IMMUNITY TO MALARIA

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MEMORANDUM

This is to certify that this thesis was composed by me. The work described in it was carried out by me, and the views therein expressed are mine.

The help I received from others in carrying out this study is acknowledged.

I also hereby declare that this thesis has not been submitted anywhere else for any purpose whatsoever.

Signed........................................

N. J. MOODY

Date........................................
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IMMUNITY IN MALARIA

SUMMARY

Section I

Literature is reviewed and the pattern of the development of immunity in a population exposed to high malarial endemicity is followed.

This is followed by a brief discussion of specific aspects of malarial immunity in an immune population.

Section II

Two major groups of Ugandan populations were included in the present study. First comprised of pregnant females and their newborns, and second of patients with Tropical Splenomegaly Syndrome.

A. Pregnant females and their newborns

1. Malarial immunity during pregnancy.

Increased parasitaemia during pregnancy, in a previously immune female was investigated to elucidate if there was any breakdown in humoral defence.

Fifty pregnant females were followed during their pregnancy and were compared with a control group of forty
non-pregnant females and seventeen males.

Concentrations of serum gamma-globulins were found to be normal during pregnancy. The levels of malarial fluorescent antibodies and their distribution in IgG, IgA and IgM were also normal during pregnancy. A reciprocal relationship between antibody levels and parasitaemia was established.

Thus the results of this study indicated no depression of humoral immunity during pregnancy.

Evidence is reviewed to support the possibility of a breakdown in cellular immunity during pregnancy, preventing lysis of ingested malaria parasites by macrophages, probably as a result of the effect of corticosteroids, which are synthesized in increasing amounts during pregnancy.

11. Intra-uterine infections.

Intra-uterine infections are known to lead to fetal synthesis of IgM and/or IgA leading to elevated levels of IgM and/or IgA in cord blood sera at birth.

In this study, elevated levels of IgM and/or IgA were detected in 18.6% of the cord blood sera tested.
Intra-uterine infections play an important role in the etiology of congenital malformations and in postnatal morbidity and mortality. It is suggested that "African macroglobulinaemia" may have its origin in the intra-uterine priming and fetal synthesis of IgM as a result of intra-uterine infections. Defining the population of the newborn likely to have had intra-uterine infection will permit further study of etiological agents and postnatal effects of such infections in Uganda population.

An attempt was made to study the possibility of malaria as an etiological agent responsible for intra-uterine infections in these neonates. There was no correlation between levels of malarial antibodies in these samples and elevated levels of immunoglobulins, neither could IgM malarial antibodies be demonstrated in cord blood sera. It was, therefore, concluded that malaria may not be one of the etiological agents responsible for intra-uterine infections in our population in Uganda.

iii. Fetal Levels of IgG.

Placental transfer of maternal IgG was demonstrated, beginning early during intra-uterine life and increasing exponentially to attain maternal levels in the fetus at term.
iv. **Congenital Malaria.**

Must be rare in an immune population as amply recorded in literature and supported in the present study, where none of the 88 neonates had parasites in their peripheral blood smear.

v. **Malarial placental parasitization and birth-weights.**

Many reports have recorded the effect of malarial placental parasitization on birth-weights. In the present study such a relationship was not obvious.

Factors known to affect birth-weights, like the sex of the newborn, its birth-rank, nutritional state of the mother and placental sufficiency seem not to have been taken into consideration in older studies.

In the present study fetal plasma proteins, especially albumin and gamma-globulins, have been demonstrated to be related to maturity at birth.

Probably the degree of parasitization, rather than just the presence of parasites in placental smears, together with other placental lesions may be more relevant to placental insufficiency leading to lower birth-weights.
vi. **Malarial antibodies in cord blood sera.**

Fluorescent antibodies were detected in significant titres in cord blood sera of immune mothers, thus at birth the neonate has a passively acquired immunity against malaria.

vii. **Malarial antibodies in colostrum.**

Fluorescent antibodies were detected in breast milk of immune mothers, and their neonates may be acquiring further protection against malaria as a result of ingesting these antibodies.

B. **Tropical Splenomegaly Syndrome.**

(a) From the following two studies it was possible to rule out the possibility of a defect in antibody response to antigenic stimuli, leading to exaggerated IgM synthesis, in TSS patients.

i. Antibody response to *E. coli* Vi antigen in TSS patients was normal and resulted in the synthesis of both IgG and IgM antibodies.

ii. The distribution of malarial antibodies in IgG, IgA and IgM in TSS patients was normal too.
(b) 7S IgM, monomeric units were detected in almost 30% of TSS patients. This may be the effect or the cause of increased synthesis of IgM in TSS patients. Presence of 7S IgM monomeric units in test sera is likely to lead to over-estimation of IgM by radial immunodiffusion technique.

(c) Immuno fluorescence study on liver biopsies has demonstrated antibody synthesis by hepatic sinusoidal lymphocytic infiltrates and the possibility that the Kupffer cells are engaged in ingesting antigen/antibody complex.

(d) Long term malarial prophylaxis has been shown to lead to clinical improvement in TSS patients. In the present study malarial prophylaxis has been shown to lead to reduction in concentrations of IgM though the IgG, IgA and MPAT seem not to be affected during the period of observation.
SECTION I - THE LITERATURE

Since more than 600 million people are still exposed to malaria, it constitutes one of the leading causes of mortality and morbidity in the tropics. It takes its maximum toll in the very young children. P. falciparum is directly responsible for the death of about 5% of the children in hyper-endemic areas and 15% in holoendemic areas. (35, 88)

Malaria has serious effects on agriculture, commerce and industry. Therefore, malaria, poverty, low population density and lack of development are inseperable in the tropics.

A. gambiae and A. funestus are the predominant vectors of malaria in Africa, south of the Sahara. The former breeds in rainwater pools and the later in swamps.

Malaria eradication is still not practicable and faces its most formidable problem in Africa. Many pilot projects in various parts of Africa, have not succeeded in halting transmission even in the small areas covered by them.

In Uganda, P. falciparum is the predominant parasite but P. malariae too is a common infection of the children. (89, 91)
The existence of acquired immunity in malaria was established by the careful observations and studies by Christophers (1924) in India, Garnham (1935) and Wilson (1939) in East Africa. Acquired immunity in malaria is characterized by the mildness of the symptoms in the immune in contrast to its deadliness in non-immunes. (37, 68, 182)

Immunity to re-infection was demonstrated in experimental animals and in paretic patients receiving malarialotherapy. For P. falciparum this resistance to reinfection was not only species-specific but often strain-specific. (17, 148-150)

Passive transfer of immunity by the use of immune serum and its IgG fraction established the humoral immunity beyond doubt. (34, 38, 39, 58)

Cellular immunity, studied extensively by Taliaferro and his co-workers, leads to phagocytosis of parasitized red blood cells by macrophages, predominantly in the spleen but also in liver and bone-marrow; which leads to hypertrophy of lymphoid-macrophage system and increased antibody synthesis. (157-159)

It must be emphasized that cellular and humoral immunity do not work independently, but combine to aid the host in defense
against the parasite.

In an immune person both the sporozoites and exoerythrocytic stages develop normally. The humoral immunity is directed towards the erythrocytic stages and acts either on mature intra-cellular forms or on merozoites liberated from red blood cells. Probably antibodies act by depressing many metabolic functions, including those on which growth and reproduction of the parasite are dependent. (71, 147)

Not all antibodies synthesized in response to malarial infections are protective in function. It is very likely that only a few or even one of the serologically detectable antigens are actually involved in functional immunity. (173)

Protective function of these antibodies can be demonstrated by ability of the immune animal to resist reinfection OR by passive transfer of immunity to infected non-immunes.

In vitro detection of these antibodies, do not reflect on the actual functional immunity, but represent the exposure of any individual to malaria parasites in the past. These reactions show considerable degree of cross-reactions without corresponding cross-protection against heterologous species.
Malarial antigen used for these tests is usually obtained from infected placentae or from heavily parasitized peripheral blood from young children or experimental animals. Recently purification and characterization of plasmodial antigens and soluble antigens have been undertaken with a view to simplifying these tests.

The tests that have been employed for the determination of malarial antibodies are:

1. complement fixation test, (53, 55, 56, 97)
2. precipitin and ogglutinin tests, (52, 54, 152)
3. indirect fluorescent antibody tests, (41, 138, 163, 171, 172)
4. sensitized tanned red cells or latex particles ogglutination tests. (2, 20, 49, 165)

Though sensitized tanned red cells ogglutination test has considerable future possibility, indirect fluorescent antibody test has been found to be malaria-specific and considerably more sensitive than complement fixation test. Precipitin and ogglutinin tests have been employed for demonstrating strain-variability in relapses during the course of chronic infections.

Malaria fluorescent antibodies appear several days after the onset of patent parasitaemia and are significantly lower in
protected persons compared to unprotected persons. This suggests that erythrocytic stage provides the antigenic stimulation and repeated and frequent infections are necessary to maintain malarial antibody at a high level. Following prolonged stimulation these antibodies may persist for many years in association with persistent asymptomatic parasitaemia.

Voller & Bray (1962) and McGregor (1965) concluded that malarial fluorescent antibodies tend to mirror the pattern of gamma-globulin levels and immunity to malaria in a population at risk. (121, 172)

In areas with high malarial endemicity, an individual passes through three phases:— (116)

i. relative insusceptibility from birth upto about the age of 3 months,

ii. acquisition of active immunity by repeated and severe infections, upto about the age of 3 years,

iii. tolerance to malaria parasites, which depends on the continuous presence of the parasite in the circulation i.e. Premunition as defined by Sergent (1956). (144)
As a result of pathological process of natural selection over several generations, a gradual transformation of highly susceptible host population to a breed with increased tolerance takes place. Thus an African has a high resistance to P. vivax infections to which presumably he has been exposed to over a considerably longer period. (19)

The relative insusceptibility, in early infancy, as evidenced by low incidence of parasitaemia, has been attributed to:

i. effective transfer of maternal IgG across the placenta, to the foetus, representing an aliquot of maternal immunological experience; (58, 116)

ii. inability of foetal hemoglobin to support plasmodial development; (74)

iii. inability of the parasite to develop when the host's diet is an exclusively milk one, deficient in PABA, an essential metabolite for the parasite; (22, 28)

iv. possible transfer of maternal malarial antibodies in the colostral IgA and (4, 161)

v. selective vector biting. (127)

Acquisition of active immunity by repeated and severe infections leads to much sickness and death among the very young
children. During this period the growth of the child is also retarded but the last ground is rapidly made good as soon as the immunity is well established, by the age of 3-5 years. (119)

It is important to consider the life cycles of the two Plasmodial species commonly encountered in Uganda. *P. falciparum* has no secondary exo-erythrocytic forms, so that chronic infections and relapses due to persistence of erythrocytic forms in the blood are rare events. However, since there are many strains, a single infection does not produce complete tolerance and repeated infections, presumably with different strains, lead gradually to a degree of tolerance which occasionally breaks down in adults.

*P. malariae* usually establishes in the liver parenchyma as exo-erythrocytic forms and lead to chronic infection and several relapses possibly due to emergency of erythrocytic stages immunologically different from those found during preceding episodes. It is, therefore, not surprising that nephrotic syndrome and Tropical Splanonomedgaly Syndrome are associated with *P. malariae* infections.

These considerations must be borne in mind when attempts at eradication and/or radical cure are undertaken in areas with
high malarial transmission.

Any temporary set-back in an eradication programme may in absence of acquired immunity in the population lead to epidemics when malarial transmission is resumed.

The early establishment of this host-parasite equilibrium serves to enhance the success of the parasite in maintaining itself in the population.

Mere finding of parasites in the blood of an immune adult does not mean that his illness is necessarily due to malaria.

Lastly, radical cure is not worthwhile and may be a disservice to the patient if the chance of reinfection is high.

Now for certain specific aspects of malaria in an immune population.

I. Congenital Malaria.

Even though placentae are often heavily infected with malaria, congenital malaria is very rare indeed. (16, 27, 33)

This may be due to efficient placental barrier preventing the malaria parasites from crossing over or due to inability of
the parasites to thrive in fetal environment.

Though many infections are known to lead to intra-uterine infections with fetal synthesis of IgM and/or IgA, in response to them, no attempts have been recorded to establish malaria as a possible etiological agent.

II. Malaria during infancy and childhood.

At birth serum gamma-globulins in African neonates are higher than those of European infants. These high levels are maintained in later life too. The evolution of gamma-globulins is similar in African and European neonates. The concentration falls gradually to reach lowest values at 3-4 months of age.

The parasite rates increase inversely in relation to serum gamma-globulins, in this age group. Repeated and severe infections follow and this intense antigenic stimulation leads to gradual acquisition of immunity with increased synthesis of gamma-globulins and hypertrophy of the spleen. This active immunity is gained at a great sacrifice of lives of young children, whose growth is also retarded until the immunity becomes well established by the age of 3-5 years (76, 118, 129, 130)
Unprotected children, in the tropics, have significantly higher concentrations of gamma-globulins by the age of 24th month of life as compared to children protected against malaria.

This constant relationship between hyper-endemic malaria and hyper-gamma-globulinaemia in children and adults has been reported in many studies from East Africa, West Africa and New Guinea. In the tropics the population carries a much higher infective load, of which malaria is one. Curtail et al. (1964) in an elegant experiment established that malarial antibody represents quite a small proportion (6-11%) of the total IgG. (46)

Both the high concentrations and rate of synthesis of gamma-globulins are reduced when individuals are protected against malaria or come to reside in non-malarious areas. (34, 143)

Hyper-gamma-globulinaemia is reflected in IgG, IgA and IgM with the most striking increases in IgM and this increase in the immunoglobulins parallels the increase in antibody titres.

The primary factor controlling immunoglobulin synthesis is the antigenic stimulation. The catabolic rates, however, vary for each of the immunoglobulins. Catabolic rate for IgG varies directly with its concentration in the serum while rates for IgA and IgM are independent of serum concentrations and proceed at
a constant rate. (13, 14, 59, 61, 179)

The size of the RES is a function of its particulate "work-load". Spleen is of prime importance when the particulate antigen is within the circulation.

