Contents:

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>I. Helminthiases</td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>Structure, molecular biology and immunology of the cuticle of parasitic nematodes</td>
</tr>
<tr>
<td>L2</td>
<td>Evaluation of a low molecular weight fraction of an Onchocerca volvulus worm extract and of a recombinant antigen (Ov16)</td>
</tr>
<tr>
<td>L3</td>
<td>Community based morbidity measurements of schistosomiasis: the role of ultrasound examinations in control programmes</td>
</tr>
<tr>
<td>L4</td>
<td>Standardisation of the use of ultrasound in schistosomiasis</td>
</tr>
<tr>
<td>II. Malaria</td>
<td></td>
</tr>
<tr>
<td>II.1</td>
<td>Interaction of Plasmodium with midgut epithelia and with salivary glands in mosquitoes</td>
</tr>
<tr>
<td>II.2</td>
<td>Development of analytical methods for the determination of antimalarial drugs in body fluids</td>
</tr>
<tr>
<td>II.3</td>
<td>Malaria chemoprophylaxis compliance and self-treatment behaviour of tourists</td>
</tr>
<tr>
<td>II.4</td>
<td>Halofantrine in the treatment of malaria: preliminary results</td>
</tr>
<tr>
<td>II.5</td>
<td>Kilombero Malaria Project, Ilakara, Tanzania</td>
</tr>
<tr>
<td>III. Leishmaniases</td>
<td></td>
</tr>
<tr>
<td>III.1</td>
<td>Biological Aspects of Phlebotomus and Leishmania</td>
</tr>
<tr>
<td>IV. Trypanosomiases</td>
<td></td>
</tr>
<tr>
<td>IV.1</td>
<td>Genetic exchange in African trypanosomes</td>
</tr>
<tr>
<td>IV.2</td>
<td>Chromatin of trypanosomes is similar to – but different from – that of higher eukaryotes</td>
</tr>
<tr>
<td>IV.3</td>
<td>African and South American trypanosomiasis, vector/parasite interactions</td>
</tr>
<tr>
<td>IV.4</td>
<td>In-vitro drug sensitivity of African trypanosomes</td>
</tr>
</tbody>
</table>
The Swiss Tropical Institute (STI), Basel, was founded in 1943 to carry out teaching and research in tropical medicine and medical parasitology, and to provide specialised medical services for the general public in Switzerland. Over the past two years, the institute has further extended its activities in development cooperation within the health sector in tropical countries, and new projects were initiated. The Institute is financed by the Canton of Basel, the Swiss Federal Government and its own income. Some research projects are also supported by grants from the Swiss National Science Foundation and other foundations. The institute is also a partner in various development projects overseas; this work is largely financed by the Swiss Development Cooperation (SDC).

The Institute maintains a library, open to the general public, which serves as a special information and reference center for tropical medicine, medical parasitology, epidemiology and Public Health. The multidisciplinary approach – teaching, basic and applied research in the laboratory and in the field, medical services and development cooperation work – is a basic feature of the institute and a prerequisite for the integration of its various activities. Each sector can profit directly and continuously from the experience gained and the results obtained in the others, thus allowing not only the development of a high level in teaching and research but also the planning of realistic policies for future activities. The organizational and administrative structures of the institute have been substantially strengthened, on the basis of a new concept, during the last four years. Special concepts have been elaborated for each sector of the institute. In 1988 a first external review of the Institute's Research Programme was carried out by a group of international experts. The implementation of the review recommendations will help to strengthen the research programme of the Institute and to develop a research policy ensuring optimal use of the Institute's resources. The present biennial research report gives an overview of the published work in 1989/90. The research activities were carried out within the framework of the five institutional research goals which concentrate on:
- Intervention in host/parasite relationships
- Chemotherapy and drug resistance
- Strengthening of diagnostics
- Disease systems research
- Health systems research.

The report has been subdivided and grouped into several sections, which concentrate on four disease complexes as well as on disease and health systems. Individual scientific reports within each section also include a substantial number of institute-wide research projects and work in collaboration with foreign institutions. A final section on teaching and postgraduate research training also reports on important changes in the teaching programme of the institute that are currently being initiated in order to better meet the future needs of students and funding bodies.
I. Helminthiases

I.1 Structure, molecular biology and immunology of the cuticle of parasitic nematodes

The cuticle of parasitic nematodes is a highly complex structure. By using immunogold and lectin-gold techniques it was possible to distinguish the cuticle layers by the distribution of the markers. On the other hand, no labelling pattern was found which led to a clear grouping of the layers into the classical morphological zones. In general, the outer surface of the epicuticle of parasitic adult worms turned out to be highly inert, since it did not react with any antibodies or lectins tested. However, immunogenic components were found on the surface of infective forms, probably in the cortical zone.

Rotary shadowing of the reduced, extracted molecules showed fibers 45 nm in length. This length is in excellent agreement with the calculated length deduced from the collagen gene sequences of *C. elegans* and *A. suum* (Fig. 2). A new model of the structural organization of the collagens of the cuticle of parasitic nematodes was proposed, where these collagen molecules occur in at least three major groups as monomers, dimers and trimers, whereby the latter two are cross-linked via non-reducible bonds.

A monoclonal antibody (MCAb 46) against third stage larvae of *Brugia malayi*, which was shown to induce an antibody dependent cell mediated cytotoxicity, recognized an antigen which was solubilized in the fraction of cuticular collagens, possibly a collagen itself. On the other hand we could show that the 30 kDa antigen of *Brugia malayi*, which is found in the cuticle (see Figure 3), is not cross-linked with cuticular collagens and can easily be recovered in a somatic fraction obtained during the isolation of the cuticle of adult worms.

