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SPRING MEETING OF THE NETHERLANDS SOCIETY FOR PARASITOLOGY

Combined postsession with the BION Working
Group on Parasitology, Nijmegen, March 19th, 1976

Dr.E.J.Ruitenbergh in the chair

1. H.J.BOS ~~and~~ A.A. van den EIJK ^{and H.J. van der KAAJ} (Laboratory of Parasitology, State
University, Leiden)

Serodiagnosis in amoebiasis

Serological methods are useful, and often necessary, tools in the diagnosis of extra-intestinal amoebiasis. Immunodiffusion (ID), in which precipitating antibodies are demonstrated according to the Ouchterlony technique, and the indirect fluorescent antibody test (IFAT), in which cultured axenic amoebae fixed on microscopic slides are used, have been applied routinely for several years in our laboratory. Both techniques, however, have some disadvantages: ID is time-consuming and IFAT is difficult to read objectively. Two new methods have been studied: the enzyme-linked immunosorbent assay (ELISA) in micro-plates and the counter immuno-electrophoresis (CIEP). Their usefulness for the serodiagnosis of amoebiasis will be demonstrated.

ELISA proved to be highly sensitive (Bos et al., 1975). Plates, coated with antigen, may be stored at -20°C until use and titers may be read visually or in a densitometer within one day. CIEP on cellulose acetate (Cellogel, Chemetron, Milaan) requires even less preparation and the test is completed within three hours. Sera from patients with liver amoebiasis (ID positive, IFA titers between 40 and 640) showed ELISA titers of 1,000 to 10,000 and between 2 and 9 precipitation lines in the CIEP. The few data available about sera from patients with intestinal forms of amoebiasis allow no definite conclusions yet, but ELISA titers tend to be lower than 1,000. Fifty percent of 26 asymptomatic Dutch carriers of Entamoeba histolytica tested, negative in IFAT and ID, had low ELISA titers, suggesting some sort of tissue-contact of amoebae in these persons. Healthy Dutch blood donors were negative in all four tests.

Bos, H.J., A.A.van den Eijk, and P.A.Steerenberg (1975). Trans.Roy. Soc.trop.Med.Hyg. 69, 440.

2. J.GOLENSER, J.P.VERHAVE, J.H.E.Th.MEUWISSEN and Miss J.HEEREN
(Laboratory of Parasitology, University of Nijmegen)

The influence of blood stages upon exo-erythrocytic forms (EEF) development in the rodent malaria.

Young (5-6 weeks old) and adult (3-4 months old) Wistar rats were inoculated intraperitoneally with 50×10^6 RBC parasitized by Plasmodium berghei. Peak parasitaemias of less than 10% and up to 50% were monitored for the adult and the young rats respectively. Control animals and infected animals received an intravenous inoculation of sporozoites at different stages of the parasitaemia (acute rise, peak, latency). Forty five hours later, the livers were removed for the estimation of the density of EEF.

In all experiments, densities of EEF in rats with a present or past parasitaemia were lower than in controls, always at a significant level. Rats heavily infected with 50/2% parasitized RBC could also prevent the development of the liver stages.

Primary parasitaemias especially caused considerable reduction of developing EEF during the rising and decreasing phases, when parasites were still observed in blood films. No EEF could be detected at all in 13 out of 18 young rats (72%) and in 17 out of 25 adult rats (68%). These results indicate that blood stages interfere with the development of EEF in liver parenchymal cells.

Two principal explanations are suggested for the results:

- a) the blood forms activate immune mechanisms cross reacting with preerythrocytic stages of the parasite
- b) the blood forms induce non-specific mechanisms interfering with the pre-erythrocytic stage.

3. M.E.M.VOSSEN, P.J.A.BECKERS and J.H.E.Th.MEUWISSEN (Laboratory of Parasitology and Institute of Electronmicroscopy, University of Nijmegen)

Electronmicroscopic studies on Pneumocystis carinii in rats treated with corticosteroids

Naturally infected Wistar rats, 6 weeks old, are treated with cortisone-acetate. After 5 weeks the lungs are fixed in situ with glutaraldehyde

2% by instillation according to Weibel. The most interesting results of the investigation will be presented.

-The occurrence of spindle figures in thin-walled organisms proves that these stages actually divide. This would explain the high ratio between the numbers of thin-walled and thick-walled stages.

