported some patients on H.D who were found to be gastric acid hyposecretors and yet they were not able to demonstrate any parietal cell antibodies in their sera(36). These mucosal abnormalities together with gastric hypo-acidity in some patients on H.D facilitate transmural shift of bacteria from the GIT. Indeed Schweinburg and coworkers were able to demonstrate increased GIT transmural migration of bacteria in uraemic dogs (37).

Other sources of infection are skin. Increased carriage of Staph. aureus on skin by patients on H.D has been reported in several studies (12, 16, 38). At the Kenyatta National Hospital (KNH), Mc'Ligeyo (39) found Staph aureus to contribute more than 60% of shunt infections. Comparable results were obtained by Rebel et al (40).

Findings by a number of investigators have indicated that there is normal to hyperactive leucopoiesis in uraemia (41,42). This has been the finding of Jensson (41) and Kloko (42) from K.N.H who noted that 66.7% of uraemic patients in his series had normal leucopoiesis and the rest 33.3% had increase leucopoiesis. Jensson (41) like Kioko (42) reported absolute and relative neutrophilia. In Kloko's study neutrophil hypersegmentation and toxic granulation were the commonest abnormalities affecting the white cell series(42). Other morphological abnormalities of neutrophils have been observed. Brogan found the neutrophils of uraemic patients to have the following abnormalities(43): They appeared to spread on glass slide giving larger than normal appearance, they had more cytoplasmic granules which were more vacuolated and the neutrophils had also long cytoplasmic processes. It is these abnormal neutrophils which were further subjected to the trauma of extracorporeal circulation during H.D.

The following functional abnormalities have been encountered; firstly in chemotaxis, in vivo studies have shown that uraemic subjects have impaired inflammatory responses compared to normal subjects. Balch and Evans (44) injected bacteria intradermally in normal and nephrectomised rabbits and compared the resulting lesions 24 hours after inoculation. The uraemic rabbits

had significantly smaller lesions. Buchanan and colleagues got similar results using microcrystalline monosodium urate (45). Secondly other studies have directly demonstrated impaired neutrophil chemotaxis (46, 47). Grover and Anderson subjected ureamic neutrophils to a chemotactic test by putting them in a chamber separated from a chemotactic stimulant by a 33  $\mu$  pore membrane filter (48). After 3 hours they quantified the number of neutrophils reaching the lower surface of the membrane filter. They subjected the same test to normal neutrophils and the result was higher in normal than uraemic neutrophils. They repeated the same procedure but instead of a known chemotactic stimulus, they used endotoxin activated uraemic serum and compared its chemotactic activity with that of endotoxin activated AB serum and the results were lower in the former than the latter. In this study they were able to demonstrate reduced chemotactic activity of both uraemic neutrophils and serum respectively.

Studies of CRF patients' neutrophil adherence in both dialysed and non-dialysed CRF patients have given conflicting results. Ruley and colleagues described increased neutrophil adherence to glass slides in patients with acute post-streptococcal glomerulonephritis(49); whereas Abrutyn and colleagues found normal adherence to nylon fibres in patients with CRF(50). In another study it was found that patients with severe uraemia had normal adherence but it became impaired during the first 120 minutes, returning to baseline value after four hours of H.D(51)

#### PHAGOCYTOSIS

Phagocytic studies done on uraemic patients both dialysed and non-dialysed have given variable results with some workers demonstrating normal (43,48,50,52) and others impaired phagocytosis (43,53,54).

Brogan (43) demonstrated normal phagocytosis in uraemic patients but he noted that phagocytosis became more depressed in the presence of endotoxin. Burleson found that impaired granulocyte phagocytosis was reversed by haemodialysis(53). In a study done by Hallgren et al in Sweden, it was found that impaired phagocytosis correlated with rising phosphate levels and was reversed after four months of maintenance haemodialysis(55).

Studies on the ability of uraemic neutrophils to kill ingested organisms have also given conflicting results. Mac-Intosh et al found impaired killing for E. coli and Staph aureus(56) whereas Abrutyn and Solomons reported normal killing for Staph aureus(50).

Uraemic serum bactericidal activity has also been studied with variable results. Montgomerie et al reported depressed killing for E. coli and Staph aureus and normal activity on Strep faecalis(54). However considering the fact that uraemic serum has increased levels of lysozymes(57) one would expect to find increased bactericidal activity for this serum. The reason for this has not been widely agreed on.

There appears to be a functional variability between neutrophils from people of African descent and that of Caucasian extraction. Lule and Kyobe (58) found that neutrophils from Africans in Nairobi had lower phagocytic ability compared with neutrophils from a population in U.K. They also demonstrated lower mean leucocyte alkaline phosphatase (LAP) scores in Africans compared with that of the same population in the U.K. Hence there is need to study neutrophil function in the African uraemic patients.

Uraemia has been found to be associated with lymphopenia (42) and B lymphocytes have been more affected than other lymphocyte types (59). It has further been shown that this lymphopenia is partially or completely reversed by H.D (60). Despite the noted lymphopenia, C.R.F patients have been found to have normal immunoglobulin A, G and M levels and renal failure per se has not been shown to affect complement levels unless it is immunological in origin(55). In a number of studies (43,47,54), complement in C.R.F was comparable with that of normal controls. However, two independent studies (61,62) have shown that there is activation of the alternative pathway of complement following blood membrane-interaction during haemodialysis.

