

1. Course of a Plasmodium berghei infection in several mouse strain.

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Plasmodium berghei (strain K 173) was kept in several mouse strains by weekly subinoculation of infected blood from the homologous strain: Swiss and germ free (G.F.) Swiss mice (bred in closed colonies); C3Hz, B10Lp mice (inbred) and congenitally thymusless mice: B10Lp Nu⁺/Nu⁺ (4th generation of crossing Nude gene into B10Lp mice). In all parasite - host combinations the infection is lethal in all mice. The course of the disease however is markedly host specific (infectious inoculum: 10⁵ parasitized erythrocytes - i.p. per mouse). Mean survival time: Swiss 12.8 ± 5.4 days; C3Hz 8.8 ± 1.3 days; B10Lp 9.7 ± 1.2 days; B10Lp Nu⁺/Nu⁺ 20.5 ± 5.2 days.

The prepatent period was 4-5 days followed by rapid proliferation of parasites in normal, oxyphilic erythrocytes until a peak number of infected erythrocytes was observed. The magnitude of peak parasitemia again is strain specific and varied from 13% (B10Lp) to 68% (G.F. Swiss) infected erythrocytes. Shortly after peak parasitemia an increasing number of polychromatophilic erythrocytes appeared in the blood of surviving Swiss and G.F. Swiss mice and of all B10Lp Nu⁺/Nu⁺ mice. In these cases the parasites switched from the oxyphilic to the polychromatophilic erythrocytes, predominantly invading and dividing in these cells until death of the host. Polychromatosis was rarely observed among C3Hz and B10Lp mice, which died at or shortly after peak parasitemia.

The mortality pattern seems to be related to the ability to induce polychromatosis: a) no polychromatosis: death at or shortly after peak parasitemia (C3Hz and B10Lp); b) polychromatosis in part of the mice: bimodal mortality pattern (Swiss strains); c) polychromatosis in all mice: increased survival (B10Lp Nu⁺/Nu⁺). In all combinations a more or less severe anemia developed shortly after patency but was not directly related to the magnitude of the parasitemia observed.

Also a severe thymic involution developed in the course of the disease. Except for G.F. Swiss mice only fragments of thymic tissue exhibiting cell death in the cortical area were found shortly after peak parasitemia.

This phenomenon might at least partly explain the immunoparalysis at peak parasitemia that was established so far among C3Hz and Swiss mice and might also prevent a protective immune response against the parasite itself being ultimately responsible for the death of the host.

All infected strains exhibited a gradual increase in splenic weight. Close to peak parasitemia a decrease in splenic weight was found, which was only temporary in those mice and strains that exhibited polychromatosis. The initial increase in splenic weight might at least partly be explained by an observed increase in haemopoietic cells and an increased activity of the white pulp. Around peak parasitemia degenerative changes are observed in the follicles of the white pulp. The marginal zone disappears suddenly, the size of the follicles is gradually reduced as well as the mitotic activity. Except for the marginal zone local cell death is not a common feature. The serum glutamic oxaloacetic transaminase (S.G.O.T.) activity also changed markedly strain specific. In C3Hz and Swiss mice a 35 fold increase was found near peak parasitemia whereas in the other strains a 2 to 7 fold increase was observed. Mice surviving peak parasitemia also exhibited much lower S.G.O.T. values. Although the pattern of the S.G.O.T. activity in the course of the infection runs more or less parallel to the pattern of parasitemia and antiparallel to the anemia quantitatively a lack of correlation is apparent.

On the other hand the magnitude of the S.G.O.T. values seems to be related to the histo-pathological changes observed in the liver, varying from an increased amount of lipid in the liver parenchymal cells (B10Lp) to severe necrosis (C3Hz). Although a strong increase in S.G.O.T. activity seems to reflect a severe pathological state it does not seem to explain death of infected B10Lp mice.

2. Epidemiology of Sarcoptes scabiei var. hominis.

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Sarcoptes scabiei var. *hominis* is an ectoparasitic mite of man that lives in burrows in the stratum corneum and causes a severe itch. Infection takes place by prolonged skin contact.

Epidemics of these mite infections are known since Medieval times, but statistical figures of occurrence are present only of the last century. In Germany four large scale epidemics are reported between 1850 up till now, with highest incidence in the years 1866, 1918, 1945 and probably 1970 (Barthelmes, 1970). Dutch data are not that complete, but here epidemics are also noted in the first and second world war, and nowadays.

Worldwide epidemics do not arise at exactly the same time. The last pandemic started in 1964 in The Netherlands and Germany, but was already noted in South Korea in 1961. In 1970 scabies has been epidemic in Eastern-Asia, Eastern and Western-Europe, Africa and South-America, but not in the United States of America and Australia (Orkin, 1971).

