tion of the experiment (fig.). Whereas before infusion goats were lethargic and ate their feed over most of the 24 h, after infusion they were alert and consumed all their feed in less than 5 h. The change in disposition and the increase in feed intake following infusion was similar to the responses in leucaena-fed steers which were changed to a diet of cowpea hay[15]. The in vitro study showed that fresh or frozen rumen fluid resulted in total degradation of DHP in 48 h whereas no degradation occurred if the rumen fluid was autoclaved.

Discussion. The dramatic increases in feed intake following infusion, together with the equally dramatic decline in DHP excretion which was maintained for 21 days until the experiment terminated, strongly implies a permanent change in the rumen microbial population of the infused goats – at least while eating leucaena. The fact that this ability could also be successfully transferred from a treated Australian goat to one of the control goats only 10 days after receiving an infusion from an Indonesian goat, is further evidence that transfer of DHP degrading ability had occurred. The increased feed intake appears to be associated with a reduction in circulating DHP, since feed intake of steers also increases dramatically when leucaena is replaced by cowpea hay in the diet[15].

The in vitro study clearly shows that the degradation of DHP is caused by microorganisms, most probably bacteria, since removing the protozoa failed to prevent degradation of DHP. It is generally assumed that rumen microorganisms are ubiquitous and that the feeding regimen and the host physiology are the major determinants of the composition of the rumen microbial populations[13]. The implication from our recent studies[10,11] that microorganisms essential for the successful metabolism of a particular toxin were not available over large areas, including all of tropical Australia, has met with considerable resistance where these results have been discussed. Differences in DHP metabolism between countries have been ascribed instead to differences in animal species, genotype, or management, to environment or leucaena composition. These possibilities are eliminated in the present experiment by transporting both animals and feed from Australia.

The results obtained in this study are believed to be the first demonstration of a persistent nutritional benefit from microbial introduction into the rumen of animals on the same feed as that of the donor animal. The situation can be compared to the absence of effective *Rhizobium* populations for nodulation of some introduced tropical pasture legumes. Introduction of *Rhizobium* into Australia from overseas has been necessary before effective symbiosis could occur[16]. In the light of these results with leucaena, the possibility should be explored that other introduced pasture plants that are toxic to ruminants in a particular country may not be toxic in their country of origin.

For intensive use of leucaena as a ruminant feed in Australia and possibly elsewhere, it appears to be necessary to introduce the specific rumen microorganisms to degrade DHP. Importing ruminants from areas known to be free of leucaena toxicity is a simple practical solution to the problem. However, in countries such as Australia, where quarantine regulations prohibit the direct importation of ruminants, the solution will be more difficult. It may necessitate the isolation and culture of the organisms in vitro as a prerequisite to their importation and release.

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Experimental alveolar echinococcosis. Suitability of a murine model of intrahepatic infection by *Echinococcus multilocularis* for immunological studies[1]

M. Liance, D.A. Vuitton, S. Guerret-Stocker, J.P. Carbillet, J.A. Grimaud and R. Houin[2]

Laboratoire de Parasitologie, Faculté de Médecine, F-94010 Créteil (France); Services d'Hépatologie et d'Anatomie pathologique, C.H.U., F-25030 Besançon (France); Laboratoire de Pathologie Cellulaire du Foie, E.R.A. 819 CNRS, Institut Pasteur, F-69007 Lyon (France), 23 December 1983

Summary. An experimental model of human alveolar echinococcosis was developed, using intrahepatic injection of *E. multilocularis* larvae in mice differing by their sensitivity to this parasite; it seems to be suitable for studying the relationship between cell-mediated immunity and a) growth of the parasite, b) development of fibrosis.
Key words. Alveolar echinococcosis; *Echinococcus multilocularis;* cell-mediated immunity; inbred mice; experimental model.

Alveolar echinococcosis (AE) is a rare and fatal parasitic disease related to the proliferation in the liver of the larval form of the cestode *Echinococcus multilocularis (E.m.)*. Rodents are natural intermediate hosts for this larval development, and foxes, dogs and cats can serve as hosts for the adult form[3].