If the insoluble cuticulin from the cortical zone of *Ascaris suum* is isolated, antibodies can be raised against it, which shows a high specificity just for the external cortical layer.

Fig. 1. Comparison of different collagen extracts of cuticles of parasitic nematodes

![Fig. 1](image1)

Fig. 2. Length of reduced, rotary shadowed collagen molecules

![Fig. 2](image2)

Fig. 3. Cuticle (C) of an adult filaria, *Brugia malayi*, after indirect immunocytochemical localization of a 30 kDa antigen (black dots); H = hypodermis; bar corresponds to 0.5 μm.

This anti-cuticulin antibody was shown to cross-react with similar structures of different filariae, indicating that proteins with similar epitopes are also present in parasitic filariae.

These studies were carried out with the financial support of the Swiss National Science Foundation (Nr. 3-164469.85). With the help of a number of organizations, it was possible to organize a workshop at the Swiss Tropical Institute on the "Structure, molecular biology and immunology of the cuticle of parasitic nematodes", from 16–20 September 1989, which allowed to discuss the most recent data.

References:


I.2 Evaluation of a low molecular weight fraction of an *Onchocerca volvulus* worm extract and of a recombinant antigen (Ov16)

In view of the low sensitivity of parasitological methods in demonstrating skin microfilariae – especially in cases with light or early infections – a reliable immunodiagnostic test would be of considerable importance. However, the poor specificity of such tests has limited their use so far.

We evaluated a low molecular weight antigen fraction of a crude adult *O. volvulus* extract in a micro-ELISA test for seroepidemiological purposes and for the diagnosis of onchocerciasis in the individual. From cross-sectional anti-longitudinal studies in a hyperendemic onchocerciasis area in West Africa we concluded that this assay detects antibodies elicited when female worms start to produce.
Fig. 1. Experimental onchocerciasis (Chimpanzee)

Fig. 2. Follow-up of the seroreactivity against the LMW antigens related to the period of exposure (residence = age) in Manamitouou (surveys 1982–1987). A) Individuals who became patent during the observation period. B) Individuals with patent onchocerciasis. Open squares = ml negative, closed squares = ml positive at time of serum collection.

Fig. 3. Dependence of sensitivity on specificity and the antigen used. (OV total: Specificity was determined using sera outside areas endemic for onchocerciasis.)

Fig. 4. Sensitivity of a recombinant O. volvulus antigen.

I. 3 Community-based morbidity measurements of schistosomiasis: The role of ultrasound examinations in control programmes

The present aim of the global strategy to control schistosomiasis is the reduction of morbidity caused by the disease. The development of safer and more efficient drugs and simple diagnostic techniques renders this aim feasible within the frame of national and regional control programmes. There is a need for the careful validation and evaluation of measurements and indicators of schistosomiasis morbidity. Among them, ultrasound was found to be a safe, non-invasive and efficient technique to detect schistosomiasis-related lesions and to assess the effect of treatment on the resolution of pathology. Three case-studies are presented in this summary to illustrate the use of ultrasound in areas of different endemicity for Schistosoma haematobium. A survey on Pemba Island, Tanzania, an area of high endemicity, assessed cross-sectionally the status of S. haematobium-related morbidity in a population within a control programme prior to intervention, revealing a sex- and age-specific pattern of pathological changes. The survey showed that ultrasound can be used to quickly assess subsamples of a population as a basis for deciding on sampling strategies for control programmes. Results from a study on Mauritius Island, where S. haematobium endem- icity is low, confirmed the usefulness of ultrasound for detecting uropathy and for demonstrating the association between the intensity of infection and urinary tract abnormalities in such areas. Positive results for egg output and haematuria were found to be risk factors for uropathy, which means that these indirect measurements do indeed have predictive potential in low prevalence areas. A study among school children in mainland Tanzania, in an area of moderate prevalence, provided information on the resolution of S. haematobium-related uropathy after treatment. Such information is crucial in defining treatment and retreatment schemes in relation to maintaining the lowest possible level of morbidity in a community. More studies to investigate resolution of pathology in different age (exposure) groups are needed. Such information will be essential to identify target groups to be covered by treatment and retreatment cycles. In intestinal schistosomiasis (S. mansoni and S. japonicum) sensitive and specific indirect morbidity measures are yet to be established, since there is no single indicator, such as haematuria in urinary tract schistosomiasis, to predict infection. Ultrasound has, however, been shown to be a sensitive and specific tool to assess morbidity. These studies outline the role of ultrasound particularly as a tool to complement and validate the indirect.

References:


Fig. 1. Comparison of S. haematobium morbidity in Pujuni (Pemba, Tanzania): results from a cross-sectional survey in 1986 of 162 males and 335 females screened by sonography and urine analysis.
morbidty control measurements that are already being carried out by existing health care services in many areas. Standardized follow-up studies over a prolonged period of time are needed to define the predictive potential of different morbidity measurements in large-scale control programmes in different endemic settings, as a basis for public health decisions.

References:


Savill L., Hatz C, Dixon H., Kisumku D., Dhunputh C., Mayombana C., Macpherson C.N.L., Mayombana C., Dhunputh C., and Tanner M: A consensus was achieved regarding definitions and methodologies, with the aim of standardizing the diagnostic techniques, especially for use in surveys. The discussions were supplemented by practical demonstrations with patients. A consensus was achieved regarding the most important basic examinations to be performed, and how to interpret them. A report containing the proposed standard protocols for ultrasound examinations for each of the three types of schistosomal infection, together with explanatory notes about definitions and methodology, has been prepared in the STI and after discussion with the other participants it was published by the WHO as a "White Paper Report" early in 1991. It is expected that the incorporation of these guidelines into future studies will provide sets of data enabling comparisons of morbidity in different endemic situations to be made.

