an adequate carbohydrate intake among the Bushmen for some days before testing (nor did we want to alter their usual dietary habits) we were initially tempted to attribute the findings to a form of "starvation diabetes." However, several features mitigated against this possibility: a retrospective dietary history (M. J. Konner, personal communication, 1971) indicated that the Bushmen, in fact, consumed fairly large amounts of carbohydrate-rich vegetable food during the week before testing; their mean fasting plasma glucose level was raised rather than low; their pattern of insulin secretion was not sluggish and persistent (Tzagournis and Skillman, 1970); nor did their mean plasma albumin level suggest severe protein undernutrition.

Was the unusual diet of the Bushmen per se responsible for their impaired glucose tolerance and subnormal insulin release? Consumption of large amounts of mannoheptulose (occurring most abundantly in the avocado), for example, will block insulin release, with resultant hyperglycaemia (*Nutrition Reviews*, 1971), but no information is available concerning its content in Bushmen "veldkos."

A pronounced stress reaction at the time of the investigation could have produced a similar metabolic pattern (Jackson *et al.*, 1970), but as none of the primitive subjects became overtly distressed during the test—a fact supported by their normal fasting growth hormone levels—this, too, seemed an unlikely explanation. Consideration was also given to the hypothesis that the significant impairment of insulin secretion reflected a degree of beta cell failure (or adaptation) induced by a variety of unfavourable environmental (and/or genetic) factors to which the Bushmen have been exposed.

Finally, the apparent similarity between certain of our results and those reported in the Central African pygmies by Merimee *et al.* (1968, 1969) and Rimoin *et al.* (1969) are of interest. Not only is the average height and weight of the two groups comparable but the pygmies also manifest abnormal glucose tolerance curves in some instances, significant post-glucose insulinopenia, and normal, suppressable, basal growth

hormone levels. To what extent the explanation given to account for these observations in the pygmy—namely, end-organ unresponsiveness to the metabolic effects of growth hormone—can be applied to the Bushmen, remains speculative for the moment.

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PRELIMINARY COMMUNICATIONS

Nephrotic Syndrome in Monkey Infected with Human Quartan Malaria

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Summary

A splenectomized aotus monkey infected with human quartan malaria (*Plasmodium malariae*) developed oedema and proteinuria. Histological examination revealed a generalized diffuse glomerulonephritis and immunofluorescent staining showed granular deposits of IgM in the glomeruli. The

pathological picture resembled that shown by human patients with the quartan malaria nephrotic syndrome.

Introduction

The association between *Plasmodium malariae* infection and the nephrotic syndrome has now been convincingly demonstrated in several areas of Africa (Gilles and Hendrickse, 1963; Kibukamusoke, Hutt, and Wilks, 1967), and the suggestion of Hendrickse and Gilles (1963) that the pathological picture might be the result of damage to the kidney by immune complexes has been shown to be correct as recent immunofluorescent observations (Allison *et al.*, 1969; Ward and Kibukamusoke, 1969; Houba, Allison, Adeniyi, and Houba, 1971) have shown the presence of complexes in the kidneys of affected children.

Experimental work in the past has been hampered by the absence of a suitable animal model, and it is only recently that *P. malariae* has been adapted to a convenient primate, *Aotus trivirgatus* (Geiman and Siddiqui, 1969). The present communication describes the development of nephrotic syndrome in an aotus monkey infected with *P. malariae*.

Materials and Methods

The strain of *Plasmodium malariae* of human origin was obtained from Dr. R. Ward, of the Walter Reed Army Institute

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of Research, Washington, D.C., U.S.A. Two adult aotus monkeys, one of them (No. 1) splenectomized, were infected by the injection of infected blood. The second animal (No. 2) was splenectomized two months after the infection had been induced. An uninfected animal (No. 3) was also observed during the experimental period. This animal received identical antimalarial therapy to that given to aotus No. 1.