Human AE is endemic in USSR, Japan, Canada and Alaska and, in Europe, it is particularly encountered in the eastern

Table 1. Percentage of positive infections, and liver weights in 4 inbred strains of mice at the end of the 4th month after an intra-hepatic injection of *Echinococcus multilocularis* cysts

Strain		Percentage of positive infections	Liver weight (g) mean ± SD	Percentage of metastases	Weight of metastases (g) mean ± SD
AKR	+ *E.m.*	100	3.49 ± 2.71	83	0.41 ± 0.28
	controls	0	1.47 ± 0.12	0	0
CBA	+ *E.m.*	72.8	2.26 ± 0.90	54	1.48 ± 0.72
	controls	0	1.28 ± 0.18	0	0
C57 BL6	+ *E.m.*	100	2.95 ± 1.73	75	2.38 ± 2.48
	controls	0	1.21 ± 0.36	0	0
C57 BL10	+ *E.m.*	31.5	1.36 ± 0.36	36	0.63 ± 0.55
	controls	0	1.11 ± 0.04	0	0

part of France and Switzerland. Observations in animals and humans suggest a role for cell-mediated immunity in the development of the parasite and/or the pathogenesis of fibrosis which is responsible for the major complications of this disease: a) granulomatous cell proliferation with histiocytic epithelioid cells, giant cells and numerous lymphocytes surrounding parasitic cysts is a predominent pathological feature of the human hepatic lesions[4]; b) in animal models, the development of the parasite has been shown to be increased by immunosuppression[5] and decreased by immunostimulation[6]; c) specific anti-*E.m.*[7] cellular immunity in experimental animals infected by *E.m.*, has been shown to increase during the first phase of infection and to be reduced in a second and definitive phase, as the parasite grows. Differences in susceptibility of natural or experimental[8–10] hosts to infection by the *E.m.* larvae, and the particular resistance of humans, may be related to the cellular immune status of the host. Moreover, the development of fibrosis around the parasitic cysts might be related to the nature and importance of the cellular infiltration in the liver[11]. In order to test these hypotheses, an animal model of AE should satisfy the following requirements; 1) to be able to reproduce as well as possible the pathological features observed in humans, i.e. liver localization and granulomatous aspect, 2) to be suitable for genetic and immunological analysis, 3) to allow comparisons among animals of different susceptibilities, 4) to be harmless for the research staff. The aim of this study is to find such an experimental model, suitable for an immunological study of AE.

Material and methods. Animals. Four inbred strains of mice were used: AKR, CBA, C57BL6 and C57BL10 (IFA-CREDO, Orléans-La-Source, France). Fifteen 8-week-old mice of each strain received a parasite inoculum; they were sacrificed at the end of the 4th month after infection. 10 mice of each strain served as controls. Mice from each group were weighed. The liver and metastases were removed and weighted after macroscopic examination. Samples were fixed in Bouin's fixative, embedded in paraffin and stained for histological analysis (hematoxylin and eosin, Masson's trichrome and silver-staining). 0.2 ml of blood was collected at the 30th and 60th days after infection to assay specific antibodies against *E.m.* using an indirect haemagglutination technique[12] with an antigen extracted from the same strain of *E.m.* as that used originally to infect the mice.

E.m. inoculum and inoculation technique. Inocula were prepared from i.p. cysts obtained after eight weeks of infection by *E.m.* in *Meriones unguiculatus*. The strain of *E.m.* was obtained from the infected liver of the natural host *Arvicola terrestris* found in 1980 in Franche-Comté (France)[13], and was subsequently maintained by successive i.p. injections in *Meriones unguiculatus*. 5 g of an homogenate of the parasite cysts were suspended in 20 ml of saline and used for infection. A direct injection of 0.1 ml of the parasite suspension was performed in the anterior lobe of the liver with a 1-ml syringe and 40 mm 8/15 needle, after a midline laparotomy, under ether anesthesia. A sham operation was performed on control mice with an intrahepatic injection of 0.1 ml of saline.