## AIMS OF THE STUDY

1. To evaluate the effect of HD on neutrophil phagocytosis in CRF patients.
2. To evaluate the effect on uraemic sera on neutrophil phagocytosis.
3. To find the effect of HD on neutrophils' ability to kill Candida albicans in CRF patients.
4. To find the effect of HD on serum complement and immunoglobulins in CRF patients.

## MATERIALS AND METHODS

### Patients and Controls

ESRD patients were those whose renal function could not sustain life without HD. Only those stabilised on 2-3 times a week dialysis were studied. Stable CRF patients were those whose serum urea was above 10mmol/l during adequate hydration but whose renal function could sustain life without any supportive dialysis. An earlier study(63) involving neutrophils in diabetics showed impaired neutrophil chemotaxis in diabetes mellitus, so patients with diabetic nephropathy were excluded from the study.

Normal healthy controls were individuals whose serum urea was less than 6mmol/l and were found normal on physical examination. They were medical workers including doctors, nurses and medical students.

The following categories of subject were excluded from the study;

- Those patients who had evidence of infection as suggested by physical examination and chest radiology.
- Patients on steroids or non-steroidal anti-inflammatory drugs, or anti-fungal drugs.
- Patients whose neutrophil function is impaired in absence of renal failure. These were diabetics, those who had a history of alcohol ingestion within 24 hours of blood sampling and patients in shock (63).

The study was conducted from 15/5/1990 to 31/12/1990. During this period 26 patients were on KNH renal unit HD programme. Only 20 of them fulfilled the above criteria and they were all recruited into the study.

Stable CRF patients were recruited from KNH renal clinic and medical wards by compiling a list of patients who had appointment to attend the renal clinic during the period of study, and those who had been admitted during the first week of the study. 83 names were obtained and the study sample was selected by picking every fourth name. The controls were selected from willing medical staff from whom every second person was picked. They were matched for sex and since most of the willing controls were medical students whose age was relatively younger, they were matched for age within 10 year age group.

After selecting the patient, an adequate history and physical examination was undertaken. A verbal consent that the patient enter into the study was obtained and blood was drawn.

## MEASUREMENT OF PHAGOCYTTIC AND CANDIDACIDAL ACTIVITY

This was done according to the methods described by Lehrer and Cline (64) and modified by Goldman and Th'ng (65) which is described below.

Peripheral venous blood (10mls) were collected from each subject with a plastic syringe from where it was put into a sterile plastic tube containing about 500 units of heparin. 1 ml of 6% dextran in normal saline was added and the red cells were allowed to sediment at room temperature for 20-30 minutes. The leucocyte supernatant was collected and centrifuged at 1,500 revolutions per minute (RPM) for 8-10 minutes. The leucocyte deposit was then removed and washed twice in Hanks balanced salt solution (HBSS) finally suspending it in 0.5 - 1 ml of HBSS. The absolute number of neutrophils was then calculated from the total leucocyte count and differential count.

## CANDIDA ALBICANS COLONIES

The Candida albican cells used were maintained on glucose peptone agar. When required the organisms were collected and suspended in HBSS at a concentration of  $4 \times 10^5$  per ml. Only cultures containing 95% viable cells as determined by methylene blue staining were used.

## PLASMA

AB Rh-ve plasma (200mls) was obtained from K.N.H blood bank. This was divided into five portions which were kept at temperature of  $-20^{\circ}\text{C}$ . Heparinised plasma from leucocyte donors was collected for resuspending the leucocytes after which the remaining amount was kept for urea, creatinine, phosphate, immunoglobulin and complement assays later. This plasma was stored at a temperature of  $-20^{\circ}\text{C}$ .

## TESTS

Phagocytic activity was tested by incubating a suspension containing Candida and neutrophils together with Candida in excess of leucocytes by a factor of 10 so that no neutrophil was denied the opportunity to test its phagocytic activity because of absence of Candida particles.

## PROCEDURE

Equal volumes of neutrophil suspension, autologous plasma and HBSS (0.25mls each) were placed in a sterile container. The solution containing Candida was added and the components mixed manually. Control tubes were set up containing:-

1. Patients neutrophils, HBSS, Candida organisms and AB plasma.
2. Normal neutrophils, patients plasma, HBSS and Candida organisms.
3. Normal neutrophils, normal plasma, HBSS and Candida organisms.
4. Patient's plasma, HBSS and Candida organisms without any neutrophils.

All the tubes were incubated at 37<sup>0</sup>C with manual mixing every 15 minutes. After 30 minutes incubation a small sample was drawn from each tube to confirm that the organisms have been ingested. A smear was then made from each mixture. It was stained and fixed. The smear was examined under a microscope using oil immersion lens, 100 leucocytes were counted and the number of Candida organisms ingested by each leucocytes was noted. After 60 minutes, sodium deoxycholate was added to each tube. This reagent dissolves the cell membrane without damaging the Candida organisms. 5 minutes after, methylene blue was added to each tube and the suspension centrifuged at 1,500 RPM for 5 minute. A wet preparation was then made from the cell sediment and 300 Candida cells were counted from each tube noting which one was alive and which one was dead. The living cells fail to take up methylene blue and could be differentiated from dead ones which stained uniformly intense blue (see plate 3). The candidacidal index i.e number of dead Candida cells out of 300 was then calculated by deduction of the number of dead Candida cells in the control tubes that didn't contain any leucocytes.

