

NEDERLANDSE VERENIGING  
VOOR PARASITOLOGIE

NETHERLANDS SOCIETY  
FOR PARASITOLOGY

Secretariaat: Lab. Medische Parasitologie  
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Amsterdam, 13 september 1976.

Aan de leden van de Nederlandse Vereniging voor Parasitologie,  
Aan de Project- en Werkgroepen van de BION Werkgemeenschap Parasitologie,

Namens de besturen van Vereniging en Werkgemeenschap nodigen wij u van harte uit tot het bijwonen van de jubileumvergadering welke op donderdagavond 30 september en vrijdag 1 oktober gehouden zal worden in het Hoofdgebouw van de Vrije Universiteit, de Boelelaan 1105 te Amsterdam. Voor de ligging van het gebouw, de parkeerterreinen en de verbindingen per openbaar vervoer, verwijzen wij u naar bijgevoegd kaartje.

De wetenschappelijke activiteiten zijn ondergebracht in vier secties, die achtereenvolgens worden voorgezeten door één van de vier N.V.P. voorzitters uit heden en verleden.

Het programma luidt als volgt:

30 September

Vanaf 19.30 uur: Ontvangst met koffie bij zaal 1A-05

20.00 - 21.50 uur: Sectie 1

20.00 - 20.40: J.D. Smyth: Problems of the in vitro cultivation of Echino-  
coccus

20.40 - 20.50: Discussie

20.50 - 21.10: F. van Knapen: Human Hydatidosis in the Netherlands

21.10 - 21.20: Discussie

21.20 - 21.40: W. Tadros en J.J. Laarman: Epidemiology and epizootiology of  
Sarcocystis and related cystforming coccidia

21.40 - 21.50: Discussie

Vanaf 21.50 uur lustrumborrel

1 October

Vanaf 9.00 uur koffie bij zaal 6A-00

9.30 - 10.50 uur: Sectie 2

9.30 - 9.45: H.J. Bos en R.J. v.d. Griend: Cell surface characteristics of  
Entamoeba histolytica probably related to virulence

9.45 - 9.50: Discussie



- 9.50 - 10.05: F.R. Opperdoes: Intracellular localization of glycerol-3-phosphate oxidase in Trypanosoma brucei  
 10.05 - 10.10: Discussie  
 10.10 - 10.25: B.A.J. Cohen, B.J. Looij en M. Wittner: Certain aspects of the in vitro cultivation of Trypanosoma cruzi  
 10.25 - 10.30: Discussie  
 10.30 - 10.45: H. Kuil: In vitro cultivation of Eimeria  
 10.45 - 10.50: Discussie

koffiepauze

- 11.15 - 12.35: Sectie 3  
 11.15 - 11.30: J.E.M.H. van Bronswijk en R.H. Rijntjes: Dutch ticks  
 11.30 - 11.35: Discussie  
 11.35 - 11.50: J. Jansen: Haemoglobin types and susceptibility to trichostrongylids of different breeds of sheep  
 11.50 - 11.55: Discussie  
 11.55 - 12.10: J.F. Sluiters: Endocrinological aspects of a parasite - host relationship: Trichobilharzia ocellata - Lymnaea stagnalis  
 12.10 - 12.15: Discussie  
 12.15 - 12.30: H.J. v.d. Kaay, J.Ph.W. Noordink en L. Boorsma: Efforts to label Plasmodium berghei in Anopheles atroparvus  
 12.30 - 12.35: Discussie

Vanaf 12.45 uur lustrumlunch

- 14.00 - 16.45 : Sectie 4  
 14.00 - 14.15: H. de Roever-Bonnet: Why did the patient die?  
 14.15 - 14.20: Discussie  
 14.20 - 14.35: J. Golenser, J.H.E.Th. Meuwissen, J. Heeren, L. James, H. v.d. Kaay: Indirect fluorescent antibody (IFA) titres against sporozoites, parasitized erythrocytes and exoerythrocytic forms of P. berghei  
 14.35 - 14.40: Discussie  
 14.40 - 14.55: A. van Zon en W. Eling: Antimalarial immunity and pregnancy  
 14.55 - 15.00: Discussie  
 15.00 - 15.15: J.H.E.Th. Meuwissen, I. Tauber, A.D.E.M. Leeuwenberg, P.J.M. Beckers, M. Sieben: Parasitological and serological observations on Pneumocystis infections in man  
 15.15 - 15.20: Discussie

15.20 - 15.45 uur: theepauze

- 15.45 - 16.00: J.C.M. Vetter en J.C.M. Klaver-Wesseling: Indirect Fluorescent Antibody Studies of Hookworm Infections  
 16.00 - 16.05: Discussie  
 16.05 - 16.20: E.J. Ruitenbergh: Immunological aspects of Trichinella spiralis infections in congenitally athymic (nude) mice  
 16.20 - 16.25: Discussie  
 16.25 - 16.40: J.P. Rotmans en A.M. Deelder: Purification of Schistosoma mansoni enzymes and the possible use in immunodiagnostic tests  
 16.40 - 16.45: Discussie

