

NETHERLANDS SOCIETY FOR PARASITOLOGY SPRING MEETING 1983

Amsterdam, March 11, 1983

Received March 28, 1983

EVALUATION OF TRANSMISSION BLOCKADE AFTER VACCINATION WITH MALE AND FEMALE GAMETES OF *PLASMODIUM BERGHEI*.

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In studies on vaccination with malarial gametes there is a need for a serological test to evaluate the state of immunity. We have immunized balb/c mice with male or female gametes, harvested from *P. berghei*-infected mice with high parasitemias/gametocytemias. Vaccination was carried out with various doses of live or formalin-fixed gametes, both intravenously and intramuscularly. Sera were taken after the second and third inoculation and a challenge-infection with blood stages was given one week after completing the vaccination scheme. Mosquitoes were fed on these mice on days 3-5, at which time the transmission in controls is known to be highest. Earlier work was confirmed, in that infections of mosquitoes fed on immunized mice were dramatically reduced, as judged by the percentage of positive mosquitoes and by the median numbers of oocysts in them.

Sera taken before challenge revealed titers with the immunofluorescent antibody (IFA) test, that were highest with homologous antigens, but still prominent with antigens of other stages, particularly asexual schizonts. This is indicative either of common antigenic determinants or of contaminants in the vaccines. Sera from mice that were intramuscularly inoculated with male gametes had lower titers than those collected after intravenous administration of the vaccine. The titer level corresponded with the chance of mosquitoes to become positive: the i.m. group showed a 2-fold reduction and in the i.v. group it was 16-fold. Ten times more male gametes per vaccination dose induced a significant raise in IFA-titers. They were also higher after using live male gametes, as compared with formalin-fixed ones, and titers continued to rise from the second to the third inoculation, and, interestingly, also after one week post vaccination. This may suggest a more successful transmission blockade when mice are challenged at least two weeks post vaccination. However, we are cautious about using IFA-titers as an expression of the state of immunity of the mice and their transmission-blocking capacity.

ENZYME HISTOCHEMICAL STUDIES ON THE EXOERYTHROCYTIC FORM OF *PLASMODIUM BERGHEI*

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Very little is known of the metabolism of intrahepatocytic stages of malaria parasites and almost all studies on these parasites have been concerned with biochemical analysis of the intra-erythrocytic stages. Only a

single and short report on some histochemical observations of *P. berghei* exoerythrocytic schizonts is available. We therefore started to (re-)examine a large number of enzymes in liver cryosections of Brown Norway rats, 31, 41 and 48 h after infection with sporozoites. The enzyme α -glycerol-phosphate-oxidase (EC. 1.1.2.1.) appeared to be present at levels higher than the surrounding tissues, in all stages examined and absent in control incubations (both without substratum and with suramine). Cytochrome-c-oxidase (EC. 1.9.3.1.) and lactate-dehydrogenase (EC. 1.1.1.27.) were demonstrable in parasites but had much lower staining intensity than the surrounding parenchymal cells. After a short incubation time succinate-dehydrogenase (EC. 1.3.99.1.) appeared to be present at lower levels of activity in the parasite, but after a prolonged incubation time it showed up to be distributed in a granular pattern. Also NADH-tetrazolium reductase (EC. 1.699.3.) and glucose-6-phosphate-dehydrogenase (EC. 1.1.4.9.) are slightly positive. Other enzymes tested were α -glucan-phosphorylase (EC. 2.4.1.1.), 5-nucleotidase (EC. 3.1.3.5.), Mg^{2+} -ATPase (EC. 3.6.1.4.), α -naphthyl-esterase (EC. 3.1.1.6.), glucose-6-phosphatase (EC. 3.1.3.9.), alkaline phosphatase (EC. 3.1.3.1.) and leucylaminopeptidase (EC. 3.4.1.1.). None of these enzymes were demonstrable in the parasites, whereas the reaction was normal in the hepatocytes.

These results allow the conclusion that the parasite inside hepatocytes may have a glycolysis, and in contrast to what was reported earlier, also a more or less active citric acid cycle. This is in full concordance with EM-studies which described cristate mitochondria and the presence of the respiratory chain enzyme cytochrome-oxidase.

We were also able to demonstrate the digestive enzyme acid phosphatase (EC. 3.1.3.2.) inside the parasite and particularly in its peripheral vacuoles. This enzyme was only demonstrable after using the "non-aqueous" membrane technique on non-fixed, frozen sections, whilst it was absent in fixed sections. These results extend earlier observations that the peripheral vacuoles are Golgi-derived and therefore may be involved in host cell digestion.

This study was supported by a grant from the Jan Dekker Stichting. We thank Helene Loermans and Elly Vitters for excellent technical assistance.

PLASMODIUM BERGHEI VACCINATION WITH PURIFIED SPOROZOITE ANTIGEN INCORPORATED IN LIPOSOMAL MEMBRANES.

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Immunisation with intact sporozoites can, under certain conditions, result in a protective immunity against sporozoite challenge. The protein which is thought to be responsible for the induction of this protectivity in *Plasmodium berghei* is now identified (Potocnjak *et al.* J Exp Med 1980; 151: 1504-1513; Vermeulen *et al.* 1982. Exp. Parasitol. 53: 199-208).

We like to present here preliminary results of an experiment in which we have purified this surface antigen (Sp53) of *P. berghei* ANKA sporozoites in such quantities that we could use the final product to immunize five mice. As starting material we used 7×10^8 purified sporozoites, which have been stored for up to one year at -70°C . Sp53 was purified using gel filtration and immunoaffinity chromatography. The purified antigen was incorporated in phosphatidyl choline liposomes according to the procedure by Gerritsen *et al.* [1978. Eur J Biochem 1980; 85: 225-261]. The lipid-protein complexes were concentrated by centrifugation for 2 hours at 150 000 g. 80% of the protein could be recovered in the liposome fraction, as measured using ^{125}I -labelled Sp53. The pellet was suspended in 1 ml of 10 mM Tris-150 mM NaCl pH 8.0 and intravenously injected into 5 balb/c mice. 5 control mice were injected with liposomes without antigen. Sera were taken regularly and examined for specific antibodies in the IFA test. One week after the first injection all sera gave positive reactions with titers up to 1:160, whereas control sera were negative throughout this and further tests.

At six weeks after the priming, when titers were almost zero, the mice received a second injection with antigen purified from 3.5×10^8 sporozoites, again presented in liposomes. One week later titers against sporozoites of up to 1:640 were detected. Using intact, viable sporozoites as antigen all sera gave positive reactions. All sera were negative, however, when asexual bloodforms were used as slide antigen.

