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ATTEMPT TO SUPPRESS IMMUNITY IN RABBITS IMMUNIZED AGAINST EIMERIA INTESTINALIS

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Résumé


Chez les lapins hyperimmunisés, il n’a pas été possible de provoquer une rupture de l’immunité, ni avec des immunodépresseurs, ni en provoquant une diarrhée avec un antibiotique, ni en infestant les animaux avec une autre espèce de coccidie.

Apart from primary coccidial infections, in rabbitry, the results of many aggressions are characterized by diarrhoea often followed by death. Thus, one often finds that the excretion of coccidia which is normally present becomes more marked. On the other hand, it has often been noticed during the course of strain purification notably in the case of Eimeria perforans (Coudert et al., 1979) that rabbits exhibiting a low level of infection by one species of Eimeria could excrete very large quantities following an experimental infection with another species.

One explanation for this could be that rabbits infected with one or several species immunize themselves to a greater or lesser extent and that this « immunity » is overidden or suppressed following an aggression (bacterial, coccidal, therapeutic). The present experiment was carried out to test this idea.

Stressor agents used, were as follows: two immunodepressors (dexamethasone + cyclophosphamide), one antibiotic (ampicillin), one strain of bacteria (Escherichia coli) and one strain of coccidia (E. piriformis).

Dexamethasone suppresses immune responses notably those which are concerned with cellular based mechanisms (Nicol and Cruce, 1961). Its depressive effect on coccidian infections in birds has been shown by several authors (McLoughlin, 1969; Long, 1970, 1971; Long and Rose, 1970) and similarly for mammals (Niilo, 1970). The mode of action of cyclophosphamide is dependent on the intervention of thymodependent cells. The central role of T lymphocytes in coccidiosis...
Fig. 1. — Experimental plan.
has been clearly established in mammals (Rose and Hesketh, 1979). Besides this, diarrhoea can appear in the rabbit when cyclophosphamide is used as a denuding agent (Rougeot and Thebault, 1970).

The rabbit is sensitive to ampicillin which is shown by digestive troubles with diarrhoea. This has been shown by several authors (Milhaud et al., 1976; Morisse, 1978; Licois, 1980).

Materials and Methods

Animals

All rabbits used had been free of coccidia for several generations (Schellenberg, 1976; Streun et al., 1979) and were therefore not immune to Eimeria. Twelve six-week-old rabbits maintained in a special building shielded from all external contamination were used for this experiment. Food (consisting of a granulated mix free of antibiotics and anticoccidians) and water were distributed ad libitum.

Coccidiae

A pure strain of E. intestinalis was obtained by the same method as those used to isolate E. perforans (Coudert et al., 1979), starting from a mixture constituting 99.3% of E. intestinalis and 0.7% of E. coecicola (Coudert, 1976). The pure strain obtained was left to spore at 26 ± 0.5 °C. The same strain was used throughout the experiment.

Immunization (fig. 1)

E. intestinalis is extremely pathogenic (Coudert, 1978, 1979); also, the first infection was minor (800 oocysts per animal); but the four following inoculations were progressively stronger (3200, 12800, 51200, 204800). Rabbits were inoculated every two weeks up to the third infection; thereafter weekly until the final fifth infection. At the same time as the fifth inoculation, two non-immune six-week-old rabbits were infected with 800 oocysts.

Measurements of acquired immunity

The indicator chosen was the excretion of oocysts. The total amount of faeces were collected from the ninth day (end of the incubation period) to the fourteenth day (more or less the last day of excretion) following each inoculation. Faeces were weighed and homogenised and two 24 g samples were removed and then treated according to the technique described previously (Licois and Coudert, 1980). Counting was achieved using a Mac-Master cell which had been modified for counting coccidia. The technical detection limit of this method is 100 oocysts per g of faecal material. In the case of a negative result, the flotation method was used which has twice the sensitivity of the above method.

