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Dr. H. J. v.d. KAAJ in the chair

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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 and 1975).

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 pCO₂, pO₂, 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.

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 foreigners, 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 heteroxenous 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-9H₂ 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. OPPERDOES (*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 (Müller, 1975). 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 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 g/cm³. Examination by electron microscopy of a fraction from this region of the gradient only showed mitochondria. 80% of the NAD⁺-linked glycerol-3-phosphate dehydrogenase was particle-bound and banded at 1.23 g/cm³ 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.

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. USA)

Certain aspects of the *in vitro* cultivation of *Trypanosoma cruzi*.

Attempts have been made to chemically define the growth promoting constituents of Bactotryptose in Bone's T-2 medium (Boné and Parent, 1963). 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 media (Jadin and Pierreux, 1960). This observation was confirmed for cultures of thymidine kinase deficient Chinese hamster cells (Westerveld *et al.*, 1971) in Ham's F-10 medium with fetal calf serum and antibiotic supplements (Bootsma and Westerveld, 1975). Miconazole proved to be toxic for the used strains of *T. cruzi* in such low concentrations (5 µg/ml), that *in vivo* experiments seem to be warranted.

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 1764 the first report of a tick in the Netherlands was published. More case reports emerged in the following centuries, but no systematic investigation has been reported. It seems that at least the genera *Argas*, *Haemaphysalis* and *Ixodes* are endemic here. Babesiosis of cattle was studied intensively (Wilson and Perie, 1965) but the ecology of the vector ticks was almost ignored. Dutch dermatologists attributed prurigo, lymphocytoma, erythema chronicum migrans and acrodermatitis chronica atrophicans to tick bites, without studying the Dutch Ixodoidea.

Foreign ticks are probably imported regularly. Jansen (1975) reported this in case of *Rhipicephalus sanguineus* (Latreille, 1806), a known vector for encephalitis virus. From the Netherlands human tick-borne-encephalitis has not (yet ?) been reported.

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 homozygote for type A are the most resistant, with types A and B are less resistant and homozygote for type B 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 between breeds of sheep is questionable.

10. J. F. SLUITERS (*Laboratory of Medical Parasitology, Free University, Amsterdam*) *Trichobilharzia ocellata* - *Lymnaea stagnalis*: endocrinological aspects of a parasite-host relationship.

Infection of juvenile *Lymnaea stagnalis* with *Trichobilharzia ocellata* causes a temporary acceleration of the growth rate. Also the ovipository activity is strongly suppressed. Before growth rate changes can be observed, a clear inhibitory effect on the development of the accessory sex organs of the host is already seen (Sluiters and Khan, in press). Preliminary results indicate that gametogenesis by itself is not influenced by the parasite. However the outgrowth of the ovotestis is inhibited. This "parasitic castration" is brought about by the mother sporocyst in the head-foot region of the snail. The effects of the parasite on body growth and reproduction could be mediated via the neuroendocrine system of the host, because removal of the neuroendocrine centres that control growth and reproduction (Geraerts, 1975) affect the snail in very much the same way. However certain differences seem to indicate other mechanisms.

11. H. J. VAN DER KAAY, L. BOORSMA AND J. PH. W. NOORDINK (*Lab. for Parasitology, University of Leiden and 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 successful marking of *P. berghei* was obtained using adenine-¹⁴C, producing oocysts with intense labelling in sections of the midgut of the mosquito, which were fed with 25 µCi or 50 µCi adenine-¹⁴C per 1 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), assuring an easy means for tracing minute amounts of ¹⁴C by using long exposure time with stripping film technique.

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 transplant 27 days after the operation the patient died due to terminal oedema of the lungs.

The pathologist found many necrotizing 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 CF reaction 1/2. some weeks later, 1/128 and 1/4 resp. However this rise is not an indication of active infection.

Free and intracellular parasites were seen in sections of brain, hypophysis, testis, heart, lungs and pancreas, none, however, in liver, spleen and lymphnodes. This can be an argument for the diagnosis "activated Toxoplasma infection". The question remains whether in this complicated case, where virus infections were excluded, toxoplasmosis is the single cause of death.

13. J. GOLENSER, J. H. E. TH. MEUWISSEN, J. HEEREN, L. JAMIS AND H. J. V.D. KAAJ
(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. macroglobulins, 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 exhibit a remarkably higher parasite rate. Such breakthroughs were frequently lethal.

A lethal breakthrough was observed among primigravidae and multigravidae, 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 useful 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.

1. *Protease*

Isolation of the enzyme from a worm-homogenate is achieved by acid precipitation, followed by gel filtration and phenylalanineagarose affinity chromatography.

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 (ACHE)*

The enzyme is isolated from a worm-homogenate by low speed centrifugation, gel filtration and affinity chromatography. The (p-aminophenyl) trimethyl ammonium-ion, a potent competitive inhibitor for the enzyme, is coupled to caproyl-caproyl-Sepharose 4-B. The enzyme is eluted by increasing the ionic strength of the eluent. The Michaelis-Menten constant of ACHE and the inhibition constant for (p-aminophenyl) trimethyl ammonium-iodide 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 application in immunodiagnostic tests.

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