The course of the infection was monitored by examination of stained blood smears taken at frequent intervals. Kidney tissue was obtained from monkey No. 1 by nephrectomy and from the other two monkeys by open renal biopsy. The material was divided and prepared for study by light microscopy, immunofluorescence, and electron microscopy. The results of the electron microscopy studies will be reported elsewhere. Material for light microscopy was fixed in 10% formaldehyde, embedded in paraffin, and sections cut at 3–4 μ . Sections were stained with haematoxylin and eosin and by the periodic-acid-Schiff/methenamine-silver method.

For immunofluorescence studies small pieces of kidney tissue were snap frozen and were sectioned on a cryostat within 24 hours. Separate sections were stained with labelled antisera to human IgM, IgG, and complement. These reagents had earlier been shown to react with these respective fractions of aotus serum. The treated sections were examined and photographed on a Zeiss fluorescence microscope using UV-blue illumination.

Serum samples were taken at the same time as the kidney tissue, and these were assayed for malarial antibody by the indirect method, with labelled antisera to human IgG or human IgM and with blood smears of *P. malariae* as antigen.

Results

Course of Infection.—The splenectomized monkey (No. 1) had a long severe infection of *P. malariae*. The parasitaemia reached a peak level of 5% after 11 weeks and then fell, only to recrudescence to another peak of 1% six weeks later. During the recrudescence facial oedema and proteinuria developed. The clinical condition at this time necessitated antimalarial treatment (chloroquine 8 mg base/kg daily for six days). In spite of this treatment, which cured the parasitaemia, the clinical deterioration continued and the animal would certainly have died. It was killed in the 20th week after infection. The intact monkey (No. 2) had a much milder infection with a peak parasitaemia, four weeks after infection, of less than 1%. The recrudescence following splenectomy, at three months postinfection, was also very mild and the blood became negative five and a half months after the infection was induced. A small amount of protein appeared in the urine but there was no evidence of oedema.

Renal Histology.—In the kidney of monkey No. 1 there was a generalized and diffuse abnormality of the glomeruli characterized by a variable proliferation of mesangial and endothelial cells. Sections stained with silver methanamine showed an increase in mesangial matrix and thickening of peripheral capillary loops (Fig. 1). Some glomeruli showed adhesions between the tuft and the capsule and others were partly sclerosed. The proximal tubules had swollen granular cytoplasm and some contained hyaline droplets. The interstitial tissue contained focal collections of lymphocytes and plasma cells with occasional eosinophils. These areas were associated with tubular and glomerular scarring. The appearances were those of a generalized diffuse glomerulonephritis with proliferative, mesangial, and membranous changes. There was also a focal interstitial nephritis which was probably not related to the major lesions. The renal biopsy of monkey No. 2 showed only mild mesangial changes and that of the control monkey (No. 3) was normal.

Renal Immunofluorescence.—Labelled anti-IgM serum gave very strong fluorescent staining of all the glomeruli in kidney sections of monkey No. 1. The fluorescence was in the form

of granular deposits (Fig. 2). This antiserum did not stain the glomeruli in the sections of the kidneys of the other two monkeys. There was no evidence of IgG, complement, or malarial antigen deposits in any of the kidneys.

FIG. 1—Typical glomerulus from monkey No. 1. There is proliferation of mesangial and endothelial cells with an increase of mesangial matrix and thickening of the peripheral capillary loops (P.A.S./methenamine-silver. $\times 380$.)

FIG. 2—Fluorescence micrograph of a typical glomerulus of monkey No. 1 stained with fluorescein-labelled antiserum to IgM. Note the granular pattern of fluorescence.

Serology.—The sera of monkeys Nos. 1 and 2 contained both IgG and IgM antibodies that reacted with *P. malariae* antigen, whereas the control was negative. Streptolysin O assays were negative for all monkeys.

The results are summarized in the Table.

