4: Separation of chloroquine from blood and its estimation using High Performance Thin Layer Chromatography

It was found necessary estimating chloroquine in blood when Plasmodium falciparum malaria failed to respond to chloroquine. To check the correct chloroquine base contact in a tablet before the drug was given to an infected person, our laboratory experience (Mwaiko, 1971) showed the base content could be determined by suspending directly the powdered tablet in O.M. HCl and subsequent centrifuging to obtain a supernatant containing the base whose concentration was then determined by ultra-violent (UV) absorbance at 231 nm.

In blood (and other body fluids like urine etc.) a more elaborate method for drug separation was needed. Precipitation of the drug was necessary, followed by extraction with an organic. High Performance Thin Layer Chromatography Technique (HPTLC) was introduced. With this method (Betschart and Steiger, 1986), chloroquine in the plasma was precipitated with 2M NaOHa. The precipitate then extracted with heptane/isooamy alcohol mixture (100:1.5 V/V) and evaporated. The residue after evaporation, was dissolved in 70% ethyl alcohol and spotted on thin layer silica gel chromatograph. The spots later were developed with toluol/diethylyamine (9:1 V/V) developing solvent system. This separation might be a suitable alternative procedure for the extraction of the drug from blood. The commonly used procedure for the extraction in alkaline-water phase by organic solvents, heptane/isooamy alcohol for example, gave the extraction efficiency of 76-78% (Betsochert and Steiger, 1986) which called for improvements.

Reference

Betschart, B. and Steiger, S. Quantitative determination of chloroquine and desethylchloroquine in biological fluids by high performance thin layer chromatography Acta Tropica 48, 125-130 (1986).


Table 1: Lethal concentrations for 50% and 95% mortalities of the citrus peel oils on C. quinquefasciatus larvae

<table>
<thead>
<tr>
<th>Citrus species</th>
<th>LC50</th>
<th>LC95</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ug x 103)</td>
<td>(ug x 103)</td>
</tr>
<tr>
<td>Bitter orange</td>
<td>50.11</td>
<td>165.95</td>
</tr>
<tr>
<td>Lemon</td>
<td>28.84</td>
<td>69.12</td>
</tr>
<tr>
<td>Orange</td>
<td>50.11</td>
<td>158.48</td>
</tr>
<tr>
<td>Mixture of Bitter orange, Lemon and Orange</td>
<td>15.84</td>
<td>56.23</td>
</tr>
</tbody>
</table>

AMANI MEDICAL RESEARCH CENTRE COMES OF AGE: ACHIEVEMENTS IN THE LAST 50 YEARS (1949 - 1999)

Lemmge, M.M., Matola, Y.G. and Kitua, A.Y.

Historical Background

Amani Medical Research Centre (AMRC) was founded in 1949 under the Colonial and Welfare Scheme initially at Ubwani in the outskirts of Muheza town, Tanga Region, Tanzania as the East African Malaria Unit (EAMU). It served British Somaliland, Kenya, Uganda, Tanganyika, and Zanzibar in the control of malaria and other vector-borne diseases. In 1951 however the EAMU was moved to the East Usambara Mountains to take over the facilities left vacant by the East African Agricultural and Forestry Research Organization (EAAFRO) which had moved to Muguga (Kenya) under the East African High Commission. As years went by, research activities were expanded to cover such other diseases as schistosomiasis and onchocerciasis. Also, there was a considerable training component for malaria field workers in basic biology and control of malaria vectors.

In 1954, the EAMU was renamed the East African Institute of Malaria and Vector Borne Diseases (EAIMVBD). Research on schistosomiasis was phased out in 1960 and later on in the 1970's bancroftian filariasis and plague were introduced as new research areas at the EAIMVBD then operating under the East African Community (EAC).