References:

II. Malaria

I.4 Standardisation of the use of ultrasound in schistosomiasis
Ultrasound has been widely used in schistosomiasis research, but comparisons between results from different places have been difficult owing to the lack of a standardized method for examination and reporting. A workshop on the Assessment of Morbidity due to Schistosomiasis using Ultrasound was therefore held in Egypt from October 1-4 1990 in Cairo, organized jointly by the WHO, the University of Cairo, and the STI. The objective was to bring together experts from different parts of the world with extensive knowledge of assessing morbidity due to schistosomiasis to discuss their experience, with the aim of standardizing the diagnostic techniques, especially for use in surveys. The discussions were supplemented by practical demonstrations with patients.

A consensus was achieved regarding the most important basic examinations to be performed, and how to interpret them. A report containing the proposed standard protocols for ultrasound examinations for each of the three types of schistosomal infection, together with explanatory notes about definitions and methodology, has been prepared in the STI, and after discussion with the other participants it was published by the WHO as a "White Paper Report" early in 1991. It is expected that the incorporation of these guidelines into future studies will provide sets of data enabling comparisons of morbidity in different endemic situations to be made.

References:

II.1 Interaction of plasmodia with midgut epithelia and with salivary glands in mosquitoes
A series of biochemical and ultrastructural studies were performed with the aim to find new information about the interaction of malaria parasites and their vectors and to identify parameters which are responsible for the differing susceptibilities amongst mosquito species and strains.

The activities of digestive enzymes, the peritrophic membrane formed around the ingested blood, and characteristics of the surface of the midgut epithelium, are factors the parasites have to deal with on their way from the gut lumen to the haemoelymph. Trypsin, aminopeptidases, and a glucosidase activities and distribution were studied in Anopheles stephensi. Except trypsin, which is only secreted into the posterior midgut lumen, these enzymes occur in the whole midgut. Aminopeptidases (major activity peak at 347 kDa) which play an important role in secondary digestion of bloodmeal proteins, are also active within the epithelium of the posterior midgut. A comparison of a susceptible and a refractory strain of An. stephensi showed differences in the relative activity of this enzyme and the amount of protein ingested. A possible inhibitory effect on malaria transmission could be attributed to the peritrophic membrane (PM). An increase of the thickness of this envelope, formed around the blood clot in the posterior midgut after each bloodmeal, was experimentally induced in Aedes aegypti. The result was a 75% reduction of the oocyst number as compared to a control group, when the mosquitoes were cyclically infected with Plasmodium gallinaceum. An important factor with regard to the malaria cycle in mosquitoes is most probably the surface of the midgut epithelium. It has to be recognized by the parasites prior to their passage through the gut wall. Lecin binding studies (see Figure) revealed a higher reactivity of the gut surface and the PM with lectins specific for GlcNAc for A. aegypti and with lectins specific for GalNAc for A. stephensi. In the latter species the P.gallinaceum cycle is interrupted in the gut although the parasites develop until the ookinete stage. The sporozoites of plasmodia have the pecularity to interact with the membranes of the salivary glands of mosquitoes and mammalian hepatocytes. The involvement of the circumsporozoite protein (CSP) in the process of salivary gland penetration

Fig. 1. Midgut epithelium of female Aedes aegypti 24 h after infection. Indirect localization of Con A-binding sites with horseradish peroxidase-gold (black dots); ic = intercellular cleft, mi = mitochondrion, rer = rough endoplasmic reticulum; bar corresponds to 0.5 µm.
was investigated by establishing an in vitro adhesion assay with salivary gland proteins immobilized on nitrocellulose. Ligated sporozoites of P. berghei bound to the salivary gland proteins of Anopheles stephensi. It was not possible to inhibit the binding by using anti CSP antibodies. These results are consistent with evidence that at least the repetitive epitopes of the CSP are not involved in this adhesion process.

References:

II. Development of analytical methods for the determination of anti-malarial drugs in body fluids

The need for simple, fast and reliable methods to determine the consumption of anti-malarial drugs for pre- and post-therapeutic monitoring as well as for epidemiological studies is widely recognized. The classical methods (e.g. Dill-Glazko test) lack a sufficient sensitivity and specificity. The introduction of new thin layer materials in the form of well defined and controlled silica particles led to a revival of interest in thin layer chromatography. We have developed a testkit based on this technique, which allows the determination of several antimarial drugs in the urine under field conditions. Small urine samples are directly applied onto HPTLC-Silica gel plates and the plates are developed in a solution of tolune- diethylamine-methanol (8:1:1) in an appropriate development chamber. Chloroquine, quinine, mefloquine, primaquine and their metabolites can be detected as fluorescent spots under long wave ultraviolet light provided by a color powered pocket lamp. The method has already been tested in several places and was shown to be reliable and relatively economical. Further studies are now being carried out to develop methods for antimarialas like mefloquine and halofantrine.

References:
Betschart B.; Thin-layer chromatography as a method for antimalarial drug determinations in body fluids under field and laboratory conditions, International Monograph Series (1990) in press.