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Concluding, we have shown that it is possible to induce a good antibody response with solubilised antigen without the use of Freund's Adjuvant. The antibodies reacted with the surface component(s) of the sporozoite and showed specificity for this developmental stage of the parasite. Although this experiment has not yet been completed, it seems clear that the application of liposomes as a presentation model for (membrane-bound) antigens offers good prospects.

MATURATION OF GAMETOCYTES IN CONTINUOUS IN VITRO CULTURE OF *PLASMODIUM BERGHEI* (ANKA)

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Recently we reported our successful *in vitro* culture of *Plasmodium berghei* (Mons *et al.* *In vitro* culture of *Plasmodium berghei*, Int Journ Par; in press). Optimisation of techniques has now resulted in the establishment of a continuous culture of this rodent malaria parasite in rat-cells. The morphology of all stages *in vitro* is comparable to normal morphology *in vivo*. After 90 days in culture parasites were still infective for rodents. Peak parasitaemias of over 20% were consistently observed. Several subcultures were initiated and showed rising parasitaemias.

Low numbers of gametocytes were produced in all cultures (between 0.5 and 8% of the parasitized erythrocytes). Direct evidence for full maturation of both micro and macro gametocytes was obtained by induction of exflagellation and fertilisation (oökinete-formation) *in vitro*.

CEREBRAL MALARIA IN MALE C57Bl/RIJ MICE INFECTED WITH *PLASMODIUM BERGHEI*

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Plasmodium berghei infection in mice is always lethal. In a well adapted *P. berghei* - mouse strain combination, and a standardized inoculum (10^5 PE/mouse) the mortality pattern is monophasic or biphasic. The cause of death in the first phase or mortality, usually early in the second week of infection, is still obscure, and so far none of the observed changes was pathognomonic as to the moment of death. Since mortality in the second week of infection usually takes place during crisis, i.e. a declining parasitaemia, proliferating parasites seem to be involved only indirectly.

Recent observations suggest that cerebral malaria may play an important role in early mortality in *P. berghei*-infected male C57Bl/Rij mice. Measurement of body temperature revealed a dramatic fall from the 7th day of infection onwards. The body temperature dropped from 37°C to values below 28°C within one or two days, suggesting a collapse of cerebral thermoregulation. Measurement of brain weight revealed an increase of almost 20% from day 7 to day 9, but of only 6% in splenectomized animals, which prevented early mortality completely.

Microscopy revealed petechial hemorrhages particularly in the cerebellum and in the calcarin area in the majority of the mice by the 9th day of infection. No such lesions were found in splenectomized mice. Sticking parasitized erythrocytes have not been found in the development of the lesions. The above observations suggest that in this particular parasite-host combination cerebral infarcts might be due to compression of the venous outflow in the affected area as a result of substantial edema. This may also explain the collapse of the cerebral thermoregulation. Parasites are only indirectly involved in as much as they induce edema. The absence of lesions in splenectomized mice suggest a parasite dependent immunological reaction, e.g. macrophage activation and the production of monokines (e.g. leukotrienes), which affect vascular permeability.

COMPLEMENT DEPENDENT DEFENCE MECHANISMS AGAINST INTRAERYTHROCYTIC *PLASMODIUM BERGHEI* PARASITES

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Phagocytosis of parasitized erythrocytes (PE) is supposed to be an important defence mechanism of the host against malaria infection. Adherence, the first step of phagocytosis, can be facilitated by the complement product C3b. Whether complement could enhance the phagocytosis and destruction of PE was studied. Adherent peritoneal exudate cells (APEC) from normal B10LP mice were incubated with radioactively labeled parasitized (*Plasmodium berghei*) erythrocytes (radioactive material was almost exclusively localized in the parasite). After incubation radioactivity associated with the phagocytic cells was determined. Results indicated that fresh normal mouse serum (NMS) enhanced the uptake of radioactive material by APEC when compared to inactivated NMS. Inactivation could be achieved by preincubation with zymosan as well as heat-treatment (30' at 56°C). Essentially the same results were achieved when guinea pig serum was used as a source of complement. To determine whether PE could activate complement, NMS was preincubated with PE and subsequently tested for its ability to facilitate the uptake of PE by APEC. It was found that preincubation of NMS with PE (but not with normal erythrocytes) reduced the capacity to enhance uptake. This phenomenon could not be explained by the presence of naturally occurring antibodies because the factor involved was heat-labile. Thus PE can activate complement. Whether this activation could lead to lysis of PE was studied in a cytotoxicity assay. When radioactively labeled PE were incubated with NMS a proportion of the radioactivity (free parasites) was released into the culture medium. Inactivation of NMS abolished this activity. Thus, complement activation resulting in opsonization and/or subsequent lysis of PE may be part of the *in vivo* defence mechanism and explains the enhanced clearance of PE over normal erythrocytes from the blood of normal mice. Whether complement is activated by the classical (immune complexes on PE?) or the alternative pathway remains to be determined.

MALARIAL IMMUNITY IS REINFORCED BY TEMPORARY IMMUNOSUPPRESSION BUT NOT BY BOOSTING

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Mice can be immunized to *P. berghei* by drug (sulfonamides) controlled infection. Protection is of the premunition type, i.e. some parasites survive in immune animals for a long time. Improvement of the effector function of this type of immunity may result in conversion of premunity to sterile immunity, which may be maintained for some months. A substantial proportion of mice lose immunity during pregnancy, apparently in relation to a pregnancy-associated immunosuppression. The percentage of mice with malaria recrudescence is higher in primigravidae than in multigravidae, indicating an improved immunity after the first pregnancy. This improvement is only achieved when some parasites are present during the first pregnancy. Moreover, improvement of immunity in parous immune mice is shown by enhanced clearance of parasites from the blood after challenge infection. Further experiments show that the need for the presence of parasites is restricted to the second half of pregnancy, the period when malaria recrudescences are observed. A similar relation between reinforcement of immunity and temporary suppression of certain immune reactions is also observed in non-pregnant immune mice after treatment with corticoids or anti-T-cell serum. Malaria immunity is not improved by repeated booster infections. In immune animals repeatedly challenged shortly before pregnancy, no reduction of the recrudescence rate is observed. In non-pregnant immune animals repeated challenges do not enhance the clearance rate of parasites from the blood. Repeated inoculations with parasitized erythrocytes in immune animals do not alter the quality of the effector function of immunity.

Improvement of the effector function of malarial immunity in pre-mune mice is achieved by an active interaction of the proliferating parasite and the temporarily suppressed immune system of the host. This improvement may be the result of reinforcement of the existing, or activation of an additional, immune response.

