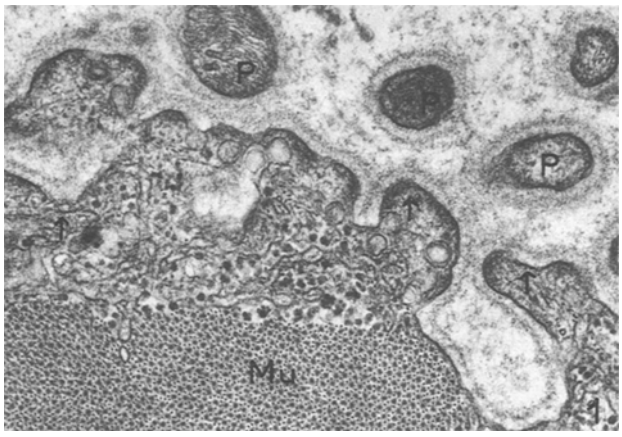

Fig. 1. Transverse section of the muscle, 124 days after denervation. The sarcoplasmic membrane is convoluted (arrows) and small muscle portions (P) are separated from the parent muscle fibre (Mu). The basal lamina is in contact with the convoluted sarcoplasmic membrane as well as with sequestered muscle pieces. Note the presence of vacuoles underneath the muscle membrane. × 39,500.

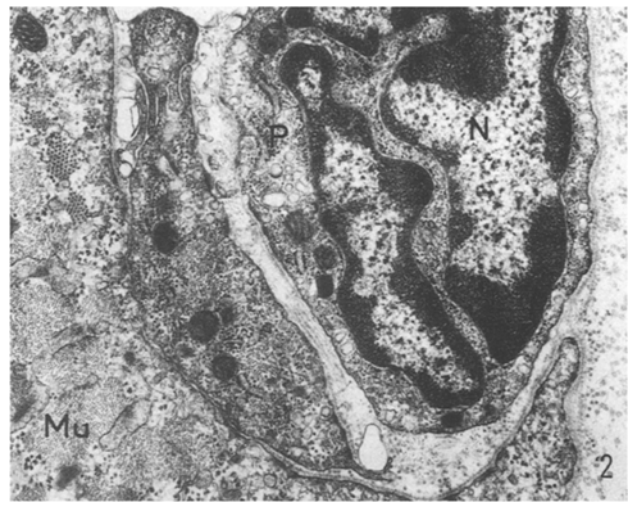

Fig. 2. Transverse section of the muscle, 115 days after denervation. A part of the muscle (P) is separated with its nucleus (N) from the parent muscle fibre (Mu). × 15,200.

lite cells is observed from the 2nd month onwards of denervation[5], whereas the sequestration of muscle portions takes place only during the 5th month or later than that.

Miledi and Slater[3] reported that denervated muscle of *Rana temporaria* atrophies after a longer period than that of rat. In *R. temporaria,* there is no sequestration of muscle portions, even after more than 4 months[6]. Thus, the results of present investigation show that the denervated muscle of *R. esculenta* atrophies earlier than that of *R. temporaria.* This observation is complimentary to our previous research where we have shown the electrophysiological and morphological differences between these 2 species of frog[2].

Birks and collaborators[6] in *R. temporaria* and Miledi and Slater[4] in rat have described the dissociation of basal lamina from sarcoplasmic membrane in the denervated muscle. They suppose that during muscular atrophy, the sarcoplasmic membrane retracts and detaches itself from the basal lamina. We never observed dissociation of basal lamina in case of *R. esculenta* even after a period of 4 months or more. Even the detached muscle pieces were lined with the basal lamina. We do not know whether to attribute this difference to the difference in animal species or to the preparation of the tissue.

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Comparative evaluation of 7 helminth antigens in the enzyme-linked immunosorbent assay (E.L.I.S.A.)

