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# IMPAIRED RENAL FUNCTION IN OWL MONKEYS (*AOTUS NANCYMAI*) INFECTED WITH *PLASMODIUM FALCIPARUM*

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*Impaired renal function was observed in sixteen Aotus nancymai 25 and 3 months following infection with the Uganda Palo Alto strain of Plasmodium falciparum. Decrease were noted in the clearance of endogenous creatinine, creatinine excretion, and urine volume while increases were observed in serum urea nitrogen, urine protein, urine potassium, fractional excretion of phosphorus and potassium, and activities of urinary enzymes. The results were suggestive of glomerulonephropathy and chronic renal disease.*

Key words: *Aotus nancymai* – *Plasmodium falciparum* – impaired renal function – pathology – Palo Alto strain

It is generally accepted that the effects of Plasmodium falciparum infection on the kidney can vary considerably from individual to individual. Some patients will evidence no adverse or only transient clinical effects, while others may progress to glomerulonephropathy, chronic interstitial nephritis, nephrotic syndrome, and end-stage renal disease.

A number of studies in human subjects and monkeys have focused on the histopathological changes in the kidney associated with acute or chronic *P. falciparum* infection (Jervis et al., 1972; Stone et al., 1972; Hutt et al., 1975; Boonpucknavig & Sitprija, 1979; Ehrich & Horstmann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988a; Aikawa et al., 1988b; Iseki et al., 1990). There is, however, little data available regarding alterations in the indices of renal function as sequelae to malaria infection. Impaired renal function has only been reported in human subjects with *P. falciparum* with nephrotic syndrome secondary to acute glomerulonephritis or renal failure but not in patients without sings or symptoms of renal manifestations (Areekul, 1987).

The purpose of this study was to determine the chronic effect(s) of *P. falciparum* infection on diagnostic indices of renal function in the owl monkey.

## MATERIALS AND METHODS

*Animals* – Quantitative urinalysis measurements used for calculating mean, standard deviation, range for each variable, and correlation coefficients were obtained from the records of 16 owl monkeys (*Aotus nancymai*) maintained by Battelle, Pacific Northwest Laboratories (BNW). They were part of an original group of fifty-four animals that had been employed in immunization studies at the Centers for Disease Control (CDC) to determine the safety, immunogenicity, and efficacy of vaccines against *P. falciparum* (Collins et al., 1991). Criteria for inclusion in the study were that the monkey was an adult, had no signs or symptoms of disease, and a history of infection with *P. falciparum*. At the time of sample collection, the animals had been in BNW facilities for at least 18 months and were believed to be well acclimated to their surroudings. Following their return to BNW, the monkeys were housed in male: female pairs, the accepted method for housing owl monkeys, and maintained according to specified guidelines (USAID, 1980; NIH, 1985). Animals were fed a balanced commercial primate diet (Purina 5040 New World Primate Chow, Ralston-Purina Co., St. Louis, MO) supplemented with fresh fruit three times a week, with water provide *ad libitum* through an automatic watering system.

*Parasite* – The Uganda Palo Alto (FUP) strain of P. falciparum was used to induce infections in *A. nancymai*. Heparinized blood was diluted in sterile saline to give $10^6$ parasites/ml. One ml of inoculum was injected intrave-

nously into each test animal. Daily thick and thin blood films were made according to the method or Earle and Perez and stained with Giemsa stain (Collins et al., 1991). Parasite counts were recorded per $mm^3$ of blood; when the percentage of red blood cells (RBCs) infected was very high, the parasite count per $mm^3$ was determined by counting the percentage of RBCs infected (1% of the RBCs infected = 40,000 parasites per $mm^3$). Maximum parasite density was designated as the primary outcome of the trial. Chemotherapeutic studies had indicated that the parasite was susceptible to chloroquine and quinine (Schmidt, 1978).

*Parasitological outcome* – Daily parasite counts were made for a 28 day period following inoculation. In the sixteen animals described in this study, maximum levels of parasitemia ranged from 16;400 to 856,000/$mm^3$. At the end of the 28 day observation period, animals had either recovered from their infections without treatment or were treated with chloroquine given orally at a dosage of 10 mg/kg of body weight for three consecutive days. All animals were subsequently treated with chlorquine to assure that they were free of infection prior to leaving CDC.

