

MORPHOLOGICAL AND FUNCTIONAL CONSEQUENCES OF THE COEVOLUTION OF RODENTS WITH GASTRO-INTESTINAL MICROBIAL ENDOSYMBIONTS

by

ELENA I. NAUMOVA

Institute of Evolutionary Animal Morphology and Ecology,
Ac. Sci. USSR, Leninsky Prospect, 33, Moscow, W-71, (USSR)

SUMMARY

In four species of muroid rodents (*Apodemus sylvaticus*, *Microtus arvalis*, *Neotoma albigula*, *Myospalax myospalax*) the stomach and hindgut morphology was studied as well as associations of bacteria colonizing the epithelium of the forestomach. Various adaptations of the rodent stomach and hindgut to digestion and assimilation of plant food are discussed with special emphasis on coevolution of rodents and their endosymbionts. The possibility of specificity and high functional specialization of forestomach symbiocenoses in different rodent species is demonstrated. An important role of the phenomenon of endosymbiosis in the phylogeny of herbivorous rodents is stressed.

INTRODUCTION

It is well known that animals (in particular mammals) utilize plant food only in cooperation with symbiotic microorganisms involved in digestion. The reason is that plants have two nutritional drawbacks : (1) plants contain great amounts of cellulose that mammals cannot digest and assimilate ; (2) protein content of plants is insufficient for animal nutritive requirements. If the inevitability of coevolution of plant-consuming mammals with microbial symbionts is obvious, the specific patterns of this coevolution in different lineages provide an open field for comparative studies.

In the course of mammalian evolution herbivory arose in different lineages showing a wide range of wonderful morphological variations of their stomach and hindgut. Comparative studies of the digestive system in herbivorous mammals have demonstrated different ecological and evolutionary trends. However, the role of endosymbiosis in the evolution of these varieties has been traditionally underestimated. In large herbivorous mammals, especially in ruminants, the ecological and functional aspects of their coevolution with endosymbionts are well investigated (HUNGATE, 1966 ; LANGER, 1974, 1986 ; HUME and WARNER, 1980 ;

HOFMANN, 1983). It should be noted here that ruminants totally depend on the symbiotic activity and, most interestingly, the ruminal symbiocenoses are polyfunctional in all cases : they are composed of a variety of bacteria and protozoa that are able to ferment not only cellulose but also starch, lipids, proteins and other nutrients. As to rodents, the functional and structural manifestations of the endosymbiosis are there still little understood.

MATERIAL AND METHODS

The structure of various stomach and gut patterns and modes of colonization of the corporal cornified epithelium by attached bacteria were investigated in four species of different genera of muroid rodents : the wood mouse, *Apodemus sylvaticus* (from West Ukraine, USSR), the common vole, *Microtus arvalis* (from suburbs of Moscow, USSR), the white-throated wood rat, *Neotoma albigula* (from Mexico) and the Siberian zokor, *Myospalax myospalax* (from Altai Mountains, USSR). From 5 to 10 mature specimens of each species were fixed by means of injection of 4 % neutral formaldehyde into the cavities of stomach and gut. The samples (5 mm by 5 mm) of cornified corpus ventriculi were washed in isotonic saccharose solution, fixed in neutral 4 % formaldehyde and subsequently processed for SEM (scanning electron microscopy). The samples of *N. albigula* corpus ventriculi were embedded in paraffin and sectioned at 7 mkm. Sections were stained with the Gram technique and the periodic acid-Schiff reaction combined with haematoxylin.

RESULTS AND DISCUSSION

The wood mouse is a widely distributed muroid rodent and is known to be a seed-eater. Seeds do not contain much cellulose, but their other components are digestible by means of endogenous enzymes. The stomach of the wood mouse (Fig. 1, A) may be classified (CARLETON, 1973) as bilocular-hemiglandular. The incisura angularis is not very deep, the sulcus on the greater curvature is not clearly developed and the bordering fold is not high. So the non-glandular corpus ventriculi, or the forestomach as many authors call it (e.g. GAERTNER and PFAFF, 1979 ; PERRIN, 1986 ; AMASAKI *et al.*, 1988) and the glandular part of the stomach are not well isolated from each other. Consequently, gastric acidity cannot be kept constant for optimal activity of symbionts and, moreover, symbionts can be easily washed out from the stomach. The symbiocenosis of the forestomach is represented by various attached bacteria (Pl. 1, A), as the food that enters the stomach of the wood mouse consists of biochemical components which can serve as a favourable substratum for the activity of proteinolytic, amylolytic and some other symbionts. But in the unisolated forestomach bacterial fermentation is not intense, so bacterial biomass is not large, and can provide the host with only a complementary source of protein.