In response to repeated erythrocytic infections, the RES and especially spleen, actively engages in phagocytosis of the parasite, host cells and cellular debris. This leads to marked hypertrophy and increased antibody synthesis. Splenectomy, in monkeys, destroys its natural resistance to the blood forms of human malaria and impairs antibody synthesis to intravenous inoculation of particulate antigen. (18, 137, 141)

III. Malaria in adults.

The development of acquired immunity changes the host-parasite relationship from one of pathological parasitism to that of efficient parasitism with a state of tolerance in the host.

Maintenance of this state of tolerance in the host depends on continuous presence of the parasite in the circulation. In the absence of secondary exo-erythrocytic stages, for *P. falciforum* this needs continuous and repeated infections all
throughout adult life and is liable to breakdown occasionally. P. malariae usually persists as exo-erythrocytic forms within the liver parenchymal cells and gives rise to erythrocytic infections from this harbour.

IV. Genetics.

Gradual emergence of a resistant population from susceptible host population, as a result of the process of natural selection over many generations has been achieved in experimental animals. (133)

The natural immunity of an African to P. vivax infection can be explained on this basis.

It is likely that infectious diseases have been the most potent agents of human natural selection in the past. Host tissues essential for parasite growth would be the most probable site of metabolic polymorphism. Malaria parasites primarily proliferate in red blood cells and depends on some of the enzymes and metabolites of the host cells for its normal metabolism. So that if the red cells deviate from the normal, suboptimal growth of the parasite may result.

Even though the affected homozygote may be at a
disadvantage, the heterozygote will be selectively favoured compared with the normal individual, in presence of high malarial transmission. Thus high gene frequencies, in the face of strong selection against homozygotes, are possible without the help of high mutation rates.

Such deviations from normal red cells are met with in Hb S, Hb E and thalassemia and also in G-6-P-Dase deficiency. High frequencies of these abnormalities are found in malarious areas. Parasite rates and mortality in affected individuals have been shown to be lower. However, these observations are not universally accepted with regard to Hb S.

Hb S and G-6-P-Dase deficiency genes are commonly met within Uganda. (6-9, 31, 66, 75, 126, 134)

Inability on the part of the parasite to utilize Hb S can be discarded as children with SCA do suffer from repeated malarial infections. Miller et al. (1956) have shown that the parasite can enter and develop readily in cells showing the sickling reaction. Therefore, the protection afforded by this abnormal Hb is more likely to be due to increased destruction of parasitized red blood cells connected with the susceptibility to sickling deformity.
Percentage of G-6-P-Dase deficient red blood cells with parasites is lower than the percentage of parasite-containing cells with normal enzyme content. Plasmodia require reduced glutathione for their growth and enzyme deficient cells with suboptimal concentrations of glutathione are likely to hinder parasite development. (98)

V. Malaria during pregnancy.

(a) The mother.

By the age of puberty acquired immunity is well established in people residing in malarious regions. Yet during pregnancy, females have higher parasite rates and densities with higher incidence in primipara than in multipara and during the 2nd trimester compared to the first and the last trimester. (27, 102, 120, 131)

This breakdown of established immunity has been suggested to be the result of:

i. stress of pregnancy,

ii. diversion of protein from immune system to the fetus,

iii. hormonal changes during pregnancy.
Crude stress like ECT has no effect on malarial immunity. Though protein intake in majority of the population in tropical countries is usually only marginally sufficient, no gross lowering in the concentrations of gamma-globulins or immunoglobulins has been recorded. Cohen et al. (1961) have reported lowest rate of gamma-globulin synthesis in a pregnant unprotected African adult, compared to others living in the same area. (30, 34)

Human placenta secretes large amounts of progesterone and estrogens and the adrenal cortex provides increasing amounts of corticosteroids. There is a general depression of immunity during pregnancy as evident from:

1. increased tendencies towards many infections
   e.g. bacturia,
2. low rates of Rh-sensitization in Rh-incompatible pregnancy,
3. non-rejection of fetal graft.

Corticosteroids have been shown to lead to increased susceptibility to infections, including experimental malaria. (32, 51, 92, 142)

(b) The Placenta.

In malaria infested placenta the intervillous
spaces are an almost solid mass of RE cells containing P. falciparum schizonts in various stages of growth, which may lead to insufficiency and lower birth weights, abortions and stillbirths. (11, 15, 16, 27, 33, 67, 90, 122, 151)

VI. Auto-immunity in malaria.
(a) Malarial anaemia.

Cell dysfunction and degeneration with necrosis arise as a result of anoxia due to inhibition of cellular respiration and oxidative phosphorylation, and not from anaemia.

Extensive studies by Zuckerman have established that blood loss, in malarial infection, is in excess of that due to direct parasitization and maximal anaemia develops when parasitaemia is waning. (185-188)

EM appearances suggest increasing breakdown in the structure of the parasitized red cells, so that red cells become progressively "foreign" to the host and are removed from the circulation by the spleen and the liver. (25)

That the non-parasitized red cells are destroyed by an auto-immune mechanism is very likely. This may be due to the
interaction of malarial antibodies with non-parasitized red
cells coated with soluble malarial antigens. (43)

(b) Black-water fever.

Auto-immune mechanism where red cell-parasite-
quinine complex forms the altered host antigen leading to severe
hemolysis and renal failure.

(c) Auto-antibodies.

Hyper-gamma-globulinaemia, in the tropics, is
associated with auto-antibodies. To record a few of the many
studies:-

i. Liver and Kidney, Curtain (1965) (47)

ii. Hearth, Gastric parietal cells and thyroid,
Shaper et al. (1968) (146)

iii. RF-like globulins, Houbal & Allison (1966) (86)
    van Tongeren (1966) (170)

iv. Cold hemogglutinins. (44, 132)

(d) Malarial nephrosis.

Early observations by Manson-Bahr & Maybury
(1927), Surdey (1931) and Giglioli (1932) had linked nephritis
with quartan malaria. (73, 108, 154)
Extensive studies by Kibukamusoke in Uganda have established the casual relationship between P. malariae and nephrotic syndrome, its clinical picture and possible auto-immune mechanism in its pathogenesis. (93-95, 180)

These findings have been confirmed by other workers.

(e) Tropical Splenomegaly Syndrome.

Adult patients with massive splenomegaly, hepatomegaly and anaemia with characteristic finding of lymphocytic infiltration of the hepatic sinusoids have been studied extensively in Uganda and elsewhere. (36)

Careful and repeated examinations have confirmed the etiological relationship with P. malariae. Hyper-gamma-globulinaemia, markedly elevated levels of IgM and malaria fluorescent antibodies further support this relationship. (62, 72, 77, 87, 109, 110, 128)

Further studies are required to demonstrate auto-immune mechanism, if any.

In conclusion, I must quote excellent reviews dealing with Immunity In Malaria. (24, 70, 160)
SECTION II

A. TECHNIQUES, MATERIALS AND PATIENTS.

Techniques

(a) Collection and storage of specimens.
(b) Analytical techniques.
(c) Purification of plasma proteins.
(d) Raising of antisera.
(e) Purification of antisera.
(f) Labelling of antisera.
(g) Removal of non-specific staining.
(h) Preparation of immunoplates.

All the biological reagents, that would normally be required for various tests and estimations were raised personally in Uganda. However, antisera and immunoplates employed for the actual estimations were purchased from commercial sources, while experiments for their preparation were being carried out.

Dr. John Fahey very kindly provided antisera against human IgG, IgA, IgM and K & L chains. These were extremely useful in controlling the purification steps.

(a) Collection and storage of specimens.

Blood obtained by venepuncture was allowed to clot at
room temperature for 2-3 hours and sera separated after overnight storage at +4°C. Sera were stored at -20°C and thawed only once when required for various estimations. All the estimations were carried out within 24-48 hours (stored at +4°C) of thawing.

Urine specimens were stored at +4°C and tested for paludrine and chloroquine, as soon as possible.

(b) Analytical techniques.

1. Estimation of total serum protein concentrations.
   i. Biuret method. Ref: Wooton (1964) (183)
   ii. Refractometer method.

2. Serum protein electrophoresis, on cellulose acetate membrane. Ref: Wooton (1964) (183)
   Quantitation by scanning with Chromosonde (Joyce & Label).


Hyland "Immunoplates" or Hoechst "Portigan plates" were employed along with the standards provided in the Hyland kits.
Concentrations of test sera were calculated on the basis of linear relationship between the logarithm of concentrations and diameter of the diffusion ring.

4. Malarial fluorescent antibodies by indirect technique. Ref:- (Collins et. al. 1964) (40)

First dilution of the serum was 1:100 followed by four doubling dilutions.

GMRT was calculated for comparing results in different groups. \[ \text{GMRT} = \text{antilog} \int_{x_1}^{x_2} f(\log x)/N \cdot \text{antilog} \int_{x_1}^{x_2} f(\log x)/N_7 \] where

- \( x \) is the individual reciprocal of titre
- \( N \) is the number of sera tested
- \( f \) is the number of sera with a given titre.

Ref:- Diggs & Sadun (1965) (50)

5. Staining of malaria parasites in peripheral blood and placental smears.
   i. May-Grundwald/Giemsa for thin smears
      Ref:- Daice & Lewis (1968) (48)
   ii. Field's stain for thick smears
      Ref:- Daice & Lewis (1968) (48)
6. Analytical gel-filtration on sephadex G-200
   i. Column size 2.5x100cms.
   ii. Sample size 1ml.
   iii. Flow-rate 7-8 nls/hour
   iv. Fractions at 30 mins. intervals.
   v. Buffer:— Tris-HCl-NaCl 0.1M pH8.0 containing sodium azide as bacteriostatic agent
   vi. Temperature: +4 °C.
   vii. Characterization — optical density at 290mu

(Unican SP 500)

Ref:— Flodin & Killander (1962) (64)

7. Urinary chloroquine

Ref:— British Pharmacopoeia 1963 p164 (23)

8. Urinary paludrine

Ref:— Gage & Rose (1946) (65)

(e) Purification of plasma proteins.

i. IgG


From pooled human plasma obtained from "expired-date"
blood from blood bank. Successive steps were:
- defibrination by recalcification,
- delipidation by dextran sulfate,
- gamma-globulin precipitation by one-third saturation with ammonium sulfate,
- DEAE - cellulose column chromatography using 0.015M phosphate buffer pH 8.0 for equilibrating as well as for eluting.

ii. IgM.

Ref: - Hijmans, Schuit & Klein (1969) (82)

Modification as follows:-

From pooled human plasma obtained from "expired-date" blood from blood bank. Successive steps were:-

- defibrination by recalcification,
- delipidation by dextran sulfate,
- dialysis against 10 volumes of 1% boric acid for 24 hours at +4°C
- precipitate redissolved in PBS and once again dialysed against 10 volumes of 1% boric acid, for 16-18 hours at +4°C
- the second precipitate redissolved in PBS was filtered on Sephadex G-200 to isolate 19S peak.
iii. IgA

Ref:— Tonsi, et al. (1965) (167)

From pooled human colostrum. Successive steps were:

- clarification by centrifugation
- decasieration by adjusting pH to 4.6 with acetic acid
- delipidation by dextrin sulfate
- column chromatography on DEAE—Cellulose, using gradient elution. Starting and equilirating buffer — 0.015M phosphate buffer pH8.0; final buffer 0.30M phosphate buffer pH8.0

Various steps in purification were controlled by immunodiffusion and immunoelectrophoresis techniques.

(d) Raising of antisera.

Rabbits, weighing at least 2kg, were injected intramuscularly with approximately 1ng of purified protein in 2-5mls of saline emulsified with an equal of complete Freund's adjuvant (Difco). One injection was repeated at three to six weeks intervals for several months, until test bleeding demonstrated potent antibodies.
(c) Purification of antisera.
   i. anti-IgG was adsorbed with polymerized colostral IgA.
   ii. antIgA and anti-IgM were adsorbed with polymerized cord blood sera.

Polymerization was carried out by the use of gluteraldehyde.

Ref:-- Avraneas & Tornynck (1967) (12)

(f) Labelling of antisera.

FIIC (RDH) was used in the proportion of 0.05ng per ng of protein.

Ref:-- "Fluorescent antibody technique" (169)

Unreacted, free FIIC, was removed by gel-filtration on Sephadex G-25.

(g) Removal of non-specific staining.

   i. Adsorption with acetone dried pigeon liver powder (Ligges). Ref:-- "Fluorescent antibody technique"

OR. ii. DEAE - Cellulose column chromatography

Ref:-- McDevitt et al. (1963) (111)
(h) Preparation of "immunoplates".

Optimum volume of antiserum required for estimating immunoglobulins in concentrations met with in adult sera was determined by the method described by Fahey & McKelvey (1965). (60)
SECTION II

B. Pregnancy and Malaria.

Introduction

For almost two decades, raised parasite rates have been reported to occur in pregnancy and recent reports from East and West Africa have confirmed this. (27, 102, 120, 131)

These studies have revealed that higher parasite rate and parasite density index in pregnancy occur during 2nd trimester as compared to the first and the last trimesters. Higher rates have been observed in primigravida compared to those in multigravida.

Factors capable of depressing established immunity are not clearly understood. It has been variously explained as being due to physiological stress of pregnancy or due to hormonal changes associated with pregnancy or a decrease in humoral immunity as a result of a decrease in gamma-globulin synthesis in pregnant females.

Malaria during pregnancy affects not only the mother but also leads to prenatal and postnatal loss of life.
No detailed investigations seems to have been carried out to inquire into the cause of this apparent breakdown of an established immunity during pregnancy.

A longitudinal study was undertaken to inquire into the possible factors that may be responsible for increased incidence of malaria during pregnancy.

New Mulago Hospital, Kampala, Uganda is situated in an area where malarial transmission occurs throughout the year with increased transmission during the wet seasons.

With the kind permission of Professor Trussell, Professor & Head of Department of Obstetrics and Gynaecology and the utmost co-operation of Dr. Sharma, Senior Lecturer, Department of Obstetrics and Gynaecology, this study was initiated in October, 1968.

It was decided to investigate the state of humoral immunity during pregnancy by estimating the following:-

1. gamma globulins concentrations,
2. immunoglobulins concentrations,
3. malarial antibody levels by indirect fluorescent antibody technique:
screen for positive reactions
b. titrations of positive specimens
c. differential titrations of some of the positive specimens.

Materials and Methods

During the period October 1968 to January 1970, 50 women attending the routine Antenatal Clinic were invited to attend the special clinic arranged for this study.