*Sample collection* – The urine samples were obtained using stainless-steel rodent metabolism cages that had been modified for small primates. Animals were weighed and placed in the metabolism cages for 16 hours after emptying of the urinary bladder by spontaneous voiding or manual expression. The 16-hour collection period included 8 hours of light and 8 hours of dark to allow for possible nocturnal or diurnal variations in excretion rates. Water, purified by a reverse osmosis system, was provided *ad libitum* by an automatic watering systwem located outside the metabolic unit to eliminate water contamination. The animals were fasted during the entire urine collection period. Samples were collected in graduated cylinders over ice. All urine voided during this time was collected. At the end of the collection period, the sample volume was determined and the entire sample submitted for quantitative urinalysis. Samples were kept capped and refrigerated until processed by the clinical pathology laboratory. Serum concentrations of creatinine, urea nitrogen, total protein, calcium, phosphorus, sodium, potassium, and glucose were also measured. Blood samples were collected by venipuncture, using a 23 ga needle, before and after each urine collection period from animals sedated with ketamine hydrochloride at 10 mg/kg body weight. Two to three milliliters of femoral venous blood was collected and put in Vacutainer™ tubes and allowed to clot. Serum was collected from the clotted sample and frozen at - 70 °C within two hours after sampling for subsequent analysis.

*Analysis* – All biochemical analyses were determined using a centrifugal analyzer that utilizes the horizontal light path principle (Cobas Fara, F. Hoffman-LaRocke and Co., Basel, Switzerland). Serum and urine creatinine were determined using a kinetic modification of the alkaline picric acid reaction (Bonses & Taussky, 1945; Larsen, 1972). Serum and urine urea nitrogen were measured by a urease method (Tiffany et al., 1972). Total serum protein was determined by a modified biuret method, while urine protein concentration was measured by the Coomassie brilliant blue dye-binding method (Gornall et al., 1949; Bradford, 1976). Total serum and urine calcium concentrations were measured by a ocresolphthalein method (Connerty & Briggs, 1966). Serum and urine phosphorus were determined using a phosphomolybdate method (Daly & Er-tingshausen, 1972). Serum sodium and potassium were measured using the centrifugal analyzer and an ion selective electrode (Cobas ISE Module), while urine sodium and potassium concentrations were determined by flame photometry (IL Model 443, Instrumentation Laboratories, Lexington, MA). Serum and urine glucose concentrations were determined by the hexokinase method (Sonowane et al., 1976). Urine alkaline phosphatase (ALP) activity was analyzed by the p-nitrophenyl phosphate method (Bessey et al., 1946), aspartate aminotransferase (AST) activity by the coupled enzymatic reaction of malate dehydrogenase and NADH (Bergmeyer et al., 1976), and N-acetyl-β-D-glucosaminidase (NAG) by the 3-cresolsulfonphthaleinyl-NAG method (Yuen et al., 1984; Stolarek et al., 1989). Urine specific gravity was determined by a refractometer calibrated against double distilled water.

Endogenous creatinine clearance was calculated from the following formula:

$$\text{Creatinine clearance} = \frac{(U_{cr})\ (^{o}V)}{(S_{cr})\ (BW)}$$

and expressed in milliliter/minute/kilogram of body weight, where, $U_{cr}$ = urine concentration of creatinine; $^{o}V$ = urine flow rate (ml/min); $S_{cr}$ =

serum concentration of creatinine; and BW - body weight (kg). Fractional excretions (FE) were calculated with the following equation:

$$FE = \frac{([x]u)\ ([Cr]s)\ 100}{([x]s\ ([Cr]u)}$$

where x is the substance in questions, and s and u represent concentrations of x and creatinine in serum and urine, respectively. Fractional excretion for a substance x in glomerular filtrate, as determined by the clearance of creatinine.

Serum and urine analyses were statistically analyzed by procedures in the Statistical Analysis System (SAS) (SAS, 1985). Evaluation of the distribution of measurements for the parameters by the Shapiro-Wilk W statistic indicated that the distributions of some analyses were not normal (Shapiro & Wilk, 1965). Therefore, the nonparametric Kruskal-Wallis Test was used to determine if measurements different between sexes or between normal animals and those previously infected with the FUP strain of *P. falciparum* (Conover, 1980).