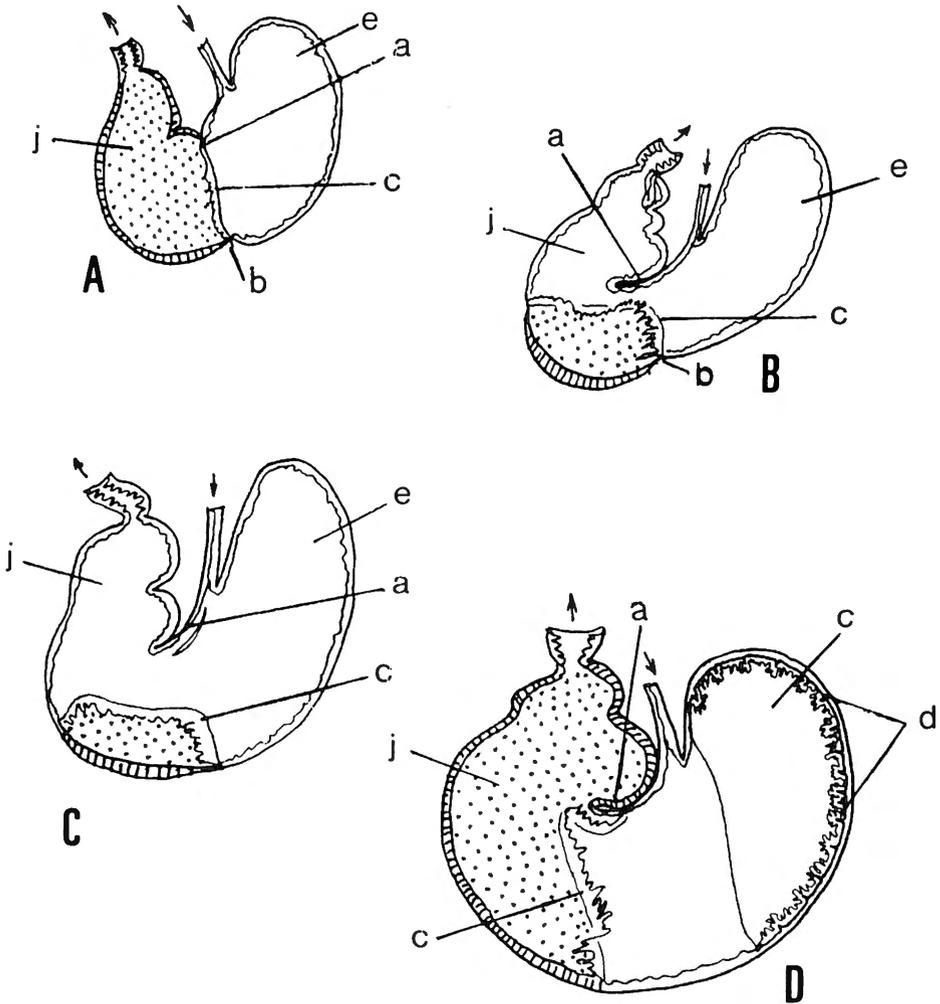
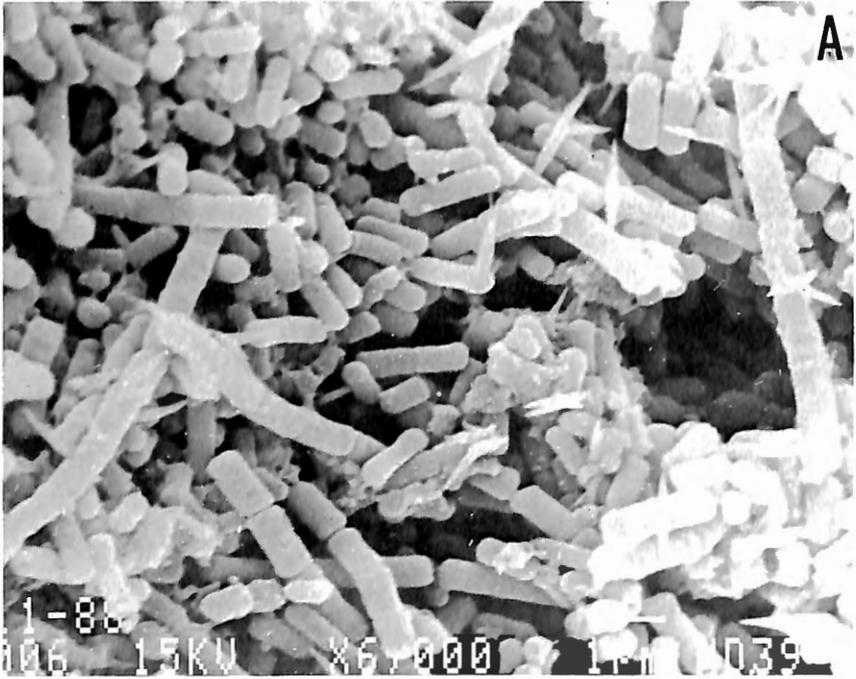