## IMMUNOGLOBULIN AND COMPLEMENT ASSAYS

These were done by single radial immuno-diffusion (66) using antibody kits supplied by Boeringer Pharmaceutical company. The method is hereupon described.

The kits are anti-human IgA; anti-IgG, anti-IgM and anti-complement antibodies with standard IgA, IgM and IgG plasma.

The anti human antibody (anti IgA, IgG, and IgM) and anti human complement were each added to agar gel and allowed to set. The wells were punched in the agar after which the test and the standard plasmas containing



known concentrations of Immunoglobulins were poured into the wells using micropipettes. The agar plates were left for 24 hours during which time the antibodies in the wells diffused out to form soluble complexes with the anti-human Immunoglobulin in the agar, until an equivalent point was reached and the complexes precipitated in a ring. The diameter of each ring was then measured. The area within the precipitin ring as measured by the square of the diameter is proportional to the Immunoglobulin or complement concentration.

For IgA, IgG, and IgM, different curves for each were drawn relating the concentration of the standard plasmas with the square of the diameter of their precipitin rings from which the concentration of the test plasmas were read against the squares of the diameters of their rings. The concentration of complement expressed as a percentage was calculated from the formula:

$$\text{Complement \%} = \frac{(\text{diameter of test ring})^2 - 4^2}{(\text{diameter of standards})^2 - 4^2} \times 100$$

where 4 is the diameter of the wells in mm.

#### ANALYSIS OF DATA

The data obtained for phagocytosis, candidacidal activity, Immunoglobulin and complement levels for the three groups was analysed by computer. The student T-test was used to evaluate the differences amongst the groups. P-values (P) were computed with confidence limits set at  $\alpha = 95\%$  and  $1-\beta = 0.8$  ( $\beta=80\%$ ).

## RESULTS

Three groups of subjects were studied:

Group 1 consisted of CRF patients on maintenance haemodialysis.

Group 2 were patients with CRF who did not require haemodialysis at the time of the study

Group 3 were normal healthy controls.

TABLE 1 SHOWS THEIR SEX DISTRIBUTION

	Males	Females	Total
Group 1	13	7	20
Group 2	14	6	20
Group 3	14	7	21
Total	41	20	61

TABLE 2 AGE DISTRIBUTION AND BIOCHEMICAL PARAMETERS

	Mean age (yrs)	Mean urea (mmol/l)	Mean creatine ( $\mu$ mol/l)	Mean phosphate (mmol/l)
Group 1	35.3	14.6	294.4	1.96
Group 2	25.1	28.1	28.1	3.0
Group 3	32.4	3.1	3.1	1.2

Phagocytosis was examined in two ways, one way was to count the number of cells out of 100 participating in the phagocytic process ie 100 - number of neutrophils ingesting no Candida cells (phagocytic index). The other way was to examine each neutrophil and quantify the number of Candida cells it ingested. The results were tabulated below.

Table 3

Phagocytic indices of groups 1, 2 and 3 in autologous and AB plasma expressed as mean  $\pm$  2SE

Phagocytic Index = (100 - number of neutrophils ingesting 0 Candida cells)

GROUP	PLASMA	
	Autologous	AB
1	49 $\pm$ 9.06	53 $\pm$ 9.65
2	36.2 $\pm$ 4.76	35.6 $\pm$ 4.40
3	48.8 $\pm$ 6.50	-

Table 4

Phagocytosis of individual cells according to the number of Candida cells ingested 1-3 cells and >3 cells (mean  $\pm$  2SE).

Group	Autologous plasma		AB plasma		Uremic plasma	
	1-3	>3 particles	1-3	>3 cells	1-3	>3
1	39.0 $\pm$ 7.10	10.2 $\pm$ 4.44	38.6 $\pm$ 7.97	11 $\pm$ 4.93	-	-
2	31.9 $\pm$ 3.83	2.6 $\pm$ 0.69	34.2 $\pm$ 3.86	2.5 $\pm$ .98	-	-
3	41.9 $\pm$ 4.95	9.6 $\pm$ 3.61	-	-	38.5 $\pm$ 4.21	9.2 $\pm$ 3.61

Table 5: Candida cells killed out of 300 by neutrophils in group 1, 2 and 3 (mean  $\pm$  2SE)

Group	Autologous plasma	AB plasma	Uremic plasma
1	34.6 $\pm$ 7.68	49.0 $\pm$ 1.64	43.2 $\pm$ 9.65
2	30.2 $\pm$ 6.55	48.1 $\pm$ 8.11	-
3	44.1 $\pm$ 4.44	-	43.2 $\pm$ 9.11

There was no significant difference between the values observed for Immunoglobulins and complement in the three groups

### Phagocytosis in autologous plasma

There

was no significant difference between phagocytosis of dialysed neutrophils and control neutrophils considering both the phagocytic indices and number of particles per neutrophil in both groups. However the phagocytic indices for undialysed patients was significantly low compared to the other groups with 95% confidence limit for the difference = 2.07 - 23.33 and  $P < 0.02$ . The phagocytic indices for control neutrophils were the same whether they were suspended in autologous plasma or in uraemic plasma. Each neutrophil in groups 1 and 3 ingested more Candida particles than group 2 on the average.