II. 3 Malaria chemoprophylaxis compliance and self-treatment behaviour of tourists

Compliance with malaria chemoprophylaxis and self-medication was studied in 477 individuals travelling to endemic areas for malaria. 78% (225/285) followed chemoprophylaxis as advised. This compliance level was the same regardless of the drugs used (Mefloquine, Sulfaadoxine-Pyrimethamine, Chloroquine). Two tourists did not start chemoprophylaxis one week before departure. 14 travellers stopped the chemoprophylaxis too soon after returning home. 31 tourists took too high a dosage of the drug. None of the tourists travelling to areas where standby medication for self-treatment is recommended acquired malaria. Two tourists travelling in West Africa contracted malaria.

References:

II. 4 Halofantrin in the treatment of malaria: preliminary results

Halofantrin is a recently developed blood schizontocidal antimalarial drug which seems to be promising for the treatment of multiresistant strains of Plasmodium falciparum. We are presently investigating the efficacy and side effects of Halofantrin in non-immune patients with uncomplicated malaria. The study is designed as an open trial. The dosage of Halofantrin is 500 mg 6-hourly. Treatment is completed after 3 doses. Starting in January 1990, 15 patients took part in the study. Their mean age was 30 years (21-47). In 10 patients P. falciparum was diagnosed, in 3 P. vivax. It was not possible to identify the species in the remaining 2 patients. When first seen, 8 patients had a parasitaemia of <1%, 7 between 1 and 10% and 2 between 10 and 25%. In all patients parasites were cleared from the blood within 5 days after treatment. The mean parasite clearance time was 69 hours (12-120), the mean fever clearance time was 46 hours (24-72). In 1 of 12 patients, who were observed over 28 days, a recrudescence was found on day 21 after treatment. 5 of 15 patients had gastrointestinal symptoms on day 1 after treatment (abdominal pain 1, diarrhoea 4, nausea + diarrhoea 1). All these side effects resolved spontaneously on the second day after treatment.

References:
Brændli B., Loveson L., Markwalder K.; Therapeutic experiences with Halofantrin in the Swiss military during the year of 1989. Swiss Malaria Foundation, Schaffhausen, Switzerland. (1990)

II. 5 Kilombero Health Research Programme

The Kilombero Health Research Programme (KHERP) is a district based research programme that was initiated in 1981. It is undertaken by the STI at the Swiss Tropical Institute Field Laboratory (STIFL) at Ifakara, southeastern Tanzania, and is financially supported by the Swiss Development Cooperation. The programme not only addresses health research issues of regional and national importance by operational and health systems research, but equally emphasizes training of health personnel and direct health service support activities. As will be presented in this report, malaria, schistosomiasis, health systems research and health planning issues form the major part of the research activities. Interested readers can obtain copies of the annual report of the KHERP programme by writing to the Department of Public Health and Epidemiology at the STI.

Kilombero Malaria Project, Ifakara, Tanzania

The Kilombero Malaria Project, which is part of the Kilombero Health Research Programme (KHERP), was started in May, 1989. Its major aims are to determine the risk factors of malaria in the Kilombero District in southern Tanzania, and to gain a better understanding of the complex relationships between parasitology, clinical features, and immunity. Data have now been collected over 18 months in two villages of the district, Namawala and Michenga. Clinical data have been collected every two weeks, parasitologically and immunologically every two months. Entomological data have been collected from selected houses every ten days, starting in January, 1990. An evaluation of the data of the first year of collection shows that although there is a distinct rainy season from March to May and a distinct dry season from August to December, there is a remarkable constancy in the malaria situation throughout the year. In both the rainy and the dry seasons prevalences of parasites in infants are more than 80%, and in adults prevalences are constant between 40% and 50% (Fig. 1). Similarly, the seropositivity rate for antibodies against the repetitive epitope of the CSP protein using synthetic (NANP)- as antigen, which is an indicator of recent infection by P falciparum parasites, is constant throughout the year. The evaluation further shows, after correction for age, an aggregated distribution of parasites and clinical attacks over persons. Thus, some persons tend to be infected more often, other persons tend to be infected less often than is expected by random infection processes (Fig. 2), i.e. some persons are more susceptible to infection than others. We do not yet know what factor contributes to this difference in susceptibility. However, it does not seem to be due to differential bitting-rates of mosquitoes: the distribution of mosquitoes over houses in the villages follows a random distribution. Each of the houses is as likely as any others to be chosen as a resting-place for mosquitoes.

A further aim of the Kilombero Malaria Project is to follow the evolution of chloroquine resistance in the district as part of the national long-term monitoring of drug resistance. This aim has been followed since 1983, when chloroquine resistance was first observed in the district. Since 1983 children under five years of age and since 1988 schoolchildren were investigated, and the chloroquine resistance of their parasites was measured in vitro. Overall, no significant increase in resistance was observed during this period. However, resistance of the parasites obtained from the under-five year olds increased, whereas resistance of parasites from schoolchildren decreased. This pattern is interpreted by us as a result of antigenic differences between resistant and sensitive parasites: If immunity develops principally against the most frequent parasite strains, then it develops the numbers of the most frequent strains will be reduced, while the rare strains may become predomi-
nant and thus be detected in the blood of immune patients. Thus, the observed resistance pattern in non-immune infants will differ from that in immune schoolchildren. In the forthcoming year, intervention studies are being initiated to compare the effectiveness of insecticide-impregnated bed nets and impregnated bags placed along the open spaces between the walls and roofs of the houses. The routine survey of clinical, parasitological, immunological and entomological parameters will continue.