## RESULTS

Table I shows the identification number, sex, time post-infection, and maximum parasitemia for the 16 monkeys included in this study. The mean, standard deviation, and range for serum and urine concentrations of creatinine, urea nitrogen, calcium, phosphorus, sodium, potassium, glucose, total protein, urine volume, and urine specific gravity in the 16 monkeys are shown in Table II. Endogenous creatinine clearance and urinary excretion data are given in Table III. Data on urinary enzyme activities are shown in Table IV. These data were compared to reference data on renal function previously obtained from 62 malaria-naive adult *A. nancymai* that had been housed and maintained in BNW facilities in the same manner as the study group (Weller et al., 1991, 1992). The nonparametric Kruskal-Wallis test indicated that significant differences in several serum and urine analyses existed between the reference population and previously infected monkeys. Those analyses that showed statistically significant differences ($P \leq 0.05$) are listed in Table V. The Kruskal-Wallis Test further revealed that significant differences in serum glucose concentrations, urine phosphorus existed between sexes for malaria-exposed monkeys only. There was no correlation between peak parasitemia and any of the serum or urine analyses studied.

## DISCUSSION

It has been well recognized for many years that renal disease occurs in association with malaria infections in man (Aikawa et al., 1988a; Chugh & Sakhuja, 1986). The acute and chronic effects of malaria infection on the urinary system, and severity of those effects, appear to vary according to the species and strain of *Plasmodium* infecting the host (Hutt et al., 1975; Boonpuchnavig & Sitprija, 1979; Ehrich

TABLE I

Animal identification, sex, months post-infection, and maximum parasitemia in 16 owl monkeys infected with *Plasmodium falciparum*

| Animal ID | Sex | Months Post-Infection | Maximum Parasitemia (/mm$^3$) |
|---|---|---|---|
| 236 | F | 37 | 172,000 |
| 243 | F | 37 | 480,000 |
| 253 | M | 37 | 592,000 |
| 264 | M | 37 | 192,000 |
| 268 | F | 37 | 420,000 |
| 269 | M | 37 | 404,000 |
| 561 | M | 37 | 204,000 |
| 569 | M | 37 | 584,000 |
| 580 | F | 37 | 650,000 |
| 585 | F | 25 | 40,500 |
| 586 | M | 25 | 16,400 |
| 588 | M | 25 | 336,000 |
| 591 | M | 25 | 369,000 |
| 610 | M | 25 | 856,000 |
| 628 | M | 25 | 40,700 |
| 640 | F | 25 | 252,000 |

TABLE II

Summary data from timed urine collectionis for 16 owl monkeys previously infected with *Plasmodium falciparum*

| Variable | Mean | Standar deviation | Range |
|---|---|---|---|
| Serum calcium (mg/dl) | 8.98 | 0.70 | 7.9-10.3 |
| Serum phosphorus (mg/dl) | 4.46 | 1.76 | 2.1-8.5 |
| Serum creatinine (mg.dl) | 0.97 | 0.20 | 0.7-1.4 |
| Serum total protein (g/dl) | 7.16 | 0.60 | 5.7-7.9 |
| Serum glucose (mg/dl) | 116.31 | 32.49 | 72-177 |
| Serum sodium (meq/l) | 151.75 | 3.96 | 142-156 |
| Serum potassium (meq/l) | 3.51 | 0.52 | 2.6-4.9 |
| Serum urea nitrogen (mg/dl) | 26.25 | 15.79 | 10-73 |
| Urine calcium (mg/dl) | 3.83 | 2.92 | 1.3-13.7 |
| Urine creatinine (mg/dl) | 32.19 | 14.53 | 7-54 |
| Urine protein (mg/dl) | 70.69 | 86.95 | 12-360 |
| Urine glucose (mg/dl) | 13.63 | 19.37 | 0-71 |
| Urine phosphorus (mg/dl) | 36.71 | 32.08 | 0.8-97.0 |
| Urine sodium (meq/l) | 13.28 | 16.65 | 1-72 |
| Urine potassium (meq/l) | 43.90 | 26.18 | 9.2-89.3 |
| Urine protein: creatinine ratio | 2.71 | 4.41 | 0.44-18.94 |
| Urine volume (ml/16hr) | 62.63 | 62.12 | 28-270 |
| Urine specific gravity | 1.010 | 0.004 | 1.002-1.015 |