### Phagocytosis in AB plasma

There was no significant difference between phagocytosis in autologous plasma and phagocytosis in AB plasma for the three groups but phagocytosis in AB plasma for non-dialysed patients was much lower than that of the two remaining groups ( $P < 0.04$ ). This implies that the impaired phagocytosis in non-dialysed patients was due to an intrinsic defect within the cells not the plasma. See tables 3-5.

The study suggests that there is a correlation between phagocytic index and serum phosphate see fig 1.

The higher the phosphate the lower the phagocytic index. But there was no relationship between urea or creatinine, and phagocytosis. see fig 2 and 3.

### Candidacidal activity

In autologous plasma the neutrophil ability to kill Candida was higher in control population compared with non-dialysed group ( $P < 0.02$ ). It was also higher than the dialysed group but the difference was not statistically significant ( $P > 0.05$ ). The difference between dialysed and non-dialysed neutrophils was also

not statistically significant but the former had higher value.

The ability to kill Candida by the cells from control group did not alter significantly when they were suspended in patients plasma.

When the cells were suspended in AB plasma, their ability to kill Candida significantly increased but there was no difference between the groups when both were suspended in AB plasma. The increase may be a property of the AB plasma not of the neutrophils.

There was no difference in the killing of Candida between neutrophil free dialysed and non-dialysed plasma.

Immunoglobulin and complement profiles were same for all the three groups.

## DISCUSSION

Immunosuppression is one of the known complications of the uraemic state. Investigators of immune function in patients with ESRD have revealed depression of cell mediated immunity and lymphocyte abnormalities (8,67,68-70) and depression of phagocytosis and chemotaxis by granulocytes (71). The findings of this study seem to agree with such studies. Indeed it shows that there is impaired phagocytosis in ESRD which is corrected by haemodialysis. There appears to be a correlation between phagocytosis and phosphate levels which has been earlier reported by Hallgren et al (55). This may explain the depressed phagocytosis in uraemic patients who are not dialysed since they have higher serum phosphate values than the other groups; but the low phagocytosis persisted even when they were suspended in normal AB plasma. In the list of uraemic toxins one finds urea and guanidino-compounds such as guanidine, methyl and dimethyl guanidine and uric acid (72) but phosphate normally does not feature on the list. Other suggested toxic compounds are creatinine, creatine, urates, guanidinosuccinic acid, aliphatic amines, some peptides and degradation products of aromatic amino acids like tyrosine, tryptophan and phenylalanine (72). Defective ion transport across membranes, decreased cellular metabolism and  $\text{Na}^+$ -and- $\text{K}^+$  stimulated ATPase activity are metabolic abnormalities that are known to occur in uraemia(72). It is no wonder therefore that this goes with decreased phagocytic activity since it is an active process that requires energy. Another biochemical abnormality of the uraemic neutrophils has been found to be increased leucocyte alkaline phosphatase (LAP) score as shown by Otieno and his group (73) but whether this has any immunological significance is yet to be determined.

Other specific neutrophil abnormalities have been identified. In CRF, the

neutrophils have been found to have toxic granulation and to be hypersegmented (42). Other workers reported abnormal cytoplasmic processes and abnormal vacuolations (43). Chemotaxis (the directional movement of neutrophils towards the particle to be ingested), has also been found to be impaired (46,47,48). Abnormal neutrophil adherence in CRF has also been described (49). These factors may help to explain the impaired phagocytic activity in CRF neutrophils. Phagocytosis is influenced by immunoglobulins mainly IgA and IgG which coat the antigens and facilitate their ingestion by phagocytes (opsonisation) (74). It is also affected by the complement system. The complement components C3a and C5a are chemotaxins which promote migration of the phagocyte to a position where there is a particle to be ingested, and C3b is also an opsonin (74). However, the differences in phagocytic activity noted cannot be explained by the role of complement or immunoglobulins since the plasma levels for complement and immunoglobulins were the same for all the three groups. The candidacidal activity of non-dialysed uraemic neutrophils was lower than those for dialysed and controls. The latter had higher value than the former but the difference was not significant. I have not been able to establish the relationship between ingestion and killing of the Candida. High phagocytic activity value did not correspond with high candidacidal value. In fact one patient on H.D had good phagocytic activity value but all the Candida cells were still alive after adding cell-membrane lysing agent. This can be explained by the variability in the contents of the lysosomes in the neutrophils which are normally responsible for killing the organisms intracellularly. After injection of an invading particle by a phagocytic cell, the particle is drawn into a vacuole, the phagosome. The phagosome then fuses with the cell lysosomes which discharge their contents into it. It is the powerful antimicrobial substances in the lysosomes- lysozyme,