Fig. 1. — Gastric morphology of *Apodemus sylvaticus* (A), *Microtus arvalis* (B), *Neotoma albigula* (C), *Myospalax myospalax* (D) : a - incisura angularis, b - sulcus on the greater curvature, c - bordering fold, d - symbiopapillae, e - forestomach (corpus ventriculi), j - antrum.

All these facts show that endogenous and symbiont types of digestion in the wood mouse supplement each other. Indeed, the diet of this rodent contains all the components that the host needs for growth and reproduction.

In the caecum the substratum for symbionts is more uniform than the one in the stomach. Owing solely to caudal disposition of the caecum in the digestive tract caecal content is as if enriched with cellulose, since digestible nutrients have been already extracted in the stomach and small intestines. So dimensions and architec-



ture of the caecum are good indices in the first line of its role in cellulose splitting in different species.

The caecum in the wood mouse is small, the ampula coli is not clearly developed ; the colon has V-shaped form (Fig. 2, A). In the hindgut of such structure the fibrous residues of seeds probably are subjected to weak symbiotic fermentation.

The common vole consumes predominantly grasses that are rich in cellulose, and so this rodent is obliged to use bacterial symbionts intensively for utilization of these components. The common vole possesses a bilocular-discoglandular stomach that differs from that in the wood mouse in the following aspects. The zone of glandular epithelium is restricted to a small area on the greater curvature ; the incisura angularis, as well as the sulcus on the greater curvature, are appreciably deeper than in the wood mouse (Fig. 1, B). Consequently, a narrow channel connecting two chambers of the stomach is formed. The high bordering fold with a toothed ridge contributes substantially to the isolation of two chambers. The conditions described above favour a more intense bacterial fermentation of nutritional components (including cellulose) because isolation of the forestomach allows the process of fermentation to be prolonged. The composition of bacterial populations colonizing the cornified epithelium is more homogeneous than in the wood mouse (Pl. 1, B) and, hence, proves more high specialization of bacterial fermentation in the forestomach. As regards the extraction of nutrients from cellulose in the forestomach, there is evidence that some species of *Microtus* apparently are similar to ruminants. For example, *M. montebelli* produces volatile fatty acids in a ratio similar to that found in ruminants (OBARA and GOTO, 1980) ; these points to bacterial cellulose fermentation. Probably in the herbivorous *M. arvalis* similar processes take place in the forestomach.

The caecum and colon in the common vole, compared to those of the wood mouse, are more complicated (Fig. 2, B). The caecum is long and sacculated ; the double colic spiral and ampula coli are well developed. As stated above these peculiarities of the caecal morphology manifest a high degree of adaptation to digestion of cellulose.