References:

III. Leishmaniases

III. 1 Biological Aspects of Phlebotomus and Leishmania

Sticky paper traps were used to demonstrate the presence of three sandfly species – Phlebotomus perniciosus, Phlebotomus mascittii and Sergentomyia minuta – in canton Ticino (Southern Switzerland). Individual concentrations of populations of P. perniciosus and S. minuta were noted in western Mendrisioto and minor Malcantone. Studies on the vertical distributions of sandflies showed S. minuta at 200–600 m, P. perniciosus at 300–600 m and P. mascittii at 400–700 m above sea level. When modified CDC light traps were used in certain biotopes, improved catches of P. perniciosus and P. mascittii were recorded. P. perniciosus isolated from Southern Switzerland was shown to be a potential vector of L. infantum. 14 days after an initial bloodmeal on an infected hamster, parasites were successfully transmitted to new hosts by the bites of several infected sandflies. The promastigote surface protease (PSP, gp63) was demonstrated to be present in promastigote forms of L. infantum in the midgut of P. perniciosus. Different clones and subpopulations of clones were analyzed for their infective potential in vitro. Long-term cultivation assays in vitro were used to test the influence of different cultivation conditions on the infectivity of promastigotes. An originally infective clone (LEM 76B-A/ST) lost its infectivity during these assays, but was able to regain it after the cultivation conditions had been changed. In addition, an originally non-low-infective clone (LEM 287-D/ST) could be forced to produce highly infective promastigotes. Infective cells were only found among stationary phase promastigotes, i.e. after the cultures had reached a maximum cell number per ml and the cell volume had clearly decreased.

References:
IV. Trypanosomiasis

IV. 1 Genetic exchange in African trypanosomes

The existence of genetic exchange by sexual or other processes in African trypanosomes in the field would have profound implications for the epidemiology of trypanosomiasis, especially in terms of the development of antigenic diversity and the spread of drug resistance. We have recently demonstrated that genetic exchange occurs in laboratory crosses of genetically and phenotypically well-characterized clones. A number of cloned progenies were recovered which exhibited hybrid characters for different genotypic and phenotypic parameters including 2-DE analysis of the protein phenotypes of parental and hybrid progeny clones. Extensive work on the frequency of hybrid formation demonstrated that genetic exchange in these trypanosomes is an non-obligatory event which occurs in parallel with development by cell fission (Fig. 1).

The sexual process of trypanosomes in the tsetse fly involves meiosis and syngamy, but the sexual stages involved are not yet known. The analysis of parasites from different stages during the life cycle in the fly revealed that midgut-derived isolates contained hybrid trypanosomes. It is the objective of current experiments to investigate -in collaboration with other groups - the mechanism of nuclear gene exchange and the parasite stages involved. This work is currently supported by the Swiss National Science Foundation, grant no. 31-25367/88.

References:

Fig. 1. Time course of hybrid formation in Trypanosoma brucei Phenotypes observed in 23 tsetse flies

IV. 2 Chromatin of trypanosomes is similar to - but different from - that of higher eukaryotes

Digestion of nuclear chromatin of Trypanosoma brucei procyclic culture forms with micrococcal nuclease yielded DNA fragments which formed DNA ladders in agarose gels, similar to those of rat liver. However, the chromatin of trypanosomes was digested more rapidly. The digestion of T. b. brucei chromatin yielded a large amount of DNA fragments of core-particle size. The number of base pairs per nucleosomal and linker DNA were identical in both species, if the digestion conditions were milder in the case of T. b. brucei.

Four major groups of proteins are involved in the organization of the chromatin. The banding patterns of these DNA-bound proteins were clearly distinguishable from those of core-histones of rat liver and calf thymus in different gel systems. Amino acid analysis revealed a composition characteristic for histone proteins.

Psoralen cross-linking of soluble chromatin at 5 mM salt concentration at pH 7 or pH 10 resulted in an irregular array of single-stranded (ss) bubbles separated by variable stretches of double-stranded (ds) DNA. The proportion of ss DNA was low compared with the ratio of ss/ds stretches in rat liver chromatin, which also showed regularly arranged nucleosomal DNA. Soluble chromatin of T. b. brucei, pre-treated with 500 mM NaCl to remove any H1 present, and psoralen cross-link at 5 mM salt, was destabilized by changes in the experimental conditions.

Chromatin of blood stream forms showed salt dependent condensation as compared to procyclic culture forms, but formed no 30 nm fiber like rat liver chromatin. The compaction seemed to be independent of histone H1. Chromatin of blood stream forms proved to be better protected from digestion with micrococcal nuclease than that of procyclic forms. Histone H1 could not be shown for blood stream forms either. It is obvious that structural and functional differences of the chromatin exist not only between T. b. brucei and higher eukaryotes but also between various stages of the life-cycle of the parasite.

References:

IV. 3 African and South American Trypanosomiasis Vector/parasite interactions

A study of the development of Trypanosoma rangeli in Rhodnius prolixus revealed that only 2–5% of the reduviid bugs become haemolymph positive in the system used. The parasites penetrate the gut epithelium in a parasitophorous vacuole and are released into the haemolymph surrounded by a zone of cytoplasm devoid of organelles (see Figure 1). The salivary glands were invaded by the trypanosomes in all bugs with a haemolymph infection, and only in these bugs did metacyclic forms of T. rangeli develop. Haemolymph infections in the bug Trypanosoma intercellulae, on the other hand, were found to be eliminated within a few days.

The localization of sugar residues by the lectin-gold technique yielded changes on the parasite surface...
Fig. 1. Trypanosoma rangeli (T) penetrating the basal lamina (BL) of the midgut epithelium (E) contained also extruded part of cytoplasm (asterisk). The flagellum (F) is directed "backwards"; bar corresponds to 1 μm.