These patients were in early stages of pregnancy thus allowing a longer period of observation.

During the same period 40 women attending Outpatients Department for minor complaints and judge to be non-pregnant from history were employed as a control group. These women in the control group were not followed up for a period of 6-8 months as were the ones in pregnancy group.

At the same time 17 men attending Outpatients Department for minor complaints were included as an additional group to compare with the females.
The pregnancy group had it explained and emphasized that they must not utilize any anti-malarials unless when prescribed at the clinic. They were instructed to report all incidences of fever with rigors and to attend the clinic any time such episodes occur and also at regular intervals which were every four weeks until 28 weeks and then every two weeks until 36 weeks, followed by weekly attendance until labour.

At each of the visit the patients received antenatal care and had a sample of blood collected for peripheral blood smears and for estimations of serum proteins, immunoglobulins and malarial antibodies.

The control groups had samples of blood taken for blood smears and estimations of immunoglobulins and malarial antibodies.

The relevant clinic information is presented in the following tables and charts.
No. 1 TABLE. Malaria and Pregnancy Study.

Age and Tribal Distribution in each group studied

<table>
<thead>
<tr>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>Non-pregnant</td>
</tr>
<tr>
<td>Age in Years</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Tribe</td>
<td>Baganda</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
</tr>
</tbody>
</table>

No. 2 TABLE. Malaria and Pregnancy Study.

Stage of Pregnancy when first seen at the Clinic

| 1st Trimester | 22% |
| 2nd Trimester | 78% |
No. 3 TABLE. Malaria and Pregnancy Study.

Gravid Status of the pregnant females

| Primipara | 24.4% |
| Multipara | 75.6% |

One of the patients was a known Hb S homozygote who aborted at weeks twenty four. Two of the patients had pre-eclampsia, one of whom developed eclampsia and had to have a LSCS.

Only one patient developed anaemia during her pregnancy and was admitted in the ward for appropriate treatment.
GRAPH I

Mean Monthly Rainfall, Kampala, Uganda.

Results

No. 4 TABLE. Malaria and Pregnancy Study.

**Incidence of Malarial Parasitaemia**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Exams.</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Non-pregnant females</td>
<td>40</td>
<td>40</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>50</td>
<td>268</td>
<td>26</td>
<td>9.7%</td>
</tr>
<tr>
<td>1st Trimester</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td>137</td>
<td>21</td>
<td>15.3%</td>
<td>4.2%</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>120</td>
<td>5</td>
<td>4.2%</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1st Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>111</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.08</td>
<td>1.00</td>
<td>1.03</td>
<td>0.71</td>
</tr>
<tr>
<td>FTPI</td>
<td>2.55</td>
<td>2.61</td>
<td>3.03</td>
<td>6.67</td>
</tr>
<tr>
<td>Afp-beta</td>
<td>0.44</td>
<td>0.54</td>
<td>0.49</td>
<td>11</td>
</tr>
</tbody>
</table>

\[ \text{No. of Pts. = 111} \]

Table 5. Fetal Maturation and Pregnancy Study

Mean Serum Protein Concentrations during Pregnancy
No. 6 TABLE. Malaria and Pregnancy Study.

Mean Immunoglobulins Concentrations during Pregnancy

mgs/100mls.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>40</td>
<td>1550</td>
<td>298</td>
<td>193</td>
</tr>
<tr>
<td>Pregnant</td>
<td>241</td>
<td>1784</td>
<td>202</td>
<td>118</td>
</tr>
<tr>
<td>Males</td>
<td>17</td>
<td>1345</td>
<td>328</td>
<td>155</td>
</tr>
<tr>
<td>Medical students-E.Af.</td>
<td>1570</td>
<td>214</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>British adults</td>
<td>1200</td>
<td>270</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>No</td>
<td>Mean</td>
<td>%</td>
<td>Mean</td>
</tr>
<tr>
<td>--------</td>
<td>----</td>
<td>------</td>
<td>---</td>
<td>------</td>
</tr>
<tr>
<td>0.5%</td>
<td>97</td>
<td>210</td>
<td>0.5%</td>
<td>129</td>
</tr>
<tr>
<td>1%</td>
<td>87</td>
<td>596</td>
<td>1%</td>
<td>212</td>
</tr>
<tr>
<td>2%</td>
<td>63</td>
<td>596</td>
<td>2%</td>
<td>178</td>
</tr>
</tbody>
</table>

Immunoextraction Concentrations during Pregnancy mg/100mls

No. 7 Table: Maternal and Pregnancy Study
No. III GRAPH. Malaria and Pregnancy Study.

Mean Concentrations of IgG During Pregnancy
No. IV GRAPH. Malaria and Pregnancy Study.

Mean Concentrations of IgA During Pregnancy
No. V GRAPH. Malaria and Pregnancy Study.

Mean Concentrations of IgM During Pregnancy
<table>
<thead>
<tr>
<th>%</th>
<th>Positive</th>
<th>% Positive</th>
<th>No. Total Exam.</th>
<th>No. Pregnant</th>
<th>Non-Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.1%</td>
<td>1%</td>
<td>1%</td>
<td>113</td>
<td>117</td>
</tr>
<tr>
<td>22%</td>
<td>97</td>
<td>11%</td>
<td>11</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>64%</td>
<td>7</td>
<td>35%</td>
<td>0</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>78%</td>
<td>182</td>
<td>78%</td>
<td>14</td>
<td>142</td>
<td>142</td>
</tr>
<tr>
<td>35%</td>
<td>14</td>
<td>35%</td>
<td>0</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

**Maternal Antithyroid Antibody Serum during Pregnancy**

- Titre 1:100 or more = Pos.
- Titre less than 1:100 = Neg.
Pregnant Females

Ponies

Non-Pregnant

Reduction of titre

Material Antibody Titres

No. 9 TRIB. Maternal and Pregnancy Study

29.1.6

14 11 7 19 29 25.2

4 4 1 4 4 4

6.0.1.5

100 200 400 800 1600
No. VI GRAPH. Malaria and Pregnancy Study

Malarial Antibody Titres
No. VII  GRAPH.  Malaria and Pregnancy Study.

Distribution of Malarial Antibodies in IgG, IgA & IgM
Discussion

i. Incidence of malarial parasitaemia during pregnancy.

Both Pingoud (1969) and Lelijved (1969) have reported more than double parasite rate during pregnancy. (102, 131)

<table>
<thead>
<tr>
<th>Percentage Mp:Positive</th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pingoud (1969)</td>
<td>48%</td>
<td>22.7%</td>
</tr>
<tr>
<td>Present Study</td>
<td>9.7%</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

Pingoud's observation that the parasite rate is dependent on the state of pregnancy, being highest in the 2nd trimester is confirmed in this study. The parasite rates observed in the present study were:

<table>
<thead>
<tr>
<th>Percentage Mp:Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester</td>
</tr>
<tr>
<td>2nd Trimester</td>
</tr>
<tr>
<td>3rd Trimester</td>
</tr>
</tbody>
</table>
ii. Possible factors that may lead to increased incidence of parasitaemia.
   
   (a) Selective vector biting,
   
   (b) Increased exposure to vector biting,
   
   (c) Depressed immunity - Humoral
       - Cellular
       - Humoral and Cellular.
   
   Selective vector biting has been demonstrated as one of the causes leading to lower incidence of parasitaemia in infants. Host seeking behaviours of the vector are complex and seem to be dependent on such factors as smell, heat and moisture of the host's body. Review of literature did not reveal any report recording selective preference of the vector of pregnant females. The same was true of the possibility that the pregnant female may be unduly exposed to vector biting. (99, 127)

iii. Serum Proteins during pregnancy.
   
   Hemodilution, specific alterations in protein metabolism, diversion of available amino-acids to the fetal needs and transplacental movement of proteins are likely to alter maternal serum proteins during pregnancy.
<table>
<thead>
<tr>
<th>Term</th>
<th>3rd Trimester</th>
<th>2nd Trimester</th>
<th>1st Trimester</th>
<th>Controled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.79</td>
<td>0.89</td>
<td>0.48</td>
<td>0.12</td>
<td>6.35</td>
</tr>
<tr>
<td>2.02</td>
<td>0.82</td>
<td>0.36</td>
<td>0.21</td>
<td>6.80</td>
</tr>
<tr>
<td>2.03</td>
<td>0.93</td>
<td>0.35</td>
<td>0.25</td>
<td>6.75</td>
</tr>
<tr>
<td>2.03</td>
<td>0.73</td>
<td>0.51</td>
<td>0.25</td>
<td>3.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ER/100 Mls</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLX</td>
</tr>
<tr>
<td>ALP</td>
</tr>
<tr>
<td>AST</td>
</tr>
<tr>
<td>ALB</td>
</tr>
</tbody>
</table>

No. 10 TABLE. BOWEN, J.C., J. CLIN. PATH., 1961, 14, 644. (77)

During pregnancy: To quote only two of the many records of changes in serum proteins.
| 1.76 | 0.89 | 0.93 | 0.30 | 3.16 |
| 1.82 | 0.86 | 0.88 | 0.31 | 3.22 |
| 1.92 | 0.78 | 0.81 | 0.32 | 3.29 |
| 1.97 | 0.73 | 0.78 | 0.31 | 3.36 |

3rd nut: middle 3.36
top .86
bottom .73
set

6/100 ins.

1965 14 678 (45)

<table>
<thead>
<tr>
<th></th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.Fr.</td>
<td>6.89</td>
<td>6.93</td>
<td>6.67</td>
</tr>
<tr>
<td>Alb.</td>
<td>3.03</td>
<td>2.61</td>
<td>2.55</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.46</td>
<td>0.54</td>
<td>0.44</td>
</tr>
<tr>
<td>Alpha-2</td>
<td>0.73</td>
<td>0.76</td>
<td>0.71</td>
</tr>
<tr>
<td>Beta</td>
<td>1.00</td>
<td>0.86</td>
<td>1.03</td>
</tr>
<tr>
<td>Gamma-Glob.</td>
<td>1.76</td>
<td>1.97</td>
<td>1.89</td>
</tr>
</tbody>
</table>

**Table:** Present Study.
<table>
<thead>
<tr>
<th>Year</th>
<th>Quarter</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.99</td>
<td></td>
<td>1.78</td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td>1.97</td>
<td></td>
<td>1.92</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>1.96</td>
<td></td>
<td>1.92</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table: Comparison of serum & urinary hormone concentrations during pregnancy.
It is, therefore, concluded that the concentrations of gamma-globulins are not decreased during pregnancy.


Gamma-globulin turnover rates were determined by Cohen et al. for Gambian Africans living in holoendemic areas and compared with those for West Africans and Europeans living in Britain.

Derived from Cohen et al. (1961) Nature 1961, 192, 733 (34)

<table>
<thead>
<tr>
<th>Turnover-Rate for Gamma-Globulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unprotected Gambians</th>
<th>5</th>
<th>169</th>
<th>139-203</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preg. Unprotected Gambians</td>
<td>1</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>Protected Gambian</td>
<td>5</td>
<td>98</td>
<td>80-113</td>
</tr>
<tr>
<td>W. Africans in Great Britain</td>
<td>4</td>
<td>50</td>
<td>42-57</td>
</tr>
<tr>
<td>Europeans in Great Britain</td>
<td>5</td>
<td>23</td>
<td>18-28</td>
</tr>
</tbody>
</table>
The turnover rate for a pregnant Gambian is lowest in the groups of Gambians studied, yet the turnover rate is more than three times the turnover rate of Europeans in Britain. This high turnover rate can hardly be inadequate for maintaining an already established immunity to malaria.

Subsequently this turnover rate has been quoted by a number of workers as being responsible for the reduced humoral immunity to malaria during pregnancy.

v. Immunoglobulins during pregnancy.

Rowe et al. (1968) reported values for IgG which appeared to be lower in pregnancy while the distribution of levels of IgA, IgM and IgD in these women were similar to those of their control group. (136)

Mendenhall (1970) observed normal or slightly elevated serum concentrations and concluded that in pregnancy, the total circulating IgG is increased. (123)

Immunoglobulin concentrations in this study have shown that the IgG is significantly higher in pregnancy, highest during the 2nd trimester when there is peak parasitaemia too.

These results are not to be interpreted as contradictory but as evidence that levels of immunoglobulins, in an immuno-
logically competent person, are controlled by the antigenic stimulation, unless there is a depression of humoral synthesis.

In this study the IgA and IgM levels are lower during pregnancy as compared to the control group, but they are not at levels which may lead to increased susceptibility to repeated infections.

The levels of IgM have progressively increased during the course of pregnancy which fact can only be interpreted as body's ability to respond to antigenic stimulation.

At this stage then it is possible to conclude that during pregnancy, the concentrations of gamma-globulins and of the immunoglobulins are not decreased to levels where one can anticipate increased susceptibility to repeated infections.

Not only that but the concentrations of immunoglobulins have been shown to be increased as a result of increased antigenic stimulation during the 2nd trimester.

vi. Malarial antibodies.

(a) Screening for malarial antibodies.

The number of positive reactions during pregnancy were
more than twice compared to the non-pregnant females. Maximum positive reactions were obtained during the 2nd trimester when peak parasitaemia was observed. As expected, unlike parasitaemia, malarial antibodies have persisted in the 3rd trimester.

(b) Titration of malarial antibodies.

Titres obtained for pregnancy group are not suggestive of diminished antibody response.

(c) Distribution of malarial antibody in IgG, IgA and IgM.

It is evident that the antibodies are distributed in these three major classes of immunoglobulins and the titres obtained are at significant levels.

From these results, it would appear that there is no impairment in antibody production against malaria during pregnancy.

Vigorous antibody response during the 2nd trimester, a period of peak parasitaemia, followed by the persistence of antibodies in the 3rd trimester when the parasitaemia is reduced seems to indicate a successful attempt at controlling parasitaemia with a reciprocal relationship between antibody levels and parasitaemia.
The technique employed measures the fluorescent antibodies and not necessarily the protective antibodies. Fluorescent antibodies do cross-react with heterologous strains and species while there is no cross immunity between these groups.

However, the development of fluorescent antibodies from birth onwards, in response to malarial infections, has been shown to follow the classical concepts to acquired immunity to malaria. (121, 172)

That the antibodies cross-react with shared antigens does not necessarily rule out the possibility of at least some of these antibodies playing a protective role.