TABLE III

Endogenous creatinine clearance and urinary electrolyte excretion data

| Determination | Mean | Standard deviation | Range |
|---|---|---|---|
| Endogenous creatinine | | | |
| clearance (ml/min/kg) | 1.70 | 0.54 | 0.38-2.56 |
| mg/kg/day | 24.15 | 7.22 | 7.56-33.37 |
| Calcium excretion | | | |
| mg/kg/day | 2.92 | 1.65 | 1.60-7.18 |
| FE (%) | 1.89 | 2.95 | 0.70-12.78 |
| Phosphorus excretion | | | |
| mg/kg/day | 32.83 | 29.28 | 0.37-108.61 |
| FE (%) | 27.81 | 19.71 | 0.58-66.96 |
| Sodium excretion | | | |
| meq/kg/day | 1.03 | 1.00 | 0.17-3.33 |
| FE (%) | 0.43 | 0.87 | 0.05-3.61 |
| Potassium excretion | | | |
| meq/kg/day | 3.13 | 1.02 | 1.65-4.66 |
| FE (%) | 41.47 | 22.85 | 17.19-113.36 |
| Clucose excretion | | | |
| mg/kg/day | 7.23 | 7.85 | 0-28.24 |
| FE (%) | 0.60 | 1.49 | 0--6.15 |

FE = fractional excretion.

& Horstmann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988a). However, the pathogenesis of renal disease in patients with malaria is still not fully understood, which has led to the use of animal models in an attempt to clarify the pathogenesis. To date the closest approach to the human situation has been achieved with owl monkeys or squirrel monkeys infected with human malarias that show clinical courses similar to man (Jervis et al., 1972; Hutt et al., 1975; Aikawa et al., 1988a, b; Iseki et al., 1990).

Owl monkeys are considered to be an excellent model for human malaria research and

TABLE IV

Urinary enzyme activity (mean values)

| Enzyme | Malaria-Exposed | Malaria-Naive |
|---|---|---|
| Alkaline phosphatase (IU/l) | 31.88 | 21.17 |
| ALP urine creatinine ratio (IU/gVCr) | 98.99 | 61.00 |
| N-acetyl-β-D-glucosaminidase (IU/l) | 2.94 | 2.00 |
| NAG urine creatinine ratio (IU/gCr) | 9.12 | 5.76 |
| Aspartate aminotransferase (IU/l) | 18.63 | 14.93 |
| AST urine creatinine ratio (IU/gCr) | 57.84 | 43.01 |

TABLE V

Comparison of significant analytes between haive and malaria-infected owl monkeys

| Determination | Naive | Malaria-Infected | P-Value |
|---|---|---|---|
| Serum calcium (mg/dl) | 9.83 | 8.98 | 0.0001 |
| Serum glucose (mg/dl) | 147.66 | 116.31 | 0.0007 |
| Serum urea nitrogen (mg/dl) | 17.74 | 26.25 | 0.0006 |
| Urine protein (mg/dl) | 41.16 | 70./69 | 0.04 |
| Urine potassium (meq/L) | 29.69 | 43.90 | 0.03 |
| Creatinine clerance (ml/min/kg) | 2.23 | 1.70 | 0.005 |
| Creatinine (mg/kg/day) | 30.49 | 24.15 | 0.003 |
| FE of phosphorus (%) | 18.27 | 27.81 | 0.007 |
| FE of potassium (%) | 24.61 | 41.47 | 0.005 |
| ALP urine creatinine ratio (IU/gCr) | 61.00 | 98.99 | 0.0001 |
| NAG urine creatinine ratio (IU/gCr) | 5.76 | 9.12 | 0.0001 |
| AST urine creatinine ratio (IU/gCr) | 43.01 | 57.84 | 0.02 |
| Urine volume (ml/16hr) | 81.94 | 62.63 | 0.05 |

FE = fractional excretion.

many researchers have reported the pathological changes of the kidney of owl monkeys infected with various strains of *P. falciparum* (Jervis et al., 1972; Hutt et al., 1975; Aikawa et al., 1988a, b; Iseki et al., 1990). None of these studies, however, has evaluated either the acute or late effects of falciparum malaria on renal function. In this current study, quantitative urinalyses were performed on owl monkeys previously infected with *P. falciparum*. Impaired renal function in these animals was suggested by significant decreases in endogenous creatinine clearance, creatinine excretion, and urine volume; and, increases in blood urea nitrogen, urine protein, urine potassium, fractional excretion of phosphorus and potassium, and urinary enzyme activities. Serum concentrations of calcium and glucose were also noted to be significantly decreased. In addition, several other analyses were found to be near statistical significance. The pathogenesis of these changes is unknown but the results are consistent with a altered renal blood flow, glomerulonephropathy, functional adaptation of residual nephrons, and tubular dysfunction.

