The mouse in experimental parasitology, and comments on the use of laboratory animals in studies of immunity to parasites

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Parasitic diseases are of the utmost importance in man and domestic animals in many parts of the world, so it is no wonder that studies of parasitic infections and diseases have been the concern of many scientists during the last one hundred years or so.

Experimental parasitology includes subjects as zoology, physiology, biochemistry, immunology and pathology and may involve studies in final hosts, intermediate hosts, and vectors. The various aspects of a certain host-parasite relationship are for obvious reasons most ideally studied in the proper host infected with the proper parasite. However, it is equally obvious that such studies in many cases are not possible due to economic, practical and ethical reasons. Thus, very early in the era of experimental parasitology it became clear that studies of host-parasite interactions whatever their nature and whenever relevant had to take advantage of laboratory animals as models. Rats, mice, guinea pigs, and rabbits are probably the species most often used in experimental parasitology.

Some may ask how it is possible to use small laboratory animals in studies of parasites indigenous to man and domestic animals. That is possible because many parasites are not adapted to live exclusively in a single host, but may have a rather broad host spectrum in a sense that at least some of the stages, often the initial ones, of the parasites are able to develop to a certain degree in an abnormal host, say the mouse, and some parasites are even able to go through a complete life cycle in an abnormal host. Another type of investigations are those in which parasitic infections indigenous to laboratory animals are studied in order to apply the results to infections with related parasites in man and domestic animals.

Parasitic infections in laboratory animals, besides being a model for studying various aspects of the parasitic infection itself, can be a tool for studying fundamental pathological phenomena. That is to say, a great number of parasitic infections provoke a variety of pathological reactions which have morphological and other features in common with similar reactions provoked in other infectious and non-infectious conditions, for example, granulomatous inflammation, fibrosis, hyperplasia and atrophy of cells and tissues.

The reason for choosing mice for parasitological research is that they are cheep, easy to handle and that they can be accomodated in large numbers in a comparatively small

space. Additionally, the organs are small which means that it is easy to recover and count parasitic stages more reliable from say the whole liver or lung, than if you recover parasites from the organs of larger animals. Of course, there are also disadvantages by using mice for experimental infections, in particular when series of blood or urine samples are required for the investigations, as such samples can only be available in limited amounts. Frequent bleedings may be hazardous to the animals and may invalidate the outcome of the experiments.

My personal experience of using mice for parasitological research started some fifteen years ago when I began to look into the immunity to the large intestinal roundworm, *Ascaris suum*, in pigs, and to study the pathogenesis of the hepatitis which the *A. suum* larvae are said to provoke in the liver of the infected pigs. In all animals infective *A. suum* eggs hatch in the intestine and the liberated larvae migrate via the blood to the liver, lung, trachea, oesophagus and back to the intestine where the larvae will mature and become adult worms in the pig, but not in other species. At that time when my interest in *Ascaris* arose the majority of researchers believed that the immunity to the *Ascaris* larvae worked in the liver of infected animals as mice, rats, and guinea pigs, whereas it was uncertain whether that was applicable to pigs.

As too little attention had been paid to the importance of the intestine as the working site of the immunity I devised a series of experiments in mice. Mice were made immune by repeated oral inoculations of infective *A. suum* eggs and the effect of the immunity was tested by challenge infections of immune mice and non-immune controls. Through several experiments in which larvae were recovered from the liver and lung, and counts were made of non-hatched eggs and liberated larvae in the intestinal lumen, it was concluded that the immunity to the initial stages of *A. suum* was most likely operating at the intestinal level in terms of a reduction of hatching eggs in the gut lumen and of a decreased mucosal penetration of the liberated larvae resulting in a decrased number of larvae commencing the extra-intestinal migration. Thus, the reason for recovering fewer larvae from the liver of immune animals was not that the larvae were killed in the liver due to immunity, but that fewer larvae reached the liver from the intestine. What is the practical relevance of that? Does the immunity in pigs operate at the intestinal level as well? That does not seem to be finally established in the literature, and there has not been the necessary economic support in this country to repeat the studies in pigs.

In other studies primary *A. suum* larvae infections were investigated in whole-body irradiated (X-rays) mice and in neonatally thymectomized mice which showed that fewer larvae migrated in irradiated mice, but that more larvae migrated in thymectomized mice compared to the controls. It was concluded that irradiated mice made a less suitable environment for the larvae and that the number of larvae starting migration in normal mice were influenced by thymusdependent factors. Again

it is uncertain whether the results apply to pigs.

