practitioners are able to interprete their results or that they refer to a specialist.
By some examples the problems and tendancies of the parasitological diagnosis of these diseases are briefly analyzed.
As for the malaria, particular emphasis is laid on the importance of the blood examination until immunological techniques for the detection of antigenes can assure the continuation of the procedure. Unless there is no room for doubt, an examination must be repeated at the proper time. The same situation applies for the stool examinations for the detection of intestinal amebiasis and lambliasis.
Taking into account the increasing resistance of *P. falciparum* to Chloroquin, the STI has introduced routine in vitro tests. Moreover, these routine in vitro tests play a more and more important role in the diagnosis of protozoa, e.g. leishmaniasis and trypanosomiasis.
Techniques for special examinations like rectal biopsy, skin-snips, urine and blood filtration are briefly illustrated.
Blood eosinophilia is often the only objective sign for a helminth infection. It could indicate either an invasion phase (mainly ascariasis, strongyloidiasis, fascioliasis) with or without Loeffler syndrome or helminths in an impasse (toxocariasis) or even helminths that are difficult to trace without appropriate laboratory techniques (strongyloidiasis, filariasis). A comprehensive anamnesis and a serological screening, which is now possible through the ELISA technique, are the basis for the diagnosis of these eosinophilia.

Serodiagnosis of parasitic diseases

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The reliability of a serological test depends primarily on the quality of the antigen used. In contrast to most serological tests for bacterial and viral diseases, defined antigens are not readily available for the serodiagnosis of parasitic diseases. In most cases crude antigen extracts or whole organisms are used. Thus, parasite serology very often lacks specificity. The problem of specificity is especially pronounced in tests for helminth diseases, where cross reactions not only occure among related organisms but also among phylogenetically divergent species. To overcome partly this specificity problem, we developed a multi-antigen ELISA screening system, which will be described here for the example of serodiagnosis of infections with tissue dwelling helminths.
The test system is characterized by the following features which distinguish it from more conventional ELISA systems:
a) relatively short (15 min) incubation times at 37 °C for sera and conjugate (conjugate: goat anti-human IgG (H + L), IgG fraction conjugated with horseradish peroxidase, Miles No. 61-230-1).
b) relatively low serum dilution (1:160 in PBS saline, containing 0.05% Tween-20).
c) relatively high concentration of the substrate and chromogen (H_2O_2 0.03%, orthophenylene-diamine 0.1%).
d) rapide and perfectly linear enzyme-reaction (stopping time ± 3 min at 20 °C, reactions stopped with 8N H_2SO_4).
e) in each horizonal row of wells, a different antigen preparation is adsorbed on the plastic surface.
f) the patients sera are then tested vertically against the different antigens.
Thus, avoiding inter-test variations and keeping low intra-test variations, small differencies between extinction values of different reactions can be recognized for one serum in a reproducible way. Since short incubation times and low serum dilutions are used, the test may preferentially detect antibodies of higher avidity, thus increasing the specificity of the test. The short incubation time with the substrate chromogen solution leads to a test system similar to the k-ELISA, enabling better quantitative measurements.
In this ELISA system, we found that, despite the lack of specificity of some antigen preparations, homologous antibody-antigen reactions give in most cases the highest extinction values, enabling the multi-antigen approach to indicate the true infection of the patient. In addition, we observed typical reaction patterns in a given helminth infection. These reaction patterns are visualized in the figure.

1. Toxocariasis patients have antibodies directed mainly against specific metabolic antigens of *Toxocara canis* larvae II. In this regard, it is interesting to note that a high sensitivity and specificity was recorded with the toxocariasis in vitro precipitation test. Our results indicate, that the humoral response of toxocariasis patients is directed predominantly against very specific antigens released in vivo and in vitro by the *T. canis* larvae II. However, some borderline reactions (cf. fig.) between toxocaral patients sera and antigens from *Dipetalonema viteae* or *Echinococcus granulosus* can be observed when the homologous toxocariasis system reveals very high extinction values. In line with the hypothesis of a specific immune response in toxocariasis patients, crossreactions with metabolic antigens from *T. canis* larvae II are rarely observed and if, to our knowledge, predominantly with sera from filariasis patients, showing very high extinction values with *D. viteae* antigens. These crossreactions, however, never exceed extinction values over 1.0 (cf. fig.).

2. The most often observed cross reactions occur with sera from filariasis and echinococcosis patients and antigens from *E. granulosus* resp. *D. viteae*. However, the homologous antibody-antigen reactions are stronger than can be observed with the cross reacting system. Thus, in most cases a good indication for an echinococcosis infection can be obtained by just comparing the extinction values of both reactions. This is less true when estinction values with *D. viteae* antigens are considered, since virtually all sera from patients with extraintestinal helminthic infections, exhibiting high serum antibody concentrations, can crossreact with *D. viteae* antigens. This problem could not be overcome to date by using antigens from other filariae. Thus, antigens from *D. viteae* serve as a good general marker for extraintestinal helminthiasis (except toxocariasis). This is also true with antigens from *E. granulosus* at lower extinction values, since crossreactions always occur with both antigens.