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PLASMODIUM BERGHEI: THE INFLUENCE OF MALARIA ON MYELOPOIESIS AND MACROPHAGE PRODUCTION IN MICE

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Granulopoiesis and macrophage production during experimental malaria (*P. berghei*) infection was studied in BALB/c, Swiss, and C57Bl/Rij mice, using a viscous (0.8% methylcellulose) tissue culture system.

In the initial period of the disease when T-cells in the spleen revealed a strong increase in number, a rise of the serum level of colony stimulating activity (CSA) could be observed. As a consequence the number of the granulocyte/macrophage progenitor cells (in vitro colony forming cells, GM-CFC) in the bone marrow increased gradually, reaching a maximum during the second week of infection.

With some delay, the spleen, too, showed an increasing myelopoiesis and macrophage production. Since the number of progenitor cells in the peripheral blood rose also, a redistribution of these cells from the bone marrow to the spleen might take place.

At the end of the second week of infection the serum level of CSA decreased, followed by a rapid drop of granulocyte and macrophage production, reaching subnormal values just before death supervened.

There was a linear correlation between the rise of GM-CFCs in the bone marrow and parasitemia in oxyphilic erythrocytes. However, when parasites resided predominantly in the young polychromatophilic erythrocytes the number of progenitor cells in the bone marrow was markedly depressed, probably as a consequence of a competition between erythropoiesis and granulocyte/macrophage production.

In conclusion: granulopoiesis and macrophage production during *P. berghei* infection may be influenced by immunological as well as by hemopoietic events.

ACTIVATION AND FERTILIZATION OF FEMALE GAMETES OF *PLASMODIUM BERGHEI* AND THEIR USE AS A TRANSMISSION BLOCKING VACCINE

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Light microscopic analysis of *P. berghei* female gametes collected in suspended animation solution, then activated and fertilized in tris-buffered gamete releasing solution revealed three morphological types:

Type I is abundant during the first 10 minutes of exposure of infected mouse blood to the ambient and is virtually indistinguishable from the mature macrogametocyte in tail- or heartblood where it completely fills its host cell. These parasites have a small pink nucleus containing a dark red spot of chromatin. Fine brown pigment could be found in the homogenous blue cytoplasm.

Type II consists of macrogametes that are observed from about 10 minutes of activation onwards. These parasites are characterized by a nucleus that increases in size, first along one axis, then at right angles to this. Pigment granules become aggregated and more dense at the edge of the cell.

Type III consists of macrogametes zygotes observed from about 20 minutes of activation onwards. These forms are characterized by a bigger round nucleus with dark red patches of chromatin on a pink background or homogeneously dark pink. Pigment is less pronounced and disperse. It is believed that type III represents fertilized macrogametes, because when isolated after selective centrifugation on a metrizamide gradient, these cells were viable and fertilized as judged by their capacity to grow into ookinets.

Type III cells we used as vaccines, either non fixed or fixed in 0.1% formalin. Three inoculations of 200.000 cells were given intravenously to balb/c mice at 7 days intervals. Ten days after the last inoculation these mice and their controls received a (challenge) infection with a mixed population of blood parasites and were exposed to mosquitoes feeding on days 3-5 after inoculation. Evaluation of transmission blocking immunity was based on the percentage of non infected mosquitoes and on the number of oocysts in positive mosquitoes, in comparison with those having fed on control mice.

With comparable parasitaemias the percentage of negative mosquitoes fed on vaccinated mice ranged from 32% to 93% and in controls from 1% to 15%. The median numbers of oocysts in positive mosquitoes

reached from 2-5 and 24-43, respectively. Both parameters indicate a very pronounced effect, which is not far from complete transmission blockade.

MEMBRANE FEEDING OF *ANOPHELES ATROPARVUS*

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Infections of susceptible *Anopheles atroparvus* with *Plasmodium berghei* via a membrane feeding technique did not consistently give good results (Van Dijken, Sluiter, 1982). Adjustments in the experimental design with regard to constancy of pH of the infective bloodmeal and of the mosquito cages are presently tested and preliminary results indicate an improvement compared to the original set up. This membrane feeding technique is currently used to obtain midgut contents for iso-electric focusing of proteases without the presence of disturbing serumproteins and haemoglobine and to test the infectivity of gametocytes of *Plasmodium berghei*. This work is partly supported by a grant from the Dutch government.

REFRACTIVENESS OF *ANOPHELES ATROPARVUS* FOR *PLASMODIUM BERGHEI*

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From a compatible combination of a wild strain *Anopheles atroparvus* and *Plasmodium berghei* a nearly one hundred percent susceptible and a refractory strain were selected by Van der Kaay and Boorsma (1977). So far the physiological basis of this refractoriness is not known. However, observations of an arrested development of the ookinetes in the midgut of refractory mosquitoes prior to penetration of the "mucoid membrane" could indicate an inhibitory effect of digestive enzymes. The research currently in progress on refractory and susceptible mosquitoes is a time-sequence study by light-microscopy of parasitic development and characterization of proteolytic iso-enzyme patterns by iso-electric focusing.

An experiment on proteolytic activity (assayed by the Azocoll method) indicates that there is no appreciable difference between refractory and susceptible mosquitoes, at least till 25 hours post infection (Experiment in collaboration with M.H. Dresden; Baylor College of Medicine, Houston). This work is partly supported by a grant from the Dutch government.

DETERGENT-SOLUBILIZATION OF ANTIGENS FROM THE TEGUMENT OF *SCHISTOSOMA MANSONI*

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Because of the major immunological role of the tegument of *Schistosoma mansoni*, characterization of tegument components is essential for a better understanding of the host-parasite relationship.

For solubilization of tegument antigens we used 1-0-n-octyl- β -D-glucopyranoside (octylglucoside), since this detergent has major advantages over the more frequently used detergents like Triton and Tween. Excess octylglucoside can be removed by dialysis and interference with the antigen-antibody reaction is minimal. Moreover, octylglucoside solubilized antigens can be used in the ELISA, since in contrast to most other detergents, octylglucoside does not prevent adherence of antigens to the surface of the microtitration trays.

As antigen preparation was obtained by gentle extraction of whole adult worms with 0.1% octylglucoside in PBS. An indirect immunofluorescence reaction on cryostat sections of adult worms showed that the extracted antigens mainly originated from the tegument. The complex antigenic composition of the extract was shown by immunoelectrophoresis against serum from infected mice and immunized rabbits, which gave up to 8 and 12 precipitation lines respectively. Analysis of the octylglucoside extract, fractionated by gel filtration, showed that the main serological activity both in infected humans and in immunized rabbits, was due to high molecular weight antigens. We compared the humoral immune response of schistosomiasis

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patients to soluble adult worm antigens and octylglucoside solubilized antigens in the DASS and the ELISA tests.