Vanaf 16.45 uur: informeel samenzijn

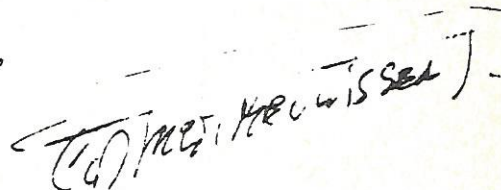
Dank zij financiële steun van Merck, Sharp en Dohme kunnen lunch en andere consumpties U gratis worden aangeboden. Wel is het noodzakelijk dat U zich voor de lunch opgeeft. Dit kan tot uiterlijk 27 september geschieden door bijgaand strookje ingevuld op te sturen naar het secretariaat van de N.V.P.

In de hoop, dat U op beide dagen aanwezig zult zijn - introducé(e)s zijn van harte welkom - verblijven wij,

hoogachtend,



dr E.A. Meuleman  
secretaris N.V.P.



dr J.H.E.Th. Meuwissen,  
secretaris BION Wgm  
Parasitologie

Bijlagen



AUTUMN MEETING OF THE NETHERLANDS SOCIETY FOR  
PARASITOLOGY

Combined session with the BION Working Group  
on Parasitology, Amsterdam, September 30th -  
October 1st, 1976

Dr.H.J.v.d.Kaay, in the chair

Communications

1. J.D.SMYTH (Dept.of Zoology, Imperial College, London, S.W.7)

Problems of the in vitro cultivation of Echinococcus

The protoscoleces of Echinococcus are unusual in being able to differentiate in either a cystic or strobilar direction depending on the environmental conditions provided. Over a period of years, a technique for the in vitro cultivation of the strobilar stage has been developed. Basically this consists of treating brood capsules with pepsin to free the protoscoleces, evaginating them and culturing them in a diphasic medium with a solid phase of coagulated bovine serum and a liquid phase of Parker 858 plus foetal calf serum supplemented with glucose and yeast extract.

Full technical details have been published (Smyth & Davies, 1974)\*.

Protoscoleces cultured in this system develop to 3-5 proglottid sexually mature adults, but histological examination has revealed that insemination does not take place, the receptaculum seminis is empty and the uterus contains only unfertilized ova. Numerous attempts to induce insemination in vitro have so far failed. The results mean, however, that a 'safe' (i.e. non-infective) adult Echinococcus can be grown in vitro and this is useful for physiological and cytological studies.

The above experiments were carried out with E.granulosus of sheep origin. When, however, the identical culture procedures were used with E.granulosus of horse origin, protoscoleces failed even to segment in vitro. Attempts to induce growth and segmentation by varying factors such as  $pCO_2$ ,  $pO_2$ , Eh, use of different serum samples, addition of growth substances etc. have so far been unsuccessful. It is concluded that the horse 'strain' of E.granulosus has different nutritional and/or physiological requirements from the sheep strain and that these are not provided by the culture system used. The implications of this will be discussed.

Unexpected results were also obtained when protoscoleces of Echinococcus multilocularis were cultured in vitro. In contrast to the results with E.



granulosus, many protoscoleces developed into (a) 'monozoic' adults i.e. sexually mature, unsegmented forms or (b) pseudosegmented forms. The problems raised by these results will be discussed.

\* Int.J.Parasit. 4: 631-644 In vitro culture of the strobilar stage of Echinococcus granulosus (sheep strain) : a review of basic problems and results. J.D.Smyth and Zena Davies.

2. F. van KNAPEN (Rijks Instituut voor de Volksgezondheid, P.O.Box 1, Bilthoven)

Human Hydatidosis in the Netherlands

Serodiagnosis of human hydatidosis in the Netherlands is performed by means of immunofluorescence and complement fixation tests. Sera of 1583 suspected individuals were examined in the years 1971 to 1975. For evaluation of results only those cases (62 patients, 74 sera) were used in which the diagnosis of hydatidosis had been confirmed by surgery and/or pathology. Of these patients 46 (74%) were foreigner, most of them originating from mediterranean countries. Eight cases (13%) were Dutch people who had been abroad for a certain period. The remaining 8 cases (13 %) were Dutch residents, who had never been abroad. Of the latter cases, 6 were over 60 years, and one of them was younger than 30 years. Together with the knowledge that 0,2% of Dutch cattle is infected at slaughter it was concluded that the life cycle of E.granulosus is, although rare, still existing in the Netherlands.