Following the collapse of the EAC in 1977, the EAIMVBD was renamed Amani Medical Research Centre to
operate under the National Institute for Medical Research (NIMR) established by an Act of Parliament No. 23 of 1979 and becoming operational in 1980. AMRC has three field stations: Muheza Field Station located at the former EAMU premises, Bombo Field Station at Bombo Hospital in Tanga Municipality and Gonja Field Station in Same, Kilimanjaro Region. Research activities at AMRC have increased to include health research systems and socioeconomic studies.

The Golden Jubilee Celebration
The AMRC on the beautiful East Usambara Mountains, North East Tanzania celebrated on reaching 50 years of its research activities the Golden Jubilee from 3-6 December 1999. The occasion took the opportunity to look back from the beginning, examine the current status in the process of preparing for the challenges of the next century. Additionally the celebration was planned to include a symposium whose theme was "Past, Present and Future of Health Research at Amani Medical Research Centre.

It was the hope that the Jubilee which happened to fall at the end of the 20th Century and the 2nd millennium would be a great source of inspiration in health research for the years to come.

The occasion was preceded by AMRC Open Day during which the general public was welcomed to witness for themselves the achievements made so far in the research undertaken by the Centre. Exhibitions were also displayed in the Laboratories and at the Conference Venue, the Amani Welfare Centre Tennis Court.

At the Symposium Poster presentations made by AMRC and other NIMR Centres/Stations and other health institutions depicting their research activities continued throughout the event including a commercial component by Zeneca Company.

The historic occasion brought together about 100 guests both from outside (United Kingdom, Kenya, Denmark and Zimbabwe) and within Tanzania (e.g. Government and Ministry of Health (MoH) officials and associated health institutions). The Guest of Honour Hon. Dr. Aaron Chiduo (MP and Minister for Health) was represented by Dr. G. Upunda (Chief Medical Officer); Capt. G.H. Mkuchika (Tanga Regional Commissioner) was represented by Mrs G. Mpaka (Regional Administrative Secretary); Mrs R. Njau represented World Health Organization Representative in Tanzania. Other representatives included: Mr. J. Ndungu, (for Zeneca Company Kenya Manager, Mr. N. Mitchellmore); Prof. B.S. Kilonzo (for Prof. Kambarage, Sokoine University of Agriculture); Institute of Traditional Medicine Director

Dr. Z.H. Mbwambo (for Prof. R.L. Mahunnah, Institute of Traditional Medicine); Dr. A. Hemed (for AMMP Project Manager); and Dr. S. Kimboka (for Managing Director, TFNC).

Also present were: The National Institute for Medical Research Council Chairman (Dr. V.M. Eyakuze); Dr. A.Y. Kitua, (NIMR Director General); Mr. L.T. Gama (Muheza District Commissioner); Dr. E.C. Magoti (MP for Musoma); Tropical Pesticide Research Institute Director; Dr. W. Marandu; Prof. M.H. Nkunya UDSM (Chief Academic Officer, USDM); Prof. J. Shao (Director and Vice Chancellor of Tumaini University of Kilimanjaro Christian Medical Centre); Mr. E. Manumbu (Director of Planning, MoH); Dr. Munyaeti (Head, HSR Unit, MoH); and health related institutions and neighbouring organizations. Other participants included invited NIMR retired officers within easy reach and community leaders.

Objectives of the Golden Jubilee
The broad objective of the Golden Jubilee celebration was to raise awareness on the worsening health of the people and its consequences on socioeconomic development on one hand, and on the other to highlight the importance of understanding disease epidemiology in devising effective sustainable control strategies in such a complex and dynamic environment.

The Jubilee’s specific objectives included the provision of a forum for scientific exchange of ideas; research results and experiences gained during the past 50 years of AMRC existence; to promote individual and institutional collaboration on health research with a view to combating ill-health in a more and cost effective approach to raise both national and international awareness on the mandate and roles of the National Institute for Medical Research; and to compile the Jubilee Proceedings and Bibliography for wider dissemination.