The twenty-four percent (24%) reduction in glomerular filtration rate (GFR), estimated by the clearance of endogenous creatinine, observed in these animals demonstrates impaired renal function consistent with progressive renal failure. It is probably due to a reduction in effective filtration pressure secondary to the constriction of afferent arterioles and other disturbances in the renal microcirculation (Boonpucknavig & Sitprija, 1979; Areekul Chantachum, 1984). Transient decreases in the clearance of endogenous creatinine have been reported in patients with falciparum malaria and rhesus monkeys infected with *P. knowlesi*, but only in acutely infected individuals (Areekul & Chantachum, 1984; Ahmad et al., 1989).

Renal proteinuria is a common finding in patients with acute falciparum malaria, as well as those that progress to nephrotic syndrome. It is the result of glomerular changes and tubulo-interstitial disease directly associated with the *P. falciparum* infection (Boonpucknavig & Sitprija, 1979; Ehrich & Horstamann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988b). The mechanism producing increased leakage of plasma protein in subjects infected with *P. falciparum* is not well established, but may be the result of increased glomerular capillary permeability related to reduced renal perfusion and malaria-induced tubular dysfunction (Boonpucknavig & Sitprija, 1979; Ehrich & Horstmann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988b). However, renal injury associated with acute falciparum infection is usually transient and will resolve without progression to chronic disease (Hutt et al., 1975; Boonpucknavig & Sitprija, 1979; Ehrich & Horstmann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988a). This view is supported by the results of a study in which renal biopsies taken from 12 patients with falciparum malaria showed glomerular mesanguial and endothelial proliferative changes and some acute tubular necrosis. Repeat biopsies taken 6 weeks later in 5 of the same patients revealed no residual pathology (Rath et al., 1990). Although the nature of the lesion producing proteinuria in the 16 animals included in this study is unknown at this time, it may be inferred from previous studies conducted in owl and squirrel monkeys that the lesion is likely to be a mesangiopathic glomerulonephropathy (Aikawa et al., 1988a, b; Iseki et al., 1990). While the morphologic features of acute glomerulonephritis will usually resolve with 8 weeks, mesangiopathic glomerulonephropaty can persist for up to 10 years after the acute syndrome (Iseki et all., 1990). This represents the later phase of renal disease in *P. falciparum*-induced glomerulonephropathy, and could explain the increased urine protein observed in these animals. The low serum calcium concentration in the malaria-infected animals is best explained by lowered serum protein related to proteinuria, although the tendency toward hyperphosphatemia which develops as GFR falls can also decrease serum calcium concentrations. (Morrison & Murray, 1981; Petithory et al., 1983; Weller et al., 1990).

As chronic renal disease progresses to renal failure, the number of functioning nephrons is reduced. In order to preserve external balance and body fluid homeostasis, the residual nephrons adjust their excretory functions in compensate for this loss. The ability of the residual nephrons to adapt depends on their ability to increase the fractional excretion of a variety of substances as the GFR falls (Morrison & Murray, 1981; Bovee, 1984). For example, as chronic renal disease develops, the residual nephrons are able to maintain potassium homeostasis by increasing their fractional excretion of potassium as the GFR falls (Morrison & Murray, 1981). The patterns of adaptation which develop for different substances as the GFR falls have been classified as: 1) no adaptation; 2) limited adaptation; and 3 complete adaptation (Morrison & Murray, 1981). Urea and creatinine are examples of substances for which no adaptation occurs, while phosphorus and potassium are examples of substances for which limited and complete adaptation occur, respectively (Morrison & Murray, 1981; Bovee, 1984). The mild azotemia, increased fractional excretion of phosphate, and increased fractional excretion of potassium observed in the monkeys included in this study is consistent with the decline in GFR and representative of all three adaptive patterns in chronic renal failure.