At intervals with low incidence of scabies endemic areas have been recorded e.g. in Kiel (Germany) in 1953-1962 (Schirren, 1970).

Acquired immunity of the host, mutation and selection in the mite populations, changes in cultural patterns such as increase of sexual promiscuity and neglect of hygiene, increase of long distance travel (by army movements, tourism, urbanisation and introduction of foreign labour) and effects of therapeutic measures have been proposed at some time to explain the intermittent peaks in morbidity of the disease.

Immunity of the host and gross genetic change of the parasite are unlikely. Massive long distance travel (war, tourism) suffices in explaining the start of the last four epidemics by dispersing the mites from endemic areas. A rapid spread within the host population has been caused by overcrowding, widespread sexual promiscuity, bad hygiene, wrong therapy and a lack of early diagnosis. The decline of the epidemics of the last century can be attributed almost exclusively to an effective therapy. In earlier times extensive bacterial infections which followed the mite attack, presumably worked in the same direction.

It is to be expected that the present day pandemic will vanish within the next ten years under the severe pressure of our therapeutic arsenal. However, to safeguard ourselves against the surprise of new epidemics, we should trace and treat endemic areas on a world-wide scale.

References:

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 Orkin, M. (1971): J. Amer. Med. Ass. 217, 593-597.
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3. Helminth infections in mediaeval Utrecht

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Material from two cesspits, located in the Stroosteeg in Utrecht, dating from the 13th and 14th century was examined for the presence of eggs of parasitic helminths. Large numbers of the eggs of Ascaris lumbricoides and Trichuris trichiura were found. These results are comparable with those of Taylor (Vet. Rec., 67, 216, 1955) and Pike and Biddle (Antiquity, 40, 293, 1966) in Winchester, Jansen and Boersema (Stadskernonderzoek in Amsterdam, 41, 1972, in press, 1975) in Amsterdam and as far as T. trichiura is concerned of Jansen and Over (Proc. I. Int. Congr. Parasitol., 2, 791, 1966) in Valkenburg on Rhine.

4. The epidemiology of Nematodirus filicollis (Nematoda: Trichostrongylidae) infections in sheep in the Netherlands.

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Material and methods see Hendriks c.s. (1975, in press).

Lambs, born in March, at pasture with a lamb-grazing history, show an increasing Nematodirus filicollis egg output from April till August after which the epg's are decreasing to almost zero in autumn.

Lambs, three months old, grazing till then on a clean pasture, and put on the infected pasture in May show an increasing egg output in June, whereas wormfree six months old lambs turned out end of September and housed beginning of November have an epg rise in October. Last two groups show decreasing epg's in October and December respectively.

Gimmers and older sheep normally produce no or very few N. filicollis eggs.

High pasture larval counts of N. filicollis are found from January till May. From the end of May till July the pasture infection decreases to low levels. From October onwards the pasture larval counts rise to the high winter level.

Autopsy in January of permanent grazing lambs shows relative low numbers of N. filicollis worms, which are almost exclusively L4 stages.

The following epidemiological pattern is constructed. In spring lambs at pasture with previous lamb-grazing pick up high numbers of N. filicollis larvae and contaminate the pasture with eggs. Their decreasing egg output in autumn is explained by acquired immunity, which is maintained during the rest of their life. Contamination of the pasture with eggs by the lambs results in increasing pasture larval counts in October, forming the source of infection for the next lamb generation; picked up by gimmers and older sheep the larvae will not develop into fertile adult worms, because of the immunity of these sheep.

Results with the wormfree lambs indicate that there is no effective "age-resistance".

Preliminary results with wormfree tracer lambs confirm roughly the described epidemiological pattern.

5. On the life-cycle of Strongyloides westeri Ihle, 1917 (Nematoda: Strongyloididae) in Shetland ponies in the Netherlands. Preliminary communication.

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The parasitic, parthenogenetic phase of the life-cycle of Strongyloides westeri takes place in the small intestine of equids (horse, pony, donkey).

Patent infections with S. westeri are seen in a large number of foals. In contrast, we never saw patent infections in adult horses and ponies. By means of examinations of milk of Shetland pony mares larvae which could be identified as S. westeri larvae were found in the sediment. These larvae have migrated already in the mare and are able to mature to parasitic females directly after arrival in the small intestine of the foal. All foals of the mares examined showed eggs of S. westeri in the faeces at the age of 14 days. Patency lasted about three months.

As a pre-natal, intra-uterine infection is not excluded by a galactogen infection a number of foals were separated from their mares 24 hours after birth and raised isolated under the utmost hygienic conditions. No worm infection could be detected in these foals during a long period of observation.