To exclude differences between the two preparations with regard to adherence to the carrier surface, we performed the DASS and the ELISA tests as sandwich assays. Non-saturating amounts of the total worm antigen-preparation and the octylglucoside extract were sandwiched between human IgG preparations and their alkaline phosphatase conjugates. The average sensitivity of the tests was higher with the octylglucoside extract, although differences between individual sera occurred.

WATERCONTACTS AND SCHISTOSOMIASIS IN A HYPERENDEMIC VILLAGE IN EASTERN ZAIRE

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Local observers collected data on the water contact behaviour of the population of Makundju on 10 different sites over a total of 128 workingdays, which were distributed in time and over the various sites. In this way observations of activities were obtained over 12.8 days in the total water contact area of Makundju. The number of contacts observed was 2145, of which 702 contacts could be combined with the parasitological data of 231 individuals. The statistical analysis was performed on the data of these 231 individuals. With bivariate statistics it was confirmed that these data were an unbiased sample of the total activity and of the level of *S. mansoni* infection of the whole population.

Three types of data were available: personal, parasitological and data on water contact behaviour. Initially, the contacts were divided into 9 types, but to be useful they were combined into household and economic activities, either within the village or in the surroundings.

Parasitological data consisted of eggcounts before chemotherapy, increase of eggcounts after chemotherapy and data on other infections and on hepatomegaly and splenomegaly. Because of these three types of data, factor analysis was performed on three factors. The first three factors explained only 32% of the total variance, which is an indication of the lack of consistent relations in the original multivariate space. Projection of the factor loads on the factor space confirms this. The 231 individuals, projected on the factor space, are distributed only by the combination of age, weight and height and the number of and duration of certain watercontacts. No evidence, therefore, is found that the intensity of the schistosome infection is related to the water contact behaviour.

REINFECTION FOLLOWING TREATMENT OF INTESTINAL SCHISTOSOMIASIS IN EASTERN ZAIRE

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In a number of communities in Maniema, Eastern Zaire, randomly chosen fractions of the population were treated with oxamniquine (either at 15 mg/kg or at 40 mg/kg). The process of reinfection was followed by regular quantitative stool examinations.

In one village (Tshamaka), where no preventive measures were taken, the egg counts were even higher than the pre-treatment counts, 20 months after oxamniquine treatment. In another village (Makundju), intensive snail control was added to chemotherapy. Egg counts remained low but re-infection did continue to take place, particularly in children. In a third village (Kakota) only focal snail control was carried out: the heavily snail-infested canal in the centre of the village was treated with molluscicides regularly and thoroughly but the other habitats outside the village were left untreated. Although the most important and most obvious transmission site was thus treated satisfactorily, reinfection of the human population was very quick, once again. Twenty months after treatment, the mean egg outputs were about the same as before treatment. Again, a quicker reinfection was observed in children than in adults.

Parts of this investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

THE SIDE EFFECTS OF PRAZIQUANTEL IN THE TREATMENT OF INTESTINAL SCHISTOSOMIASIS IN EASTERN ZAIRE

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Praziquantel was administered to 90 persons in a *S. mansoni* endemic village in Eastern Zaire. Half of them excreted more than 600 epg while the geometric mean egg count varied from 542 to 810 epg in three different pre-treatment surveys.

Side effects occurred in a large part of the treated subjects. The most important observation was the occurrence of bloody diarrhoea in 56% of the treated subjects. An additional 23% complained of abdominal pains without producing bloody stools. Vomiting was observed in 15%.

These side effects became manifest shortly after treatment: in one third of the cases the first portion of bloody diarrhoea was produced within 30 minutes after treatment; in two thirds of the cases within 1 hour. There was no correlation with the occurrence of occult blood prior to treatment and these side effects were seen in all age groups.

The mean egg counts prior to treatment were significantly greater in those with bloody stool production than in those without.

| | geometric mean egg count |
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| no complaints | 230 |
| abdominal complaints but no bloody diarrhoea | 684 |
| abdominal pains and bloody diarrhoea | 1439 |

It is not clear why the side effects of bloody diarrhoea and abdominal pains are much more pronounced in Maniema, Zaire, than has been recorded elsewhere.

IMMUNOLOGICAL COMPATIBILITY OF SCHISTOSOMES AND THEIR INTERMEDIATE HOSTS

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A schistosome can only establish itself and develop in its snail host if it is not affected by the latter's internal defence system. This phenomenon may be brought about by 1) suppression of defence activities and 2) evasion of recognition by the snail's defence system. The latter strategy is feasible if the parasite's surface is inert physically and chemically, or disguised by snail-like molecules. Aim of the present study was to investigate the occurrence of snail-like antigens on the parasite's surface in the schistosome - snail model *Trichobilharzia ocellata* - *Lymnaea stagnalis*. Juvenile snails were exposed to 75 miracidia and 25 days thereafter the headfoot region (containing mother sporocysts) and the digestive gland area (daughter sporocysts) were fixed. Paraffin sections were stained immunocytochemically (immunofluorescence and PAP method) using rabbit antisera to *L. stagnalis* haemolymph fractions. Snail tissue was stained intensely; unlike host material, the parasite (mother as well as daughter sporocysts) was not stained at all. Positive material, occasionally found closely associated with the surface of the parasite appeared to be host tissue (amoebocytes and connective tissue). Thus it is likely that in the investigated developmental stages, *T. ocellata* does not use shared antigens to evade defence activities of *L. stagnalis*. Before final conclusions can be drawn in favour of other evasion mechanisms, the parasite should, during its intra-snail development, be frequently investigated at regular time intervals; in addition to the antisera used in the present study, antisera to snail tissue antigens should be used.

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ANALYSIS OF POLYSACCHARIDE ANTIGENS OF ADULT SCHISTOSOMA MANSONI USING MONOCLONAL ANTIBODIES

A.M. Deelder, D. Helaha, D. Kornelis, Y.E. Fillié and M.J.A.J. Engels. *Laboratory of Parasitology, University of Leiden, The Netherlands*

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The polysaccharide antigens of *Schistosoma* constitute a serologically important group of at least 8 different antigens, which have partially been characterized. Two of these antigens, the circulating cathodic antigen (CCA), have been demonstrated to be major circulating antigens and both antigens have been shown to be present in body fluids and tissues of infected hosts. A further characterization of this group of antigens, including immunopathological aspects of these antigens, is needed. As the production of mono-specific antisera against these antigens by conventional methods has proven to be difficult, we have produced monoclonal antibodies against several of these antigens.