Protective antibodies can only be tested by passive transfer of immunity in susceptible individuals infected with malaria. In this instance it would mean administration of maternal gamma-globulins or IgG to infants.

It must be concluded that the results obtained rule out the possibility of a depression in humoral immunity during pregnancy to malaria.
Transfer of passive immunity to malaria by immune
mother to her newborn has been demonstrated and has been
accepted as a major factor preventing establishment of malaria
in the newborn up to the age of three months. (121, 172)

The results of this study have demonstrated no
depression of humoral immunity and, therefore, one must look
for evidence of depression in cellular immunity and if possible
inquire into its cause.

In the present study no attempt has been made to study
 cellular immunity to malaria during pregnancy.

Cellular immunity plays an important role in immunity
to malaria where phagocytosis and subsequent lysis of malaria
parasites by the RES cells occur during parasitaemia.

Nature in its repeated experiment of pregnancy seems to
provide a clue regarding the possibility of depression of
cellular immunity in pregnant females.

Human placenta when infected with malaria parasites
contain large macrophages in the intervillous spaces with
ingested malaria parasites, segmenting and growing within them.
In normal placentae the intervillous space is lined entirely by trophoblast and contain nothing but blood.

Excellent studies by Garnhan (1938) have established beyond doubt that the macrophages are derived from lymphocytes and conveyed to the placenta by the blood stream. (67)

In severely infected placentae the contents of intervillous spaces are almost solid masses of RE cells which seems to lead to poor fetal nourishment.

Garnham has further demonstrated a normal phagocytic activity in these macrophages by injecting vital dyes into pregnant subjects.

For more than 30 years infected human placenta has been utilized as a source of malarial antigens for their use in sero-diagnosis of malaria.

Human placenta, therefore, seems to provide suitable culture requirements for the growth and multiplication of malaria parasites.

Also it is difficult to find any reason to account for the fact that there is no comparison between the intensity of
placental infections and the degree of parasitization of the corresponding peripheral blood.

It seems necessary to believe that rest of the circulatory system is immune to infection and yet at the same time, in the same individual placentas far from being immune, exhibits massive infection.

This can hardly be due to sluggish blood flow within the intervillous spaces as blood flow within RES organs is sluggish compared to the general circulation.

In infected placentae phagocytosis seems to proceed normally but subsequent lysis of ingested material seems impaired.

One must inquire into the cause of this inability on the part of the macrophages to lyse the malaria parasites after successful ingestion.

RE cells seen to provide specific nursery for a number of parasites where inability to lyse the parasites, after ingesting then leads to an establishment of infection.

The higher incidence of parasitaemia in primigravida
and in later stages of pregnancy seems to indicate a hormonal mechanism which may be responsible for this depression in cellular immunity.

During pregnancy, human placenta secretes large amounts of progesterone and estrogens and the adrenal cortex provides increasing amounts of corticosteroids. Though there is a concomitant increase in transcortin, in late pregnancy the unbound cortisol levels, in plasma, approach values found in Cushing's syndrome. (32)

The effect of steroid hormones on infections other than malaria is well established. The effect of corticosteroids on immunity is dose dependent. It affects the RES function which permits persistence and even proliferation of bacteria within macrophages and may even lead to involution of thymus and other lymphatic organs. With high doses of corticosteroids the antibody synthesis may also be depressed. (51, 92)

Recently serum from pregnant females has been shown to stabilize leucocyte lysozymes and to prevent the discharge of their lytic contents. (80)
Experimentally, the administration of corticosteroids has been shown to lead to increased parasitaemia without cellular reaction as evidenced by the size of the spleen, which is smaller in cortisone-treated monkeys than in control animals.

Histologically the spleens of cortisone-treated monkeys reveal enormous macrophages within splenic sinusoids with ingested malaria parasites. A histological picture similar to that of infected placentae. (142)

Taking the above observations into consideration with the fact that there is no evidence of a depression in humoral immunity, it is very tempting to suggest that there is, during pregnancy, a depression in cellular immunity.

Increased parasitaemia during pregnancy seems to be due to depression in cellular immunity with inability on the part of macrophages to lyse ingested malaria parasites. This inability to lyse ingested material may be due to the effect of steroid hormones on the macrophages.
C. INTRA-UTERINE INFECTIONS.

Introduction

Even in the recent past it was widely held that mammalian fetus was incapable of an immunologic response which was thought to be switched on with the event of birth.

Yet the development of the human lymphoreticular system commences with evagination and caudal migration of the 3rd and 4th branchial arch epithelium, during early embryonic life.

At birth, even in the most premature infants skin graft rejection can be elicited and the capacity of antibody responses involving all of the immunoglobulins is present in the human fetus as early as the 5th or 6th month of gestation. The effect of antigenic stimulus in fetal life, on lymphoreticular system leads to the development of germinal centres, plasma cells and secondary follicle formation.

The dominant response to an antigenic stimulus in fetal life is that of IgM antibodies; just as, even during adult life, in the primary response IgM antibodies almost inevitably appear before IgG antibodies.
Intrauterine infections have provided a special opportunity to examine the response of the human fetal immunopoietic system to a single infecting agent prior to exposure to the general environment.

The presence of IgM and/or IgA in the cord blood serum is then the result of active synthesis by fetus. Only the maternal IgG is transported across the placenta to the fetus. This transportation is not dependent on molecular size since other maternal serum proteins having molecular weights less than that of IgG do not cross the placental barrier and are found in fetal sera only because of fetal synthesis. (145)

Intense antigenic stimulus of the fetus does lead later on to synthesis of IgG by the fetus as demonstrated by the presence of fetal type, non-maternal Gm phenotype IgG in cord blood sera.

Maternal bleeding into the fetal circulation can lead to contamination of fetal serum with IgM and/or IgA.

By repeating the estimation of IgM and IgA a few days after birth, the maternal contamination can be distinguished from fetal synthesis. Half-life of IgM and IgA are 5-6 days only,
so that maternal IgM if present in cord blood serum will decrease remarkably within a few days after birth.

In one series it was thought probable that 60% of the cord blood sera containing elevated IgM were due to placental leak. (125)

The techniques that can be employed for estimation of cord blood sera IgM are:— (145)

i. single, radial diffusion, in antibody incorporated ogargel,

ii. latex ogglutination,

iii. elector-immunodiffusion.

Of these the radial immunodiffusion has been used most widely. Most of the commercial immunodiffusion plates have amounts of anti-serum incorporated in the gel which are suitable for estimation of IgM in adult sera.

At very low concentrations encountered in cord blood sera, the linear relationship between logarithm of concentration with diameter of ring, does not hold any more. So that the reproducibility of the method is not satisfactory.
Ideally the agar plates should have anti-serum in amounts suitable for estimations of cord blood IgM. However, if the same worker utilizes the same batch of immunoplates for a single project, the detection of elevated IgM levels in cord blood sera, can pin-point high-risk infants.

The significance level of IgM concentration in cord blood serum is held at equal to or greater than 20mg/100mls. From the points discussed above it would appear that this significance level needs to be reconsidered.

Further study of these high-risk infants by the identification of specific antibodies and/or antigens will assist in defining the etiological agents responsible for intra-uterine infections.

The bacterial, protozoal and viral infections that have already been shown to cause intra-uterine infections of the fetus are:— (146)

i. syphilis.

ii. Toxoplasmosis

iii. cytomegalic virus

iv. rubella virus

v. herpes virus
The effects of intra-uterine infections on the fetus depend on the stage of pregnancy. Effects during first trimester are congenital malformations.

In the 2nd and 3rd trimesters the fetus appears to be protected against such effects by maternal IgG and by its own immunologic defences leading to synthesis of IgM.

Intra-uterine infections in the very late stages of pregnancy may not be recognized by the presence of IgM in cord blood serum.

Intra-uterine infections may persist for remarkably long periods, with elevated IgM levels and persistence of IgM antibodies, after birth.

Fahey & Robinson (1963) pointed out that rates of gamma-globulin and immunoglobulin synthesis are determined largely by response to antigenic stimuli of the environment.

Socio-economic factors have been shown to affect the rate of detection of elevated levels of IgM in cord blood sera. Population in developing countries are constantly at high risk with regards to parasitic infections.
In this maternal environment the intrauterine fetal infection rate is likely to be high too.

McFarlen & Udeozo (1968) have reported high concentrations of IgM and IgA in cord blood sera in the rainy seasons than in the dry season in Nigeria. During wet seasons incidence of malarial, helminthic and viral infections is higher. This led then to suggest these infections as being responsible for increased levels of cord blood IgM in Nigeria. (114)

Materials and Methods

100 paired maternal and cord blood samples, at the time of delivery, were collected during the period of December, 1968 to January, 1969 from mothers attending the labour ward.

The second batch of paired maternal and cord blood samples collected during the month of May, 1969 for the study of malarial placental infections was a selection of twenty paired sera on the basis of malarial antibody levels in these samples.

Lastly, seven maternal and cord blood samples collected during the pregnancy and malaria were also included in this study.
As mothers were discharged from the labour ward within a day, after delivery, no attempt was made to repeat immunoglobulin estimates after a few days.

Samples of blood were also obtained from 23 fetuses by cardiac puncture, soon after abortion or delivery. Nineteen patients were admitted to the hospital for inevitable abortions between 16-28 weeks of gestation and 4 cases admitted for spontaneous premature labour and infants after delivery were fresh stillborns, the duration of pregnancy varied between 28 to 32 weeks.

The period of gestation was determined on the basis of the maternal L.M.P. and by Haase's Rule.

IgG, IgA and IgM were estimated on these samples.
**Results**

No. 14 TABLE. Intra-uterine Infections Study.

**Analysis of Cord Blood Levels of IgM and IgA**

<table>
<thead>
<tr>
<th>No.</th>
<th>Total</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated IgM (equal to or greater than 20ng/100mls.)</td>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td>Elevated IgA (equal to or greater than 11ng/100mls.)</td>
<td>14</td>
<td>97</td>
</tr>
<tr>
<td>Elevated IgM and IgA</td>
<td>18</td>
<td>97</td>
</tr>
</tbody>
</table>

No. 15 TABLE. Intra-uterine Infections Study.

**Immunoglobulins in Maternal and Cord Blood Sera**

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th></th>
<th>Neonate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>S.D.</td>
<td>No.</td>
</tr>
<tr>
<td>IgG</td>
<td>105</td>
<td>1452</td>
<td>642</td>
<td>124</td>
</tr>
<tr>
<td>IgA</td>
<td>98</td>
<td>229</td>
<td>154</td>
<td>32</td>
</tr>
<tr>
<td>IgM</td>
<td>105</td>
<td>138</td>
<td>91</td>
<td>95</td>
</tr>
</tbody>
</table>
No. VIII GRAPH. Intra-uterine Infections Study.

Distribution of IgM Concentrations in Cord Blood Sera
No. IX GRAPH. Intra-uterine Infections Study.

Distribution of IgA Concentrations in Cord Blood Sera
No. X GRAPH. Intra-uterine Infections Study.

Log Normal Probability Chart for Cord Blood IgM
No. XI GRAPH. Intra-uterine Infections Study.

Log Normal Probability Chart for Cord Blood IgA
No. 16 TABLE. Intra-uterine Infections Study.

Relationship between Malarial Antibody Levels in Maternal and Neonate sera. To Elevated Cord Blood IgM and/or IgA.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>IgA</th>
<th>IgM</th>
<th>Maternal</th>
<th>Neonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>17</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>13</td>
<td>400</td>
<td>Neg.</td>
</tr>
<tr>
<td>39</td>
<td>0</td>
<td>13</td>
<td>Neg.</td>
<td>400</td>
</tr>
<tr>
<td>41</td>
<td>0</td>
<td>13</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
<td>10</td>
<td>1600</td>
<td>Neg.</td>
</tr>
<tr>
<td>46</td>
<td>0</td>
<td>10</td>
<td>400</td>
<td>Neg.</td>
</tr>
<tr>
<td>47</td>
<td>0</td>
<td>12</td>
<td>1600</td>
<td>400</td>
</tr>
<tr>
<td>56</td>
<td>0</td>
<td>10</td>
<td>800</td>
<td>Neg.</td>
</tr>
<tr>
<td>63</td>
<td>0</td>
<td>13</td>
<td>200</td>
<td>Neg.</td>
</tr>
<tr>
<td>67</td>
<td>0</td>
<td>12</td>
<td>800</td>
<td>Neg.</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>22</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>15</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>17</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>20</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>26</td>
<td>15</td>
<td>22</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>12</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>12</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>37</td>
<td>0</td>
<td>8</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>38</td>
<td>0</td>
<td>12</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>55</td>
<td>0</td>
<td>6</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>Serum No.</td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cord Blood IgG in mg/100 ml

Revised cord blood IgM and/or IgA

Relationship between Maternal Antibody Levels in Neonate Serum to

- No. 17TABLE
- Inter-nuture Inctective Study
No. 18 TABLE. Intra-Uterine Infections Study.

IgG Concentrations in Premature Fetuses

<table>
<thead>
<tr>
<th>Period of Gestation</th>
<th>No.</th>
<th>IgG conc. in mg/100mls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-20 weeks</td>
<td>6</td>
<td>160 ± 114</td>
</tr>
<tr>
<td>21-24 weeks</td>
<td>7</td>
<td>205 ± 88</td>
</tr>
<tr>
<td>25-28 weeks</td>
<td>6</td>
<td>368 ± 250</td>
</tr>
<tr>
<td>29-32 weeks</td>
<td>4</td>
<td>423 ± 164</td>
</tr>
</tbody>
</table>

No. 19 TABLE. Intra-Uterine Infections Study.

IgA Concentrations in Premature Fetuses

<table>
<thead>
<tr>
<th>Period of Gestation</th>
<th>No. tested</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-20 weeks</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>21-24 weeks</td>
<td>7</td>
<td>Nil</td>
</tr>
<tr>
<td>25-28 weeks</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>29-32 weeks</td>
<td>4</td>
<td>Nil</td>
</tr>
</tbody>
</table>
No. 20 TABLE. Intra-uterine Infections Study.