While the percutaneous infection doubtless plays an important role in the life-cycle of S. westeri, it can be concluded from these experiments that the first infection in foals can be a galactogen one. It seems likely that this mode of infection occurs in a large number of cases after which, during patency, the percutaneous way and less so the indirect oral infection become more important.

6. Cyclical transmission in goats and mechanical transmission in mice with Trypanosoma vivax.

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Until recently it was a widely accepted fact that Trypanosoma vivax could only be established in laboratory animals if supplementary to the parasite inoculation, normal sheep serum was given. Desowitz et al. succeeded in this way to adapt a T. vivax strain to rats, mice and rabbits. In 1972 and 1973 Leeftang isolated from cattle in Northern Nigeria two field strains of T. vivax (Y58 and Y486) by direct subinoculation into mice. With these two mouse isolates up till 100 passages or more were made.

A preliminary study was done in our laboratory. One goat was infected with strain Y58 after 15 mouse passages and another with Y486 after 40 mouse passages. From these goats cyclical transmissions to four other goats were performed with Glossina morsitans and with Glossina tachinoides. At various intervals after infection blood from each of these four goats was inoculated into mice.

Some of the results were:

1. Both strains could be transmitted cyclically in goats by G. morsitans and G. tachinoides after several subpassages in mice.
2. At dissection trypanosomal stages could only be detected in the proboscis of the flies.
3. When transmitted by G. tachinoides both strains caused higher mortality in mice than when transmitted by G. morsitans.
4. Parasitaemia in mice was often high ($10^4/\text{mm}^3$ blood or more) and one to three relapses occurred mostly.
5. Only seven subpassages were made (in normal mice without serum supplement) because of the many mice needed for the experiments. It is however reasonable to assume that more passages could have been made. Furthermore, 7 T. vivax passages in mice is already more than what has ever been achieved.

7. The sandwich immunofluorescent complement fixation test in Trypanosomiasis.

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A comparison was made of the development of antibody titres during experimental infections with *T. congolense*, *T. brucei*, *T. vivax* and *T. evansi* obtained by the indirect fluorescent antibody test (IFAT) and the sandwich immunofluorescent complement fixation test (sandwich IFCT). The animals used for the experimental infections were sheep, goats, donkeys, dogs and cattle.

First the effect of complement components C_{1q} , C_4 and C_3 of human AB-serum and C_3 of guinea pig serum on the trypanosoma species and the specificity in binding of these various components to the complex of antigen-antibody was tested.

All the trypanosoma species reacted similarly. Even without serum, trypanosomes were stained aspecifically when C_3 of human and C_3 of guinea pig serum were used, while the C_4 component gave false positive reactions in the presence of negative serum. The C_{1q} component, however gave specific results in all positive homologous sera obtained in the course of experimental infections. Undiluted negative sera never gave false positive reactions and titres of $\geq 1:1$ were therefore considered as positive (see tables). The ultimate sandwich ICFT was as follows: $\{ \text{(trypanosomal antigen + antibody)} + \text{human } C_{1q} \text{ from AB-serum} \} + \text{rabbit produced human anti } C_{1q}] + \text{FITC antigamma globulin rabbit.}$

The interpretation of titre level of the IFAT and sandwich ICFT in Trypanosoma infections.

Reciprocal titres of:		Interpretation:
IFAT	sandwich ICFT	
40.80	very weak fluorescence of undiluted serum	dubious
≥ 160	≥ 1	specific
≥ 320	≥ 32	highly positive

In general the titres of the sandwich ICFT were lower than those of IFAT. The course of these titres ran, however, more or less parallel.

8. Mechanization of Complement Fixation Test for the serodiagnosis of Toxoplasmosis.

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Serodiagnosis of toxoplasmosis is mainly based on the combination of two serological methods, one of them being the complement fixation test.

Routinely complement fixation tests are carried out by macro methods in tubes. This involves much manual labour. Furthermore this type of test is hard to mechanize. Experiments were carried out to perform the C.F. test as a micro method in micro trays. The advantages of this method being that reduced quantities of reagents and smaller amount of serum are needed, easy handling, less expensive equipment and reduction in manpower. The major advantage is, however, that these trays can be handled very adequately by semi-automated equipment.

First the reliability of this micro method was compared with the macro method hitherto applied. The reliability of the micro method seemed to compare more favourably with the immunofluorescence test than that of the macro method. The micro method involved considerably less manpower than the macro method. The latter method offers therefore an interesting alternative for routine screening purposes.

9. Effect of intestinal flagellates on immune responses in mice.

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In the literature no adverse effects to the host of "normal" numbers of intestinal flagellates (Hexamita muris) are described.

It is known, however, that stress factors (crowding, low temperatures) render mice more prone to intestinal pathology in relation to intestinal flagellates.