3. WEDAD TADROS and J.J.LAARMAN (Laboratory of Parasitology, University of Amsterdam)

Epidemiology and epizootiology of Sarcocystis and related cystforming coccidia.

Observations were presented on the incidence of different Sarcocystis species in cattle, sheep, domestic and wild swine as well as rabbits and different rodents in the Netherlands. The epidemiology and epizootiology of muscular sarcosporidiosis and Sarcocystis-induced intestinal coccidiosis were discussed in the light of the implications of 1) the obligatorily hetero-xenous life cycle of Sarcocystis and Frenkelia, 2) the predator-prey relationship of the final and intermediate hosts, 3) the ecological and territorial parameters of the intermediate and definitive hosts, 4) the internal maturation of the coccidian oocyst, 5) the prolonged patency of excretion by the final host, 6) the apparent lack of protective immunity in the final host,



7) the stimulus inducing excystation of the sporocysts, 8) the host-specificity of the asexual and sexual stages of the parasite.

Bovine Sarcocystis was pinpointed as the main source of human Sarcocystis-induced coccidiosis. Data were presented of the parasitological and serological investigation of representative samples of the Dutch population. Speculations were made on the source of muscular sarcosporidiosis in primates, including man, with special reference to the recent finding by the authors of mature diasporic, tetrazoic isosporan oocysts together with muscular sarcocysts in the skeletal muscle of a Cercopithecus aethiops. The influence of the migratory habit of the buzzard on the incidence of Frenkelia-infection in bank voles in the breeding and overwintering territories of the avian definitive host were discussed. Comparisons were made with the conventional monoxenous coccidia as well as with Toxoplasma and Besnoitia, both facultatively heteroxenous parasites.

4. H.J.BOS and R.J.van de GRIEND (Laboratory of Parasitology, University of Leiden)

Cell surface characteristics of Entamoeba histolytica probably related to virulence.

The ability of cultured strains of E.histolytica to cause ulcers in the cecum of guinea pigs and rats or necrosis in the liver of the Syrian hamster decreases rapidly upon the elimination of the accompanying bacterial flora. One strain, HK-9, seemed to have lost its pathogenicity entirely, whereas the extent of necrosis caused in hamster liver by other axenic strains depended on the size of the inoculum. The pathogenicity of the HK-9 strain became manifest again after addition of extra cholesterol to the medium. Its virulence could be raised remarkably by two subsequent passages through a sensitive host (hamster). The resulting HK-9H<sub>2</sub> strain differed from the original HK-9 strain in at least three aspects:

1) toxicity to guinea pig leucocytes, 2) concanavalin A induced agglutination, and 3) antigenic composition, as revealed by immuno-electrophoresis. A quantitative model has been developed for measuring toxicity, a characteristic that is based apparently on contact-dependent lysis. Evidence exists indicating that properties of the plasma membrane, such as surface charge, may be involved in this phenomenon. ConA induced agglutination is known to depend on the mobility of membrane-associated receptors.

A clue to understanding the pathogenic mechanism of the amoeba may lie in the study of how its toxicity helps to overcome the cellular reactions in the host.



5. F.R. OPFERDOES (Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam)

Intracellular localization of glycerol-3-phosphate oxidase in Trypanosoma brucei.

In bloodstream trypanosomes glycerol-3-phosphate oxidase (GPO) is thought to be localized in the microbodies (1). Cell fractionation experiments are hampered by the fact that mitochondrial and microbody activities are completely suppressed. Therefore, Trypanosoma brucei was systematically screened for unequivocal marker enzymes for the different cell organelles. We found oligomycin-sensitive ATPase and  $\text{NAD}^+$ -linked glycerol-3-phosphate dehydrogenase as markers for mitochondria and microbodies, respectively. In cell fractionation GPO, malate dehydrogenase, isocitrate dehydrogenase and oligomycin-sensitive ATPase behaved similarly. The activity profiles on isopycnic sucrose gradients were identical with maximal activity at a density of  $1.17 \text{ g/cm}^3$ . Examination by electron microscopy of a fraction from this region of the gradient only showed mitochondria. 80% of the  $\text{NAD}^+$ -linked glycerol-3-phosphate dehydrogenase was particle-bound and banded at  $1.23 \text{ g/cm}^3$  in sucrose. This fraction of the gradient contained the microbodies (as judged by electron microscopy). The specific activity of GPO was less than 3% of that in the mitochondrial fraction. We conclude that in T. brucei GPO is located in mitochondria and not in microbodies.

(1) Müller, M. (1975) Ann. Rev. Microbiol. 29, 467-483.

6. B.A.J. COHEN and B.J. LOOIJ (Dept. of Med. Microbiology, Erasmus University Rotterdam) M. WITTNER (Dept. of Pathology and Parasitology, Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y. U.S.A.)