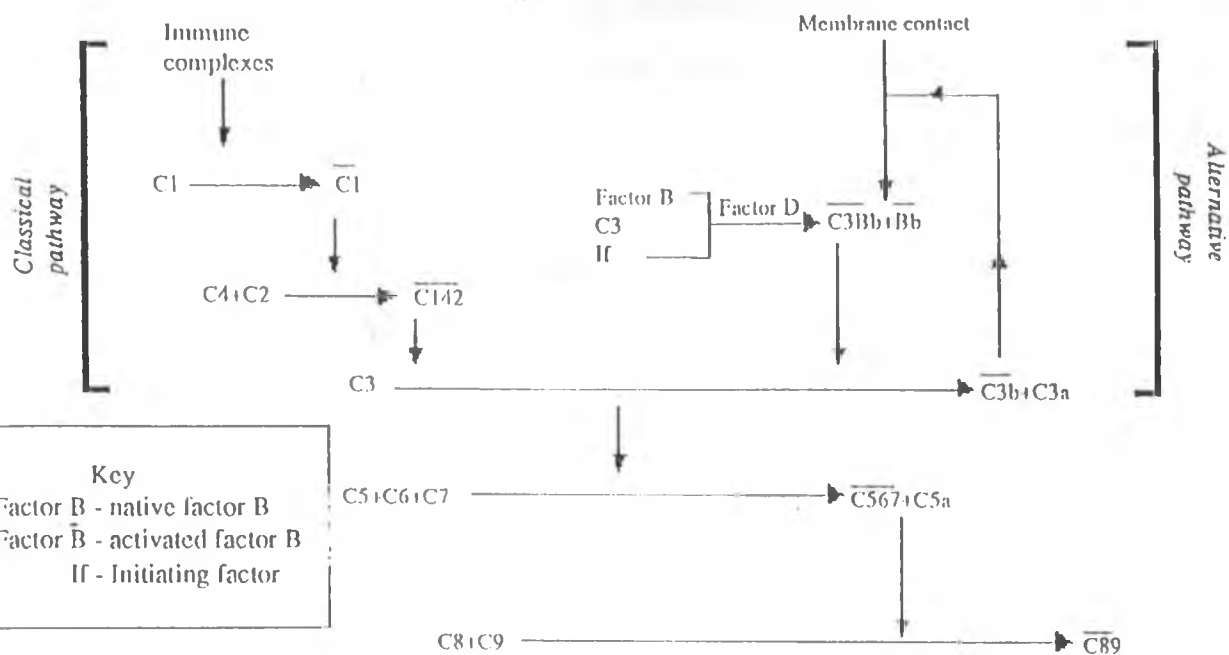


hydrogen peroxide, myeloperoxidase, lactoferritin and cationic proteins which then kill the invading particle (75). It appears that the activity of lysosomal contents did not correspond with phagocytic activity. Earlier studies using bacteria as test particles have shown variable results. One study (56) demonstrated impaired killing for E. coli and Staph. aureus while another one showed normal killing for Staph aureus (50). Similar variable findings have been made with the bactericidal activity of serum alone. One study (54) found that uraemic serum had depressed killing for E. coli and Staph. aureus, increased killing of B. subtilis and normal activity for Strep. fecalis L forms. It would therefore appear that increased, decreased or normal killing will depend on the organism in question.

Majority of studies have found the levels of the major immunoglobulins to be normal in CRF patients (56,76,77). Other studies have focused on the synthesis of antibody in response to specific antigenic challenge in CRF and haemodialysis patients. The antigens used were tetanus toxoid (2) Influenza virus vaccine (78) and endotoxin (79), and the response was normal. However recent studies (80,81) involving administration of pneumococcal vaccine to haemodialysis patients showed lower response compared to normal subjects and the levels achieved declined quickly.

Studies (61,62) have shown that there is activation of the alternative pathway of complement after blood-membrane interaction.

**Figure 4**



The pathways of complement are illustrated above (74)

Activation of the complement pathway should theoretically lower the levels of circulating native C3 and C5 but studies done in this respect have not found this to be so (43,47,54). The reason has not been explained. My study had made similar findings in that C3 levels in the three groups were not different. C5 assays have not been done due to lack of reagents.

More findings have been added to the list of immunological abnormalities in uraemia and haemodialysis. Ruiz et al (82) found that macrophage  $FC_\gamma$  - receptor function was impaired in CRF and was partly corrected by haemodialysis.  $FC_\gamma$  receptors are important in the clearance of IgG-coated particulate antigens and immune complexes. Nina et al (83) suggest that this  $FC_\gamma$  receptor deficiency was the major cause of immunosuppression in these patients. Furthermore preliminary studies with Interleukin-2 administered in conjunction with hepatitis B vaccine have suggested that this cytokine markedly enhances the host response of patients with uremia (84). Other abnormalities found are impaired

Interferon production (67,85), nutritional deficiencies such as protein malnutrition and zinc and pyridoxine deficiency (83) and mobilisation of iron stores by deferoxamine which has been associated with a marked risk of mucormycosis and perhaps other infections (86).

### CONCLUSION

This study suggests that there is impaired neutrophil phagocytosis in stable CRF and it seems with dialysis in ESRD the phagocytic activity improves to normal levels. It also suggests that there is impaired neutrophil ability to kill Candida albicans in CRF which persists even when haemodialysis treatment is introduced. The decreased phagocytic activity in stable CRF is not dependent on plasma but intrinsic cellular factors possibly morphological abnormalities and metabolic defects described (43,72).

Rising phosphate levels tend to reduce the neutrophil phagocytic activity but the latter is not affected by plasma urea or creatinine. Plasma levels for IgA, IgG and IgM and complement components C1q, C3 and C4 were the same in normal subjects as in CRF whether or not on dialysis.