The measurement of urinary enzyme levels has been used as a non-invasive procedure to detect and monitor renal disease and to assess tubular function in man and some animals (Ragan, 1989; Stonard, 1990). Enzymuria often occurs before any change in other indices of renal function (Brobst et al., 1986). Among the urinary enzymes which have proved to be most useful in evaluating renal integrity are alkaline phosphatase (ALP) and aspartate aminotransferase (AST) which are located on the luminal brush-border region of the proximal renal tubule, and N-acetyl-β-D-glucosaminidase (NAG) a lysosomal enzyme which has high activity in the renal proximal convoluted tubule. Therefore, selective measurement of enzyme activities in urine can be used to detect the site of renal injury (Stonard, 1990). Variations in urine volume can make the quantification of urine enzyme activities difficult, thus, enzyme activities are often expressed as a factor of creatinine excreted to correct for fluctuations in urine volume (Bishop et al., 1991). The urinary values for ALP, AST and NAG, expressed as IU/g creatinine, in the reference population of owl monkeys were similar to normal values reported for the dog (Reusch et al., 1991), cat (Bishop et al., 1991), horse (Brobst et al., 1986; Brewer et al., 1991), and man (Ragan, 1989; Endo et al., 1990). Significantly increased activities of all three urinary enzymes were detected in the malaria-infected owl mon-

keys, suggesting renal damage and impaired tubular function (Table V).

In summary, impaired renal function was detected in 16 owl monkeys (*A. nacymai*) 25 and 37 months following infection with *P. falciparum*. These data appear to run contrary to the view that renal injury associated with uncomplicated acute falciparum infection is transient and does not lead to chronic renal disease (Boonpucknavig & Sitprija, 1979; Ehrich & Horstmann, 1984; Chugh & Sakhuja, 1986; Aikawa et al., 1988a). The results described herein would suggest that a subclinical pathological process, characterized by chronic progression, persisted in the kidneys of these owl monkeys following resolution of their parasitemias. They also suggest the need for long term follow-up of animals utilized in malaria research, and prospective studies to evaluate the relationship between histopathological changes in the kidney and alterations in renal function over time.

## REFERENCES


AHMAD, S.H.; DANISH, T.; FARIDI, M.M.; AHMAD, A.J.; FAKHIR, S. & KHAN, A.S., 1989. Renal function in acute malaria in children. *J. Trop. Pediatr., 35:* 291-294.

AIKAWA, M.; BRODERSON, J.R.; IGARASHI, I.; JACOBS, G.; PAPPAIOANOU, M.; COLLINS, W.E. & CAMPBELL, C.C., 1988a. *An atlas of Renal Disease in Aotus Monkeys with Experimental Plasmodial Infection*. American Institute of Biological Sciences, Washington, D.C.

AIKAWA, M.; JACOBS, G.; WHITELEY, H.E.; IGARASHI, I. RISTIC, M., 1988b. Glomerulopathy in squirrel monkeys with acute *Plasmodium falciparum* infection. *Am. J. Trop. Med. Hyg., 38:* 7-14.

AREEKUL, S., 1987. Renal clearance, tubular reabsorption and urinary excretion of albumin in monkeys infected with *Plasmodium knowlesi. Southest Asian J. Trop. Med. Pub. Hlth, 18:* 59-65.

AREEKUL, S. & CHANTACHUM, Y., 1984. Studies on the glomerular filtration rate and extracellular fluid volume in rhesus monkeys infected with *Plasmodium knowlesi. Southeast Asian J. Trop. Med. Pub. Hlth, 15:* 364-370.

BERGMEYER, H.U.; BOWERS, G.N.; HORDER, M. & MOSS, D.W., 1976. Provisional recomendations on *IFCC* methods for the measurement of catalytic concentrations of enzymes. Part 2. *IFCC* method for aspartate aminotransferase. *Clin. Chim. Acta, 70:* F19-29.

BESSEY, .O.A.; LOWRY, O.H. & BROCK, M.J., 1946. A method for the rapid determination of alkaline phosphatase with 5 cubi millimeters of serum. *J. Biol. Chem., 164:* 321-329.

BISHOP, S.A.; LUCKE, V.M.; STOKES, C.R. & GRUFFYDD-JONES, T.J., 1991. Plasma and urine biochemical changes in cats with experimental immune complex glomerulonephritis. *J. Comp. Pathol., 14:* 65-76.