Certain aspects of the in vitro cultivation of Trypanosoma cruzi

Attempts have been made to chemically define the growth promoting constituents of Bactotryptose in Boné's T-2 medium (1). It was established after dialysis and ultrafiltration, that Bactotryptose may be replaced by its fractions with Molecular Weights of 500 and less. Chemical analysis of these fractions and their subsequent replacement with a mixture of chemicals as indicated by the analysis results, did not lead to a fully defined medium for T. cruzi. The growth promoting constituents of Bactotryptose, albeit undefined, appear to have relatively simple structures, in view of their Molecular Weights.



5-Fluorocytosine was found to be an effective fungicidal additive without impairing ITMAP-943, Maryland, Tehuantepec or Tulahuen strains of T. cruzi in T-2, GLSH and GLSH-SF (2) media. This observation was confirmed for cultures of thymidine kinase deficient Chinese hamster cells (3) in Ham's F-10 medium with fetal calf serum and antibiotic supplements (4). Miconazole proved to be toxic for the used strains of T. cruzi in such low concentrations. (5 ug/ml), that in vivo experiments seem to be warranted.

#### References

1. Boné, G.J. & Parent, G. (1963) - J. Gen. Microbiol. 31, 261-266
2. Jadin, J. & Pierreux, G. (1960) - Ann. Soc. belge Méd. Trop. XL, 6, 903-906
3. Westerveld et al (1971) - Nature New Biol. 234, 20-24
4. Bootsma D & Westerveld, A. (1975) - Personal communications

7. H. KUIL (Institute for Tropical and Protozoan Diseases, Utrecht)

#### In vitro cultivation of Eimeria

A short review of the work on the growth of Eimeria in cell cultures is given.

Own experiences with the cultivation of Eimeria tenella of the fowl are reported, concentrating on the following aspects :

- type of cells used and initiating of the cell culture
- incubating conditions
- stages of the parasites used to infect the cell culture and preparation of these stages
- development of the various stages of the parasite in cultured cells

Applications of the in vitro cultivation of Eimeria are discussed briefly, such as:

- studies on the morphology and biology of the parasites growing without the influence of the host
- studies on the effect of the parasite on the host cell
- assessing the viability of parasite
- testing of drugs on anticoccidial activity
- studies on the action of anticoccidial drugs
- immunological studies on Eimeria grown in vitro.



8. J.E.M.H. van BRONSWIJK and R.H. RIJNTJES (Laboratorium voor Minibiologie, Dermatologisch Instituut, Rijksuniversiteit Utrecht)

#### Dutch ticks

In 1964 the first report of a tick in the Netherlands was published. In the neck of a boy a dogtick was found (probably Ixodes ricinus). More case reports emerged in the following centuries, but no systematic investigation has been reported. The scanty data indicate that at least the following species are endemic here : Ixodes ricinus (L. 1758), I. hexagonus Leach 1815, I. trianguliceps Birula 1895, I. vespertilionis Koch 1844, Argas vespertilionis Latreille 1802 and Haemaphysalis punctata Canestrini & Fanzago 1877.

At the veterinary faculty of the State University of Utrecht Babesiasis of cattle was studied intensively (Wilson & Perié 1965, Adamu 1974) but the study of the ecology of the vector ticks was almost ignored. Dutch dermatologists attributed prurigo, lymphocytoma, erythema chronicum migrans and acrodermatitis chronica atrophicans to tick bites (L.H. Jansen 1975), but until very recently this did not induce further studies on the Dutch Ixodoidea.

Foreign ticks are probably imported regularly. J. Jansen (1975) reported this in case of Rhipicephalus sanguineus (Latreille 1806). This species, together with some of our endemic ticks, are vectors for encephalitis virus in e.g. Scandinavia, Germany, Czechoslovakia, Austria and Switzerland (Radda 1973). From the Netherlands human tick-borne-encephalitis has not (yet ?) been reported. Recently we were able to start a survey on the Dutch ticks and their host relationships.

#### References:

- Adamu, N.M. 1974. The seasonal incidence of ixodid ticks of cattle in Northern Nigeria and in The Netherlands, with particular reference to their role in the transmission of bovine piroplasms. Thesis, Ahmadu Bello University.
- Jansen, J. 1975. Entomol. Ber. 35 : 1-3.
- Jansen, L.H. 1975. Huid- en geslachtsziekten. Utrecht (Oosthoek, Scheltema & Holkema).
- Radda, A. 1973. Zeitschr. Angew. Zool. 60 : 409-461.
- Wilson, S.F. & N.M. Perié 1965. Tijdschr. Diergeneesk. 90: 1443-1459.