As regards the abovementioned pathogenesis of the hepatitis induced in consequence of the larval migration through the liver, sequential studies of the development of the early liver changes in primary *A. suum* infections in mice indicated, that the lesions were not only macroscopically different, but obviously also histologically and pathogenetically different from those in pigs. In mice the liver damage seemed to be primarily due to parenchymal anaemic necrosis caused by a blocking of the sinusoids by the larvae, whereas the damage in pigs as judged from the available literature seemed to be primarily due to a mechanical trauma caused by the larvae. In both species the involvement of immunological factors can not be excluded. It can thus be concluded that the mouse might be a useful model for studying the immunity to the initial stages of *A. suum*, but not to study the pathogenesis of the liver lesions in pigs.

In another series of experiments the intestinal pathology in mice infected with the trematode *Echinostoma revolutum* was studied. *E. revolutum* is an intestinal trematode in ducks and geese, but it can also infect man and other mammals including mice. The infection in mice is an example of a parasite capable of developing from infective stage to adult parasite in a foreign host. Experimental infections in mice have been used mostly for studying intestinal, homologous immunity to parasites and for studying cross-immunity between heterologous parasite species. The experiments which were carried out in collaboration with the Danish Bilharziasis Laboratory revealed that the intestinal pathology is dominated by a marked villous atrophy, crypt hyperplasia, and some hypertrophy of the muscle layers.

These changes were found to be thymus-independent and were not influenced by oral application of sulfathiazole and parenteral injections of prednisolone indicating that bacterial overgrowth and inflammatory reactions, respectively, were not involved in the development of the mucosal lesions as seen in other cases of villous atrophy etc. in parasitic infections. Additionally, the lesions did not bear pathogenetical resemblance to similar morphological lesions seen in coeliac disease and tropical sprue in man. We are thus left with the impression that the villous atrophy in *E. revolutum* infections in mice is due to a mechanical destruction of the villi during the feeding of the trematodes which are notorious tissue feeders, and that the crypt hyperplasia is a natural sequel to the villous damage. These experiments may be an example of how studies of artificial host-parasite systems can broaden the insight into basic pathological phenomena shared by more natural host-parasite relations and various non-infectious conditions.

Finally, I would like to refer to a recent study which shows another aspect of using mice in experimental parasitology. WHO had shown some interest in investigating the absorption of chloroquine, an anti-malaria drug for peroral use, from the intestine of *A. suum* infected pigs. The reason for this was that infections in man with a closely related para-

site, *A. lumbricoides*, are prevalent in most malaria-haunted areas, and one might suspect that the absorption of chloroquine in malaria patients suffering at the same time from ascariasis would be impaired due to mucosal damage caused by the worms, or in other words, the anti-malaria treatment would be less efficient. For many reasons, however, field studies of that kind in human beings were not possible, and an alternative test system was looked for. In that respect *A. suum* infected pigs were thought to be a useful model as mentioned above, but again several practical problems made these investigations impossible for the time being. It was then suggested that the absorption of chloroquine could be examined in *E. revolutum* infected mice with pronounced mucosal damage in the instestine. The underlying idea of the study was, that if no decreased absorption was seen in these mice with heavy mucosal damage, it would be unlikely to see an effect in man infected with the much less damaging *A. lumbricoides*. Consequently, absorption studies in pigs and/or man could be spared or at least would need careful reconsideration.

In collaboration with the Department of Clinical Pharmacology, Huddinge University Hospital, Huddinge, Sweden, experiments were devised in which mice heavily infected with *E. revolutum* were dosed orally with chloroquine about eight weeks after the infection. Serial killings over a week of dosed, infected and dosed, but non-infected mice showed that there was no significant difference between the two groups as regards absorption rate and blood concentration of the drug. Admittedly, more investigations are necessary before it can be generally accepted that the absorption of chloroquine is not significantly impaired in human beings with ascariasis. Certainly, it might be a field of study where the use of laboratory animals is profitable.

It was mentioned in the introduction that laboratory animals have frequently been used as models in parasitological research, and as a conclusion to the present review I would like to comment very briefly on the general use of small laboratory animals in parasite immunology which has been of the greatest interest for many years.