IgM Concentrations in Premature Fetuses

<table>
<thead>
<tr>
<th>Period of Gestation</th>
<th>No. tested</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-20 weeks</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>21-24 weeks</td>
<td>7</td>
<td>Nil</td>
</tr>
<tr>
<td>25-28 weeks</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>29-32 weeks</td>
<td>4</td>
<td>1 (22mg/100mls)</td>
</tr>
</tbody>
</table>
No. XII GRAPH. Intra-uterine Infections Study.

Relationship between Maternal and Cord Blood IgG
No. XIII GRAPH. Intra-uterine Infections Study.

IgG Concentrations in Relation to Period of Gestation
No. XIV GRAPH. Intra-uterine Infections Study.

Logarithm of IgG Concentrations in Relation to Period of Gestation
No. XV GRAPH. Placenta and Malaria Study.

Distribution curve of birth-weights
Discussion

Edozien (1962) detected IgM by IFA in pooled cord blood sera of Nigerian newborns and McFarlane and Udeosu (1968) have shown elevated levels of IgM and IgA in cord blood sera of Nigerians. (58, 114)

It has been repeatedly documented that African infant at birth has a higher spleen weight than the Caucasian infant and also higher levels of gamma-globulins. These differences can be due to genetic differences in the two races. However, intra-uterine infections can stimulate the lymphoreticular system into greater development and fetal synthesis of IgM, IgA and even IgG.

Higher levels of IgM have been shown to persist in the sera of newborns for as long as two years after birth. African macroglobulinemia may have its origin in the intra-uterine priming and fetal synthesis of IgM, so that IgM response becomes more vigorous than in the non-infected fetus. (Huntley, et.al.1969)

In view of the importance that intra-uterine infections, play a part not only in causing congenital malformations but also in postnatal morbidity and mortality, the first step is to
define the population at risk.

Intra-uterine infections can be detected by antibodies screen, for a number of infective agents, of cord-blood sera.

However, the estimation of cord blood immunoglobulins will permit the study of infants who are at high risk in regards to development of clinical and subclinical postnatal infections.

The results of this study have shown a high incidence of elevated IgM and IgA in cord blood sera. This must be compared with results obtained elsewhere with same socio-economic background of the population.

Sever et al. (1969) have reported a very low (0.8%) frequency in a middle class Caucasian population, compared to 10% from Alford et al.'s (1969) study of the population composed primarily of low income Negro group. (5, 145)

Hardy et al. (1969) found the incidence of only 11% for Negro newborns compared to 21% for the Caucasian group. (78)

Therefore, it is unlikely that genetic factors play an important role. Rate of infection, even intra-uterine, seems to depend on the infective environment of the population.
Fetus in Uganda population seems to have increased rate of intra-uterine infections because his mother is exposed to infective environment.

Malaria forms most important infection in Uganda and the incidence of malaria during pregnancy is increased compared to in the non-pregnant state. Also placental parasitization occurs commonly, with higher incidence of placental parasitization when compared with peripheral blood parasitaemia.

Thus malaria may play an important part in intra-uterine infections.

The cord blood sera from the pregnancy and malaria study group were all negative for fluorescent antibodies using monospecific fluorescent anti-IgM.

There are differences in reactivity of different batches of such biological reagents and these differences may be due to the presence of 7S IgM antibodies in cord blood sera compared to 19S IgM antibodies in adult sera.

More work needs to be carried out before malarial can be ruled out as a possible infective agent, in our environment, leading to intra-uterine infections.
However, the results presented here seem to indicate that malaria is not playing a significant role in the etiology of intra-uterine infections in our population in Uganda.

IgG is transferred from mother to the fetus. This transfer across the placenta is an active and specific process. It is, therefore, not surprising to find higher levels of IgG in cord blood than in maternal blood. (124)

In the cord blood of African neonates the IgG concentrations have been reported to be lower than in the maternal blood.
<table>
<thead>
<tr>
<th>p</th>
<th>p</th>
<th>642</th>
<th>710</th>
<th>550</th>
<th>210</th>
<th>256</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>1452</td>
<td>2496</td>
<td>2100</td>
<td>1131</td>
<td>0.1%</td>
</tr>
<tr>
<td>124</td>
<td>106</td>
<td>45</td>
<td>45</td>
<td>38</td>
<td>38</td>
<td>78</td>
</tr>
</tbody>
</table>

Material and Cord Blood IgG in mg/100ml

- No. 21 Table: Intraperitoneal Injections Study

Material and Betal p. (114) Narayan et al. 1968 1966

Material and Betal p. (124) Narayan et al. 1968 1966
Michaux et al. (1966) have explained this apparent difference between African and Caucasian pattern of IgG transfer across the placenta on the relationship the bisectrix had with their regression line. The relationship was such that for maternal IgG levels exceeding 1500mg/100mls, the fetal IgG concentrations would be lower than the maternal levels. Since African mothers usually have higher IgG levels than Caucasian mothers, African neonates are likely to have lower IgG levels than their mothers.

In the present study the maternal IgG concentration is lower than 1500mg/100mls, yet the fetal IgG level is lower than maternal IgG.

As evidenced by the values quoted in the above table the deviation from mean value is greater for African neonates than for Caucasian neonates (124). African mothers usually receive less antenatal care and are exposed to greater risk of infections. It is likely that transfer of IgG across the placenta may not be efficient under these conditions and therefore, the neonates have lower than maternal levels of IgG.

The transfer of IgG to the fetus begins early during intra-uterine life and seems to proceed slowly at first,
increasing exponentially, so that in the last few weeks of intra-uterine life the IgG levels increase rapidly to reach maternal levels at term.

Similar observations have been reported by Hobbs & Davis (1967) on a larger number of premature fetuses. (83)

The presence of elevated IgM levels in a fetus of gestational age of 32 weeks must be indicative of fetal synthesis in response to intra-uterine infection.
SECTION II

D. PLACENTA AND MALARIA

Introduction

The placenta acts as the source of nutrients for the growing fetus and also forms a protective barrier against adverse influences in the maternal circulation.

Blood films from infected placentae contain remarkable numbers of malaria parasites in sporulating stage. It is, therefore, important to study the effects of placental parasitization on fetal nutrition and efficiency of placental barrier in preventing congenital malarial infections.

Blacklock & Gordon (1925) were the first to suggest that malarial infection of the placenta might adversely affect the outcome of pregnancy. (16)

Malaria during pregnancy may lead to increased incidence of abortions, stillbirths and neonatal mortality, through its effect on maternal health, and by causing relative placental insufficiency, directly on fetal nutrition.

Garnham (1949) found malaria as the causative factor in only 13% of the total number of abortions and rarely of
stillbirths. (69)

Others have also been unable to demonstrate any relationship between malarial infection of the placenta and the incidence of abortions, stillbirths or neonatal mortality.

Malarial parasitization of the placentae has been reported as having an effect in lowering birth-weights and thus increasing the incidence of prematurity.

Since the first report by Bruce-Chwatt in 1952, several reports from West Africa have confirmed the association of placental parasitization with prematurity.

McLaren from East Africa (1962) was the first to point out that past reports had not taken into consideration, factors well known to be affecting birth-weights. Sex of the neonates, their birth-rank and nutritional status of the mother as well as of the fetus must be taken into account when effect of malarial placental infection on birth-weight is being studied.

This was followed by a detailed report by Jelliffe (1968) where some of these factors were taken into consideration and placental infection was shown to lead to increased incidence of prematurity.
Materials and Methods

Eighty-eight paired maternal and fetal blood samples were collected at birth from mothers delivering at Mulago General Hospital during the month of May, 1969.

Blood smears were also obtained from the mother, the newborn and the placenta, at the same time. The smears from placenta were made from three different sites by deep cuts on the maternal side of the placenta.

Maternal and fetal serum proteins were estimated and serum protein electrophoresis carried out, and malarial fluorescent antibodies were titred on all sera.

The blood smears were stained and examined microscopically for malaria parasites.

Of the eighty-eight cases, maternal age was recorded in eighty-two. Mean age was 23 years and tribal distribution was 62.3% for the Baganda and 37.7% were made up of other tribes residing in Buganda.

Eighty-six mothers delivered single babies and 2 delivered twins. The male to female ratio was 1.15 and only one of the neonates was transferred to Special Care Unit, because of prematurity.
Results

No. 22 TABLE. Placenta and Malaria Study.

Malaria parasites in the blood smears at birth

<table>
<thead>
<tr>
<th></th>
<th>No. examined</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>84</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Placenta</td>
<td>88</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Neonate</td>
<td>88</td>
<td>Nil</td>
<td>0</td>
</tr>
</tbody>
</table>

No. 23 TABLE. Placenta and Malaria Study.

Birth rank and malarial placental infections

(84/88 where birth rank was recorded)

<table>
<thead>
<tr>
<th>Birth rank</th>
<th>Infected</th>
<th>Not infected</th>
<th>Total</th>
<th>% infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>15</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>13</td>
<td>18</td>
<td>27.6</td>
</tr>
<tr>
<td>3+</td>
<td>5</td>
<td>42</td>
<td>47</td>
<td>10.6</td>
</tr>
</tbody>
</table>
No. 24 TABLE. Placenta and Malaria Study.

Birth-weights in gms. and malarial placental infection

<table>
<thead>
<tr>
<th></th>
<th>Infected</th>
<th></th>
<th>Not infected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>S.D.</td>
<td>No.</td>
</tr>
<tr>
<td>Males</td>
<td>7</td>
<td>3321</td>
<td>318</td>
<td>38</td>
</tr>
<tr>
<td>Females</td>
<td>7</td>
<td>2773</td>
<td>481</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>3047</td>
<td>484</td>
<td>70</td>
</tr>
</tbody>
</table>

No. 25 TABLE. Placenta and Malaria Study.

Maturity at birth and malarial placental infection

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Not Infected</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>Not Infected</td>
</tr>
<tr>
<td>Males</td>
<td>Nil</td>
<td>4</td>
</tr>
<tr>
<td>Females</td>
<td>1*</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

*had to be cared for in Special Care Unit.
No. 26 TABLE. Placenta and Malaria Study.

Singleton birth-weights in gms. and sex of the neonates

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>46</td>
<td>3106</td>
<td>443</td>
</tr>
<tr>
<td>Females</td>
<td>40</td>
<td>2987</td>
<td>466</td>
</tr>
</tbody>
</table>

No. 27 TABLE. Placenta and Malaria Study.

Singleton birth-weights in gms. in relation to birth-rank

<table>
<thead>
<tr>
<th>Birth Rank</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>3392</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>2889</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3066</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3270</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3129</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3455</td>
<td>2</td>
</tr>
<tr>
<td>6+</td>
<td>6</td>
<td>3049</td>
<td>7</td>
</tr>
</tbody>
</table>
Marturity in relation to fetal serum proteins

- ALP
- ATPase-2
- beta-Gamma-glutamyl transpeptidase
- 5' nucleotidase
- No. 20 TFRB
- Plasma and maternal study
Maturity in relation to maternal serum proteins

No. 3 Table: Percenta and Maternal Study

<table>
<thead>
<tr>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.28</td>
<td>1.08</td>
<td>0.57</td>
<td>2.61</td>
<td>2.09</td>
<td>3.27</td>
<td>7.45</td>
<td>2.53</td>
<td>0.61</td>
</tr>
<tr>
<td>2.28</td>
<td>1.01</td>
<td>0.65</td>
<td>2.57</td>
<td>2.53</td>
<td>7.45</td>
<td>2.53</td>
<td>0.61</td>
<td>2.53</td>
</tr>
<tr>
<td>2.24</td>
<td>0.94</td>
<td>0.65</td>
<td>2.53</td>
<td>2.53</td>
<td>7.45</td>
<td>2.53</td>
<td>0.61</td>
<td>2.53</td>
</tr>
<tr>
<td>2.42</td>
<td>1.38</td>
<td>0.96</td>
<td>2.59</td>
<td>2.53</td>
<td>7.45</td>
<td>2.53</td>
<td>0.61</td>
<td>2.53</td>
</tr>
</tbody>
</table>

In Case

Birth weight
No. XVI GRAPH. Placenta and Malaria Study.

Distribution Curve of Maternal Malarial Fluorescent Antibodies
No. XVII GRAPH. Placenta and Malaria Study.

Placental Infection and Birth-Weights

A. Jelliffe 1968
B. McLaren 1962
C. Bruce-Chwatt 1952
D. Archibald 1956
E. Archibald 1958
F. Mody 1970
No. 32 TABLE. Placenta and Malaria Study.

**Fetal and Maternal malarial fluorescent antibodies**

<table>
<thead>
<tr>
<th></th>
<th>GMRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>175</td>
</tr>
<tr>
<td>Fetal</td>
<td>154</td>
</tr>
</tbody>
</table>

**Discussion**

i. Congenital malaria.

Not even one out of the eighty-eight heel-prick peripheral blood smears, from neonates at birth, showed the presence of malaria parasites, though fourteen placentas were infected with malaria and one mother showed malaria parasites in her peripheral blood at the time of delivery.

This may be due to placental ability to prevent the passage of malarial parasites from crossing from maternal to fetal side or failure on the part of any parasites that may cross over to thrive in the fetal tissue.
<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>No. Examined</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacklock &amp; Gordon  (16)</td>
<td>1925</td>
<td>158</td>
<td>Nil</td>
</tr>
<tr>
<td>Garnham (67)</td>
<td>1938</td>
<td>400</td>
<td>Nil</td>
</tr>
<tr>
<td>Garnhan (69)</td>
<td>1949</td>
<td>145</td>
<td>Nil</td>
</tr>
<tr>
<td>Bruce-Chwatt (27)</td>
<td>1952</td>
<td>332</td>
<td>Nil</td>
</tr>
<tr>
<td>Cannon (33)</td>
<td>1958</td>
<td>117</td>
<td>Nil</td>
</tr>
<tr>
<td>Spitz (151)</td>
<td>1959</td>
<td>576</td>
<td>Nil</td>
</tr>
<tr>
<td>Jelliffe (90)</td>
<td>1968</td>
<td>570</td>
<td>1</td>
</tr>
</tbody>
</table>

This confirms the rarity of congenital malaria, at least in newborns of immune mothers.

ii. Placental parasitization.

Placenta showed 16% rate of malarial infection. The rate of placental infections reported varies in different reports and is likely to depend on malarial endemicity as well as availability and use of anti-malarials in any population.

Anti-malarials are generally available throughout East Africa and are self-administered or prescribed at Outpatients Clinics for "fevers".