9. J.JANSEN (Institute of Veterinary Parasitology and Parasitic Diseases, State University, Utrecht)

Haemoglobin types and susceptibility to trichostrongylids of different breeds of sheep

As pointed out earlier (Jansen, Summ. 2d Europ.Multicoll.Parasitol., 84, 1975) Frisian milch-sheep are far more susceptible to trichostrongylids and trichostrongylosis than Texel sheep.

In the literature (e.g. Evans et al., Austral.J.Agric.Res., 14, 549, 1963; Bradley et al., Amer.J.Vet.Res., 34, 729, 1973) susceptibility to trichostrongylosis is correlated with the haemoglobin types of the sheep in such a way that sheep with type AA are the most resistant, with type AB are less resistant and with type BB are the most susceptible.

Sixty-seven Frisian sheep from our flock had 34% AA, 49% AB and 17% BB whereas 75 Texel sheep had 0% AA, 11% AB and 89% BB as their haemoglobin types.

Therefore the said correlation between susceptibility to trichostrongylids and trichostrongylosis and haemoglobin types in sheep is questionable.

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

Endocrinological aspects of a parasite - host relationship :

Trichobilharzia ocellata - Lymnaea stagnalis

In the central nervous system of the hermaphrodite snail Lymnaea stagnalis various groups of neurosecretory cells and two pairs of endocrine organs can be distinguished. On the base of extirpation and implantation experiments the male and female activity of the snail is thought to be controlled by different centra. Furthermore the production of a growth hormone has been demonstrated (Geraerts, 1975). Exposure of juvenile Lymnaea stagnalis to miracidia of Trichobilharzia ocellata results in a temporary acceleration of the growth rate and also inhibits the development of the reproductive tract of the host. This "parasitic castration" is brought about by the mother-sporocyst in the head-foot region of the snail (Sluiters and Khan, in press). Parasitic infection and extirpation experiments affect the snail in much the same way, suggesting an influence of the parasite via the centres concerned. However certain differences appear to indicate other mechanisms.

References :

Geraerts, W.P.M. Thesis, Free University, Amsterdam 1975.

Sluiters, J.F. and A.Khan, Trop.Geogr.Med. In press.



11. H.J.van der KAAY<sup>1</sup>, L.BOORSMA<sup>1</sup> and J.Ph.W.NOORDINK<sup>2</sup>  
(<sup>1</sup>Lab.for Parasitology, University of Leiden, <sup>2</sup>Institute for Plant  
Protection Research (I.P.O.), Wageningen)

Efforts to label Plasmodium berghei in Anopheles atroparvus

True relapses in malaria infections may be caused by either "secondary" exoerythrocytic forms of the parasite in the liver or, as more recently suggested by Garnham (1967), by dormant sporozoites. The latter remaining latent for some time in parenchymal cells of the liver prior to undergoing full development. A labelled parasite could be valuable in tracing and subsequent study of early exoerythrocytic development and or study of dormant stages in the vertebrate host. Various approaches to label Plasmodium berghei during the sporogonic cycle in Anopheles atroparvus are being discussed.

A succesful marking of Plasmodium berghei was obtained using adenine-<sup>14</sup>C, producing oocysts with intense labelling in sections of the midgut of the mosquito, which were fed with 25  $\mu$ Ci or 50  $\mu$ Ci adenine-<sup>14</sup>C per ml in a 5% honey solution. Only the former produced sporozoites infective for the vertebrate host (TBGif mice).

In addition it is reported, that the label of A.atroparvus remained detectable in the next generation (eggs, larvae and adults), providing an easy means for tracing dispersion of released specimen in an area of study.

References :

- Garnham, P.C.C. 1967. Relapses and latency in malaria.  
Protozoology 2, 55-64 (Suppl. to J.Helm., dec. 1967).

12. H.de ROEVER-BONNET (Dept.of Parasitology, Royal Tropical  
Institute, Amsterdam)

Why did the patient die ?

A renal transplantation was performed in a 26 year old man, suffering of the syndrome of Alport.

After many complications, which made it necessary to remove the transplantat 27 days after the operation the patient died due to terminal oedema of the lungs.

The pathologist found many necrotizising foci in different tissues, containing toxoplasma parasites and concluded : "the patient died of an activated toxoplasma infection, though the dye test and the complement fixation reaction had never been positive".

But on the day of the operation the dye test titre was 1/16, the c.f. reaction 1/2, some weeks later, 1/128 and 1/4 resp. . However this rise is not



an indication of active infection.

In slides of brain, hypophysis, testis, heart, lungs and pancreas free and intracellular parasites were seen, none in liver, spleen and lymphnodes. This can be an argument for the diagnosis "activated Toxoplasma infection". But the question remains if in a complicated case like this one, in which virus infections were excluded, toxoplasmosis is the only cause of death.