Results. The percentage of mice having a positive infection in each strain is shown in table 1. The mean liver weight was increased, due to the presence of parasitic mass. Parasitic lesions appeared as a white-yellow mass present only in the infected hepatic lobe in C57BL10 strain and usually extending to other hepatic lobes and to the peritoneal cavity in the other strains, particularly in the AKR and C57BL6 strains (fig. 1). Peritoneal lesions were the most frequent metastatic localizations of the disease; pulmonary metastases were observed in two mice of the AKR strain. The frequency and weight of metastases are shown in table 1. Macroscopic examination isolated one strain particularly 'resistant' to *E.m.* infection: C57BL10. On the other hand, two strains were very 'sensitive' to infection: C57BL6, characterized by considerable metastatic lesions, and AKR, characterized by a significant primary liver lesion associated with frequent but discrete metastatic localisations. CBA mice could be considered as hosts of 'intermediate sensitivity' to *E.m.* infection. Histological examination disclosed the typical lesions observed in human Alveolar Echinococcosis: parasitic vesicles surrounded by a granulomatous infiltration with epithelioid cells at the position of contact with the *E.m.* germinal layer (fig. 2A), numerous macrophages and giant cells (fig. 2B), and lymphocytes at the points of contact with the liver parenchyma (fig. 2D) A this stage, ongoing fibrosis was observed in the histiocytic infiltrate, even distant from the primary parasitic lesions; fibrosis was more conspicuous in C57BL10 mice (fig. 2C), but nevertheless it was present in all the strains under study. Protoscolices were present in parasitic vesicles in the 'sensitive' strains and were

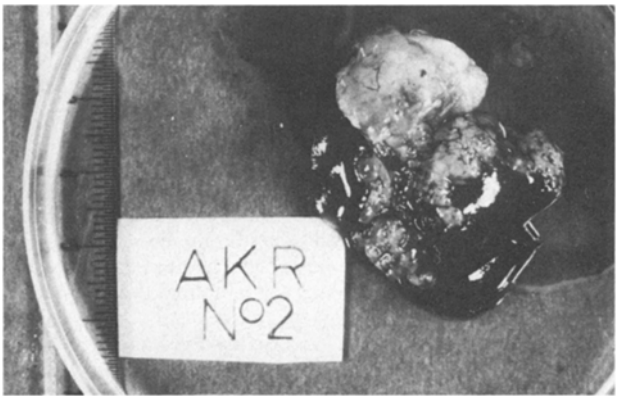

Figure 1. Macroscopic aspect of the experimental hepatic alveolar echinococcosis in a mouse of the AKR strain.