### COMMENT

The findings in this study suggest that there is impaired neutrophil and normal B lymphocyte function in chronic uremia as indicated by depressed phagocytic activity, and normal immunoglobulin levels in uraemic subjects. Depressed phagocytic activity calls for care in the handling of these patients while doing any procedures to avoid unwarranted infections. In the event of infection, care should be taken while selecting the right antibacterial therapy. Dialysis should be started early when indicated.

Earlier studies (22, 24) have demonstrated a high incidence of TB in these patients. This implies that there is impaired T lymphocyte function. In a

community where there is a high incidence of TB, a study of this aspect of immune defence is suggested.

## LIST OF ABBREVIATIONS

1. AMREF - African Medical and Research Foundation.
2. CRF - Chronic Renal Failure.
3. ERSD - End-stage Renal Disease.
4. GIT - Gastro-intestinal Tract.
5. HBSS - Hanks Balanced Salt Solution.
6. HD - Haemodialysis.
7. K.N.H. - Kenyatta National Hospital.
8. MTB - Mycobacterium tuberculosis.
9. Rh-ve - Rhesus negative.
10. R.P.M. - Revolutions per minute

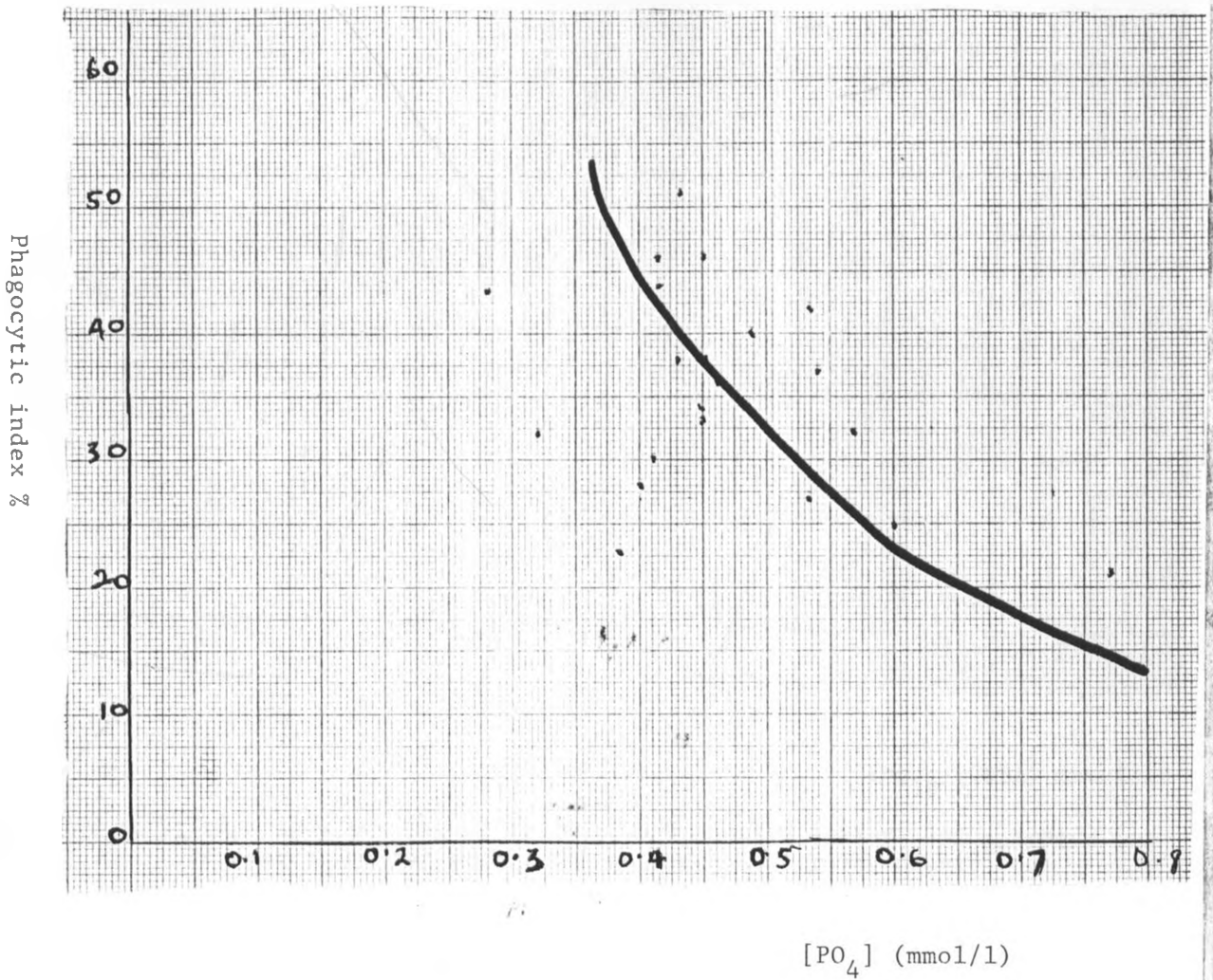
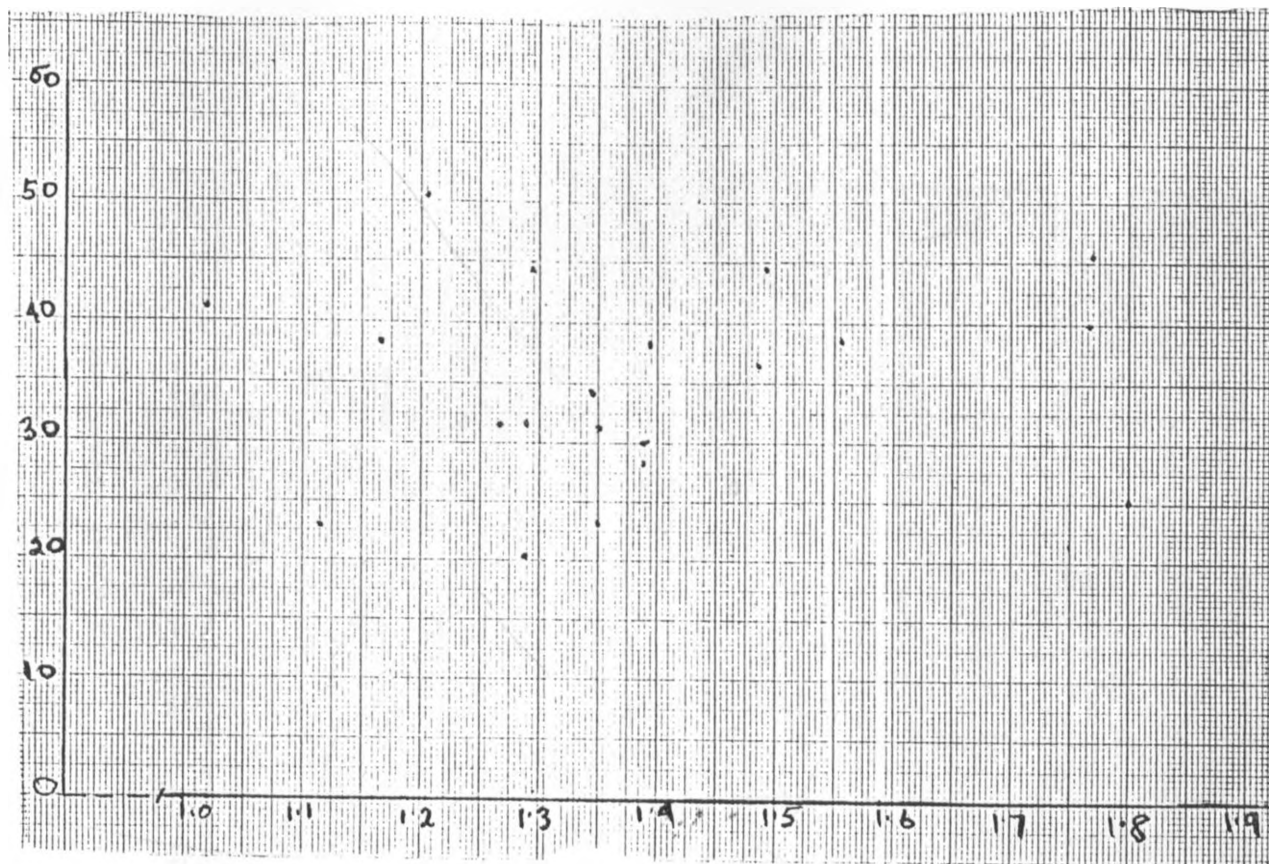