13. J.GOLENSER, J.H.E.Th.MEUWISSEN, J.HEEREN, L.JAMES and H.v.d.KAAY (Laboratory of Parasitology, University of Nijmegen and Laboratory of Parasitology, University of Leiden)

Indirect fluorescent antibody (IFA) titres against sporozoites, parasitized erythrocytes and exoerythrocytic forms of P.berghei

IFA studies are reported with plasmodial antigens from three different stages of P.berghei malaria: sporozoites (SP), exoerythrocytic schizonts in rat liver (EEF) and parasitized rat erythrocytes (SCH-schizonts). Two series of specific sera were applied: sera from adult rats with a blood induced infection (series A) and sera from rats immunized against sporozoites by mosquito bites and protected against parasitemia by chloroquine (series B).

In series A antibody titres with all three antigens were seen, but those with SCH were the highest at any stage of the infection. Superinfection with parasitized rat blood did not change the titre. In series B sera collected from rats after a single exposure to infected mosquitoes, showed only titres with SPA, from day 3 onwards. After a second exposure titres to all three antigens developed. Cross reactivity with the heterologous antigens in series B was clearly less than in series A. Anti P.berghei sporozoite antibodies did not crossreact with P.vivax sporozoites.

14. A.van ZON and W.ELING (Department Histology and Cytology, Katholieke Universiteit Nijmegen)

Antimalarial immunity and pregnancy

It is well established that the relapse rate is higher in pregnant than in non-pregnant women in holo-endemic malarious areas. This holds also for an observed reduced immunological capacity during pregnancy (e.g. blast-cell-transformation; reduced amounts of immunoglobulins). Also, a temporary involution of lymphatic organs (mouse model) as well as the presence of immunosuppressive components (e.g. macroglobulines, chorionchonadotropin) are concomitantly observed.



In order to explain the higher parasite rate in pregnant women not only a reduced immunological capacity for newly introduced antigens but also for secondary responses may be considered. As in the human model, in the experimental malaria (Plasmodium berghei)-mouse model, pregnant females exhibited a remarkably higher relapse rate, which was frequently lethal.

A lethal breakthrough was observed among primigravidae and multi-gravidae, in the latter case after temporary parasitaemia during previous pregnancies, which was suppressed after delivery.

15. J.H.E.Th.MEUWISSEN, Ilse TAUBER, Anna D.E.M.LEEUWENBERG, P.J.M.BECKERS, Marianne SIEBEN ( Laboratory of Parasitology, University of Nijmegen )

Parasitological and serological observations on Pneumocystis infections in man

Over 500 lung tissue specimens were parasitologically examined for Pneumocystis, 38 infections were found, especially but not exclusively in patients with a deficient immune system.

Serum samples of about 600 "normals" and 117 children with acute lymphatic leukemia (ALL) were examined with an indirect fluorescent antibody test (IFA). The age related data of "normals" suggest that nearly 100% of children are infected during the first two years of life. ALL patients have significantly higher mean IgG antibody titer levels than normals, and groups of ALL patients with a clinical picture suggestive of Pneumocystis pneumonia have significantly higher IgG titer levels than groups of ALL patients without these symptoms.

Nevertheless the diagnostic value of the IFA test is limited; serological follow up can be usefull however. Groups of children with ALL have lower mean IgM antibody titer levels irrespective of their clinical picture. The results are discussed and a hypothesis is given about a possible role of macrophages in this infection.

16. J.C.M.VETTER and J.C.M.KLAVER-WESSELING ( Laboratory of Parasitology, University of Amsterdam )

Indirect Fluorescent Antibody Studies of Hookworm Infections

The indirect fluorescent antibody technique demonstrated antibodies against hookworms (Ancylostoma ceylanicum, A.braziliense and A.caninum) in serum from dogs infected with these parasites, when cryostat sections of infective larvae and adult worms were used.



These antibodies caused an intense fluorescence of the cuticle. Against living infective larvae and adult worms no antibodies were detectable.

When they were dead, however, antibodies were found, which caused fluorescent precipitates on the whole body surface.

17. E.J. RUITENBERG (Rijks Instituut voor de Volksgezondheid, Bilthoven)

Immunological aspects of Trichinella spiralis infections in congenitally athymic (nude) mice

Trichinella spiralis (T.s.) adult worms are expelled from the gut of the host by an immune mechanism, which was shown to be T-cell dependent. Therefore, experiments were performed to study whether the accompanying cellular gut response was also T dependent.

Cells which are possibly involved in the host defense are : macrophages (for antigen processing); plasma cells (for production of specific antibodies); small lymphocytes (for production of lymphokines); mast cells/globule leucocytes (histamine release); eosinophils. Production of gut plasma cells and specific antibodies to T.s. was T dependent. Induction of gut mast cell formation and degranulation to globule leucocytes during a T.s. infection occurred only in thymus-bearing mice. In congenitally athymic mice T.s. infection failed to induce a gut mast cell response. Both gut and blood eosinophils increased during a T.s. infection, the phenomenon being T dependent.