It is remarkable that placental infection depends on the parity of the mother, just as peripheral parasitaemia has
been shown to be marked in first pregnancies.

Cannon (1958) attributes this negative correlation between the frequency of infection of the placenta and the parity of the mother, to increasing resistance to infection simply due to the increasing age of the women at each successive parity and correspondingly higher degree of immunity.

Yet acquired malarial immunity is well established by the age of puberty.

It is likely that the effect of increase in steroid hormones during first one or two pregnancies may lead to more marked depression of a well-established immunity as compared to subsequent pregnancies.

iii. Placental parasitization and birth weights.

The effect of placental parasitization on birth weights seems to be negligible, if any, in this series.
Summary of Published Reports

- No. 37 Table: Percente and Matara Study
No. XVII GRAPH. Placenta and Malaria Study.

Distribution Curve of Fetal Malarial Fluorescent Antibodies
These results are presented graphically as mean birth-weights with one standard deviation on either side.

Therefore, placental infection can be implication as a factor in lowering birth weights and increasing the incidence of prenaturaty.

Even in the present study the only neonate that needed to be transferred to Special Care Unit had placental parasitization.

However, the mere presence of malaria parasites in placental smears cannot be expected to lead to placental insufficiency.

Placenta, like any other organ in the body, must have reserve capacity and therefore, either heavy parasitization or other superimposed lesion is likely to lead to a degree of insufficiency that may cause lowering of birthweights.

This seems to be indicated from McLaren's study and in the present study.

It is probably more relevant to think of placental insufficiency only when there is heavy parasitization with or without other lesions like placental infarction.
iv. Birth-weights in relation to other factors.

Tables 26 and 27 suggest that birth-weights depend on the sex and birth rank of the neonates.

Therefore, the effects of placental parasitization on birthweights must be unravelled only when such factors are taken into account.

That placental parasitization does reduce birth-weight even when these factors are ignored is likely to be due to higher endemicity and heavy placental infections, over-riding effects of other factors.

v. Prematurity and serum proteins.

The results of fetal serum proteins show a definite increase in albumin and gamma-globulins with increasing maturity at birth, as judged by birth-weights.

This relationship has been reported by a number of workers. (88, 129, 180)

It is interesting to note that fetal serum albumin is synthesized by the fetus from aminoacids actively transported across the placenta by specific carrier systems. The availability of maternal aminoacids for fetal synthesis is likely to be
dependent on maternal nutrition. Thus it is possible that fetal serum albumin concentrations may reflect on maternal nutritional status as well as fetal maturity.

On the other hand fetal gamma-globulin is derived from maternal gamma-globulins, where selective transfer of IgG molecule takes place across the placenta. Therefore, fetal serum gamma-globulin concentrations may depend on placental sufficiency.

Corner (1963) has emphasized the need for a test of placental sufficiency. It is tempting to suggest that further study of transport of aminoacids and proteins across the placenta may help in devising such a test. (42)

vi. Transfer of maternal malarial antibodies across the placenta

This is clearly demonstrated in the present study and is in support of earlier reports by Voller & Bray (1962) and McGregor et al. (1965). Therefore, at birth the newborn of an immune mother has a certain degree of passively acquired immunity against malaria. (121, 172)
SECTION II

E. MILK AND MALARIA

Introduction

Infants, of immune mothers, in their first 3-4 months of life very seldom suffer from malarial infections.

Garnham (1949) found that at the end of the 3rd month of life, only 10% of children are infected with malaria, when we should expect 55.8% of the children to be infected, as calculated from the index of infective density. 969)

This relative resistance to malaria infection, in early infancy is probably due to a combination of factors listed below:

1. Transfer of passive immunity, from the mother across the placenta, (58)

2. high Hb F content of newborns' erythrocytes, (74)

3. deficiency of PABA in milk, which is normally the diet to exclusion of others, at this age, (22, 28)

4. selective vector biting. (127)

Firstly, transfer of passive immunity across the placenta occurs by selective transfer of IgG. Edozien (1962) has demonstrated antiparasitic effect of cord blood IgG of newborns of immune mothers. In the previous chapter specific fluorescent
antibodies have been demonstrated in cord blood of newborns of immune mothers

Next factor may make erythrocytic development of malaria parasites, in the presence of high concentrations of Hb F, less feasible than in the adult erythrocytes with Hb F. Other genetically different hemoglobins have been postulated as providing immunity against malaria on the same basis.

Thirdly, milk is deficient in PABA which is an essential metabolite for malaria parasites. Therefore, when infants are on exclusively milk diet with PABA requirement for malaria parasites may not be met with thus creating an environment unsuitable for their development. Some of the anti-PABA drugs have been shown to be effective anti-malarials.

Lastly, selective vector biting has been demonstrated by Muirhead Thomson (1951) who showed that mosquitoes bite infants less frequently than older children and adults.

Recent report by Adler and Fonner (1965) of possible transfer of antibodies through milk of immune mice suggests that PABA deficiency in milk diet may not be the entire explanation of the observed protective effect of milk against malaria. (4)
Maegraith et al. (1952) concluded that milk contains something that can inhibit or restrict the development of the asexual phase of P. berghei in rat. (107)

This was then confirmed in monkeys by Bray & Garnhan (1953). Hawking reversed this protective effect of milk diet by addition of PABA in the diet. He also drew attention to the widespread belief in Arab countries that milk diet protects against malaria. (22, 79)

Terry (1956) and Adler & Fonner (1965) have demonstrated that milk from immune mothers increases resistance against malaria, an effect which cannot be reversed by addition of PABA. (4, 161)

Materials and Methods

Thirty specimens of breast milk were collected by manual expression from 12 immune mothers after delivery over as many days as possible.
No. 34 TABLE. Milk and Malaria Study.

Summary of Colostral Specimens Collected

<table>
<thead>
<tr>
<th>No. of specimens collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>During the 1st day after delivery</td>
</tr>
<tr>
<td>&quot; 2nd &quot;</td>
</tr>
<tr>
<td>&quot; 3rd &quot;</td>
</tr>
<tr>
<td>&quot; 4th &quot;</td>
</tr>
<tr>
<td>&quot; 5th &quot;</td>
</tr>
<tr>
<td>&quot; 6th &quot;</td>
</tr>
<tr>
<td>&quot; 7th &quot;</td>
</tr>
<tr>
<td>&quot; 8th &quot;</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

Each specimen was centrifuged at 8,000rpm/30min. at +4°C and de-lipideated. Double immunodiffusion technique was used for detecting IgG, IgA and IgM in these specimens. Screen was carried out for presence of malarial antibodies at a dilution of 1: 100 using polyvalent fluorescent-labelled anti-gamma-globulin antiserum. Eight of these specimens were selected to cover the different days of collection and were then titred for malarial antibodies using nonspecific anti-IgA fluorescencelabelled antiserum.
Results

IgA was present in all specimens collected while IgG and IgM though present in majority of specimens tended to disappear with increasing time after delivery.

No. 35 TABLE. Milk and Malaria Study.

IgG and IgM in Colostral Specimens

<table>
<thead>
<tr>
<th>During the 1st day after delivery</th>
<th>IgG Positive</th>
<th>IgM Positive</th>
<th>Total No. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; 2nd   &quot;</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>&quot; 3rd   &quot;</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>&quot; 4th   &quot;</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>&quot; 5th   &quot;</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&quot; 6th   &quot;</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&quot; 7th   &quot;</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&quot; 8th   &quot;</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
No. 36 TABLE. Milk and Malaria Study.

**Malarial Antibodies in Colostrum**

Screening for malarial fluorescent antibodies

1:100 dilution positive = positive reaction

1:100 dilution negative = negative reaction

<table>
<thead>
<tr>
<th>No. Examined</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>30</td>
</tr>
</tbody>
</table>

No. 37 TABLE. Milk and Malaria Study.

**IgA Malarial fluorescent antibodies in Colostrum**

Titration using nonspecific anti-IgA fluorescein-labeled anti-serum.

<table>
<thead>
<tr>
<th>Collected during the 1st day after delivery</th>
<th>No.</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1600</td>
</tr>
<tr>
<td>2nd day</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>3rd day</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>4th day</td>
<td>2</td>
<td>200 &amp; 200</td>
</tr>
<tr>
<td>5th day</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>6th day</td>
<td>Nil</td>
<td>-</td>
</tr>
<tr>
<td>7th day</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>8th day</td>
<td></td>
<td>400</td>
</tr>
</tbody>
</table>
Discussion

It is, therefore, possible to conclude from these results that an immune mother secretes in the breast milk malarial antibodies as detected by fluorescent antibody technique and that though they may belong to IgG and IgM group of immunoglobulins, they certainly belong to IgA group.

In external secretions, IgA is the predominant immunoglobulin whereas it forms only a minor component in the serum.

The IgA of external secretions differs from the serum IgA in being present in polymeric form in varying proportions and in having an extra "secretory piece".

Syntheses of IgA by the glands of GIT, RT and GUT seems to be carried out by plasma cells present locally in these glands. Recovery from certain infections of these systems is better correlated with the levels of local antibody than with serum antibody.

In case of the mammary gland it appears that highly selective transport of serum IgA occurs with local synthesis of "secretory piece".
Whether the human mammary gland is similar in this respect to those of animals and selectively transports serum IgA or whether it synthesizes IgA locally is not clear.

Adinolfi et al. (1966) has demonstrated the presence of iso-hemagglutinins and antibodies against E. Coli in colostrum. However, colostral secretory antibodies after active immunization has not been recorded. Therefore, the demonstration of malarial antibodies in the colostrum is interesting as these antibodies are acquired as a result of repeated infections. (3)

The secretory IgA may play an important role in most immune defence against several micro-organisms and virus and in regulating the normal flora of mucous membranes.

Clearly for secretory anti-malarial IgA to play a role, during early infancy, in providing passive immunity to the newborn must depend on its being absorbed from GIT. IgA is resistant to gastro-intestinal enzymes and to reducing agents and it is possible that the GIT of the newborn may be specially adapted to absorption of intact IgA molecules. Though increase in levels of gamma-globulins has been shown to be insignificant in breast-fed infants it is possible that specific antibodies in significant amounts are acquired in this manner. (166, 167).
SECTION II

F. TROPICAL SPLENOMEGALY SYNDROME.

Introduction

Adult patients presenting with massive enlargement of spleen are very commonly seen in clinical practice in tropical developing countries.

Chronic malarial infections have been recognized as being one of the many causes of massive splenomegaly in these countries.

Sawdry (1955) reported malaria and malnutrition as two factors common in a group of patients in whom other infections were ruled out, as the cause of enlarged spleen. In his patients, spleen puncture failed to reveal malarial pigment or parasites. (62)

Chaudhrhi et al. (1956) described the characteristic finding of hepatic sinusoidal lymphocytic infiltration in this syndrome. (36)

That these spleens were immunologically competent in responding to particulate antigen injected intravenously, a situation analogous to erythrocytic malarial infections, was demonstrated by McFadzen & Tsang in 1956. (112, 113)
Recently in Uganda a team of workers initiated a
detailed investigation of this syndrome and established the
following picture. Reports from other tropical countries
confirming this picture enables us to summarize common features
(Hutt, 1966 and Pitney, 1968). (87, 132)

CLINICAL

Spleens were enlarged to below the umbilicus with some
discomfort and pain and acute episodes of pain presumably due
to splenic infarcts.

Patients complained of weakness and lassitude and gave
a past history of repeated low-grade fevers.

Hepatomegaly and anemia were usually present with the
later becoming marked during pregnancy in female patients.

Majority of patients were in 2nd and 3rd decade, when
first attending the hospital.

HEMATOLOGY

Normocytic, normochronic anemia was a constant feature
with decreased red cell survival and increased plasma volume.
Usually also neutropenia and thrombocytopenia were present.

**PATHOLOGY**

**Spleen:** Though massively enlarged showed no characteristic picture. Splenic cords were packed with lymphocytes and plasma cells. No malarial pigment or parasites were seen.

**Liver:** Majority of patients had varying degrees of sinusoidal lymphocytic infiltration as the characteristic finding. Majority of the infiltrating cells had the histological appearances of mature lymphocytes, lying in proximity of hypertrophic Kupffer cells. No malarial pigment or parasites were seen.

There was no histological or biochemical evidence of impaired hepatic parenchymal function.

**PARASITOLOGY AND IMMUNOLOGY**

Painstaking, repeated examinations of peripheral blood smears revealed *P. malariae* in 9 out of 20 cases (45%), as opposed to only 4% in the control group comprising of hospital in-patients.
Fluorescent malarial antibodies were detected in high titres.

Hyper-gamma-globulinaemia, with increases in concentrations of IgG, IgA and IgM was always present with IgM at remarkably high levels.

There was a higher incidence of RF and cold hemoglutinins in these cases.

These findings suggest that repeated *P. malariae* infections in some individuals of a population, exposed to heavy malarial transmission, leads to a syndrome characterized by:—

i. massive splenomegaly

ii. hepatomegaly

iii. anemia, and usually neutropenia and thrombocytopenia

iv. hepatic sinusoidal infiltration with lymphocytes

v. hyper-gamma-globulinemia, with remarkable increase in IgM

vi. high levels of fluorescent malarial antibodies

vii. relative scarcity of erythrocytic malarial infections.
As to why only some individuals of the population should develop this syndrome is not clear. The explanation may be differences in host and/or parasite.

That the host is immunologically competent seems self-evident. Marked hypertrophy of RES, in liver and spleen, hyper-gamma-globulinemia, high levels of specific antibodies and relative scarcity of erythrocytic infections and lack of acute clinical episodes speak for themselves.

Protection by heterozygote Hb AS and/or G6PDase deficiency in those that do not develop TSS appears to be less likely than previously believed. There appears to be little difference in the incidence of these two genetic defects in those that develop TSS and those that do not develop the syndrome (Personal communication Dr. P. Stuiver).

Malnutrition and low socioeconomic background have been well recorded. However, it is more than likely that chronic ill-health can lead to lowered earnings and therefore malnutrition.

In Uganda, the immigrant tribes (Rwanda/Rundi) originally from low areas of malarial endemicty predominate amongst these patients. These are non-immunes being exposed to heavy malarial
transmission at a later age with malnutrition, low socio-economic background and increased incidence of many other parasitic infections as associated factors.