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For the production of hybridoma's SP2/0 mouse myeloma cells were fused with spleen cells of BALB/c mice, which were either infected with *S. mansoni*, or immunized with a trichloroacetic acid (TCA)-soluble fraction of adult *S. mansoni* or with a TCA-soluble fraction of serum of rabbits, heavily infected with *S. japonicum*. In this way about 200 hybridoma's were selected which reacted with TCA-soluble antigens of *S. mansoni* in the ELISA. The reactivity of these antibodies was further analyzed by incubation of antibodies in an IFA on Rossman's fixed paraffin section of adult *S. mansoni* worms. The following fluorescence patterns were observed: a) staining of the cells lining the schistosome gut; a number of these antibodies were found to be directed against CCA; b) staining of the schistosome gut and a number of parenchymal cells; these antibodies were found to be directed against CAA; c) a staining of the parenchyma; d) a granular staining of the parenchyma with subtegumental concentrations of granula.

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APPLICATION OF MONOCLONAL ANTIBODIES FOR THE DETERMINATION OF CIRCULATING ANODIC ANTIGEN (CAA) OF *SCHISTOSOMA MANSONI*

A.M. Deelder, Y.E. Fillié, M.J.A.J. Engels, D. Helaha and D. Kornelis. *Laboratory of Parasitology, University of Leiden, The Netherlands*

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The best characterized circulating schistosome antigen is the so-called circulating anodic antigen (CAA), which is a proteoglycan. For quantification of CAA-levels and for immunopathological studies so far monospecific antisera have been used, which have been raised by immunization of rabbits and sheep with a trichloroacetic acid (TCA)-soluble fraction of adult *S. mansoni* (AWA-TCA). In an attempt to improve the quality of such antisera, we have produced monoclonal antibodies against CAA. For the selection of such antibodies, antibody activity of hybridoma's produced by fusion of SP2/0 mouse myeloma cells and BALB/c spleen cells of mice immunized with a TCA-soluble fraction of serum of *S. japonicum* infected rabbits was tested in an IFA on Rossman's fixed paraffin sections of adult *S. mansoni* worms. Antibodies which showed a distinct fluorescence of the schistosome gut were tested in the immunoelectrophoresis against AWA-TCA. So far, six monoclonal antibodies which show precipitating anti-CAA activity in the IEF have been isolated. These antibodies, all of which are of IgM isotype, show in the IFA a distinct fluorescence of the cells lining the schistosome gut, as well as a distinct fluorescence of a disperse population of cells in the parenchyma.

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For the determination of CAA-levels, monoclonal IgM isolated from *in vitro* cultured hybridoma in serum-free medium, has been used in the indirect haemagglutination. Using AWA-TCA as reference sample, a lower detection level of 50 picogram CAA/ml serum was observed. The IHA could be used successfully for the follow-up of CAA-levels in *S. japonicum* infected rabbits.

APPLICATION OF MONOCLONAL ANTIBODIES FOR THE DETERMINATION OF CIRCULATING CATHODIC ANTIGEN (CCA) OF *SCHISTOSOMA MANSONI*

A.M. Deelder, Y.E. Fillié, D. Helaha and D. Kornelis. *Laboratory of Parasitology, University of Leiden, The Netherlands*

A major circulating antigen of *Schistosoma* is the circulating cathodic antigen (CCA), also known as antigen "M". This polysaccharide antigen has been demonstrated in body fluids (including urine) and tissues of infected hosts. As production of high-titered, monospecific anti-CCA antisera by conventional immunization had shown no results, we have tried to produce monoclonal antibodies against CCA. In a number of fusions, hybridoma's were produced by fusion of SP2/0 mouse myeloma cells and spleen cells of BALB, c mice, which had been infected with *S. mansoni* or immunized with a trichloroacid (TCA)-soluble fraction of serum of *S. japonicum* infected rabbits. Anti-CCA activity of the produced monoclonal antibodies was determined by an IFA on Rossman's fixed paraffin section of adult *S. mansoni* worms and subsequent analysis of all those monoclonal antibodies, which showed gut fluorescence, in the immunoelectrophoresis against TCA-soluble worm antigens (AWA-TCA). In this way seven monoclonal antibodies which show precipitating activity in the IEF have been isolated. All these monoclonal antibodies, which obviously recognize a repeating determinant of CCA, are IgM antibodies. In the IFA they show a strong fluorescence of the cells lining the schistosome gut.

For the determination of CCA-levels, IgM of clone line 24-2E5-A was coupled to tanned sheep erythrocytes and used in the indirect haemagglutination reaction. Using AWA-TCA as reference sample, a lower detection level of 5 nanogram CCA/ml serum was obtained. The IHA could be successfully applied to determination of CCA serum and urine of *S. mansoni* infected golden hamsters.

FITC-labeled anti-CCA antibody was used for demonstration of CCA in tissue samples of liver, spleen and kidney of *S. mansoni* infected mice.

AN EXPERIMENTAL MODEL TO STUDY THE CAUSAL RELATIONSHIP BETWEEN BILHARZIA AND BLADDER CANCER: *IN VITRO* ORGANOTYPIC CULTURE

P.K. Das^{1,2}, H. Tubing¹, H.C. Walvoort² and G. Hodges⁴ ¹Former International Immunology Training Center, Amsterdam, ²Division of Laboratory of Pathology, the National Institute of Public Health, Bilthoven, ³Depts. of Pathology and Dermatology, Academic Medical Center, University of Amsterdam, The Netherlands, ⁴Department of Pathology, Imperial Cancer Research Fund, London, U.K.J.

Various aetiological factors have been described in explaining the causal incidence of bilharziasis (*Schistosoma haematobium*) associated bladder cancer [1]. Since severe continuous immunopathological responses of the schistosome infected hosts usually result in immune-mediated injuries to liver, bladder, kidney [2] an immunological mechanism should also be considered to be an important facet to cause bladder cancer. Nevertheless the role of *Schistosoma haematobium* in the genesis of bladder cancer remains essentially studied at a limited scale at the *in vitro* experimental level. This has led to an *in vitro* investigation aimed at establishing whether bilharzia infection is a cofactor or a direct carcinogen. This present communication reports: 1) the establishment of an organotypic culture of the bladder of a laboratory animal host for *S. haematobium* (i.e. hamster) and 2) cytotoxic effects of parasite products and induced immunological components of the host on these bladder explants.