A possible mechanism for the expulsion of T.s. from the gut will be discussed.

18. J.P. ROTMANS and A.M. DEELDER (Laboratory for Parasitology, University of Leiden)

Purification of Schistosoma mansoni enzymes and the possible use in immunodiagnostic tests

Our aim has been the isolation of schistosomal enzymes and their subsequent biochemical and immunological characterization and immunodiagnostic application. Research has, so far, been concentrated on two enzymes, a protease (Sauer and Senft, 1972) and acetylcholinesterase (ACHE).

1. Protease

Isolation of the enzyme from a worm-homogenate is achieved by acid precipitation, followed by gel filtration and phenylalanine-agarose affinity chromatography. Gel electrophoresis and isoelectrofocusing



show a manyfold purification of the enzyme, on a protein base. The enzymatic activity of the resultant preparation, as determined by incubation with its natural substrate, hemoglobin, is not increased. Immunoelectrophoresis of whole worm-homogenate against rabbit anti enzyme immune sera shows one major and one minor precipitation arc. Immunofluorescence and immunoperoxidase tests, using a rabbit immune serum, indicate a localization of the enzyme under the tegument. Preliminary results indicate the usefulness of this enzyme as an antigen in the DASS-system for the immunodiagnosis of experimental infections.

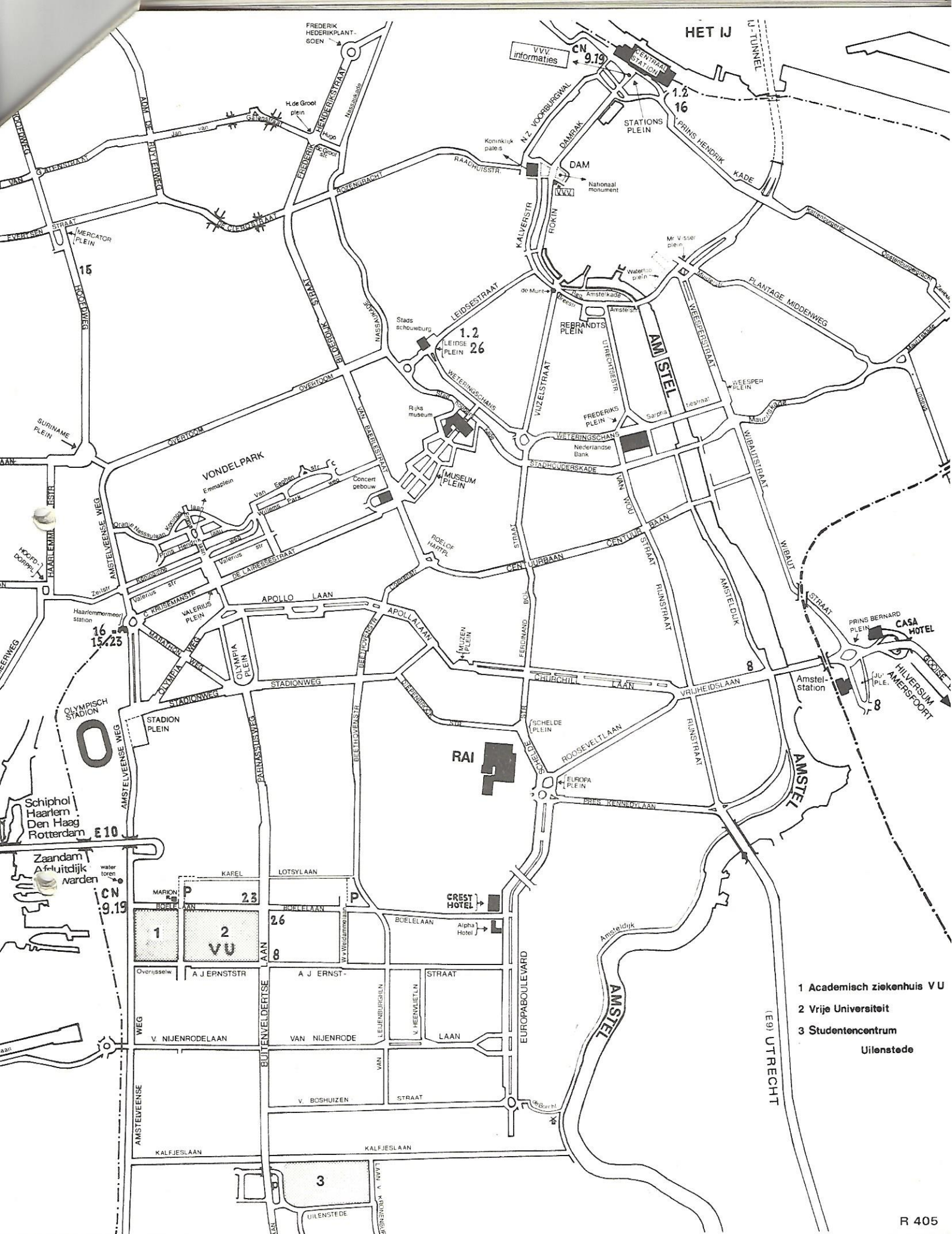
## 2. Acetylcholinesterase

Apart from its possible use as a specific antigen, this enzyme is of interest because it seems to be the target of the schistosomal drug hycanthone (Hillman and Senft, 1975). ACHE is a membrane-bound enzyme, which can be isolated from a worm-homogenate by low speed centrifugation (6000 g). The specific enzyme-activity in the supernatant (15 mU/mg protein) is increased twofold by ammoniumsulfate precipitation. Subsequent gelfiltration increases the activity to about 100 mU/mg protein. Affinity chromatography is used for further purification of the enzyme.

The (p-aminophenyl) trimethylammonium-ion, a potent competitive inhibitor for the enzyme, is coupled to caproyl-caproyl-Sepharose 4B. Delipidation by hexane-extraction and lipase-action is essential for binding the ACHE to the affinity support. The enzyme can be eluted by increasing the ionic strength of the eluent. The increase in specific activity is only moderate (2-5 fold). The Michaelis-Menten constant of ACHE and the inhibition constant for (p-aminophenyl) trimethylammoniumiodide have been determined:  $1.0 \cdot 10^{-4}$  M and  $1.5 \cdot 10^{-4}$  M, respectively.