Therefore, the present study was undertaken to investigate whether normal numbers of intestinal flagellates could exert adverse effects on some immune responses of the host using a number of bioassays with which the various functions of the immune system could be studied. Experiments were carried out in mice harbouring H. muris, whereas absolutely flagellate-free mice served as controls.

Flagellates had the following effects on the immune responses. Flagellate-infected animals proved to have a higher resistance towards Listeria monocytogenes (increased macrophage function). Antibodies towards tetanus toxoid were produced to a lower extent in flagellate-infected animals. Antibodies towards type 3 pneumococcal polysaccharide (SIII) were produced to a lower extent in flagellate-infected animals.

The results suggest that flagellates do interfere with the immune responses of the host. This seems to imply that in using animals to study the immune response to parasites in general, the flagellate status of the experimental animals should be known.

10. Bio-assays to study possible immunosuppressive effects of parasitic infections.

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In order to test possible immunosuppressive effects of parasitic infections various in vivo models were studied. The models included:

- a. T-cell function tests
- b. T-helper cell function test
- c. B-cell function test
- d. Macrophage function test

After comparative studies the following model systems were selected:

- a. T model
 - 1) Mean survival time of skin allograft
 - 2) Mean survival time of heart allograft
- b. T/B model: haemagglutination titer 28 days after immunization with 5 Lf tetanus toxoid
- c. B model: haemagglutination titer 5 days after immunization with type 3 pneumococcal polysaccharide (SIII)
- d. Macrophage model: spleen count of Listeria monocytogenes 2, 4 and 6 days after intraperitoneal challenge.

(P10)

A comparison ^{between} the properties of the mitochondrial adenosine-triphosphatase from mammalian cells and from the trypanosomatid Crithidia luciliae

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Since energy yielding reactions in trypanosomes could provide susceptible targets for new trypanocidal drugs we have started to study the properties of the ATP-synthesizing machinery of trypanosomes in a search for differences between host and parasite, which could be exploited in the development of new trypanocides.

Mitochondria isolated from Crithidia luciliae exhibit respiratory control. Oxygen consumption is sensitive to ADP, oligomycin and uncoupling agents. Stimulation of the respiration by ADP is abolished by atractyloside and bongkrekic acid, which inhibit the exchange of adenine nucleotides in mammalian mitochondria. From these results it is concluded that C. luciliae mitochondria possess an adenine nucleotide translocator which resembles the translocator found in the inner-mitochondrial membrane of mammals in its sensitivity to these inhibitors.

The mitochondrial preparation contains an ATPase activity which is stimulated by magnesium and by the presence of the uncoupler FCCP. This ATPase is in part sensitive to oligomycin and to dicyclohexylcarbodiimide (DCCD), whereas the P_i -ATP exchange reaction catalyzed by these mitochondria is completely inhibited by FCCP and both oligomycin and DCCD, inhibitors of oxidative phosphorylation in mammals.

Differences between the trypanosomal and the mammalian ATPase were found in two respects: i) The ATPase of C. luciliae is not inhibited by the natural occurring ATPase inhibitor from beef heart, which inhibits the ATPase from animal and fungal mitochondria; ii) Aurovertin, a potent inhibitor of oxidative phosphorylation in mammalian mitochondria, which exerts its action by binding to the F_1 -ATPase, had no effect on the P_i -ATP exchange or on the ADP-stimulated respiration of C. luciliae mitochondria.

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The structure of kinetoplast DNA

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1. We have developed a modified isolation procedure that yields DNA networks from Crithidia luciliae, containing more than 90% closed circular duplex DNA as shown by several criteria (1). Mini-circles with a contour length of 0.76 μ m can be isolated from these networks after controlled shear degradation in a French press.

2. Degradation of the intact DNA network and of isolated mini-circles, with the restriction endonucleases Hap_{II} (from Haemophilus aphrophilus), Hind_{II+III} (from H. influenzae, strain d) and Alu_I (from Arthrobacter luteus) yielded the following results:

a) DNA of non mini-circular base sequence is integrated in the network. The total molecular weight of this DNA is at least 15×10^6 .

b) The mini-circle population is heterogeneous in base sequence, but this heterogeneity only involves a minor part of the molecule.)

(There are at least 10, but probably many more sequence classes, differing in less than 10% of their sequence.

3. Electrophoretic analysis on agarose slab-gels has shown that there is also size heterogeneity in the mini-circles of C. luciliae. Three different classes are present, slightly differing in electrophoretic mobility and presumably, therefore, in size.

The micro-heterogeneity of the mini-circles, which has also been observed for Trypanosoma brucei kinetoplast DNA in this laboratory (2) is of interest for two reasons:

a) It proves that the replication of these circles cannot involve a single master template.

b) It argues against Simpson's suggestion (3) that the mini-circles code for rRNA, since rRNA cistrons are highly conserved in evolution.

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