The bladder explants have been cultured in Waymouth culture medium containing hydrocortisone, ascorbic acid, foetal calf serum according to the modification of the method of Hodges *et al* [3]. Upon light microscopy it has been observed that the mucosal architecture remains intact during *in vitro* cultivation up to 119 days with the exception of the following alterations: a) epithelial flattening, loss of surface oriented cell polarity, no recognisable umbrella cells, b) squamous metaplasia (intracellular bridges) without keratinisation. Furthermore, in this preliminary study explants treated with either *S. haematobium* hatching fluid (HF) in combination with i) anti-HF antibody and complement and ii) anti-HF antibody, and iii) complement, or iv) antibody or v) HF alone have shown a pronounced urothelial response (notably loss of epithelial lining) only when exposed to the first combination. This could be suggestive of an

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immunologically mediated hypersensitivity reaction. In this respect, a relationship between the miracidial secretion products of *S. haematobium* and the generation of bilharzia associated bladder cancer has been suggested in literature [4]. Our preliminary experimental data indicate that the organ culture model may serve as a valid system for further investigations on the role of such parasitic products either alone or with carcinogenic compounds in the development of bladder carcinoma.

We are grateful to Dr. D. Salim of University of Cairo, Egypt for the supply of *S. haematobium* eggs. The present work forms part of a recently started cooperation between the University of Amsterdam and Cairo Cancer Institute.

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"SELF CURE" IN RAT SCHISTOSOMIASIS - IS IT AN IMMUNOLOGICAL PHENOMENON?

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A dramatic spontaneous decline of worm burden of *Schistosoma mansoni* infected rats occurs between the 4th and 8th week after initial exposure [1]. The reason for this worm expulsion, called "self cure" phenomenon are poorly understood. Recently we reported that the schistosome grown in rats possess the ability to attach host antigens to its epidermis (coating phenomenon) as is the case with *S. mansoni* grown in mice and monkeys [2]. The present communication attempts to expand the study in order to find out whether or not the immune mechanisms function more competently in the rat than in man, hamster and mouse in dealing with this trematode infection. The studies have been carried out by observing infection patterns in rNu, rNu + rNu and Sprague Dawley rats (Spr) and relevant immunological parameters. The following has been observed:

1. Cercarial penetration in rNu, rNu is significantly higher than that + rNu and Spr.
2. Although the recovery of worms in the 4th and 6th week post infection is significantly higher in rNu, rNu than in the other two groups, spontaneous worm elimination in the former group of immune deficient rats nevertheless is markedly evident.
3. The capacity for acquisition and shedding of host proteins by worms recovered from all the group of rats, as demonstrated by the method described in [2], is similar.
4. In spite of the fact that antibodies present in the immune sera from + rNu and Spr are able to bind tegument components of the adult worms recovered from the hamster, such binding fails to kill the worms even in the presence of complement, whereas such immune sera do not bind schistosomula at all.
5. *Schistosoma* specific IgE antibody was demonstrated in + rNu and Spr, but not in rNu, rNu.
6. Pathological examination indicates that the intact parasite is contained within the liver and lung tissue of rNu, rNu without any killing or a focal reaction and eosinophil infiltration. Destruction of parasites in a focal reaction with the infiltration of mononuclear cells has been observed in + rNu rats.

The hypothesis is put forward that "self cure" mechanism in rat schistosomiasis is due to other factors in addition to immunological ones.

The technical assistance of the section of animal care of the Division of Microbiology and Pathology of the National Institute of Public Health has been highly appreciated. The present investigations were carried out in the framework of a recently started cooperation between the University of Amsterdam and the Cairo Cancer Institute.

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INTESTINAL PARASITES IN THE INHABITANTS OF A POOR QUARTER IN CARTAGENA, COLOMBIA

H.A.F.M. Jansen and E.A. Meuleman. *Laboratory of Medical Parasitology, Free University, Amsterdam, The Netherlands*

A single stool specimen was examined from each one of 104 persons who were representative for 32 families (counting in all 257 persons). These 32 families constituted a random sample of the population of San Pedro-Torices, a lower socio-economic quarter (4500 inhabitants; 560 families) in Cartagena.

Applied techniques were: direct microscopic preparation; Ridley's formol-ether concentration method; Baermann's concentration method; Fülleborn's charcoal culture method and Harada-Mori's test tube culture method.

Of the persons examined 82.7% appeared to be infected with a minimum of 1 and a maximum of 7 different parasitic species. The most frequently occurring species and their prevalence rates were: *Giardia lamblia* 21.2%, *Entamoeba histolytica* 13.5%, *Entamoeba coli* 31.7%, *Iodamoeba bütschlii* 9.6%, *Ascaris lumbricoides* 44.2%, *Trichuris trichiura* 64.4%, *Hymenolepis nana* 5.8%, *Necator americanus* 7.7% (which in the given circumstances is strikingly low!) and *Strongyloides stercoralis* 5.8%.

The true infection rates are undoubtedly higher, because not all cases will have been revealed due to: 1) limitations of the techniques; 2) fluctuations in the excretion of protozoa; 3) influence of self-medication.

The high prevalence rates are due to a high transmission level which appeared to be caused by poor personal hygiene and bad feeding habits (related to the low educational and economic level), as well as by deficiencies in the living conditions and public sanitary provisions, such as the lack of sufficient latrines, of an adequate and safe water supply, of a sewage system and of facilities for garbage disposal or regular garbage collection.

This study was facilitated by the co-operation existing between the Laboratory of Parasitology, University of Amsterdam (Prof. J.J. Laarman) and the Department of Microbiology and Parasitology, University of Cartagena (Prof. E. Rodriguez).

IN VITRO EXSHEATHMENT OF INFECTIVE HOOKWORM LARVAE OF ANCYLOSTOMA CANINUM

J.C.M. Klaver-Wesseling, J.C.M. Vetter, E.N. Schoeman and H.W. Wauters. *Laboratory of Parasitology, Royal Tropical Institute, Amsterdam, The Netherlands*

Exsheathment of the third stage is an important phenomenon in the life of parasitic nematodes. The stimulus for this process is mostly provided by the host.

It was shown, that the third stage larva of *A. caninum* exsheaths within 3 minutes when it is immersed in serum of dogs at 37°C.

When dog serum was passed through ultrafiltration membranes with different cut-off molecular weights, the exsheathment factor appeared to have a molecular weight of 5000-10000 Dalton. The factor was heat labile and susceptible to treatment with trichloroacetic acid.

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These results suggest that exsheathment of the third stage larva of *A. caninum* can be caused by a polypeptide with a molecular weight of 5000-10000 Dalton.

ESCAPE MECHANISM OF INFECTIVE ANCYLOSTOMA CANINUM LARVAE FROM HOST IgG, IgM AND COMPLEMENT FACTORS

R.S. Lee On, M.B.M. Teunissen and J.C.M. Vetter. *Laboratory of Parasitology, Royal Tropical Institute, Amsterdam, The Netherlands*

Previous experiments showed, that infective larvae of *Ancylostoma caninum* might be able to escape from their host immune system by shedding antibodies and complement from their surface.