BONSES, R.W. & TAUSSKY, H.H., 1945. On the colorimetric determination of creatinine by the Jaffé reaction. *J. Biol. Chem., 158:* 1265-1267.

BOONPUCKNAVIG, V. & SITPRIJA, V., 1979. Renal disease in acute *Plasmodium falciparum* infection in man. *Kidney Int., 16:* 44-52.

BOVEE, K.C., 1984. Functional responses to nephron loss, p. 531-553. In K.C. BOVEE, *Canine Nephrology.* Harwal Publishing Company, Philadelphia.

BRADFORD, M.M., 1976. A rapid sensitive method for the quantitation of microgram quantitites of protein utilizing the principle of protein-day binding. *Anal. Biochem., 72:* 248-254.

BREWER, B.D.; CLEMENT, S.F.; LOTZ, W.S. & CRONWALL, R., 1991. Renal clearance, urinary excretion of endogenous substances, and urinary diagnostic indices inn healthy neonatal foals. *J. Vet. Inter. Med., 5:* 28-33.

BROBST, D.F.; CARROL, R.J. & BAYLY, W.M., 1986. Urinary enzyme concentrations in healthy horses. *Cornell Vet., 76:* 299-305.

CHUGH, K.S. & SAKHUJA, V., 1986. Renal involvement in malaria. *Int J. Artif. Organs, 9:* 391-392.

COLLINS, W.E.; ANDERS, R.F.; RUEBUSH, T.K.; KEMP, D.J., et al., 1991. Immunization of owl monkeys with the ring-infected erythrocyte surface antigen of *Plasmodium falciparum. Am. J. Trop. Med. Hyg., 44:* 34-41.

CONNERTY, H.V. & BRIGGS, A.R., 1966. Determination of serum clacium by menas of orthocresolphthalein complexone. *Am. J. Clin. Path., 45:* 290-26.

CONOVER, W.J., 1980. *Practicl Nonparametric Statistics,* Secon Edition, John Wiley & Sons, Nes York.

DALY, J.S.& ERTINGSHAUSEN, G., 1972. Direct method for determining inorgamic phosphate in serum with the "Centrifichem". *Clin. Chem., 18:* 263-265.

EHRICH, J.H.H. & HORSTMANN, R.D., 1984. Origin of proteinuria in human malaria. Trop. Med. Parasit., 36: 39-4.

ENDO, G.; SHUN'ICHI, H. & KIYOTA, I., 1990. Urinary N-acetly-β-D-glucosaminidase activity in lead-exposed workers. *J. Appl. Toxicol., 10:* 235-238.

GORNALL, A.G.; BARDAWILL, C.J. & DAVID, M.., 1949. Deterination of serum proteins by menas of the biuret reaction. *J. Biol. Chem., 177:* 751-766.

HUTT, M.S.; DAVIES, D.R. & VOLLER, A., 1975. Malarial infections in *Aotus trivirgatus* with special reference to renal pathology. II. P. falciparium and mixed malaria infections. *Br. J. Exp. Pathol., 56:* 429-48.

ISEKI, M.; BRODERSON, J.R.; PIRL, K.G.; IGARASHI, I.; COLLINS, W.E. & AIKAWA, M., 1990. Renal pathology in owl monkeys in *Plasmodium falciparum* vaccine trials. *Am. J. Trop. Med. Hyg., 43:* 130-138.

JERVIS, H.R.; SPRINZ, H.; JONSON, A.J. & WELLDE, B.T., 1972. Experimental infection with *Plasmodium falciparum* in *Aotus* monkeys. II. Observations on host pathology. *Am. J. Trop. Md. Hyg., 21:* 272-281.

LARSEN, K., 1972. Creatine assay by a reation-kinetic principle. *Clin. Chem. Acta, 41:* 209-215.

MORRISON, G. & MURRAY, T.G., 1981. Electrolyte, acid-base, and fluid homeostasis in chronic renal failure. *Med. Clin. N. Amer., 65:* 429-447.

N.I.H., 1985. Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council. *Guide for the Care of Use of Laboratory Animals.* Bethesda, National Institutes of health.

PETITHORY, J.C.; LEBEAU, G.; GALEAZZI, G. & CHATY, A., 1983. Hypocalcemia in malari: study of correlations with other parameters. *Bull. Soc. Pathol. Exot. Filiales, 76:* 455-462.