At the beginning the optimism of the research workers was prevailing as regards the possibilities of getting ways and means to cope on immunological grounds with parasitic infections. The optimism was over long periods strongly supported by the success in the fields of bacteriology and virology where vaccines against some of the most serious diseases became available to the benefit of man. The success was made possible largely because of the development of basic immunology and the fact that the antigen structure of bacteria and virus is rather simple, which has made it »easy« to localize the functional antigens and antibodies. The same success in the battle against parasitic diseases is long in coming and even the most advanced immunological techniques developed over the last couple of decades have been of little or rather no use in regard to production of vaccines, and countless are the laboratory animals included in these studies.

The almost total failure could call for many explanations. Some of the most conspicuous are, that (1) metazoan as well as protozoan parasites have a far more complex antigen structure than bacteria and virus, that (2) many parasites have a variety of developing stages each with its own antigenic characteristics, that (3) some parasites are capable of changing their antigenicity during the infection possibly as a response to the antibody production of the host, and that (4) infections with parasites are characteristically long or even chronic. The latter means, *i.a.* that socalled sterile immunity, known in many bacterial and viral infections, is practically non-existing in parasitic infections.

There is a huge gap between the great number of immunological observations done in experimental infections in laboratory animals and the progress made in the understanding of immunity to parasites in man and domestic animals. The present situation is, by and large, that the optimism has given way to a state of pessimism and irresolution, and many parasitologists are beginning to realize that when you wish to study the immunity to a certain parasite in say a cow, you have to study the parasite in the cow and not in, *e.g.* a rabbit or a mouse. It is more clear today that the latter infections are artificial and may in no way reflect what is happening in the natural host. It is my personal opinion that the parasitological potential in any sense should be concentrated in fewer institutes engaging interdisciplinary scientists, and that they should predominantly study the parasites in their natural hosts. In other words, I advocate a decreased employment of laboratory animals in these investigations as they appear to be of limited value.

Finally, I dare say that another main reason for the general failure to produce vaccines against parasitic diseases and to obtain important information about other immunological aspects of host-parasite relations, may be that too much attention has been paid to immunological studies, and that the other aspects of parasitology, *viz.* anatomy, physiology, biochemistry and pathology, apart from immunopathology, have been comparatively neglected. Seemingly, far too long have these latter subjects been outshined by the fascination of immunology and immunopathology. A better knowledge of non-immunological host-parasite interrelations may very well prove to be a short cut to a better understanding of the immunological relations and through that, to a greater chance of coping more satisfactorily with the parasites on an immunological basis. The employment of laboratory animals in these studies may be justified provided that the underlying idea of the experiments is carefully considered and their purposes clearly defined.

Sammendrag

Anvendelse af mus i den eksperimentelle parasitologi, og kommentarer til brugen af forsøgsdyr til studier af parasitimmunologi

I indledningen anføres, at eksperimentelle, parasitologiske studier ofte ikke er gennemførlige i parasitternes naturlige værter, men at man i stor udstrækning har måttet benytte forsøgsdyr.

Der gives en kort oversigt over nogle af forfatterens arbejder, hvor mus har været anvendt til studier af forskellige parasitologiske forhold. Det nævnes, at forsøg i mus har vist, at immuniteten mod larver af svinets spolorm, *Ascaris suum* sandsynligvis virker i tarmen. Den primære larvevandring i mus synes afhængig af en intakt thymus, og røntgenbestrålede mus er dårlige værter for larverne.

Infektioner i tarmen med trematoden, *Echinostoma revolutum*, giver villusatrofi og krypthyperplasi. Det er sandsynligt, at forandringerne skyldes traumatisk påvirkning fra ormene. Andre undersøgelser viser, at absorptionen af anti-malariamidlet klorokin ikke er nedsat fra forandrede tarme.

Det bemærkes, at når det i al væsentlighed ikke er lykkedes at fremstille vacciner mod parasitære sygdomme, skyldes det, dels parasitternes komplekse antigenstruktur, dels at der nok er fokuseret for meget på rene immunologiske studier og for lidt på andre aspekter af parasitologiske discipliner som f. eks. anatomi, fysiologi, biokemi og den egentlige patologi. Endelig fremføres, at forsøg i højere grad skulle udføres på de naturlige værter, og at brugen af forsøgsdyr er af ringe værdi på dette område.

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