-In the precyst of the parasite 2 parallel membranes showing the unitstructure become apparent; they seem to demarcate several nuclear masses surrounded by some cytoplasm. This might indicate how intracystic bodies are formed.

-Tubular elements, 200-260 A in diameter, have been found just below the innermembrane of the wall of the intracystic bodies.

4. J.BUYS and E.J.RUITENBERG (National Institute of Public Health, Bilthoven)

Application of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of sleeping sickness in man

An Enzyme-Linked Immunosorbent Assay (ELISA) was presented for the serodiagnosis of human sleeping sickness. A crude extract of a Trypanosoma brucei suspension which was purified from all blood components was used as antigen. In rabbits experimentally infected with T.brucei or T.rhodesiense both homologous (anti T.brucei) and heterologous (anti T.rhodesiense) trypanosoma antibodies could be detected with ELISA using T.brucei as antigen. The sensitivity of ELISA was comparable with that of the immunofluorescence (IF). Human sera of patients with sleeping sickness were examined with ELISA and IF. Both methods yielded comparable results. Cross reactions were only observed with a serum from a patient in which to Leishmania were detected. No cross reactions were observed in sera of patients with malaria, toxoplasmosis, schistosomiasis and echinococcosis.

ELISA represents a good alternative for immunofluorescence in the serology of african trypanosomiasis and may be particularly suitable for mass screening purposes.

5. A.M.HAGENAARS, F.van KNAPEN and J.H.FRANCHIMONT (National Institute of Public Health, Bilthoven)

Preparation of enzyme-labeled antibodies (for ELISA)

antibodies

For labeling enzymes to antibodies (conjugation) one can choose different methods. Most frequently used are the methods of Nakane and Avrameas.

The Nakane method delivers a high molecular conjugate which gives good results in a performance test.

We used the conjugate for examination on T.spiralis antibodies in slaughter pigs. For quality control of conjugates a performance test is needed besides extinction measurements, enzyme-activity measurements and antibody-activity measurements.

6.F.LEENSTRA, A.ELGERSMA and E.J.RUITENBERG (National Institute of Public Health, Bilthoven)

Immunopathological studies of the intestinal tract in helminth infections.

Recently, Ferguson and Jarrett (Gut 16 (1975) 114) suggested nonspecific histopathological gut changes to be thymus-dependent. This hypothesis was tested in two helminth infections: the nematode Trichinella spiralis and the cestode Hymenolepis diminuta. Studies were performed in congenitally athymic (nu/nu) mice and their thymus-bearing heterozygous littermates (+/nu).

The non-specific histopathological changes were judged on villus/crypt ratio and mitosis index; for the specific (= immunological) reaction the number of plasma cells was used as criterion.

T.spiralis induced non-specific histopathological reactions both in nu/nu and +/nu mice, whereas plasma cells were only induced in the +/nu mice. H.diminuta did not induce non-specific histopathological changes, whereas both in +/nu and nu/nu mice plasma cells were formed. Formation of plasma cells to both helminths could be correlated with serum antibody production.

In conclusion the immunological reaction was shown to be dependent on the immune status of the host, whereas the non-specific histopathological changes were not, i.e. they were thymus-independent.

7. A.M.DEELDER (Laboratory of Parasitology, University of Leyden)

Circulating antigens and antigen-antibody complexes in Schistosoma mansoni infections

Previous investigations by others have demonstrated the presence of a schistosome derived antigen in the sera of mice and hamsters heavily infected with Schistosoma mansoni. Our investigations showed the presence of two circulating schistosome derived antigens, probably both polysaccharides, in heavily infected hamsters.

One antigen was an anodic high molecular weight substance, while the second antigen was a cathodic low molecular weight substance. Both antigens could be demonstrated in ~~hamster serum~~, while the cathodic antigen could be found also in hamster urine.

The anodic antigen could be demonstrated in the epithelial cells of the schistosome gut. Two schistosome-derived heat-labile antigens, probably both proteins, could be demonstrated in hamster urine. Circulating antigen-antibody complexes in mice sera could be shown by means of counterimmunoelectrophoresis.

Further research will primarily be concerned with the development of sensitive assays for the demonstration of circulating antigens in human S.mansoni infections.