IV. In vitro Drug Sensitivity of African Trypanosomes

Two in vitro test methods were developed to determine the drug sensitivity of bloodstream forms of Trypanosoma brucei isolates. The incorporation test uses [3H]thymidine uptake to evaluate the effect of existing drugs or new compounds on trypanosomes. The test is carried out in a microtiter plate using a serial dilution of the drugs. After the addition of a defined number of bloodstream forms the plate is incubated for 16 to 24 hours, before radiolabel is added and the plate is incubated for another 8 to 16 hours after which the trypanosomes are harvested in cell harvester and incorporation determined. Another test, the photometric assay, is carried out in a similar way but without the addition of label. Uninhibited trypanosomes produce significant amounts of pyruvate which lowers the pH of the medium and changes the colour of the medium. After 72 hours the plate can be read in an ELISA reader. By comparing the decreases of extinction in test cultures with those in control cultures, \(IC_{50}\) values (drug concentration causing a 50% inhibition) can be determined. Minimal inhibitory concentrations can be read by eye. With the incorporation test the sensitivity of Trypanosoma b. gambiensis and T. b. rhodesiense isolates was determined. Nine West African T. b. gambiensis isolates showed a very similar sensitivity to Suramin, pentamidine and melarsoprol, even two 'resistant' isolates which caused relapses after melarsoprol treatment (Fig. 1). A similar observation was made with T. b. rhodesiense isolates from East Africa including so-called drug resistant isolates. However, there is a trend towards the T. b. rhodesiense isolates classified as being resistant to show higher \(IC_{50}\) values than the other isolates (Fig. 1). These results seem to indicate that there are at present no drug resistant T. b. gambiensis strains in the field, whereas for the agents of the rhodesiense type of sleeping sickness development of resistance is being noticed. Relapses after melarsoprol treatment may be explained by the reduced capacity of the drugs to pass the blood-brain barrier and by reinfestation of the bloodstream from the CNS. In another study, the sensitivity of T. congolense isolates, resistant and sensitive ones, was determined to diminazene and isometamidium. For diminazene the most sensitive population was 40 times as sensitive as the least sensitive population. For isometamidium the sensitivities were in a similar range with the exception of two isolates from wild animals which were 10-104 times more sensitive than the isolates from cattle. There was a good correlation between our results and the observed in vivo sensitivities. Using the photometric assay, a series of organometallic drugs were tested for trypanocidal activity against the three subspecies of Trypanosoma (T) brucei Complexes of pentamidine with Pt, Ir and Rh were found to be the most active ones showing activities comparable to that of pentamidine. In addition, the toxicity of the Pt- and Ir-pentamidine complexes was ten to twenty fold lower than that of the uncomplicated drug, so the complexes might be candidates for use as safer trypanocidal drugs.

References:


Modeps, W., Rudin, W., Hecker, H.: Surface coat synthesis and turnover from epimastigotes to bloodstream forms of Trypanosoma brucei. Parasitology (submitted).

Rudin, W., Schwarzenbach, M., Hecker, H.: Binding of lectins to culture and vector forms of Trypanosoma rangeli. Tiers 19-20 (Protoc.


References:


V. Disease systems research

V.1 Investigation of the prevalence of Giardia lamblia in domestic dogs of Basel

A cross-sectional study was carried out in order to determine the prevalence rate of Giardia sp. amongst domestic dogs in Basel in March/April and August 1990. This study was undertaken in the context of a project to investigate the role of Giardia sp. as zoonoses in the area of Basel. Faecal samples were examined for helminths and protozoa. They were also tested for the presence of human Giardia lamblia antigens using rabbit antibodies to human Giardia lamblia in an ELISA test. These data were required for estimating the possibility of a zoonotic transmission of species of the Giardia intestinalis group to human beings, especially children, in a Swiss town. Species of the Giardia intestinalis group are also found in reptiles, birds and mammals. They cannot all be distinguished morphologically. Two areas of the city were chosen for sampling the dog faeces. Some of the samples were collected from the ground, and others from the bins for dog faeces which have been installed by the local government in an effort to keep public places clean. The study showed a considerable prevalence rate of Giardia sp. cysts of at least 5.5% in the dog faeces, but the density of cysts of Giardia sp. in each positive sample was very low. (Apart from Giardia sp., Trichuris vulpis (13%) and other organisms were also found). The ELISA test could not detect any antigens in the microscopically positive samples, using the cut-off point generally used at the STI for this test on samples from patients. The reason for this is not yet understood.

The possibility that Giardia sp. found in dogs are also infective for humans cannot be excluded at present; these results indicate a risk of zoonotic infection for children in close contact with domestic dogs, in spite of the low density of the parasite in the faeces. To obtain more information about the prevalence rate of Giardia lamblia in children and about possible zoonotic transmission by dogs, a cross-sectional and a case/control study among children in kindergarten are now being planned.