Even those members of these tribes born in Uganda have not been exposed to the selective influence of heavy malarial transmission over generations, leading to increased genetic resistance against malarial infections. Such selective factors have been shown to have built up almost complete resistance against P. vivax in the West African Negroes (Bray, 1958). (19)

It seems possible that TSS is as a result of an exaggerated response, but an effective one to malarial infections.

The parasite itself can give rise to repeated relapses as P. malariae has persistent exo-erythrocytic stages.

Antigen variation in chronic P. knowlesi infections has been demonstrated by Brown & Brown (1965) (25, 26) and by Voller & Rossan (1969). (174-177)

Antigenic variation in trypanosomes leads to remarkable increases in IgM concentrations. Similarly elevated concentrations are found in TSS too.
It is, therefore, necessary to attempt to demonstrate antigenic variations, if any in patients with TSS.

The present study was undertaken, inspired by Professor Hutt and in co-operation with Dr. Stuiver to study IgM in this syndrome.

It is hoped to continue this work on even larger numbers as the results so far have been interesting and may reveal significant defects in host/parasite relationships.

First study was undertaken, with the co-operation of Dr. John Zeigler, to inquire into the ability of TSS group of patients to synthesize antibodies in IgG and IgM fractions of their sera, subsequent to E. Coli Vi antigenic challenge. (184)

Second one was to study the distribution of malarial antibodies in IgG and IgM fractions. Preliminary results on fractions obtained by DEAE - Cellulose chromatography seemed to indicate that main anti-malarial activity in these patients was absent in IgG but present in IgM fractions.

The problem of loss of antibodies during fractionation was considered and Dr. Voller's advice sought for guidance. Dr. Voller very kindly suggested a better alternative of using monospecific fluorescent antisera for differential titration of malarial antibodies.
The third study was to seek for nonomeric IgM units in 7S peaks of Sephadex G-200 gel filtration carried out on patients' sera.

Next, was to apply immunofluorescence, using fluorescent anti-gamma-globulin antisera on liver biopsies fixed and processed by Sainte-Marie (1962) technique. This technique was recently learnt during personal visit to Gamalaya Institute, Moscow, from Professor Abelev and Dr. Englegardt.

Finally, a group of patients attending weekly "Big Spleen Clinic" were observed longitudinally. The patients were divided into three at random and were placed on one of the three lines of treatment:

i. Placebo group

ii. Paludrine group (100mg. daily)

iii. Primaquine (15 mg. daily for 14 days) followed by Chloroquine (300 mg. base/weekly) group.

Dr. Stuiver followed up these patients clinically and collected samples of blood (for IgG, IgA, IgM and MFAT determinations) and urine (for chloroquine and paludrine detection) from each of the patient at regular intervals.
The code of the treatment received and information regarding clinical changes were provided by Dr. Stuiver when the laboratory estimations outlined in these studies were completed.

i. The ability to synthesize antibodies after E. Coli Vi antigenic challenge.

Materials and Methods

3 controls and 5 TSS patients were given an intramuscular injection of 100ug of E. Coli Vi antigen.

Blood samples were collected two weeks later from all, except for one TSS patients who was bled at the end of two months.

Sera were fractionated by step-wise elution on DEAE-cellulose (20x1cm.) as follows:

Serum sample, 1 ml., dialyzed overnight with starting buffer DEAE - cellulose column, equilibrated with starting buffer. Stepwise elution using 25 mls. of the various strength of phosphate buffers.

0.015M pH 8.0  -  No protein eluted
0.08M pH 8.0  -  IgG peak eluted
0.15M pH 8.0  -  pooled the eluted peaks.
0.3M pH 8.0  -  Considered as IgM peak.
Vi antibody titres in these two fractions, from each serum, were determined by hemagglutination technique (Landy & Lamb, 1953). (100)

Results

No. 38 TABLE. Tropical Splenonegaly Syndrome Study.

Antibody response to E. Coli Vi antigenic challenge

<table>
<thead>
<tr>
<th>Controls</th>
<th>TSS Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial No.</td>
<td>IgG peak</td>
</tr>
<tr>
<td>536/68</td>
<td>1:120</td>
</tr>
<tr>
<td>534/68</td>
<td>1:60</td>
</tr>
<tr>
<td>530/68</td>
<td>1:630</td>
</tr>
<tr>
<td>379/68</td>
<td>1:120</td>
</tr>
</tbody>
</table>

*Blood sample after 2 months.

Discussion

The primary response to an antigenic challenge usually results in production of antibodies in IgM class followed by IgG type of antibodies.
Since patients with TSS have very high levels of IgM the ability to respond in this manner was tested by challenging the patients with E. Coli Vi antigen.

The results of this experiment have ruled out the possibility of an abnormal response of exclusive synthesis of IgM type of antibodies in TSS patients.

It is clear that TSS patients are immunologically competent to respond to an antigenic stimulus by synthesizing both IgG and IgM types of antibodies.

ii. The distribution of malarial antibodies in IgG and IgM.

**Materials and Methods**

(a) DEAE - Cellulose fractions obtained from the group studied in the E. Coli Vi antigen stimulation study were utilized for the determination of fluorescent malarial antibodies.

Results indicated activity in IgM fractions and not in IgG fractions, but also suggested the possible loss of antibodies on the column.

(b) Dr. Voller's suggestion of differential titration using nonspecific fluorescent antibodies was tried next on a group of eleven patients, from the "Big Spleen Disease" Clinic.
No. 39 TABLE Tropical Splenomegaly Syndrome Study.

Distribution of MEAT in IgG and IgM Fractions

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>IgG peak</th>
<th>IgM peak</th>
<th>Serial No.</th>
<th>IgG peak</th>
<th>IgM peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>536/68</td>
<td>Neg</td>
<td>Neg</td>
<td>42</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>534/68</td>
<td>Neg</td>
<td>Neg</td>
<td>128</td>
<td>Neg</td>
<td>1:200</td>
</tr>
<tr>
<td>530/68</td>
<td>Neg</td>
<td>Neg</td>
<td>486/68</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>379/68</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>376</td>
<td>Neg</td>
<td>1:200</td>
</tr>
</tbody>
</table>
No. XIX GRAPH. Tropical Spleenogengaly Syndrome Study.

Distribution of MPA in IgG, IgA and IgM
Discussion

Results of differential titres show that malarial antibodies are randomly distributed in IgG, IgA and IgM classes. Therefore, there appears to be no abnormality in the synthesis of malarial antibodies leading to synthesis only in a single class of immunoglobulins.

That it is necessary to try out different techniques and be cautious in the interpretation of results obtained is emphasized in this experiment.

DEAE - Cellulose chromatographic fractionation was satisfactory for studying antibody response to Vi antigens challenge. That it was not satisfactory for malarial antibodies may be due to low levels of these antibodies in unfractionated sera and due to disproportionate loss of these antibodies on the column. This latter possibility can be negated by carrying out differential titration for malarial antibodies using nonspecific antisera.

From these two experiments it is possible to rule out abnormality of antibody synthesis, in TSS patients, which may lead to exaggerated or exclusive synthesis of antibodies in IgM class.
iii. 7S IgM units.

Materials and Methods

1ml aliquote of sera from 28 patients and 2 controls were fractionated by gel filtration on G200 column (2.5x100cm) eluting with 0.1M pH 8.0 Tris-HCl-NaCl buffer containing sodium azide as a preservative.

Sera were stored at -30°C and were thawed only once, at the time of the run. All sera were filtered within 1-3 weeks of collections. Separation was carried out at +4°C at buffer flow rate of 7-8 nls/hour and fractions were collected at 30 mins. intervals.

Optical density was measured at 280nm, Unicam SP 500 and plotted against number of each fraction. The three peaks were pooled separately, always discarding one fraction between the 1st and 2nd peaks and one more between 2nd and 3rd peaks.

Pooled fractions were concentrated by per-evaporation and immunodiffusion by Ochterlony technique carried out against anti-IgM.

Results were considered positive for monomeric IgM units if a precipitin line was observed between 7S peak and anti IgM.
Results

No. 40 TABLE. Tropical Splenomegaly Syndrome Study.

<table>
<thead>
<tr>
<th></th>
<th>7S IgM +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>TSS patients</td>
<td>28</td>
</tr>
<tr>
<td>Controls</td>
<td>2</td>
</tr>
</tbody>
</table>

No. 41 TABLE. Tropical Splenomegaly Syndrome Study

7S IgM units in TSS sera, in relation to Malarial prophylaxis

<table>
<thead>
<tr>
<th></th>
<th>7S IgM + ve</th>
<th>7S IgM Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malarial prophylaxis</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Placebo</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>
No. XX GRAPG. *Tropical Splenomegaly Syndrome Study.*

*Sephadex G-200 Gel Filtration Normal Pooled Serum*
No. XXI GRAPH. Tropical Splenomegaly Syndrome Study.

Sephadex G-200 Gel Filtration Control Serum
No. XXII GRAPH. Tropical Splenomegaly Syndrome Study.

Sephadex G-200 Gel Filtration TSS Serum
No. XXIII GRAPH. Tropical Splenomegaly Syndrome Study.

Sephadex G-200 Gel Filtration TSS Serum
No. XXIV GRAPH. Tropical Splenomegaly Syndrome Study.

Sephadex G-200 Gel Filtration TSS Serum
No. 1 PHOTOGRAPH. Tropical Splenomegaly Syndrome Study.

Immunodiffusion pattern to demonstrate the presence of 7S IgM
No. 42 TABLE. Tropical Splenomegaly Syndrome Study.

7S IgM units in TSS sera, in relation to height of 19S peaks

<table>
<thead>
<tr>
<th>Height of 280nm</th>
<th>7S IgM Positive</th>
<th>7S IgM Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.6-1.2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 1.2</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

No. 43 TABLE. Tropical Splenomegaly Syndrome Study.

IgM concentrations in relation to heights of 19S peaks

<table>
<thead>
<tr>
<th>19S peak</th>
<th>IgM Concentrations in mg/100mls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 280nm</td>
<td>No.  Mean  Range</td>
</tr>
<tr>
<td>&lt; 0.6</td>
<td>5   115  40-156</td>
</tr>
<tr>
<td>0.6-1.2</td>
<td>6   205  46-422</td>
</tr>
<tr>
<td>&gt; 1.2</td>
<td>3   691  586-838</td>
</tr>
</tbody>
</table>
Discussion

Experimentally induced malaria, in human volunteers, leads to increases in concentrations of the three principal immunoglobulins; IgG, IgA and IgM, with highest percentage increases in IgM concentrations, (Abele, et al., 1965 and Tobie, et al., 1966). (1, 164)

The same is true for natural malarial infections (Turner & Voller, 1966 and McFarlane & Voller, 1966) and in patients suffering from TSS. (115, 168)

The 19S peak of serum proteins contains IgM, beta-lipotin and alpha_2-macroglobulin; so that it is not possible to quantify IgM from the height or area of the 19S peak.

The height of 19S peaks of TSS sera varied from near normal to very high and roughly correspond with the IgM concentrations, as determined by radial immunodiffusion technique.

The presence of 7S IgM has no relation either with the height of 19S peak or with the antimalarial treatment received by the patient.

Rowe, et al. (1968) has pointed out the discrepancy between the concentrations of 19S proteins after ultra-centrifugation
and the IgM as determined by radial immunodiffusion technique. (136)

Fahey & Mckelvey (1965) have emphasized that radial immunodiffusion technique is less reliable for the estimation of a protein if the protein exists in a polymeric and monomeric forms in the same sample. (60)

Therefore, for the following reasons the IgM concentrations in TSS sera, as estimated by radial immunodiffusion technique, are likely to be an over-estimation:

(a) Commercial immunoplates contain anti-serum optimum for estimation of concentrations of IgM met with in normal Caucasian adults. Use of such plates for estimation of higher levels, met with in populations exposed to higher infective load in tropical developing countries, is likely to be less satisfactory.

(b) Logarithmic relationship between concentrations and diameters of diffusion rings will lead to even bigger errors when estimating extremely high levels in TSS sera.

(c) Presence of 7S IgM units, as demonstrated in this study, will add significantly to such errors and will lead to significant over-estimation even when estimations are carried out on sera appropriately diluted.
It would seem advisable to utilize immunoplates containing antisera optimum for the higher levels without dilution of the patients' sera in order that the results may not be grossly unreliable.

Significance of 7S IgM units in TSS sera can be explained on the basis of one of the two, possibly related factors.

Increased concentrations of IgG and IgM are due to increased synthesis. Such high levels of IgG lead to increased rate of catabolism.

Higher concentrations of IgM do not lead to increased rate of catabolism, so that the rate of synthesis may not be as high as the high levels of IgM may suggest. (14, 61, 179)

Increased synthesis of IgM may lead to abnormal synthesis. Hyper-gamma-globulinaemia, due to any cause, is usually associated with increased incidence of auto-antibodies. Therefore, 7S IgM units may be due to defective synthesis because of increased rates of synthesis.

On the other hand, one can speculate that the basis defect in synthesis leading to synthesis of 7S IgM units may be the cause, rather than the effect of increased rate of synthesis.
Further work is required to investigate these points as well as to establish if the presence of 7S IgM is encountered in conditions other than TSS.

iv. **Immunoflorescence study on liver biopsies.**

**Materials and Methods**

Needle liver biopsies were obtained from six TSS patients and two patients suffering from other disease formed the control group.

Biopsies were fixed immediately in cold (+4°C) 95% ethanol and processed by Sainte-Marie technique (1962). 3μ thick sections were cut and serial sections stained with H & E and fluorescent anti-gamma-globulin.

Mr. Matt Findlay, Chief Technician, Department of Pathology, Macerere Medical School, kindly assisted in processing these biopsies.

**Results**

Saline control showed no fluorescence. H & E sections confirmed previous descriptions of HSL infiltrations. The appearances of the fluorescent staining was sufficiently consistent in biopsies obtained from TSS patients and those features were absent in other biopsies.
No. II PHOTOGRAPH. Tropical Splenomegaly Syndrome Study.

Normal Liver Biopsy H & E Section
No. III PHOTOGRAPH. Tropical Splenomegaly Syndrome Study.

TSS Liver Biopsy H & E Section
No. IV PHOTOGRAPH. Tropical Splenomegaly Syndrome Study.