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Summary. 112 sera from Europeans with parasitologically proven helminthiasis were tested in the enzyme-linked immunosorbent assay (E.L.I.S.A.) against 6 crude extracts of various helminths (2 of adult worms: *Dipetalonema viteae, Fasciola hepatica;* 3 of eggs: *Ascaris suum, Toxocara canis, Schistosoma mansoni;* and of *Echinococcus granulosus* scolices) and against bovine hydatid fluid. Each serum was tested simultaneously at a fixed dilution of 1:160 against all antigens. Extensive cross-reactions were observed, leading to the conclusion that non-purified helminth antigens, even in combination, are of limited value for reliable serodiagnosis in E.L.I.S.A.

The enzyme-linked immunosorbent assay (E.L.I.S.A.) has already been used for serological investigations of several helminth diseases[1,2]. In most cases sera from people living in endemic areas and control sera from nonparasitized individuals have been evaluated. The specificity of E.L.I.S.A. as a tool for individual serodiagnosis has not yet been adequately analyzed.

In this study various non-purified helminth antigens were evaluated in E.L.I.S.A. using sera from Europeans who had been carefully examined for parasitic diseases. Multi-parasitic infections were rarely encountered. As crude helminth antigens were used cross-reactions were to be expected. It was however of interest to determine whether characteristic reaction patterns of sera in this multi-antigen test system could be of help in the orientation of further clinical and serological examinations in cases of unexplained blood eosinophilia.

Materials. 112 sera were obtained from Europeans, most of whom had visited tropical countries. On the basis of parasitological findings, 96 sera were divided into 9 groups (table 2). In addition, 16 sera with positive toxocariasis serology, as well as 60 sera from people parasitologically

Table 1. Antigens

Antigen	Abbreviation	Source of antigen	Extraction	Proteinconcentration for coating (µg/ml)
Ascaris suum	As	Embryonated eggs (Cypess et al.[7])	Borate buffer (0.1 M, pH = 8.6)	2
Toxocara canis	Tc	Embryonated eggs (Cypess et al.[7])	Borate buffer (0.1 M, pH = 8.6)	2
Dipetalomena viteae	Dv	Adult females from golden hamsters (Weiss und Degrémont[9])	Barbiturate buffer (0.05 M, pH = 8.4)	5
Echinococcus granulosus	Eh	Hydatid fluid from bovine lung cysts	Dialysed against barbiturate buffer (0.05 M, pH = 8.4)	2
	Es	Scolices from bovine lung cysts	Barbiturate buffer (0.05 M, pH = 8.4)	5
Schistosoma mansoni	Sm	Eggs isolated from hamster liver (Weiss et al.[3])	Sodium carbonate buffer (0.06 M, pH = 9.6)	5
Fasciola hepatica	Fh	Adult worms from bovine livers	Barbiturate buffer (0.05 M, pH = 8.4)	5

Table 2. Reactivity of 112 sera (diluted 1:160) from parasitologically proven helminth cases in E.L.I.S.A.

Diagnosis	Number of sera reactive[a]/total	Number of sera reacting with: As[b]	Tc	Dv	Eh	Es	Sm	Fh
Ascariasis	4/23	1	2	2	2	–	–	–
Ascariasis/Trichuriasis	1/7	–	–	1	–	–	–	–
Trichuriasis	–/7	–	–	–	–	–	–	–
Ancylostomiasis[c]	4/6	1	1	3	1	–	–	–
Strongyloidasis[c]	2/7	1	1	2	2	–	–	–
Visceral larva migrans[d]	16/16	1	16	–	1	–	–	–
Filariasis[e]	18/19	ND	11	17	12	–	–	–
Hydatid disease	8/8	–	1	2	8	6	–	–
Schistosomiasis[f]	3/17	ND	–	1	–	–	3	–
Fasciolasis	2/2	2	ND	2	2	2	–	2
Total	58/112	6	32	30	28	8	3	2

[a] Reactive with at least one antigen; [b] for abbreviations of antigens see table 1; [c] in some cases additional intestinal helminths; [d] sera from cases with positive IFAT serology on *Toxocara canis* larvae; [e] sera from cases with onchocerciasis (9), wuchereriasis (3), loasis (2) and *D. perstans* (5); [f] sera from cases with intestinal (13) and vesicular (4) schistosomiasis. ND, not done.

and clinically free of helminth infections were tested. 98 sera from patients living in Lapo, Ivory Coast, where schistosomiasis is endemic, were tested against schistosomal antigen; 63 sera from persons living in Ahondo, Ivory Coast, in an area with endemic onchocerciasis, where transmission of schistosomiasis is not observed, were tested against the filarial and schistosomal antigen preparations (for details see Weiss et al.[3]).