3. Antibodies from patients with fascioliasis crossreact very strongly with antigens from *D. viteae* and *E. granu-*

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losus. This could experimentally be confirmed. With fascioliasis patients it is almost a rule, that anti-*Fasciola hepatica* antibodies react with antigens from *D. viteae* and *E. granulosus*, and with lower extinction values and much less often also with antigens from *T. canis* and *S. mansoni*. This contrasts the extremly high specificity (98%) of the *F. hepatica* total worm extract antigens present on the plastic surface. This antigen is the most specific antigen in our multi-antigen ELISA plate. One exception, however, has to be mentioned. Sera from paragonimiasis patients show the same reaction pattern as sera from fascioliasis patients. Sera from clonorchiasis patients generally do not crossreact with antigens from *F. hepatica* or if so, only in the borderline range (cf. fig.).

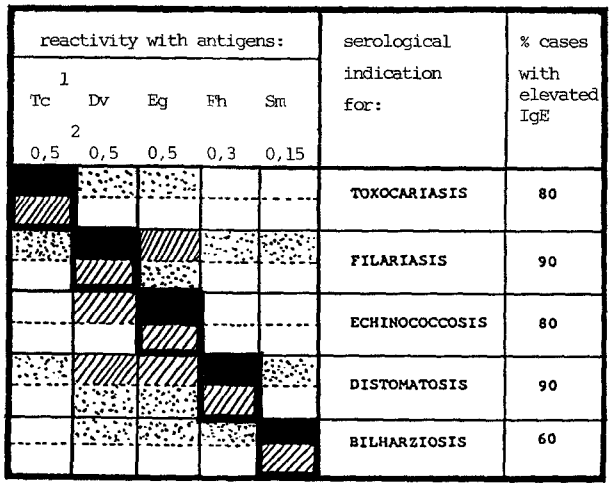
4. The sensitivity and specificity of the antigens from *Schistosoma mansoni* is high for patients with *S. mansoni* infections (about 90%). The sensitivity with patients having urinary schistosomiasis drops to about 60%, as also experienced by other authors. The simoultaneous use of antigens from *S. mansoni* and *S. haematobium* can improve the reliability of the schistosomiasis serodiagnosis with ELISA.

The multi-antigen ELISA has been proved in our laboratory to be a very valuable screening system for patients with unexplained blood eosinophilia and negative results in stool examinations. All ELISA-reactive sera are further analyzed with the indirect fluorescent antibody test (IFAT), which is more specific but less sensitive. In addition, abnormal fluorescence with a certain antigen can confirm the suspicion of crossreaction in ELISA with the corresponding soluble antigen. We found the appreciation of fluorescence of specific anatomical structure to be quite important in order to properly interpret the results. This potency of IFAT is still under investigation.

Since the serum concentration if IgE antibodies are often raised with helminth infections, we include in our helminth screening ELISA a semiquantitative IgE determination (using a monoclonal anti-IgE catching antibody from Hybritech). Values between 200 and 2000 kU/l are recorded as positive, and values over 1000 kU/l are recorded as strong positive. Patients with elevated IgE values can be further examined for specific IgE against antigens from various helminths by the Radio Allergo Sorbent Test (RAST), which proved to be more specific than ELISA or IFAT.

Using a sensitive multi-antigen ELISA as a screening procedure allows a routine serodiagnostic laboratory to become more efficient. More expensive and time consuming methods are the only applied to confirm results on a reduced number of samples, for which sufficient time will be available for labor and more careful evaluation. The overall impact is a more cost-effective service and a higher quality report for the clinician.

Typical reaction patterns of sera from different helminthiasis patients and various helminthic antigens;
sera moderate (▨) or strongly (■) positive for homologous antigens are contained within the darker borders.



1) Antigens: Tc = *T. canis, in vitro* derived metabolic antigens from larvae II; Dv = *D. viteae*, total worm extract; Eg = *E. granulosus*, antigens from hydatide fluid of bovine lung cysts; Fh = *F. hepatica*, total worm extract; Sm = *S. mansoni*, total worm extract.
2) Positive threshold value for the different antigens (extinction values at E_{492nm}; equals upper limit of normal population values).

⩾ 4× positive threshold = ■

2–4× positive threshold = ▨

1–2× positive threshold = ⁘
cut off = □

Round Table Discussions

Discharge to the environment of viruses in wastewater and sludge

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The many varieties and forms of viruses which may occur in nature were described, and the main types illustrated. Almost all living things are associated with specific viruses, including hosts as diverse as mankind and bacteria. Under suitable circumstances, any virus which exists within the catchment of a sewage works may eventually find its way into the sewage. The number of different types which may therefore be present is potentially very large, though many derive from hosts other than man and, due to the host specificity typical of viruses, the vast majority pose no problem to human health. However, some viruses from plant or animal sources distributed through contaminated sewage can be of agricultural (and therefore of commercial) importance.

The problems which face us in human health arise mainly from a large and diverse group known as the human enteric viruses. These are excreted in very large numbers by infected persons and are universally present in sewage. They show a marked ability to survive for long periods and can resist extremes of pH, the effect of organic solvents and bile salts. Conversely they are relatively sensitive to high temperatures and dessication. Infection takes place when the virus is ingested, possibly in food or water. The main route of transmission however is probably directly from person to person either by the oral-oral route or the fecal-oral route. The minimum infective dose is believed to be as little as one virus particle.