V.2 Paediatric Cryptosporidiosis in the region of Basel: A case/control study

In 1988 a prospective case/control study was performed in urban and periurban areas of Basel to investigate the epidemiology and clinical manifestations of Cryptosporidium sp., an intestinal coccidian parasite. 455 children under 17 years with diarrhoea, who attended paediatric and general practices, participated in the study. Cysts of the parasite were detected in stool samples of 21 (4.6%) patients using auramine-fluorescence and modified Ziehl-Neelsen staining. Comprehensive viral, bacterial and parasitological studies were performed. Another 10 infected people were found during separate investigations of outbreaks in a kindergarten and in two families. Serial stool samples were performed to determine the period of oocyst shedding. The mothers of each case and of two healthy controls were interviewed, using a standardized questionnaire with 122 questions concerning risk factors and signs of the illness. In comparison with healthy controls, prior contact with a person suffering from diarrhoea showed a relative risk estimation of OR = 65 (s. graph). Travelling in warm countries and contact with sick animals are also high risk factors with a 5 times higher risk (OR = 5.2 and 4.9, respectively). The consumption of contaminated food and "child specific" behaviour, e.g. playing on the ground, were of minor importance.

V.3 Identification of high risk communities

(KIHERE Programme, Ifakara, Tanzania)

Priority setting in health planning: Identification of communities at high risk for communicable diseases and chronic schistosomiasis as an example

The setting of priorities in the health sector not only requires prevalence and incidence data, but needs to rank the public health impact of the various diseases and conditions for a community, a district or the national level. This implies further that the perception of priorities by the communities, the demand, needs to be incorporated in such assessments besides the biomedical measurements, the normative needs. Experience with community diagnosis, with malaria, schistosomiasis and other communicable diseases, in particular diseases with their main signs and symptoms are often well recognized and perceived by the community members, and that this knowledge offers an important source of information for the setting of local priorities and for disease and disease control monitoring. Furthermore, matching control strategies with community disease perception will ensure the sustainability of control programmes within the existing health services based on primary health care principles. An initial approach in the Kilombero District, (southeastern Tanzania) where the diagnosis is not known, demonstrated the health status of a community and compared it to the priorities as perceived/felt by the population. This information was then used for the planning and implementation of schistosomiasis control at community level. But such a comprehensive approach, as well as large-scale, district-wide parasitological screening, would be too expensive and time-consuming for national level investigations on the communities at highest risk for schistosomiasis. A further project therefore assessed the potential of the available health statistics and of simple community-based questionnaires for the rapid identification of district level priorities in health problems, with urinary schistosomiasis as the first target disease. Self-administered questionnaires, distributed by existing administrative channels to village party chairman, headteachers and schoolchildren, showed good diagnostic performance for the quality assessment of urinary schistosomiasis endemicity. At a cost 26 times below that of the WHO-recommended parasitological screening strategy, the schoolchildren’s questionnaire allowed the screening of 75 out of 77 schools of a rural Tanzanian district in 6 weeks, and the exclusion of schools of low prevalence for urinary schistosomiasis with over 90% confidence. The questionnaires for headteachers and party chairman made it possible to assess the felt importance of a specific disease, e.g. urinary schistosomiasis, among whom was schistosomiasis. The priority rank of schistosomiasis for control was strongly correlated with the prevalence rate in the community of egg output due to the disease, i.e. above a prevalence rate of 30%, the headteachers ranked schistosomiasis among the top four health problems to be solved (see figure). As the questionnaires were not focused on schistosomiasis and health alone, the results also allow the priority of health among all the other community development problems to be appraised. This questionnaire thus contributes important information for planning at district level. This questionnaire approach was validated in another Tanzanian district resulting in a comparably high diagnostic efficiency. It was further extended to a two-step approach. In a first step high risk communities are identified by school-based questionnaires administered through the education system. In a second step, the headteachers use reagent sticks to detect haematuria leading to the identification of schistosomiasis-related morbidity and subsequent treatment at the individual level. This two-step approach relied entirely on the existing education system and allowed the screening of a rural district of 15,000 km2 with 350,000 inhabitants and 164 schools within 4 months at a cost of only US$ 3000. These findings gave the basis for a WHO-TDR supported comparative evaluation of the questionnaire approach in seven different endemic areas of Africa (Cameroon, Congo, Ethiopia, Malawi, Tanzania, Zambia, Zimbabwe) involving local biomedical and social science research teams.
VI. Health systems research (KIHARE Programme, Ifakara, Tanzania)

VI.1 Value for money? The efficiency of primary health care facilities in Morogoro region, Tanzania

A series of projects undertaken in the Morogoro region (south-eastern Tanzania) investigated the functioning of an existing district health care delivery system, as a basis for drawing conclusions about how to improve its efficiency in providing care for the population. These projects are being collaboratively undertaken by the Tanzania Ministry of Health, the London School of Hygiene & Tropical Medicine and the Swiss Tropical Institute. Financial support is provided by ODA and SDC. These investigations include an assessment of the costs of primary level health facilities (dispensaries, health centers), the quality of diagnosis and patient care, as well as a comparison between costs and quality of care. A sample of 41 government and private dispensaries, 15 voluntary agency (mission) dispensaries and four health centers, representing one third of both types of unit within the region, are under study. First a structural assessment and a calculation of the average costs per unit are undertaken and presented to the district and regional authorities as an initial picture of the existing health care delivery system. These data were compared with the allocations made in the health sector. It appears that allocations are a poor guide to likely expenditure, but that expenditure is not less than allocations in each district. The reasons for under- spending seem likely to be that resource constraints at district level lead district administrators responsible for all sectors to cut resources, in different ways from what was originally planned, owing to new priorities or price increases. Usually funds for transport and staff allowances are cut, which contributes to the undermining of health services and the morale of the health workers. Salaries are generally paid, and drugs are received (due to donor assistance to the Essential Drug Programme) despite the fact that drug funds are often underspent and are probably diverted to other district needs. Overspending in one district reflects more efforts to support the health sector, but it is not clear to what extent this is at the expense of other districts. Allocations and expenditures appear to be unfairly shared between districts, and expenditures bear little relation either to initial budgets or approved allocations. Moreover, expenditure is not easily linked to performance. Indicators such as drugs used or services provided which would enable the efficiency of resource use to be monitored.