FIG 1: Graph showing phagocytic indices % plotted against  $\log_{10}$  serum phosphate concentration for stable CRF patients.

Correlation = -0.41

$p < 0.05$



Log<sub>10</sub> [UREA] (mmol/l)

FIG 2: Graph showing phagocytic indices % plotted against log<sub>10</sub> urea concentration for stable CRF patients. No relationship.

Correlation = 0.12

p > 0.05





PLATES

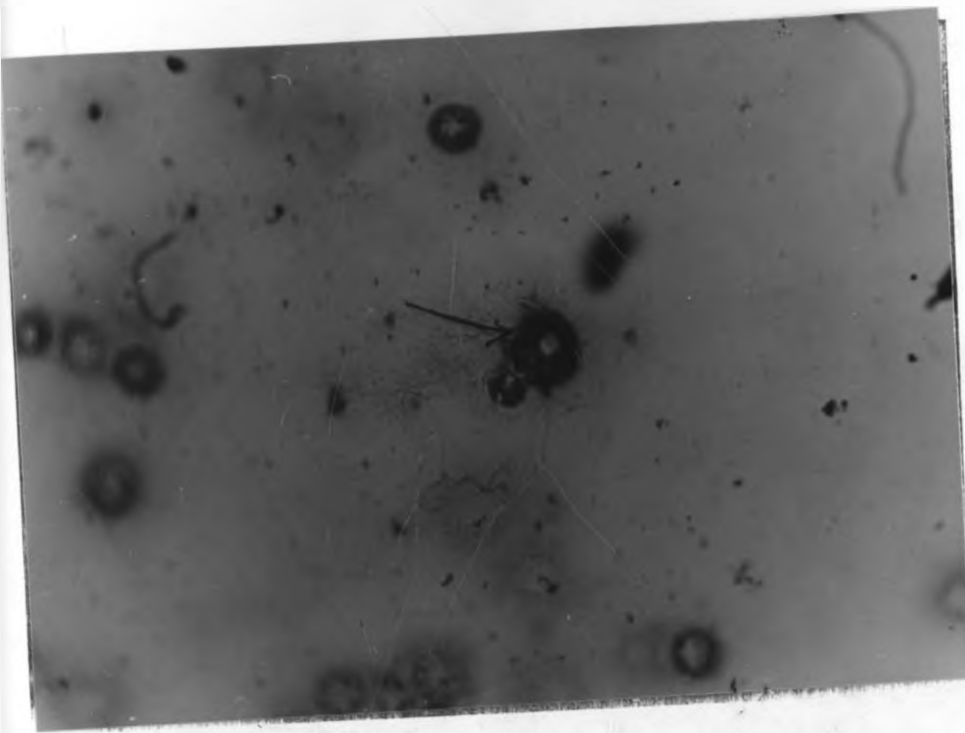


Plate 1

A single neutrophil can ingest few candida particles like the one above in plate 1 which ingested 2, or many like the one below in plate 2 which ingested 6. The neutrophils are indicated by the arrows.

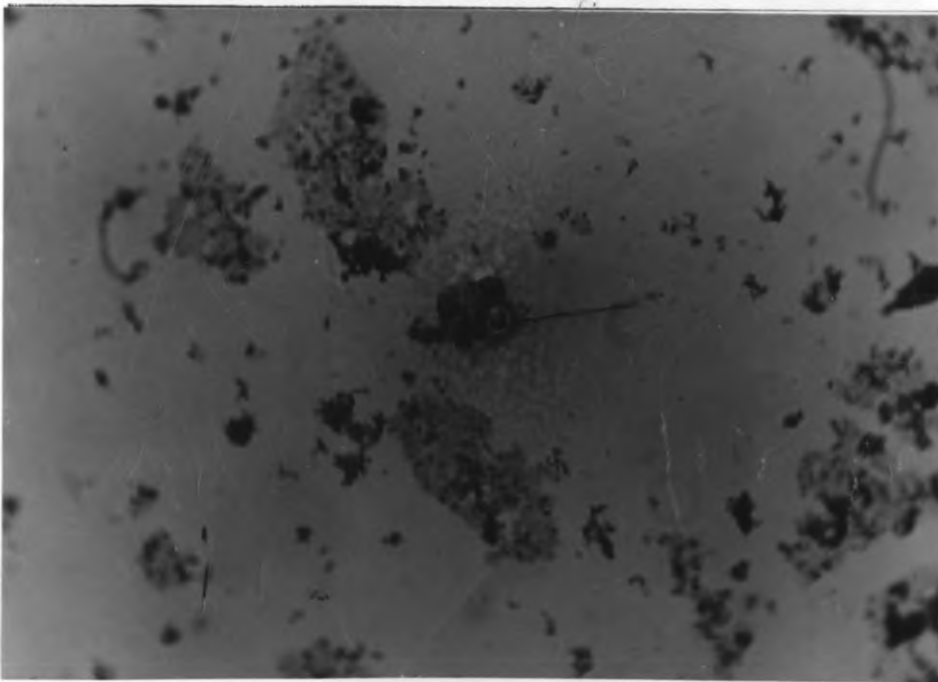
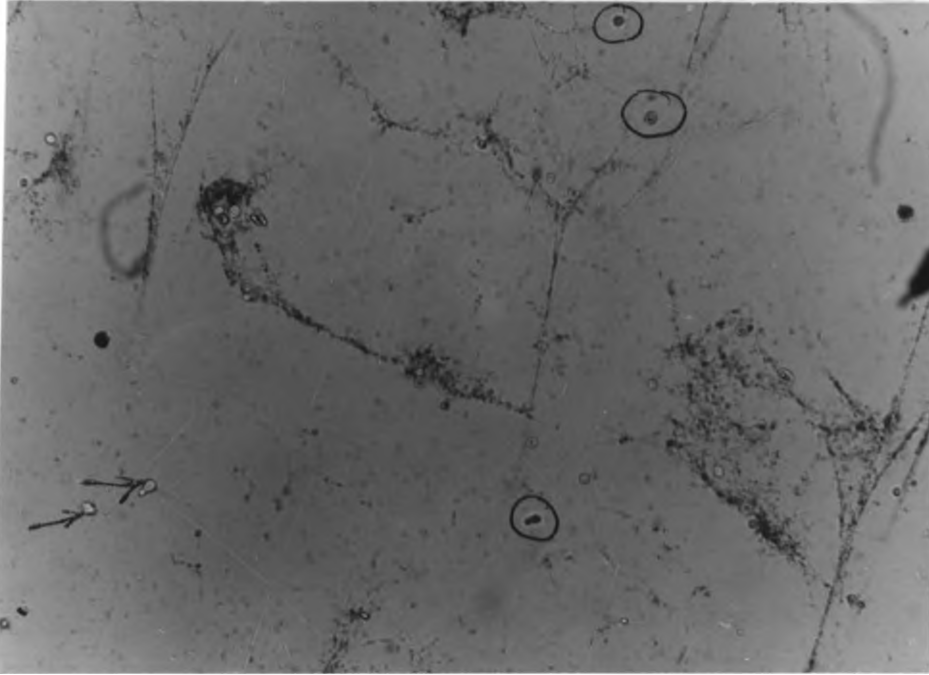


Plate 2

**Plate 3**

*Candida albicans* cells. The live cells (indicated by arrows) appear white while the dead ones (in circles) take up methylene blue and appear intense blue after staining with the dye.



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