8. J.P.ROTMANS (Laboratory of Parasitology, University of Leyden)

Characterization of enzymes present in the gut of Schistosoma mansoni

Since schistosomes have to regurgitate, the enzymes present in the gut of Schistosoma mansoni worms, will get into the circulation of the host. These enzymes, present in the worm's vomit, probably elicit a high immunological response. Our aim is to isolate and characterize these enzymes, since purified antigens may yield better results in immuno-diagnostic tests (e.g. DASS and ELISA), than a more complex crude "whole worm" antigen preparation. One can expect a decrease in the degree of cross-reactivity with other helminth parasites, and possibly between the various species of Schistosoma. Moreover certain antigens from the worm's gut might be useful in the diagnosis of infections in an early stage.

From collected living Schistosoma mansoni worms, vomit is obtained

by exposure to a cold-shock. The collected vomit is freed from insoluble material, desalted and lyophilized. This crude preparation is tested for several enzyme activities, both in solution and after immuno-electrophoresis. A high acid phosphatase activity could be detected. Furthermore a malate dehydrogenase, a lactate dehydrogenase and a protease were present. Immuno-electrophoresis against mouse antiserum reveals up to 7 precipitation arcs, the identification of which is under way.

For isolation of the various enzymes, especially affinity chromatography seems suitable. Coupling of purified antigens to agarose beads, will enable the isolation of specific antibodies from antisera. These antibodies might be used in immunodiagnosis of circulating antigens, and to study the cellular- and subcellular localization of enzymatically active antigens in the parasite.

9. D.M.LYARUU, A.BIJ DE VAATE, C.P.H.GAASENBEEK, H.J.OVER and J.F.SLUITERS
(A cooperative research between Lab.Med.Par. Free University, Amsterdam,
C.D.I., Dep.Par., Lelystad and IJsselmeerpolders Dev.Auth., Lelystad)

Transmission ecology of schistosome dermatitis in the Netherlands

From two ditches known for the occurrence of schistosome dermatitis data concerning the transmission of the disease and the ecology of the snail intermediate hosts were collected.

In 1975 on a weekly (later fortnightly) basis, at fixed sampling points on standard plots, snails were collected and subsequently shed for cercariae, measured and returned to the original place. During the observation period Lymnaea stagnalis, L.ovata, Planorbis corneus, P. planorbis and Valvata piscinalis were the dominant species. Only in L.stagnalis, L.ovata and P.corneus were ocellate furcocercariae observed.

In relation to population density the infection rate was low, L.stagnalis being by far the most important reservoir.

As a result of the analysis of snail population dynamics it might be concluded that large L.stagnalis shedding cercariae in spring and summer must have been infected the previous year and that snails shedding cercariae in late summer were infected in spring.

The last mentioned group consisted of smaller snails and cercarial production was on a lower level. Obviously the first group makes the main contribution to the occurrence of swimmer's itch.

10. J.F.SLUITERS and A.KHAN (Laboratory of Medical Parasitology, Free University, Amsterdam)

Influence of infection with Trichobilharzia ocellata on the development of the reproductive tract of Lymnaea stagnalis

Trichobilharzia ocellata infection of juvenile Lymnaea stagnalis causes a temporary acceleration in the growth rate and has an inhibitory effect on the fecundity of the host. Dissection after 5 months shows a poor development of the female and male accessory sex glands (Sluiters et al., in press). In order to find when the parasite exercises this influence, a time-sequence study was carried out with juvenile snails exposed to 4 miracidia with appropriate controls. Groups of 10 snails were sacrificed on day 12, 19, 33 and 83 post exposure. Although on day 12 no growth rate changes could be observed, a clear inhibitory effect on the development of both the female and male part of the reproductive tract is already seen macroscopically at dissection. Obviously this is brought about by mother sporocysts in the heat-foot region of the snail. Histological examination of the ovotestis and accessory glands is currently in progress.

Sluiters, J.F., C.C.M.Brussaard-Wüst and E.A.Meuleman. Proc. IInd Eur.Multicoll.Parasitol. Trogir, 1975. In press.

11. A.A.ABRAHAMS and E.A.MEULEMAN (Laboratory of Medical Parasitology, Free University, Amsterdam)

Infection with Schistosoma mansoni in Biomphalaria pfeifferi of different sizes.