Normal Liver Biopsy Immunofluorescence
No. 7 PHOTOGRAPH. Tropical Splenomegaly Syndrome Study.

TSS Liver Biopsy Immunofluorescence
Discussion

Hepatic sinusoids are dilated and outlined by fluorescence with only occasional dark bodies with peripheral fluorescence being seen within the sinusoids.

It appears that the Kupffer cells lining the hepatic sinusoids are containing within them gamma-globulins, either free or as antigen/antibody complex. Normally Kupffer cells are engaged in removing from circulation particulate antigens or antigen/antibody complexes or denatured antibodies. From the present study it is not possible to conclude whether the ingested material is antigen/antibody coupled OR denatured antibodies.

The dark bodies with peripheral fluorescence appear to be the lymphocytes, seen in H & E preparations within the hepatic sinusoids. Histologically these cells appear to be mature lymphocytes and therefore probably not equipped to synthesize gamma-globulins. However, the immunofluorescence shows quite clearly that histological appearance in light microscopy may be quite misleading as regards the function of these cells.

The close proximity between the Kupffer cells and the lymphocytes leads one to speculate that the lymphocytes are present in this site in order to receive processed information
from Kupffer cells necessary for antibody synthesis.

v. Effects of malarial prophylaxis in TSS

Materials and Methods

Patients attending "Big Spleen Clinic" under Dr. Stuiver were randomly divided into three groups so as to receive one of the following type of treatment:

(a) Placebo, 7 patients
(b) Paludrine, 6 patients, 100mg/daily
(c) Primoquine, 15mg daily for 14 days, followed by chloroquine, 500mg weekly, 8 patients.

Dr. Stuiver followed the clinical status of these patients and collected a specimen of blood and urine, usually at monthly intervals from each of the patient.

MFAT and IgG, IgA and IgM were estimated on the separated sera, stored at -30°C until required for analysis, and the urines were tested qualitively for chloroquine and paludrine.

None of the information regarding the clinical status of the patients or the type of treatment received by them was made known to me, until these estimations and tests were completed.
<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>P</th>
<th>Mean</th>
<th>S.D.</th>
<th>P</th>
<th>Mean</th>
<th>S.D.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>141</td>
<td>153</td>
<td>298</td>
<td>683</td>
<td>155</td>
<td>150</td>
<td>1345</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Campocuqano Group</td>
<td>134</td>
<td>274</td>
<td>121</td>
<td>1026</td>
<td>4511</td>
<td>23</td>
<td>1219</td>
<td>4512</td>
<td>16</td>
</tr>
<tr>
<td>Panadurine Group</td>
<td>207</td>
<td>20</td>
<td>027</td>
<td>225</td>
<td>121</td>
<td>1087</td>
<td>4222</td>
<td>27</td>
<td>158</td>
</tr>
</tbody>
</table>

Results

- Immunoglobulins in Relation to Maternal Prolactin in NSS
- No. 44 Mammalian Spontaneous Syndrome Study
No. XXV GRAPH. Tropical Splenomegaly Syndrome Study.

Mean Values of IgG in TSS in Relation to Duration of Malarial Prophylaxis
No. XXVI  GRAPH. Tropical Splenomegaly Syndrome Study.

**Mean Values of IgA in TSS in Relation to Duration of Malarial Prophylaxis**
No. XXVII GRAPH. Tropical Splenomegaly Syndrome Study.

Mean Values of IgM in TSS in relation to Duration of Malarial Prophylaxis
No. XXVIII GRAPH. Tropical Splenomegaly Syndrome Study.

Mean Values of Spleen Sizes in Relation to Duration of Malarial Prophylaxis
PLACEBO GROUP

No. XXIX Graph. Tropical Splenomegaly Syndrome Study.

Concentrations of IgG in each patient in relation to duration of treatment.
PLACEBO GROUP

No. XXX GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgA in each patient in relation to duration of treatment
PLACEBO GROUP

No. XXXI GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgM in each patient in relation to duration of treatment
PLACEBO GROUP

No. XXXII GRAPH. Tropical Splenomegaly Syndrome Study.

Spleen size in each patient in relation to duration of treatment
 PALUDRINE GROUP 

No. XXXIII GRAPH. Tropical Splenomegaly Syndrome Study.

Concentration of IgG in each patient in Relation to Duration of Treatment.
PALUDRINE GROUP

No. XXXIV GRAPH. Tropical Splenomegaly Syndrome Study.

Spleen size in each patient in relation to duration of treatment
PALUDRINE GROUP

No. XXXV GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgM in each patient in relation to duration of treatment
PALUDRINE GROUP

No. XXXVI GRAPH. Tropical Splenomegaly Syndrome Study.
Spleen size in each patient in relation to duration of treatment
PRIMAQUINE/CHLOROQUINE GROUP

No. XXXVII GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgG in each patient in relation to duration of treatment.
No. XXXVIII GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgA in each patient in relation to duration of treatment
PRIMAQUINE/CHLOROQUINE GROUP

No. XXXIX GRAPH. Tropical Splenomegaly Syndrome Study.

Concentrations of IgM in each patient in relation to duration of treatment
PRIMAQUINE/CHLOROQUINE GROUP

No. XL GRAPH. Tropical Splenonegaly Syndrome Study.

Spleen size in each patient in relation to duration of treatment
No. 45 TABLE. Tropical Splenomegaly Syndrome Study.

**MFAT in each of the Three Groups of TSS Patients**

<table>
<thead>
<tr>
<th>Reciprocal of Titres</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>1600</th>
<th>GMRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>13</td>
<td>970</td>
</tr>
<tr>
<td>Paludrine</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>14</td>
<td>1252</td>
</tr>
<tr>
<td>Primaquine/Chloroquine</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>1425</td>
</tr>
<tr>
<td>19.4</td>
<td>7</td>
<td>38</td>
<td>47.4</td>
<td>18</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>------</td>
<td>---</td>
<td>----</td>
<td>------</td>
<td>----</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>33.3</td>
<td>5</td>
<td>15</td>
<td>32.3</td>
<td>34</td>
<td>32.3</td>
<td>34</td>
</tr>
</tbody>
</table>

**Urinary Cholesterol**  
**Bilirubin**  
**Cholesterol**  
**Phenol**  
**Group**  
**Phenol group**  
**Urinary Phenol**  
**Urinary Phenol**  
**Urinary Phenol**  
**Urinary Phenol**  

**Table**  
**Proposed Sphenoid Syndrome Study**  
**No. 4.6 TABLE**
Discussion

The improvement in clinical status, with decrease in spleen sizes and increase in hemoglobin concentrations, in TSS patients on long term malarial prophylaxis has been reported (Watson-Williams & Allan, 1968 and Stuiver et al. in press) (153, 181)

This study was undertaken to elucidate changes in malarial immunity in TSS patients as a result of long term malarial prophylaxis.

Normal West Africans living in holoendemic malarious areas when on malarial prophylaxis have been reported to show the following changes:— (34, 143, 178)

i. decrease in hyper-gamma-globulinaemia

ii. decrease in rate of synthesis of gamma-globulin

iii. decrease in malarial antibody titres.

The results of the present study have shown definite differences in TSS patients on malarial prophylaxis compared to those on placebo. Further differences are seen in those receiving primaquine/Chloroquine when compared to those receiving paludrine.

(a) Spleen size

Spleen is an important organ for development and
maintenance of malarial immunity, both acquired and natural.

Bruce-Chwatt (1956) has shown that spleen size is directly related to the age of maximum intensity of malaria in African children. When their spleen weights were compared with those of Caucasian children, the African children have spleens of higher weights even at birth and this difference is maintained throughout life. (29)

Spleen by its phagocytic activity forms an important organ for clearing circulating malarial antigen. This in turn stimulates cellular proliferation and increased production of antibodies. (Taliefero, 1937, 1949 and 1956) (155, 156, 158)

The chimpanzee displays a natural resistance to the erythrocytic forms of human malaria parasites but allows multiplication and growth of these forms when splenectomized (Bray, 1957). (18)

Therefore, malarial prophylaxis by reducing the antigenic load in these patients should lead to diminution in splenic size and this expectation is met with in these trials.

Experimental effects of splenectomy are strongly indicative of lowering of immunity to malaria by inability to
remove circulating erythrocytic forms of the parasites and by producing lower amounts of antibodies to circulating particulate antigens (Saslaw & Carlisle, 1964). (141)

Until observations prove to the contrary, in human malaria, splenectomy in malarious areas must be undertaken cautiously and only when other measures have failed to improve the patients' status.

Improvement following long term malarial prophylaxis is likely to reduce the need for splenectomy in most cases.

(b) Anaemia, Thrombocytopenia and Neutropenia.

Enlargement of spleen leads to sequestration of blood leading to a redistribution of blood and altered hemodynamics. Enlargement of spleen, therefore, leads to anaemia, neutropenia and thrombocytopenia due to this redistribution, increased phagocytic activity and increased plasma volume.

No unequivocal evidence is available to support autoimmunity as an etiological factor in anaemia due to malaria even after extensive studies by Zuckerman (1957 - 1961).

(c) Immunoglobulins.

Concentrations of IgG, IgA and IgM are higher in TSS
patients when compared with the control group.

That the concentrations of IgG have not decreased inspite of malarial prophylaxis, over a period as long as a year, seems to indicate that antigenic stimulus responsible for these elevated levels has persisted.

The explanation of this apparent persistence of the antigenic stimulus may reside in either host behaviour or parasite persistence.

The possibility of auto-immunity as a result of chronic, excessive antibody synthesis cannot be ruled out. If such a state were to develop malarial prophylaxis, which will reduce malarial antigenic load may not affect antibody synthesis.

The parasite may be harboured in exo-erythrocytic forms within the body and lead to repeated relapses.

The decrease in concentrations of IgM following malarial prophylaxis seems to indicate fewer relapses with parasitic antigenic variations or fewer reinfections.

That the spleen size diminishes in parallel with the decrease in IgM concentrations does not necessarily mean that spleen is the main site of IgM synthesis. It is more likely
that both the spleen size and levels of IgM may be dependent on antigenic load.

(d) **Malarial fluorescent antibodies.**

Despite differences in type of therapy in the three groups of patients there are no differences in the titres of malarial antibody, over a period of about a year.

The antibody titres do not depend on the size of a single dose of an antigen. If persistent exo-erythrocytic forms are leading to repeated relapses the antibody titres will continue to remain high. The possibility of auto-antibodies cross-reacting with malarial parasites cannot be excluded at this stage.

Exo-erythrocytic forms have so far not been demonstrated in these patients either in the spleen or in the liver. Further careful attempts to locate such forms of parasites possibly with the use of fluorescent antibody techniques will help in better understanding of the pathogenesis of this syndrome.

(e) **Urinary chloroquine and paludrine.**

It is hoped that once a month testing of urines for these drugs will provide indication about patients' faithfulness to his therapeutic regime.
This seems too great an expectation because:

i. Urines must be tested more frequently as chloroquine can be detected for a period of 5-8 days after oral ingestion and paludrine for 24-48 hours only.

ii. Test for chloroquine is not specific and may give a positive reaction with other drugs excreted in the urine, including paludrine.

iii. Occasional self-medication and therapy at Outpatient Clinics with chloroquine for vague illness is common in our population.

Faster response to paludrine compared to primaquine/chloroquine therapy is difficult to explain as paludrine is less effective than chloroquine against erythrocytic forms. Probably the difference is due to a lesser risk of failure to take the drug if it is to be taken once every day compared to when it is to be taken once every week.

Follow-up studies are difficult to carry out in our part of the world. This study has been possible only because of hard work and single-mindedness of Dr. Stuiver, and I wish to express my gratitude to him once again.
Sague's (1970) paper (in press) has been brought to my attention by Dr. Hamilton during his visit to East Africa. In this work long term proguanil therapy has been demonstrated to lead to reduction in spleen size and serum IgM concentration. (130)
CONCLUSIONS.

i. The breakdown of a previously established immunity during pregnancy is not due to depression in the humoral mechanism. It is my opinion that this reduced resistance is due to a defect in cellular mechanism, with ingestion of malaria parasites by macrophages and failure to lyse them as a result of increased concentrations of unbound-fraction of corticosteroids, during pregnancy.

ii. The transfer of IgG across the placenta occurs early in pregnancy and increases exponentially with the period of gestation. The levels found in the fetus depend on maternal levels as well as placental sufficiency.

At term, the levels of IgG found in African neonates are usually lower than in their mothers. In my opinion this difference in the pattern, in the transfer of IgG in Africans and Caucasians, is not due to genetic differences.

iii. There is a high incidence of intra-uterine infections in Ugandan neonates. The etiological agents remain to be identified but I believe that malaria parasites is not
one of them.

iv. The neonate of an immune mother also acquires specific malaria antibodies contained in the IgG fraction of maternal antibodies.

Congenital malaria is rare in an immune population though it occurs occasionally in the non-immune. I am of the opinion that congenital malaria is uncommon even in non-immune population because the parasites are unable to thrive in the fetal environment. In an immune population this is further reinforced by the transfer of maternal antibodies.

v. Placental parasitization by malaria parasites can lead to lowering of birth-weights if the infestation is heavy enough. I believe that with the decrease in transmission and increasing use of anti-malarials this factor will be responsible for fewer cases of prematurity, than in the past.

vi. The colostrum of immune Ugandan mothers contain IgA malaria antibodies. It is my opinion that neonates' immunity to malaria is further reinforced by the ingestion and absorption of these antibodies.
vii. I believe that 7S-IgM units are synthesized in these patients as a result of increased rates of synthesis. These monomeric units must be responsible for an over-estimation of IgM concentrations met with in TSS.

viii. The Kupffer cells in TSS liver are ingesting denatured antibodies and/or antigen/antibody complex. The hepatic sinusoidal lymphocytes are engaged in antibody synthesis. In my opinion the lymphocytes receive "processed" information for antibody synthesis from their close partners, the Kupffer cells.

ix. The TSS patients have an adequate and normal immune mechanism. In my opinion the basis of this syndrome is the persistence of exo-erythrocytic forms of malaria parasites, giving rise to repeated relapses, possibly due to antigenic variations in the erythrocytic forms.

The high levels of IgM met with in these patients sera are likely to be due to such relapses.

Long term malaria prophylaxis in TSS leads to clinical improvement and reduction in high levels of IgM as a result of a reduction in the relapse rates.