Introduction Remarks
The AMRC gave introductory remarks before the opening session. Subsequently, Dr. A.Y. Kitua (NIMR Director General) invited the NIMR Council Chairman to introduce the guest of Honour for the opening ceremony.

Opening Speech by the Guest of Honour Hon. Dr. Aaron Chiduo, MP and Minister for Health
In the opening speech read on his behalf by Dr. G. Upunda, Chief Medical Officer stressed the vital importance of research in generating new knowledge and tools useful in solving existing health problems and to reduce disease burden, provision of better health services and the improvement of population health and well being.
He congratulated in particular AMRC for the active establishment of collaborative links and networking with other health institutions and other sectors. The Minister mentioned that as we enter into the new millennium, he was convinced that research will take a leading role to pave way for the health of the people and confidently said that we shall be able to face the challenges ahead and that ministerial support will be available.

The Minister stated that until late, research findings are sometimes unknown where they are needed most. He said that he was most pleased to note that NIMR has advanced in this respect, and congratulated NIMR for the issuance half yearly of the Tanzania Health Research Bulletin which presents results in a simplified manner tailored for the layman. He urged that this be kept up, improve its quality, ensure a wider and timely circulation.

On behalf of Dr. W. Mwambazi (WHO representative to Tanzania), Mrs. R. Njau congratulated the government for the foresight and worthwhile investment in medical research and that it cannot be overemphasized that in sub-Saharan Africa where the burden of disease takes a heavy toll on the GNP, human capacity building in health, management, development and training is paramount.

Dr. A.Y. Kitua, NIMR Director General briefed the audience on the Role of NIMR in Promoting Health. In his briefing “Confidently Leaping into the New Millennium” Dr. Kitua outlined the intention to boost NIMR activities as a whole in order to adhere to the Parliamentary mandate and in so doing ensuring entrance into the New Millennium Confidently. The Government and the Ministry of Health were urged to ensure that funds are available for research for the improvement of health.

The AMRC Director, Dr. M.M. Lemnge gave a synopsis of AMRC Activities dwelling on the following areas:-

1. AMRC Profile, Constraints and Capacity Building

AMRC is one of the two Research Centres of NIMR. Despite its location in beautiful scenic wooded ridges with a mixed tropical forest, it is not easily accessible by road nor any mode of telecommunication. AMRC operates under four scientific departments and two supportive departments. Acquisition of a sound academic background is a pre-requisite to good research and all Research Scientists are encouraged to pursue studies leading to Masters and PhDs. In this respect AMRC has 19 Scientists (6 with Ph.Ds, 5 Ph.D. candidates are in their final stages of completion and 8 scientists have Masters Degrees. There is, however, an imbalance in the disciplines. She urged the government to consider better incentives for researchers to avoid brain-drain.

2. Major Achievements and Services to the Community and Government

AMRC was the first on the African continent to work on the seroepidemiology of malaria in North East Tanzania.

AMRC in collaboration with LSHTM were the first in East Africa to demonstrate that synthetic pyrethroids (e.g. permethrin) insecticide treated bednets (ITNs) reduce malaria disease.

Additionally, AMRC was the first in Africa to show that ICON, a synthetic pyrethroid (lamdacyhalothrin) intradomiciliary spraying reduces malaria disease due to its impact on the vectors.

Scientists from AMRC have shown that proguaunil is an effective and safe antimalarial for pregnant women.

Mapping out malaria parasite sensitivity patterns to chloroquine and other antimalarials in different areas of Tanzania was a function of AMRC. In addition, is part of a national team to monitor the efficacy of different antimalarials in health facility - based study at 8 sentinel areas in Tanzania mainland. NIMR paid scientists and technicians have continued since 1980, to pursue research in clinical malaria including risk factors for severe and complicated malaria at Teule Hospital, Muheza.