The indirect fluorescent antibody test (I.F.A.T.) was applied in this study for toxocariasis, using pepsin digested 2nd stage larvae of *Toxocara canis* as antigen[4].

Enzyme-linked immunosorbent assay (E.L.I.S.A.). The antigens used and their preparations are listed in table 1. The soluble *Ascaris* and *Toxocara* egg antigens were kindly provided by the Center for Disease Control (Atlanta/Georgia, USA). Prior to extraction, all worm materials had been stored at −70 °C. The soluble *Ascaris* and *Toxocara* egg antigens were stored in lyophilized form at 4 °C, whilst the other soluble antigens were stored in small aliquots at −70 °C. All antigens were diluted with 0.06 M sodium carbonate (pH = 9.6), containing 0.01% merthiolate, to the final protein concentration used for sensitizing the microtiter plates (table 1). The E.L.I.S.A. was performed as a microassay and the testprocedure was essentially the same as described by Walls and Palmer[5].

Results. In preliminary experiments the optimal dilutions for the different antigens were determined by testing a battery of selected sera at various dilutions. The concentrations of protein (determined with the Lowry method) in the different antigens used for coating microtiter plates are given in table 1.

In a next step, 60 sera of patients parasitologically and clinically free of helminth diseases were tested. As 4 out of these sera reacted at a dilution of 1:80 with hydatid fluid antigen, in all subsequent screening tests sera were diluted to 1:160.

The reactivities of our antigen preparations in relation to 10 groups of sera from Europeans with various helminth infections are summarized in table 2.

In addition, 63 sera from children living in an area of the Ivory Coast with endemic onchocerciasis were tested with the *Dipetalonema viteae* antigen. Of these 57 (90%) were reactive.

Our preparation of soluble egg antigens from *Schistosoma mansoni* (Sm) exhibited a poor sensitivity: only 3 out of 17 sera from Europeans with schistosomiasis wer found to be positive. The same antigen gave better results when 98 sera from Africans were tested: 43% of these sera reacted at a dilution of 1:160. The sensitivity was increased to 95% at a dilution of 1:80 but 16% cross-reactions with sera from Africans free of schistosomiasis were observed.

Discussion. When analyzing the patterns of reactivity of 7 antigens, we observed extensive cross-reactions for those antigens exhibiting a high sensitivity (> 90%): this was the case for the *Toxocara canis, Dipetalonema viteae* and *Echinococcus granulosus* hydatid fluid antigens. Since only 2 sera from proven cases of fascioliasis were available, the sensitivity of the very specific *Fasciola hepatica* antigen could not be judged conclusively. Other antigens with a high specificity, such as *Echinococcus granulosus* scolices and *Schistosoma mansoni*, exhibited limited sensitivities.

In contrast to our results, Weiland and Schwarzhuber[6] observed a high sensitivity for toxocariasis sera, using larval *Ascaris suum* antigens. In agreement with us, these authors noted a low specificity for their larval *Toxocara canis* extract. Cypess et al.[7] reported no cross-reactions between larval *Toxocara canis* antigen and *Trichinella* and *Wuchereria* sera after preabsorbing the sera with the larval

Ascaris antigen. The use of secretory antigens from *Toxocara* larvae seems to be very promising for a specific enzyme-immunoassay[8].

The *Dipetalonema viteae* antigen has not been used before in the E.L.I.S.A. but was of value in the I.F.A.T.[9] and in immuno-electrophoresis. However, like other antigens used for serodiagnosis of filariasis, its specificity represents a major problem (for review see Ambroise-Thomas[10]).

The high sensitivity of our hydatid fluid antigen is consistent with previous reports[11,12]. Extensive cross-reactions, particularly with filariasis sera, were striking, in agreement with the recent findings of Ambroise-Thomas[13]. Such cross-reactions have also been oberved in the I.F.A.T. using frozen sections of scolices (unpublished observations). In contrast we found the soluble antigen from scolices to be more specific but slightly less sensitive in E.L.I.S.A. (table 2). These results are in agreement with those obtained with the indirect haemagglutination test[14].