Controlled attack trials using immune animals (fig. 1)

Two trials were carried out successively on the same rabbits:

- two weeks after the 5th immunization:
  a) two rabbits were given an intramuscular injection of 4 mg/kg of dexamethasone and 15 mg/kg of cyclophosphamide three times in succession at 12 h intervals.
  b) two rabbits were treated with the same way and rhythm with 5 mg/kg of ampicillin.
  c) two rabbits were infected with 10^5 Escherichia coli isolated from a rabbit which had died of diarrhoea.
  d) the remaining animals were control animals.
- one month after the 5th immunization:
  a) with 2400 oocysts of a pure strain of E. piriformis,
  b) with 2400 oocysts of the strain of E. intestinalis which was used for immunization,
  c) with a mixture of 2400 E. piriformis and 2400 E. intestinalis,
  d) the other animals were used as non-inoculated controls,
  e) six non-immune rabbits of the same age as the preceding ones, issued from the same litters but raised in another building to avoid any possibility of contamination were inoculated in groups of two under the same conditions as a, b and c.

The different methods of infecting and treating are shown in figure 1.

Results

Acquisition of immunity

The pattern of excretion of oocysts following each immunization is shown in table 1. It
is evident when comparing the results from cages 1 to 6 (first numeration) and those of cages 7 and 8 that the inoculum did not lose its virulence throughout the duration of the experiment.

From the second inoculation onwards, the excretion was 1000 times less and after the 4th infection, it was no longer detectable.

**Attempt to terminate immunity**

Animals inoculated with *E. piriformis* alone or together with *E. intestinalis* as well as the rabbits treated with ampicillin had diarrhoea although no clinical symptoms were seen in the rabbits which received immunodepressors or the bacterial inoculum.

From table 2, it is clear that in no case was immunity suppressed: no immune animal excreted *E. intestinalis*. On the other hand, the fact that the animals were immunized against *E. intestinalis* did not affect the multiplying power of *E. piriformis* whether this species had been inoculated alone or in association with *E. intestinalis*.

**Discussion**

Few experiments have been carried out on the rabbit with respect to the acquisition of immunity against intestinal coccidia. However, Norton et al. (1979) recently showed that a single inoculation with 100 oocysts of *E. flavescens* (Coudert and Norton, 1979) or *E. irresidua* was able to provide animals with slight protection against a second infection.

In the present experiment it is noticeable that with a bigger inoculum of *E. intestinalis* (800 oocysts) the excretion of oocysts following each successive reinfection diminished rapidly to the point where it was no longer detectable after the 4th infection. Similar results have been obtained in other mammals, for example the rat (Rose and Hesketh, 1979; Rose et al., 1979). In poultry, the acquisition of this immunity as judged by pathogenic capability or by oocyst excretion seems to be variable and more or less rapid, depending on the species of *Eimeria* inoculated (Joyner and Norton, 1973, 1976; Rose, 1974).

It is noteworthy that in the course of this experiment no death due to coccidiosis was observed whereas in non-immune animals the level of mortality would have become marked
from the second infection onwards (Coudert, 1979). All attempts to suppress immunity failed whether immunodepressors, « toxic » antibiotic, colibacillis or another coccidia were used. It is worthy of note that the experimental conditions used were especially unfavourable for depressing immunity. This would have manifested itself by an excretion of oocysts. On one hand, in fact, it has been shown that dexamethazone (Niilo, 1970), or betamethazone (Long and Rose, 1970), administered at the onset of the symptoms caused by the first inoculation, provokes an aggravation of illness and an increase in the oocyst output; but one may consider that the rabbits were hyperimmunized. On the other hand the attempts to attack were carried out after the end of the theoretical excretion due to the 5th infection; this was done in order to check the hypothesis which maintains that parasitic stages are blocked within the organism.

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Summary

A single infection with *Eimeria intestinalis* is sufficient to develop an immunity which strongly reduces the excretion of oocysts and the clinical signs of illness following subsequent infections. In hyperimmunized rabbits, it was not possible to break immunity either with immunodepressors or by provoking diarrhoea with an antibiotic, or by infecting animals with another species of coccidia.

References


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