In collaboration with LSHTM and DANIDA - ENRECA programme have been involved in community - based interventions by use of freely distributed ITNs. Other collaborative projects have been: Operation of a free mobile clinic at Magoda/Mpapauy villages in Muheza from 1994. DANIDA - ENRECA programme and Teule Hospital (Muheza) with DBL have been involved in community and hospital - based studies on surveillance for filarial fevers and management. AMRC provides laboratory and clinical services to the Amani community. Staff at AMRC have participated in teaching at workshops, Vector Control Training Centre and continues research involving immunology, Health Systems Research, Social and sociobehavioural studies.

Last but not least, AMRC provides technical support to the MoH e.g. malaria epidemics etc.
AMRC mainstay of research have been on the parasitological, epidemiological, entomological and clinical aspects of the diseases mentioned. Research now is geared to immunology, molecular biology, pharmacology, health economy and other systems of the above diseases. At least 13 reports on the above have been produced.

AMRC plans to do research activities covering 9 specific objectives as outlined in the NIMR - Strategic Plan for 1999 - 2002. The intention is to concentrate on some neglected areas such as traditional medical practices, ethnobotany and phytochemistry. A number of other research priorities were reported and some of the research activities will be on a collaborative basis with academic institutions both within and outside Tanzania. To this effect some such collaboration has already been initiated.

December 5, 1999 was the official research Open Day especially for the invited officials, and other institutions. This was sponsored by WHO. The official guests were most fascinated by what they viewed in the laboratories. The two days symposium was also governed by scientific sessions during which scientific presentations were made. These were made by AMRC staff and other health-related institutions. They covered: Malaria Epidemiology and Case Management; Malaria Vector Biology and Control, Lymphatic Filariasis Epidemiology, Vector Biology and Control of Plague, Onchocerciasis Epidemiology and Control and Health System Research. A total of 14 scientific presentations were made. The sessions generated great interest and valuable discussions. More than 20 posters were displayed.

At the climax of the Jubilee, one of the highlights was a grand reception at which 9 former AMRC Directors or their representatives were awarded certificates of Recognition and presents for their distinguished services at AMRC during various periods. The late Dr. Bagster D. Wilson (1949 - 1959), Dr. G. Pringle (1960 - 1965), Dr. Jan Leijveld (1966 - 1970), the late Mr. Philip Wegesa (1970 - 1977), Dr. S.E. Temu (1971 - 1983), Dr. G.L. Mwaiko (1983 - 1985), the late Dr. S.G.M. Iraire (1985 - 1991), Mr. Y.G. Matola (1992 - 1995), and Dr. K.J. Njunwa (1995 - 1998).

The forum congratulated Dr. (Ms) M.M. Lemnge, the current AMRC Director, for being the first lady Director and for excellent organization of the 50 years anniversary.

Various speeches were made by some of the past Directors or their representatives and some former AMRC employees. Some reminisced happy memorable moments whilst others remembered dangerous and health threatening working moments, and indeed it was a very jovial moment. Those unable to attend for unavoidable circumstances sent mementos which wished AMRC another 50 brilliant years. Invariably all of them and their families had very fond memories of Amani and co-workers.

The closing session was officiated by the Muheza District Commissioner Mr. Leonidas Gama. He reiterated the importance of research and wished AMRC a successful 50 more years of hard work.

At the session, the Chief Medical Officer, Dr. G. Upunda thanked Amani for the 50 years of hard work and perseverance which has brought the Centre to its current status. He said AMRC should strive to the dissemination of research findings to the general population as well.

Acknowledgements
We would like to thank Dr. V. Eyakuze, NIMR Council chairman, for his active participation throughout the celebrations. Thanks also go to Dr. M.N. Malecela, NIMR DRT, for her efforts to secure funds for Research Open Day from WHO and the Ministry of Health. The extra efforts of the organizing committee, and all Amani Staff members, are greatly appreciated.

The AMRC greatly acknowledges with thanks all those who generously contributed resources for a successful celebration. UNICEF and London School of Hygiene & Tropical Medicine are thanked for financial support towards the production of a bibliography on 50 years of research at Amani.

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