The indirect fluorescent assay was applied to demonstrate IgM, IgG and complement factor C₃ on the surface of the larvae. The larvae were incubated in serum, that was obtained from dogs four weeks after subcutaneous infection.

With this technique, we found IgM, IgG and C₃ on the cuticle of infective larvae. However, they appeared to be able to shed both antibodies completely. Regarding to C₃, this process was only accomplished towards a part of the outer membrane. When the larvae were studied with a scanning Electron Microscope, we found that the remaining fluorescence was likely to come from lateral, longitudinal gutter-like structures. When sodiumazide, a known inhibitor of protein synthesis, was added to the testmedium, the larvae no longer succeeded in shedding these serum components.

These experiments are only a start, so much work is yet to be done before the exact evasion mechanism of these hookworm larvae from their host immune response is fully understood.

THE KILLING OF EXHAUSTED HOOKWORM LARVAE (ANCYLOSTOMA CANINUM) BY A FRACTION OF DOG LEUCOCYTES

J. de Vries, S. Cramer and J.C.M. Vetter. *Laboratory of Parasitology, Royal Tropical Institute, Amsterdam, The Netherlands*

It has been demonstrated, that when infective larvae of *A. caninum* are incubated in serum and leucocytes from immune dogs, a short period of adherence occurs. After this period of attachment the cells are shed from the surface.

Submission of the infective larvae for four weeks to a 20°C - 37°C temperature rhythm exhaust their energy resources, thus decreasing their ability of shedding. The effect of different leucocytes could be examined on these exhausted but living larvae.

In combination with serum of an immune dog, the total dog leucocytes population from either immune or normal dogs could adhere onto the larvae and kill them. When the leucocytes of both kind of dogs were fractionated in subpopulations by centrifugation on a Ficoll-Paque cushion, the pellet, containing mainly neutrophils, had no killing capacity. In contrast with this, the interface, which remained on the Ficoll-Paque cushion, had a considerable effect on the larvae coated with complement and/or antibody. It did not matter if these cells were obtained from a normal or an immune dog. Both interfaces contained eosinophils and mononuclear cells.

Further investigations will be done to determine which cell in the interface has the killing capacity.

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MIGRATIONS OF EOSINOPHILS TOWARDS INFECTIVE LARVAE OF *ANCYLOSTOMA CANINUM*

G. Peters, M. Teunissen, E. Schoeman and J. Vetter. *Laboratory of Parasitology, Royal Tropical Institute, Amsterdam, The Netherlands*

Infection of immune dogs with *Ancylostoma caninum* is attended with: 1) an increase of neutrophils in the parasitized tissue within several hours; 2) an increase of eosinophils in blood at the same time; 3) an increase of eosinophils in the parasitized tissue after one or two days.

It was suggested, that after the interaction of neutrophils with infective larvae a factor was released that was chemotactic for eosinophils.

A longterm adherence of about 24 hours, as it appears in the infected skin, could only be accomplished in vitro by inhibition of the worm's metabolism by low temperatures. This was established by using cryo-preserved larvae.

For the in vitro adherence of neutrophils to the surface of the larvae, antibody and/or complement were needed. Eosinophil migration with both factors present was more than was expected with each factor individually tested.

After this prolonged interplay, an eosinophil chemotactic factor (E.C.F.) was demonstrated with a modified Boyden-chamber in a culture medium (RPMI 1640) in which the eosinophil migration occurred.

Further investigations are carried out to determine the exact character of the factor.

THE EFFECTS OF PRODUCTS FROM LARVAL STAGES OF *TRICHOILHARZIA OCELLATA* ON UNINFECTED *LYMNAEA STAGNALIS*

J.J. Mellink and W. van den Bovenkamp. *Laboratory of Medical Parasitology, Free University, Amsterdam, The Netherlands*

It has been suggested that larval stages of *T. ocellata* inhibit the response of the targets of the reproductive hormones in *L. stagnalis* by humoral effects (Sluiters *et al.*, 1980). In order to demonstrate the production of such humoral factor(s), three experiments were carried out, with appropriate blanks and controls. In the 1st, juvenile snails were twice weekly injected with culture media incubated with *in vitro* transformed mother sporocysts (cf. Mellink and Van den Bovenkamp, 1982). Inhibition of snail reproductivity could not be demonstrated. In the 2nd, adult snails were similarly treated, but again inhibition of snail reproductivity did not occur. However, in the 3rd experiment, when juvenile snails were twice weekly injected with haemolymph from parasitized snails, snail oviposition tended to be delayed. The latter results are in agreement with aforementioned hypothesis. Moreover, snail reproductivity inhibiting factor(s) may not be produced *in vitro* as *in vivo*. If so, *in vitro* culture methods could be in need of further improvements for use as a research tool in this respect. Injections of adult snails with miracidia extracts accelerated snail oviposition.

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AN INVESTIGATION ON THE OCCURRENCE OF GASTRO-INTESTINAL HELMINTHS OF FREE-LIVING *COLUMBA LIVIA DOMESTICA*, *STREPTOPELIA TURTUR*, *STURNUS VULGARIS* AND *PASSER DOMESTICUS* IN ROTTERDAM ZOO

R. Koster, *Utrecht*

Thirteen *Columba livia domestica*, 10 *Streptopelia turtur*, 38 *Sturnus vulgaris* and 60 *Passer domesticus*

were examined. Results: In *C. livia domestica*, *Ascaridia columbae* and *Capillaria obsignata* were found. In 11 animals no parasites were seen. Four *Str. turtur* harboured *A. columbae*, *C. obsignata* and *Hymenolepis* sp. In 11 *S. vulgaris*, *Prosthynchus genitopapillatus* and *Porrocoecum ensicaudatum* were found. All *Passer domesticus* proved to be negative.

The maximum number of worms per animal was 3.

Prosthynchus genitopapillatus was first found in the Netherlands.

It is concluded, that the examined birds were not a factor in the occurrence of worminfections of the zoo-animals.

THE "OSTERTAGIA" GROUP (NEMATODA: TRICHOSTRONGYLIDAE) AND POLYMORPHIC SPECIES

J. Jansen. *Institute of Veterinary Parasitology and Parasitic Diseases, State University, Utrecht*

Indications and even some evidence is accumulating about the occurrence of polymorphic species in the "Ostertagia" group of the Trichostrongylidae. As it seems that different morphs of the same species belong to different genera there are reasons to argue that some genera have to fall into synonymy with others.

Examples of presumed polymorphism are the combinations *Ostertagia ostertagi* - *Skrjabinagia lyrata*, *Ostertagia leptospicularis* - *Skrjabinagia kolchida* and *Teladorsagia davtiani* - *Stadelmannia circumcincta* - *Stadelmannia trifurcata*. Reasoning along the same lines also the combinations *Spiculopteragia boehmi* - *Rinadia mathevossiani* and *Spiculopteragia asymmetrica* - *Apteragia quadrispiculata* can presumably be regarded, based on the more or less fixed distribution of numbers of the worms, as polymorphic species.