RAGAN, H.A., 1989. Markers or renal function and injury, p. 321-343. In W.F. LOEB, F.W. QUIMBY (eds). *The clinical Chemistry of Laboratory Animals.* Pergamon Press, New York.

RATH, R.N.; PATEL, D.K.; DAS, P.K.; MISHRA, A.C.; SAHU, R.N. & MOHANTY, G., 1990. Immunopathological changes in kidney in *Plasmodium falciparum* malaria. Indian *J. Med. Res., 91:* 129-132.

REUSCH, C.; VOCHEZER, R. & WESCHTA, E., 1991. Enzyme activities of urinary alanine aminopeptidase (AAP) and N-acetyl-beta-D-glucosaminidase (NAG) in healthy dogs. *Zentralbl Veterinardmed A 38:* 90-98.

SAS, 1985. *SAS User's Guide: Statistics, Version 5.* SAS Institute Inc., Cary, North Carolina.

SCHMIDT, L.H., 1978. *Plasmodium falciparum* and P*lasmodium vivax* infections in the owl monkey (*Aotus trigirgatus*). II. Responses to chloroquine, quinine, and pyrimethamine. Am. *J. Trop. Med. Hyg., 27:* 703-717. UI 78254613.

SHAPIRO, S.S. & WILK, M.b., 1965. An analysis of variance test for normality (complete samples). *Biometrika, 52:* 591-611.

SONOWANE, M.; SAVORY, J. CROSS, R.E.; HEITGES, M.G. & CHESTER, B., 1976. Kinetic measurement of glucose with a centrifugal analyzer. *Clin. Chem., 22:* 1100-1101.

STOLAREK, I.; HOWEY, J.E. & FRASER, C.G., 1989. Biological variation of urinary N-acetyl-β-D-glucosaminidase: Practical and clinical importance. *Clin. Chem., 35:* 560-563.

STONARD, M.D., 1990. Assessment of renal function and damage in animal species: A review of the current approach of the academic, governmental and industrial institutions represented by the animal clinical chemistry association. *J. Appl. Toxicol., 10:* 267-274.

STONE, W.J.; HANCHETT, J.E. & KNEPSHIELD, J.H., 1972. Acute renal insufficiency due to falciparummalaria. *Arch. Intern. Med., 129:* 620-628.

TIFFANY, T.O.; JANSEN, J.M.; BURTIS, C.A.; OVERTON, J.B. & SCOTT, C.D., 1972. Enzymatic xenetic rate and end-point analyses of substrate by use of a GEMSAEC fasta analyzer. *Clim. Chem., 18:* 829-834.

U.S. Agency for International Development, 1980. *Guidelines for the Care and Use of Aotus Monkeys Used in Malaria Studies.* Agency for International Development, Washington, D.C., U.S.A.

WELLER, R.E.; BUSCHBOM, R.L.; RAGAN, H.A.; BAER, J.F. & MALAGA, C.A., 1990. Relationship of serum total calcium and albumin and total protein in owl monkeys (*Aotus nancymai*). *J. Med. Primatol., 19:* 439-446.

WELLER, R.E.; MÁLAGA, C.A.; BUSCHBOM, R.L.; BAER, J.F. & RAGAN, H.A., 1991. Protein concentration in urine of owl monkeys. *J. Med. Primatol., 20:* 365-369.

WELLER, R.E.; MÁLAGA, C.A.; BUSCHBOM, R.L. & RAGAN, H.A., 1992. Renal clearance and excretion of endogenous substances in the owl monkey. *Am. J. Primatol. 28:* 115-123.

WELLER, R.E.; WIERMAN, E.L.; MÁLAGA, C.A.; BAER, J.F. & LeMIEUX, T.P., 1991. Battelle primate facility. *J. Med. Primatol., 20:* 133-137.

YUEN, C.T.; KIND, P.R.N.; PRICE, R.G.; PRAILL, P.F.G. & RICHARDSON, A.C., 1984. Colorimetric assay for N-acetyl-β-D-glucosaminidase (NAG) in pathological urine using the ω-nitrostyryl substrate: the development of a kit and the comparison of manual procedure with the automated fluorimetric method. *Ann. Clin. Biochem.,* 21: 295-300.