The diet of the white-throated wood rat mainly consists of green parts of plants, like in the common vole. The stomach of this rodent is bilocular-discoglandular, but the chambers are less isolated than those in the common vole (Fig. 1, C). So both the diet and the morphology of the stomach are very similar in the common vole and the white-throated wood rat. Hence, one can expect similarities in symbiocenoses with the same functions in the stomachs of these species. Rather

PLATE 1

Specific associations of symbionts colonizing the cornified epithelium of the forestomach. A : *Apodemus sylvaticus* ; B : *Microtus arvalis* (scanning electron micrographs).

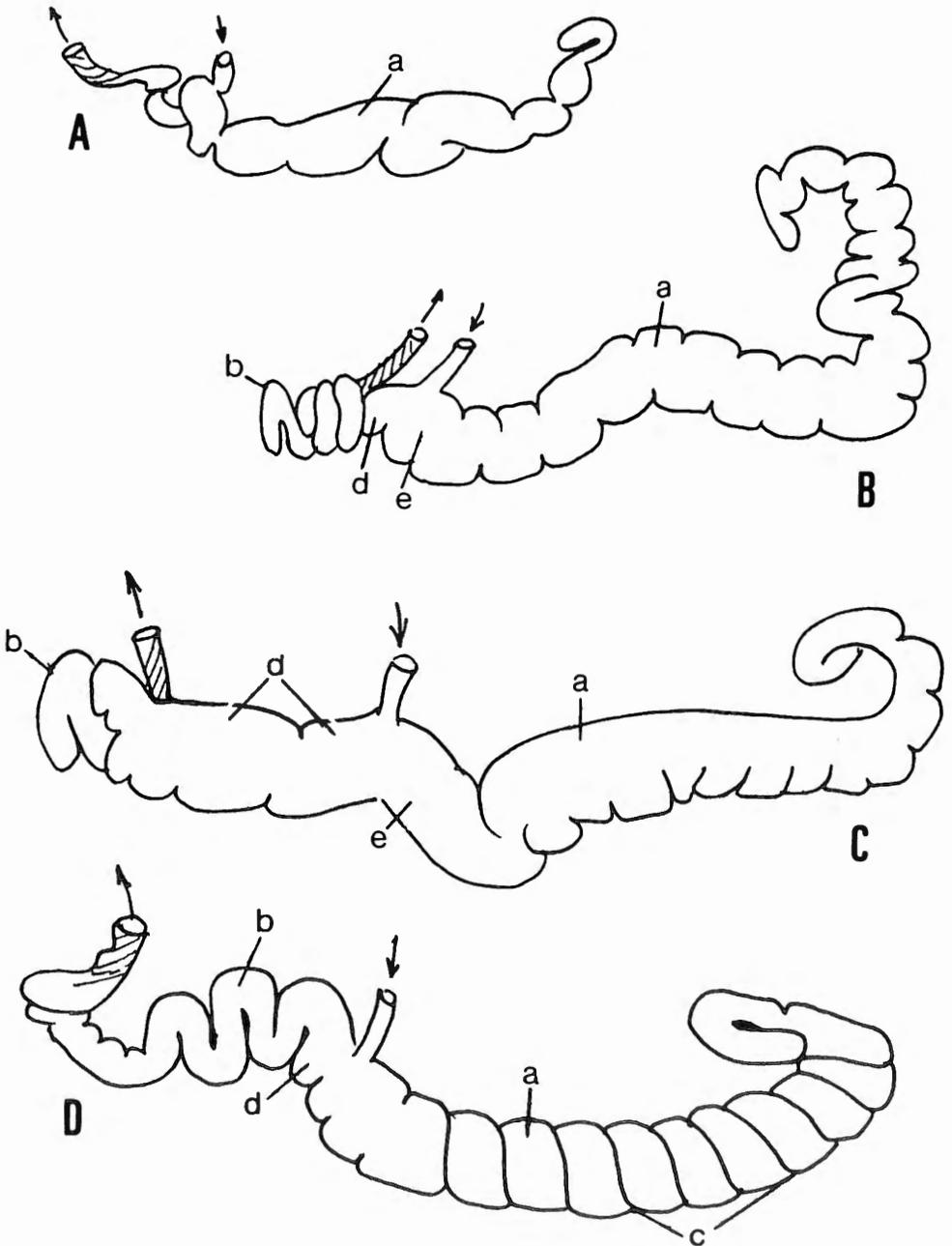


Fig. 2. — Hindgut morphology of *Apodemus sylvaticus* (A), *Microtus arvalis* (B), *Neotoma albigula* (C), *Myospalax myospalax* (D) : a - caecal body, b - colic spiral, c - spiral fold, d - ampulla coli, e - ampulla caecalis.

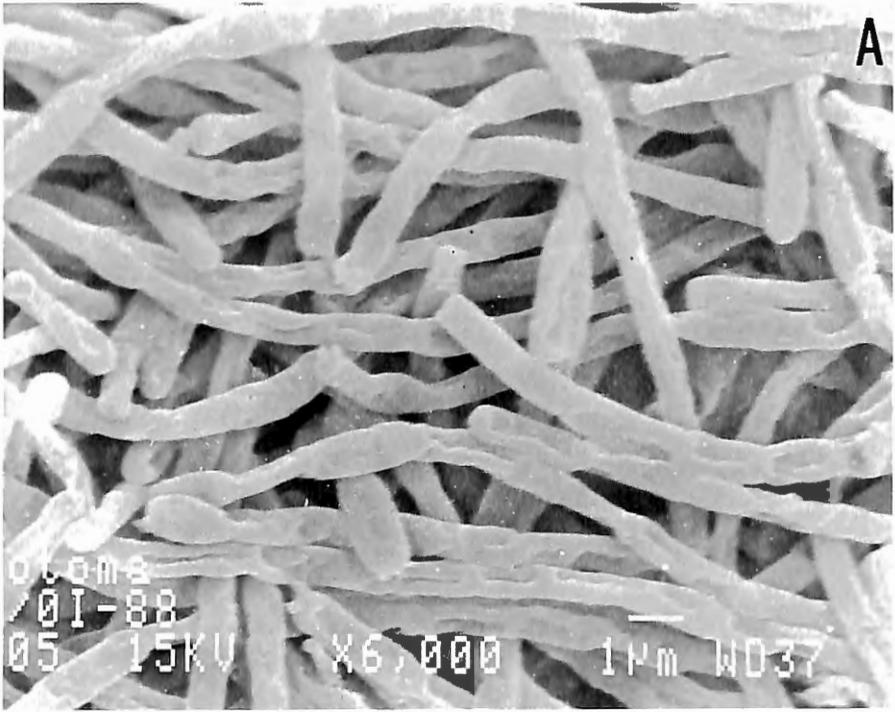
unexpectedly, together with common cocci and bacilli filiform bacteria were found colonizing the wide surface of the cornified stomach epithelium of the latter species (Pl. 2, A). Such an association of attached bacteria gives grounds to believe that the main function of symbiocenosis in the white-throated wood rat differs from its supposedly cellulolytic function in the common vole. In these two cases the different ways of coevolution of rodents with endosymbionts are not reflected conspicuously in the morphology of the stomach but mainly manifest themselves in the different associations of attached bacteria, that probably produce different digestive enzymes.

The caecum of the white-throated wood rat is long and voluminous, has a sacculated body; the ampulla caecalis is moderately pronounced; the colon is V-shaped, the ampulla coli is long and wide (Fig. 2, C). According to the complicated morphology of the hindgut in this rodent it is the place of more intense fermentation of cellulose.

In the Siberian zokor, an underground rodent consuming bulbs, rhizomes and roots, as well as green parts of plants, unusual endosymbiosis was discovered. The stomach of this rodent is unilocular-hemiglandular, but the cornified epithelium of the forestomach forms papillae (Fig. 1, D). Earlier investigators used to explain these complications in the structure of the stomach as adaptations to consumption of food rich in cellulose, as it is observed in ruminants (for review see CARLETON, 1971), but recent investigations, as stated below, disproved this explanation.

