2. When compared to purified virus preparations, there was an excess of gp52 in the milk, in agreement with the isolation of membrane fractions enriched in gp52 during the purification of milk-borne MMTV (CALBERG-BACQ *et al.*, 1976). However, amounts of p28 and gp52 released in the milk vary in parallel to each other.

3. A follow-up in six different mice of the viral antigens produced in the course of the lactation shows that amounts of both antigens are very low at the beginning of lactation; they reach a maximum value on the 7th-8th day (10 times higher) and decrease during late lactation up to weanling.

4. Major amounts of antigens, with the highest p28/gp52 ratio, are more frequently detected during the 5th-6th nursing periods; this time is also the average age for the appearance of tumours.

5. Infection appears however to be better transmitted by young mice, since mice borne from a second or a third delivery are those which produce the highest amounts of antigens in their milk when adults. This is related with the observation in the Swiss colony that a selection over more than eight generations of mice borne from the 3rd to the 6th delivery does not statistically enhance the viral expression in the milk.

These results illustrate that viral expression depends on numerous factors, one of which is certainly the cyclic activity of the mammary gland, as the number of infected cells increase both from one lactation to another and in the course of one lactation. Other factors must account for the decrease in viral expression after the 5th-6th delivery and the variation in the infectivity of virus transmitted to the offspring. It might be related with a progressive immunization of the mouse, since circulating antibodies against gp52 have been detected in infected Swiss mice (KOZMA, 1982). In contrast to high-cancer-incidence strains, the Swiss strain exhibits various degrees of infection, and thus appears a suitable material for the study of the factors involved in this process.

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Natural infection of Swiss mice by the mouse mammary-tumour virus (MMTV) : 2. Studies on the pathway of infection.

The various events occurring between ingestion of virus-carrying milk by sucking mice and excretion of the virus by the mammary gland of the adult are not clearly understood. A long time of nursing appears to be necessary to obtain a high incidence of tumours, and no viral particle is detected in organs other than the gut content before the adult age (for review see MOORE *et al.*, 1979). The gut of the mouse, immature at birth, is able to absorb macromolecules, either selectively or not, up to the 14th day (MORRIS, 1980). This feature could be important in the process of the natural infection by MMTV. This work investigates the uptake of the main viral antigen by the gut and their persistance in the digestive tract.

1. The viral material ingested was followed by immunoperoxidase-staining and micro-immunoenzyme assays of gp52, the main envelope glycoprotein, and p28, the main core protein of MMTV. These latter assays were also performed after ingestion of milk enriched in viral antigens using Cr_2O_3 as a marker for the alimentary bolus migration (HAINAUT *et al.*, 1983).

Amounts of both gp52 and p28 decrease during intestinal transit, p28 being more rapidly digested than gp52. The antigens are however destroyed to a much larger extent in the gut of the adult than in that of the newborn mice. Both p28 and gp52 are found in the duodenum and small intestine. Moreover, the viral antigens are clearly observed in very large supra-nuclear vacuoles inside the epithelial cells of the distal part of the gut.

2. Preparation of tissues from different parts of the gut were examined by transmission electron microscopy (TEM) to look for intact viral particles. A comparative study of Peyer's patches of newborn and adults was also carried out by scanning-electron-microscopy (SEM) and by TEM, since different results point to the importance of the lymphoid cells in the dissemination of infection.

Virus-like particles are detected in the contents of the gut up to the distal part of the jejunum, and in close association with the apical membrane of the enterocytes of the duodenum and the jejunum. They are sometimes present in sites where a pinocytotic "coated vesicle" is initiated. By SEM, Peyer's patches of the newborn are much smaller than that of the adult, and "M" cells are less differenciated. By TEM, large dendritic cells are observed inside the lymphoid follicles of both adult and newborn mice. The membranes of these cells form a characteristic network surrounding the lymphocytes. In the adults, virus-like particles are observed in intercellular spaces between dendritic cells and lymphocytes.

In conclusion, complete particles can reach the intestine. The viral material could then be either destroyed or taken up in the epithelial cells by endocytosis so that the intestinal epithelium might serve as a portal of entry for MMTV in the sucking mouse. Moreover, virus-like particles are observed in close association with gut-associated lymphoid cells, which are known to migrate to the mammary gland during pregnancy and lactogenesis (PHILIPPS-QUAGLIATA *et al.*, 1983).

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