The sensitivity of our *Schistosoma mansoni* egg antigen was lower than that found by other workers[15,16] and reacted differently with sera from Europeans and from Africans. The low reactivity of some of our schistosome sera from Europeans was confirmed by tests at the Center for Disease Control (Atlanta/Georgia).

In conclusion we can say that using crude antigens and testing 1 single serum dilution, E.L.I.S.A. was inadequate for specific immunodiagnosis. For further improvements a quantitative evaluation either by photometry and/or by titration could be considered. However, preliminary studies with our antigens have shown that a higher specificity can only be achieved together with a considerable loss in sensitivity. Before purified antigens are available, a further possibility for improvement might be to test sera after absorption with different cross-reacting antigens. Initial results are promising: the extensive cross-reactivity between some filariasis and echinococcosis sera could be abolished by preabsorption with the respective antigens.

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The effect of BCG-vaccination on vaccinia virus infections in mice

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Summary. Pretreatment with BCG yielded a high degree of protection against experimental vaccinia virus infections in mice. *Corynebacterium parvum* and *Aristolochia* acid were less protective; other immunostimulants were ineffective.

After application of the attenuated *Mycobacterium bovis* (BCG), an increase of nonspecific resistance against some malignant tumors has been demonstrated[1-3]. Some authors have investigated the effect of BCG against viral infections[4-7]. While studying experimental vaccinia virus infections in mice, we have compared BCG with other immunostimulants.

Material and methods. Animals: NMRI-mice, 15-25 g, were kept under conventional conditions. Immunostimulants: Levamisole (Janssen, Düsseldorf), 2.5 mg/kg s.c. once a week; Isoprinosine (Röhm Pharma, Darmstadt), 50 mg orally, daily; *Corynebacterium parvum* (Mériéux, Lyon, Lot No.R 0185), 500 µg daily s.c.; AS-3 (Madaus, Cologne), ampoules of 2 ml containing 0.461 mg *Aristolochia* acid, 0.5 ml daily s.c.; Echinacin® (Madaus, Cologne), ampoules of 2 ml containing 0.1 mg extract of *Echinacea purpurea*, 0,5 ml daily s.c.; BM 12.531 (Boehringer, Mannheim), 1-carboxamido-2-cyanaziridin[8], 50 mg/kg every 3rd day s.c.; BCG (dry vaccine Connaught, medac, Hamburg). The reconstituted vials contain 1×10^7 viable bacteria per ml.

The potency of the vaccine was tested on Lowenstein-Jensen-agar, using serial dilutions; furthermore, viable BCG were counted in the spleen cells from mice[9]. Doses of BCG: 0.1 ml once i.p. One group received a single dose of 0.5 ml orally with a gastric tube.

Virus: The neurotropic vaccinia virus strain Ma1[10] was grown on the chorio-allantoic membranes of embryonated eggs. The infectious titer came to 4.5×10^8 pox-forming units (pfu)/ml. The ID_{50} after intracerebral (i.cer.) inoculation (inoculum 0.05 ml) was $10^{2.8}$ pfu/ml according to Spaerman-Kaerber[11]. After i.p. infection it was 10^4 pfu/ml (inoculum 1.0 ml). In i. cer. infections a $100 \times ID_{50}$ was applied, in i.p. infections a $500 \times ID_{50}$.

Experimental procedure. Groups of 25 mice each were given one dose of BCG either 7 or 12 days prior to the infection. *C. parvum*, BM. 12.531, Levamisole, Isoprinosine, Echinacin or AS-3 were initiated 3 days prior to the infection. In other groups the immunostimulants (except BCG) were started 6 h after the infection. All groups were monitored daily for deaths, starting with the 2nd day after infection. The experiments were terminated after 30 days. In the surviving animals either a challenge infection with $100{,}000 \times ID_{50}$ or serological investigations were performed: the pooled serum of 5 animals was examined for