Papillae in the forestomach of the Siberian zokor do not look like papillae in the rumen. In the latter papillae consist only of the host's tissues including all mucosae layers and submucosa; their surface is covered with various attached bacteria (SOKOLOV *et al.*, 1989). To the contrary, in the Siberian zokor papillae consist of the tissues of two different organisms viz. only of cornified epithelium of the host's stomach and bacterial bodies that are embedded in these epithelial cells (Pl. 2, B). This gave me grounds to name these unusual structures «symbiopapillae» (NAUMOVA, 1981). Similar structures have been described in detail in four other species of rodents that belong to different systematic groups. These species are: *Tachyoryctes splendens*, Rhizomyidae (RAHM, 1976; ZINGG, 1978); *Mystromys albicaudatus*, Cricetidae, Cricetinae (MADDOCK and PERRIN, 1981); *Cricetomys gambianus*, Muridae, Cricetomyinae (PERRIN and KOKKIN, 1985); and *Cryptomys damarensis*, Bathyergidae (the latter species is mentioned by many authors, but it has not been investigated sufficiently).

Bacteria colonizing the stomach epithelium in the Siberian zokor belong to the gram-negative group as well as those in *Tachyoryctes*. High amylolytic activity was discovered in bacteria inhabiting forestomach papillae in *Mystromys* and *Cricetomys* (PERRIN, 1987). Supposedly, the symbiopapillae in Siberian zokor, having the same structure as in the species listed above, produce amylase or some other enzymes duplicating functions of the host's endogenous enzymes. It is explainable as a result of the phenomenon of the function transfer (KOKSHAYSKY, 1980) which implies that microbial and endogenous enzymes are interchangeable in the course of evolution. The formation in rodents' forestomachs of symbiocenoses with enzyme-systems duplicating those of the host, is biologically significant in develop-



ment of the internal trophic chains in the course of evolution (NAUMOVA, 1981, 1989), viz. in enriching the poor plant food with microbial protein. The incorporation of bacteria into the epithelium of papillae prevents them from being washed out from a forestomach that is poorly isolated morphologically. Together with exfoliating superficial cornified epithelial cells bacterial bodies making up an appreciable biomass (see Pl. 2, A), get into the stomach cavity and are digested as the usual food.

The caecum of the Siberian zokor is voluminous and possesses a unique structure for rodents viz. the spiral fold (Fig. 2, C) that assists the circulation of the caecal content and the retention of symbionts in it. It is very likely that these symbionts produce cellulase, as the Siberian zokor consumes much cellulose with the food.

CONCLUSIONS

It is well known that different parts of a plant have a different biochemical composition. Whereas large grazing herbivorous mammals, for example ruminants, consume almost the whole plant (therefore much cellulose as well), small rodents are able to choose their food more precisely and to select the most palatable parts of plants. That is why rodents can provide a more homogeneous substratum for specific activities of their symbionts. Importantly, many rodents have retained predatory habits (LANDRY, 1970) and so complementary animal diet can compensate for the deficiency in nutritional plant protein. These two circumstances determine the differences in the ways of coevolution between hosts and endosymbionts as one can observe it in rodents and ruminants. The described examples illustrate particular traits of a coevolutionary process between muroid rodents and their endosymbionts. First of all, in the forestomach of rodents a high specialization of symbiocenoses may be formed in contrast to ruminants having only polyfunctional symbiocenoses. Secondly, because of the phenomenon of function transfer in the digestion of rodents, these animals depend less on the activity of their endosymbionts than ruminants do. Furthermore, evolutionary morphological transformations in the digestive system of rodents should be considered as adaptations not only to the particular feeding habits but also to the endosymbionts, which are the primary consumers of food entering the forestomach and simultaneously producers of valuable nutrients for the host.

PLATE 2

A : *Neotoma albigula* (scanning electron micrographs) ; B : *Myospalax myospalax*, histological section through symbiopapilla, haematoxylin and eosin, obj. 20, oc. 7, a = bacterial mass, b = cornified epithelium.

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