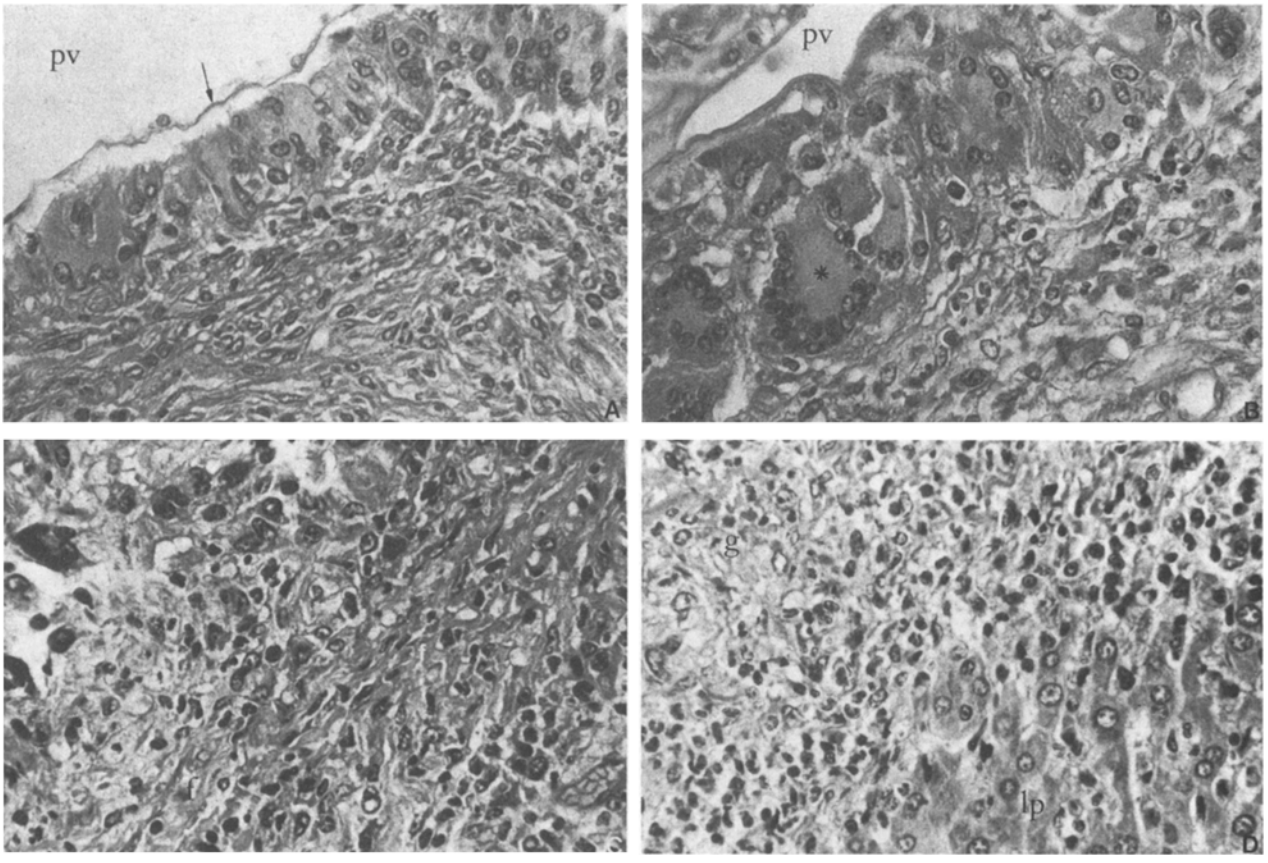

Figure 2. Pathological changes in the liver of mice at the end of the 4th month after an intrahepatic injection of a suspension of *E.m.* cysts. *A* Epithelioid cells at the contact of *E.m.* germinal layer (↓) (C57BL6 strain, HES × 40) (pv: parasitic vesicle). *B* Macrophages and giant cells (*) in the cellular infiltrate surrounding a parasitic vesicle (pv) (C57BL6 strain, HES × 40). *C* Fibrosis (f), fibroblasts and macrophages in the granuloma, distant from the parasitic vesicles (C57BL10 strain, Masson's trichrome, × 40). *D* Lymphocytes at the periphery of granuloma (g) (C57BL10 strain, HES × 40) (lp: liver parenchyma).

particularly numerous in AKR mice. They were absent in the parasite lesions of the C57BL10 strain.

There was no relationship between 'resistance' and 'sensitivity' of a strain, expressed by the percentage of positive infection or parasitic mass, and the mean specific antibody titer obtained in this strain at the first and second months after infection (table 2).

Discussion. The murine experimental model of AE tested in this study appears to fulfill the requirements that we have mentioned previously. It reproduced with a good accuracy and reliability the pathological changes observed in humans. An injection in portal or coecal veins could be considered as a good way to reproduce exactly the natural mode of infection; however, the direct intrahepatic injection of the inoculum allows a study of the noninfected liver lobes and of possible changes in portal spaces of the normal liver. As in humans, the granulomatous infiltration was very extensive and the nature, phenotype, and functional activity of the cells involved in this infiltrate could be easily studied.

Previous experimental models used the i.p.[8,14], or s.c.[14,15] routes of infection with parasite larvae. These techniques can be used to study the development of the parasite and to perform histopathological evaluation of changes in lymphoid organs[16,17] and cellular infiltration in draining lymph nodes[15,18]. However it has been shown that the liver can be considered as a very peculiar organ from an immunological point of view[19] and that the portal drainage of an allograft[19] as well as the intrahepatic development of a graft-versus-host reaction[20-22] were associated with a certain degree of tolerance. Cell-mediated immunity appears to be an important factor of resistance or sensitivity to infection with *E.m.*[3-5], and changes in lymphoid organs during AE have been shown to be very similar to those observed in graft-versus-host reactions[16-18]. For these reasons, an animal model of AE for immunological studies should reproduce the natural liver site of the initial development of the parasite. The natural route of infection with segments and onchospheres of *E.m.* given by mouth to rodents, although reproducing the 'natural' lesions[23,24] is particularly harmful for the research staff; as AE is still incurable and fatal, such a risk must be considered unethical.