Summary of Findings on Experimental Animals

	Monkey No. 1	Monkey No. 2	Monkey No. 3
Splenectomized:	At time of infection	2 months after infection	Not
Infection	<i>P. malariae</i> Severe	<i>P. malariae</i> Mild	Control —
Course of infection	Oedematous	Normal	Normal
Clinical appearance at time of biopsy	700 mg/100 ml	30 mg/100 ml	<10 mg/100 ml
Urinary protein	<200 units	<200 units	<200 units
Streptolysin O titre	Membrano-proliferative glomerulonephritis	Mild mesangial changes only	Normal
Renal histology			
Immunofluorescence staining of kidney sections for:			
IgM	++	—	—
IgG	—	—	—
Complement	—	—	—
<i>P. malariae</i> antigen	—	—	—
Serum malarial antibody titres:			
IgM	80	320	<20
IgG	320	1,280	<20

Discussion

Previous workers have shown that rhesus monkeys infected with *P. cynomolgi* develop transient complexes of immunoglobulins and complement in their kidneys but that these resolve spontaneously and do not lead to the classical nephrotic syndrome (Ward and Conran, 1969). However, *P. cynomolgi* infections are more analogous to vivax malaria in man, and the latter has not been found to be associated with nephrosis. In the only other studies of this type rodent malarias have been used in N.Z.B. mice, which develop late renal disease spontaneously. Such mice when infected with *P. berghei* developed transient proteinuria a month after infection but the onset of their late renal disease was delayed (Greenwood and Voller, 1970).

Deposits of immunoglobulin and of complement were heavier in the malaria-infected mice than in the control mice, and the time course suggested a malarial aetiology rather than a later onset of the usual N.Z.B. renal disease.

Though a non-malarial aetiology cannot be excluded, the nephrosis observed in the splenectomized aotus monkey infected with *P. malariae* resembles the nephrotic syndrome of human quartan malaria. The results of the streptolysin-O assays seem to rule out the possibility that intercurrent streptococcal infections played any part as a cause of the nephrosis.

We have not observed oedema in any normal aotus monkeys nor in over 50 animals infected with *P. falciparum*. Many of these animals have received antimalarial regimens identical with that given to the affected monkey (No. 1) described here.

The clinical, histological, and immunofluorescent observations are all consistent with the hypothesis that the kidney lesion was caused by the deposition of immune complexes in the glomeruli. In many human cases of quartan nephrosis IgG, IgM, complement, or all three have been detected in the glomeruli (Allison *et al.*, 1969; Ward and Kibukamusoke, 1969; Houba *et al.*, 1971). The granular appearance of the IgM deposit in the kidneys of the aotus monkey corresponds with that of IgM deposits in the human kidney. The apparent absence of detectable *P. malariae* antigen is not particularly surprising; it has been found in only a minority of the human cases (Houba *et al.*, 1971).

We did not observe any of the tubular fluorescence reported by Houba *et al.* (1971) but this may reflect the state of the disease at the time we obtained the kidney material.

In view of the relative infrequency of the appearance of nephrotic syndrome in areas where *P. malariae* is very common it seems unlikely that nephrosis will be a regular feature of induced infections in normal aotus monkeys. However, it may be that the nephrotic syndrome reflects a peculiar immunological status of the affected individual, and that the quartan malaria infection acts as a "catalyst."

The occurrence of the syndrome in a splenectomized aotus monkey suggests that it may be possible, using this model, to determine the immunological conditions that give rise to the nephrotic syndrome in human *P. malariae* infections.

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MEDICAL MEMORANDA

Rapid Propagation of Thrombus in Deep Vein Thrombosis

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It is known that when a thrombus begins in the soleal venous sinuses or femoral vein it may propagate up the iliofemoral segment. Such a thrombus is large enough to

produce severe pulmonary embolism if it breaks free. Though it is accepted that there is a certain degree of urgency in treating these cases in order to prevent pulmonary embolism, there is little information about how rapidly thrombus can propagate up the femoral vein in man.

This case report shows that thrombus propagation may occur at a rapid rate, for within one hour a thrombus spread from the lower third of the femoral vein to above the inguinal ligament, putting the patient in imminent danger of a massive, possibly fatal, pulmonary embolism.

Case History

The patient, a woman aged 69, was undergoing routine investigations for a cerebral artery aneurysm and had had a carotid arteriogram taken under general anaesthesia. Five days after this

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