To find out whether the age of the host affects the infection rate, two experiments were carried out, in which snails of 2, 5, 9 and 12 mm were individually exposed to 1 and 20 miracidia, respectively. In the first experiment no differences in behaviour, and sites and frequencies of attachment of the miracidia were observed. Also no clear differences in penetration frequencies were found between the first three groups, although a tendency to higher penetration numbers existed in the 5 mm group. No penetration was observed in snails of the 12 mm group. Development after penetration, as established by cercarial shedding, was equally high in the first three groups (88% in total).

In the second experiment 4 randomly selected snails per group, which had been fixed for light microscopy 1 week after exposure, were studied for the presence of developing and degenerating mother sporocysts. The findings roughly confirmed those concerning penetration and development of the first experiment.

12. R.F.E. DE WIT and J.E.M.H. VAN BRONSWIJK (Department of Dermatology, State University Utrecht, The Netherlands)

The harvest mite, Neotrombicula autumnalis (Shaw 1790) (Trombiculidae; Acari)

In Western Europe the eight legged adult mites (velvet mites) live predatory on small insects in the soil, where they also hibernate. From June to September the eggs are laid. After hatching, the six legged larva (harvest mite) feeds for a few days on a vertebrate host. The harvest mite is widely distributed in Western Europe and the British Isles. The larva of Neotrombicula autumnalis is not host specific; it has been found on mice, voles, cattle, sheep, horses, birds, reptiles and even man. On different hosts it has different praedelection sites: on voles the ears are attacked, in man attachment of the larvae is particularly seen where clothing is tight.

The histological reaction of the skin of several hosts is quite similar, and consists of a stylostome with a more or less dense cellular infiltrate.

The clinical symptoms of man may vary from hardly recognisable red spots to itching furuncle-like lesions, forming yet a rather specific picture that for unknown reasons is very often misdiagnosed by general practitioners.

13. J.L.GEROLD (Laboratory of Parasitology, University of Leyden)

The house-leaving behaviour of mosquitoes investigated in the laboratory.

With the twin-funnel test differences of house-leaving habits have been established between anopheline species, sub-species and selected strains. However, Anopheles sacharovi collected in Turkey gives rise to the expectation that still other characteristics than those measured in the

twin-funnel test are underlying to the house-leaving behaviour. We studied therefore modifications of the twin-funnel test and evidence is found for additional characteristics which would be of importance to the house-leaving behaviour:

1. Under condition that neither air-currents nor other attractantia are present, anopheline mosquitoes find an escape port in a plane exclusively by chance and no evidence is found for an active search of the plane.
2. Anopheles atroparvus strains AN and BN stay 50 percent of the period of activity in the lower 1/3 part of the test cage and 20 percent of the period of activity in the upper 1/3 part of the test cage. This can be influenced by illumination from above but scarcely by illumination from below.
3. After irritation by DDT empty first day females will be attracted by light. This phototropy can be evaluated at individual mosquitoes by measuring the preference of a mosquito to one of two differently illuminated surfaces. The value obtained varies with varying size of the escape port. This can be explained by the accompanying varying of the distance of the escape port to the less illuminated surface.
4. No adaptation of the attraction to light is observed during 21-minutes lasting test periods.

14. J.J.MELLINK and A.E.M.GILLIÉRON (Laboratory of Medical Parasitology, Free University, Amsterdam)

Onchocercosis in the area of the Kainji Simulium Control Scheme (Nigeria)

The prevalence of onchocercosis in the area under review was assessed by a survey encompassing virtually the entire populations of about 30 villages (ca 3,000 subjects) chiefly within the inner perimeter of the area after 5 years of extensive control operations during which on the average an appraised 90% reduction of the vector species population(s) was achieved. The results of the skin biopsies were evaluated by methods as presented by Knüttgen and Büttner (1969), which were applied to nodule and blindness rates as well. Detailed analysis resulted in the discernment of 3-4 parts of differing (high) endemicity.

Notwithstanding the limitations posed by the lack of comparable pre-control figures, a definite effect of the measures taken on the incidence

[project

[This was

of onchocercosis in the area could be demonstrated, although to a lesser extent than anticipated. The degree of protection secured for the immigrant population - the main purpose of the control project - must have been satisfactory, but certainly not absolute.

Knüttgen, H.J. and Büttner, D.W., Z. Tropenmed.Parasitol., 20 (1969) 303.