Sigmodon hispidus (Cotton rat) and *Meriones unguiculatus* have often been used in experimental studies of AE mostly because of their particular sensitivity to infection by *E.m.*[10,14,23,25]. They are well adapted for pharmalogical evaluations but not

Table 2. Specific anti-*Echinococcus multilocularis* antibody titers after intrahepatic infection by *E.m.* in 4 inbred strains of mice (indirect hemagglutination test)

Strain	30th day after infection (titer*)	60th day after infection (titer*)
AKR	1/160	1/2560
CBA	1/160	1/2560
C57 BL6	1/1280	1/5120
C57 BL10	1/640	1/5120

* Titers obtained from a pool of sera from the infected mice of each strain.

suitable for immunological studies. Various sensitivities to infection can be obtained by using different inbred strains of mice. Some discrepancies in the results according to the so-called 'sensitivity' or 'resistance' of a given strain of mice[8,9,23,26] could be due to differences in the genetic background of these strains in the different countries. The 'sensitivity' of our strain of C57BL6 mice appeared to be higher compared to that of mice[23,26,27] used in previous studies.

The major interest of the comparison of the immune reactions in strains differing in their resistance to the infection by a parasite has been emphasized[28]: our experimental model of AE allows such a comparison between 'resistant' and 'sensitive' strains; the analysis of different pathological forms of sensitivity (frequency versus weight of metastases) could also be performed. Moreover, the mouse represents a fairly good model for immunological studies since its immunological status is well known; monoclonal antibodies for phenotype determinations are readily available; inbred stains are genetically well defined and recombinant and congenic mice can be obtained in order to study the relationship between resistance to *E.m.*, development of cellular immunity and genetic control. Our observations in C57BL6 and C57BL10 mice respectively 'sensitive' and 'resistant' to infection by *E.m.* although sharing the same H2 (b) determinants argue against a direct link between H2 and resistance to the parasite. Expression of cell-mediated immunity, and natural or acquired resistance to mycobacterial infection, have been shown to be controlled by, at least, two different genes, one being H2 linked and the other one not. Such relationships have to be studied in alveolar echinococcosis.

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Parenteral soya bean fat emulsions potentiate the hepatotoxicity of *E. coli* endotoxin in suckling rats

K.M. Heinonen, V. Kataja and M. Laitinen

Children's Hospital, University of Kuopio, SF-70210 Kuopio 21 (Finland), 8 March 1982

Summary. A dose of soy bean fat emulsion which was injected i.v. in suckling rats accumulated in the cells of liver parenchyma, both in hepatocytes and in reticuloendothelial cells. Subsequent i.p. injection of *E. coli* endotoxin was followed by extensive liver tissue necrosis and increased activities of serum aspartic and alanine aminotransferase. These signs of liver damage were markedly more pronounced than those observed after the administration of *E. coli* endotoxin only.
Key words. Rat, suckling; *E. coli* endotoxin; endotoxin; hepatotoxicity; soya bean fat emulsion.

Parenteral fat emulsions are widely used in parenteral nutrition. They provide high amounts of energy in small volumes of fluid, without inappropriately high osmotic loads[1]. Parenteral soya bean fat emulsions also contain sufficient essential fatty acids[2]. However, recent observations have confirmed that there is accumulation of lipid pigment in hepatocytes and reticuloendothelial cells during the parenteral use of soya bean fat emulsions in young children[3-5]. Lipid pigment deposition has been shown to persist for years after parenteral infusion of fat[5]. During and after parenteral fat infusion, various liver disturbances have been reported in human beings[6-9]. Whether or not this is caused only by the parenteral fat emulsion, or by the latter in combination with other factors, has not been thoroughly evaluated. A potentially important hepatotoxic factor, often associated with parenteral feeding, is infection[3,6-9]. In this paper, we present observations on liver damage associated with the use of parenteral fat emulsions, alone and in combination with experimental endotoxinaemia, in suckling rats.

Material and methods. 10 litters, each containing four suckling male Wistar rats, 15 days old, were divided into four groups of 10 animals. There were no significant differences (±1 SD) between the mean weights of the animals in the 4 groups (table). The groups were treated as follows.

Group I. Each animal received a parenteral injection of a 20%