Household based interviews and discussions with community leaders, patients and health workers aimed at assessing the quality of care. Consultation practice is stronger on history taking than on physical examination. There is little explanation of the illness, treatment and the use of the prescribed drugs. Although the health workers have the adequate skills, diagnostic tests are not routinely done owing to substantial shortages of reagents and supervision.

Prescribing and dispensing are deficient in poor health facilities. Doctors suffer from the illicit drug selling that results from the difficult general economic conditions and the lack of a motivation and incentive scheme for health workers. These problems are perceived and openly discussed by the health workers, the community leaders and the villagers. There is a general willingness of the community to pay for health services if the quality of care can be improved. However, a definition of "quality" is not easily reached as the perception patterns of felt needs and demand are quite heterogeneous.

Within the frame of activities at regional level, several small subsidies on determinants of health planning and service and of the management of the health workers. Salaries are generally paid, and drugs are received.
situation, the Kilombero District, south-eastern Tanzania. Earlier studies on the epidemiology of the endemic diseases prevailing in the district provided the relevant data for this adaptation. The model tests the existing health system and/or specific planning options with regard to the allocation of health staff, facilities and management funds with the following outcome measures:

(i) number of patients that are not treated and (ii) the number of death due to non-treatment. The model deals with a series of different data sets that take into consideration (i) topography, (ii) demography, (iii) disease pattern and its incidence as well as case fatality rates, (iv) the available resources, and (v) the available facilities and their costs, and their curative and preventive capacities. Models of the probability the people which seek care with a given condition/disease at the various levels of care finally link to the generated incidence data with the health care system, leading to the outcome variables of the number of patients not treated and the number of deaths due to non-treatment.

The HRAM allows to discuss and plan health services, to readjust the plans based on the modeled outcome and to test the sensitivity of the various options for readjustment with regard to reduce morbidity and mortality within a situation of limited resources.

Tanner M. From the bench to the field: control of parasitic infections within primary health care, Parasitology 99, 5, 81–92 (1988)

In 1988 the Institute started to reorganize its teaching and training activities and initiated the development of an integrated postgraduate teaching and training programme in Medical Parasitology, Tropical Medicine, Health and Development. In conjunction with modern teaching methods including student-oriented instructional techniques, structured courses with compulsory and optional modules have been introduced.

The teaching programme now has two main parts: a course on Medical Parasitology, within the curriculum of Biology I of the University of Basel, and a special Teaching Programme for Tropical Medicine, Health and Development which is open to medical and biological students from Switzerland and elsewhere, including the Third World. Specific information is available from the Institute's secretariat of Teaching and Training.

In addition to teaching within the Institute, individual staff members of the Institute have taught in a number of Universities and the Federal Polytechnics in Switzerland as well as in courses run by international organizations and institutions abroad. The Institute provides supervision and training in research methods for MSc and PhD degrees in Medical Parasitology and in Tropical Medicine, Health and Development for students of biological sciences and medicine (Fig. 1).

This programme is closely linked with the research programmes of the Institute as outlined in the scientific reports above. Research projects involve both laboratory work and applied field research activities in Switzerland and overseas. In 1989 and 1990, 15 students obtained a MSc and 9 students received a PhD or MD. The development of postgraduate Research Training 1982–1990 is shown in Figs. 2 and 3.

Fig. 1. Number of MSc, PhD & MD students at STI 1980–1990 (Incl. external students)

Fig. 2. Number of MSc degrees obtained at STI 1980–1990.

References
Kilombero Health Research Programme-Health Systems Research

References
Within the Institute's special Programme on Tropical Medicine, Health and Development, several courses have been given each year:

- Refresher courses in Tropical Medicine for medical practitioners and laboratory technicians
- General Tropical Course for interested laypeople
- Diploma in Tropical Medicine, Health and Development for medical doctors and nurses. This intensive three months' course started in April each year. In 1989/90, 32 medical doctors and 17 nurses passed the diploma examination.

Doctoral theses

Barth, Phillip: Isolation eines von HDL (High Density Lipoprotein) verschlie- denen trypanolytischen Faktors aus normalem Humanserum. Untersu- chungen über den lytischen Effekt von normalem Humanserum an Trypanosoma brucei

Beck-Matti, Max Christian: Prospek- tive Studie über Zusammenhänge zwischen Eisenstoffwechsel, Anämie und Malaria bei Malnutrition und deren Rehabilitation

Boulanger, Nathalie: Studies on the involvement of circumsporozoite
proteins in the interaction of plasmo- dial sporozoites with the mosquito salivary glands

Lengeler, Christian: Individual and community diagnosis of urinary schis- tosomiasis and their relevance for disease control: a study in an endemic area of Southeastern Tanzania

Mnzava, Abraham Peter: Epidemiology and control of malaria transmission by residual house spraying with DDT and Lambda cyhalothrin in two populations of the Anopheles gambiae complex in Tanga region, Tanzania

Modespacher, Urs-Peter: Immunocyto- chemische Untersuchungen an meta- zyklischen Trypanosoma brucei brucei (Protozoa, Kinetoplastida)

Petzold, Franziska: Zur Populations- dynamik von Galba truncata (Müll.) und deren Infektion mit Fasciola hepatica (L.) in einem endemischen Voralpengebiet der Schweiz

Russbach, Rémi: Blood transfusion in relation to malaria endemic and aids epidemic