As a workable nomenclature is suggested to use the term morph: *Ostertagia ostertagi* m. *ostertagi*, *O. o. lyrata*, *O. leptospicularis* m. *leptospicularis*, *O. l. m. kolchida*, *Teladorsagia circumcincta* m. *circumcincta*, *T. c. m. trifurcata*, *T. c. m. davtiani*, *Spiculopteragia boehmi* m. *boehmi*, *S. b. m. mathevossiani*, *S. a. symmetrica* m. *asymmetrica*, *S. a. m. quadrispiculata*.

THE SPRING RISE PHENOMENON IN SHEEP AND THE TIME OF LAMBING

J. Jansen. *Institute of Veterinary Parasitology and Parasitic Diseases, State University, Utrecht*

The spring rise of faecal strongylid egg output occurs mainly in lactating ewes. As it was argued recently that the phenomenon was not related directly to parturition and lactation but resulted mainly from the egg production of worms which resumed development after a period of inhibition, more or less by coincidence at the same time as the lambing period of the ewes, Frisian milch sheep ewes lambing in the normal period, March, showing a normal spring rise were compared with ewes lambing at the end of May and the beginning of June.

In the older late lambing ewes a slight true spring rise in the spring was seen and a clear post-parturient rise in three of twelve animals whereas the late lambing yearling ewes all showed a significant rise during lactation in contrast to the barren yearling ewes which did not.

So it is clear that postponement of parturition means also in many instances postponement of the spring rise. The groups of ewes lambing outside the normal period and showing a spring rise during lactation were reinfected naturally during summer, whereas the other animals were not. This suggests that disturbance of the normal epidemiological cycle of trichostrongylid infections might interfere with a normal build up of immunity.

CYTOPHOTOMETRIC INVESTIGATIONS ON SEXUAL AND ASEXUAL MULTIPLICATION OF SPOROZOA

J.P. Overdulve and A.W.C.A. Cornelissen. N.B. Meeting at Nijmegen, 22 October 1982

In spite of their small size and unicellular character Sporozoa are true eukaryotes – just as their hosts are. Their nuclear DNA is presumed not circular but linear, not naked but enclosed within a nuclear envelope, bound to histones and spiralized into nucleosomes. Their DNA content hardly differs more from that of mammals than it does from that of the equally unicellular prokaryotic bacteria (cfr.: *E. coli*: 0.004 pg, *Isospora (T) gondii* (1c): 0.1 pg, mammals (1c): about 3 pg, equivalent to 4×10^6 , 10^8 and 27×10^8 bp, respectively). As a consequence Sporozoa, just like higher eukaryotes but in contrast to bacteria, exhibit extensive somatic cell differentiation. In Sporozoa, this cell differentiation, although mostly as little reversible as in higher eukaryotes, is not a "choice forever" finally leading to the cell's death, but part of a cyclic event, the life cycle. Increased knowledge of the genome and of nuclear events and mechanisms of selective gene expression in Sporozoa certainly will result in better understanding of phenomena such as host and host cell specificity, evasion of the host's immune response and premunity, and drug resistance, and may open new ways for blocking transmission and for control of the diseases caused by them.

There are 2 quantitative methods to achieve this goal: 1) molecular biology and biochemistry, 2) quantitative cytochemistry. The latter approach has the advantage over the former, that individual cells are studied instead of large quantities of cells. Thus, inaccuracies caused by cellular impurity (contamination with cells of other organisms, especially of the host; insufficient separation of different stages) and those inherent to classical cell counting (inhomogeneity; Poisson distribution) are avoided. However, it needs time, often expensive equipment, and above all extreme specificity and sensitivity of cytochemical reactions.

The so-called Feulgen staining reaction for DNA, if performed under appropriate conditions, meets the latter requirements. Therefore we have used it to measure the DNA content of all stages (except sporoblasts) in the life cycle of *Isospora (Toxoplasma) gondii* and of several stages of the related Sporozoa *Sarcocystis*, *Eimeria* and *Plasmodium*. Individual nuclei, after fixation, hydrolysis in 5N HCl at room temperature for 1 hour, and staining with pararosaniline (SO_2), were measured with an automated microfluorometer in 2 different ways: 1) by measuring the total amount of fluorescence emitted by individual nuclei during excitation with U.V. light (direct fluorometry), and 2) by scanning cytophotometry of microphotographic negatives of single nuclei, produced either by fluorescence photography or by absorption photography using green light ($\lambda = 560$ nm). Resulting histograms were analyzed with a computer program describing cell division.

Results and conclusions were as follows:

1. Absolute haploid (1c) amounts of DNA are: 99 fg in *I. (T.) gondii*, 76 fg in *Eimeria acervulina* and *E. tenella*, 27 fg in *Plasmodium berghei* and 216 fg in *Sarcocystis cruzi* or 90 , 70 , 25 and 197×10^6 bp respectively.
2. All stages of *I. (T.) gondii*, including sporozoites are haploid, except the zygote; hence meiosis occurs during sporulation. Combined with the results of infections in cats with clones derived from sporozoites or from later stages in the lifecycle (always both types of gametes formed) it proves that sexual dimorphism is a phenotypic cell differentiation of genetically identical cells, triggered by some final host factor.
3. The pre-gamontic merozoites M2 and M3 of *I. (T.) gondii* are already sexually differentiated, i.e. are in fact macro- and microgametoblasts, respectively. M2's are always strictly haploid but start already to form a nucleolus. M3's, on the contrary, never contain a nucleolus but start DNA replication immediately.
4. Fertilization in *I. (T.) gondii* does not take place until the macrogamete has grown to its maximum size.
5. Macrogametes and zygotes of *I. (T.) gondii* contain an extra amount of DNA of 8% above the haploid and 17% above the diploid value, respectively, possible the result of amplification of genes coding either for rRNA or for histones, not of so-called cryptomitotic nuclear division (Sinden, 1982). Therefore, we consider the gametes in which Sinden (1982) found evidence of mitotic activity to be in fact microgametes and not – as he believed – macrogametes.

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photography or by
are analyzed with a

acervulina and *E.*
25 and 197 × 10⁶ bp

ence meiosis occurs
ed from sporozoites
proves that sexual
gered by some final

ifferentiated, i.e. are in
but start already to
plication immedia-

its maximum size.
above the haploid
genes coding either
982). Therefore, we
be in fact microga-

Reference

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Parasitol 1982; 84: 1-11.