Subsequent research will be concerned with further characterization of these and other enzymes and their use in immunodiagnostic tests.





- 1 Academisch ziekenhuis VU
- 2 Vrije Universiteit
- 3 Studentencentrum
- Uilenstede



Nederlandse Vereniging  
voor Parasitologie,  
Subsidiekommissie  
Engweg 44  
BUNNIK

Aan de leden van de Nederlandse  
Vereniging voor Parasitologie

Utrecht, 30 augustus 1976

De subsidiekommissie wil nog eens wijzen op de mogelijkheid een subsidie te krijgen voor kosten welke samenhangen met bezoeken aan wetenschappelijke kongressen.

In aanmerking voor subsidie komen die leden van de Vereniging die ingepast zijn beneden schaal 130 van het Rijksbezoldigingsbesluit of volgens een daarmee overeenkomende honorering.

Gesubsidieerd kan worden tot 50% van de reëel begrote bedragen voor eis- en verblijfkosten, etc., met een maximum van f. 250,-- mits 15% van deze kosten door de aanvrager zelf betaald wordt.

Indien u in aanmerking wilt komen voor deze subsidie, kunt u een voorlopige aanvraag, waarin reisdoel en globaal begrote kosten vermeld zijn, aan onze kommissie toezenden.

Namens de Subsidiekommissie,

drs H. Kuil,  
penningmeester N.V.P.



NEDERLANDSE VERENIGING  
VOOR PARASITOLOGIE

NETHERLANDS SOCIETY  
FOR PARASITOLOGY

Aan wie het betreft,

Het is mogelijk dat voor sommigen afstand en/of reiskosten een bezwaar vormen om zowel op 30 september als 1 oktober aan alle activiteiten deel te nemen.

Derhalve verzoek ik zowel diegenen in Amsterdam en omgeving die gedurende de nacht van 30 september - 1 oktober een of meer logé(e)s kunnen herbergen, als diegenen die van een dergelijke mogelijkheid gebruik zouden willen maken, mij daarvan schriftelijk b.v. via het lunchstrookje, of telefonisch (020-5483975, evt. 's avonds 794816) op de hoogte te stellen.

Voor degenen voor wie de dubbele reiskosten een bezwaar vormen, bestaat de mogelijkheid een verzoek tot een bijdrage te richten aan de penningmeester, drs. H. Kuil, Engweg 44, Bunnik.

In de hoop op uw medewerking, respectievelijk u van dienst te kunnen zijn,

hoogachtend,



E.A. Meuleman.



Ondergetekende: .....

Adres: .....

geeft zich op voor de lunch tijdens de vergadering van de N.V.P. en BION Werkgemeenschap Parasitologie op 1 oktober 1976.

(Inzenden vóór 27 september 1976 aan de secretaris van de N.V.P.; Lab. Med. Parasitologie, Faculteit Geneeskunde, Vrije Universiteit, Postbus 7161, Amsterdam).

*Handtekening*

Handtekening:

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