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Individual Evolution of Digestive Tract Colonization of Holoxenic Mice by *Candida albicans*

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Oral administration of various concentrations of *Candida albicans* to 6-day-old mice established colonization of the digestive tract without mortality. After being weaned (day 21), the development of colonization was studied in a group of mice by daily counting of the number of CFU contained in the feces of each animal. Two features concerning the development of colonization were noted. The course of colonization in individual mice was highly erratic and lead to either transitory or sometimes long-lasting colonization. These results show the importance of a dynamic study of colonization, a condition necessary for any experimental study.

The experimental model of Pope et al. (13) makes it possible to obtain colonization of the digestive tract of outbred mice with *Candida albicans* when a suspension of blastospores is administered orally to 4- to 6-day-old mice. Compared with other models (germ-free, specific-pathogen-free adult mice or outbred adult mice previously treated with substances such as antibiotics, immunosuppressors, or irradiation alone or in combination), this model mimics perfectly the behavior of a healthy carrier. It, therefore, appears to be the best model to use to study mechanisms which favor the occurrence of digestive candidiasis, its persistence, and outcome.

An analysis of all the studies performed with this model (6, 8, 9, 12, 13) showed extreme differences in the behavior of mice within the same test group; with death occurring in some animals and colonization of the digestive tract occurring in others. In colonized mice, significant variations in the duration of digestive tract colonization (between 4 and 10 weeks) and its intensity are noted, with these parameters being measured at certain times during its development. The variations observed by comparing successive groups of animals over time make it difficult to assess the process of healing or aggravation. It is interesting to follow the course of infection in colonized mice individually to undertake a kinetic study of the level of *C. albicans* colonization in the digestive tract. Daily evaluation of colonization by the enumeration of organisms in homogenates of different segments of the gastrointestinal (GI) tract is not possible because mice must be sacrificed for such evaluations. For this reason, the population of *C. albicans* present in the final portion of the gastrointestinal tract was evaluated by performing colony counts on fecal pellets. Reliability of this method has been demonstrated in numerous publications dealing with the antagonistic effect exerted by the intestinal flora of the mouse against various bacteria and fungi ingested from the environment (2) and the modulation of this effect by different agents (1, 3–5).

MATERIALS AND METHODS

Strains. This study was carried out with a clone of *C. albicans* 6311 isolated from hemodialysis fluid and maintained in the laboratory by successive subcultures on Sabouraud glucose agar. However, the virulence of five other clones was also compared with that of clone 6311. The five other clones were isolated from five *C. albicans* strains obtained from the University Hospital, Lille, France (four of these strains were isolated from lesions on buccal or vaginal mucous membranes, and the fifth was isolated from a bedsore). Suspensions of these different clones were freshly prepared in sterile deionized water from cultures grown on Sabouraud glucose agar for 24 h at 37°C. Counts were performed by using a Thoma cell counting chamber.

Animals. The breeding stock was composed of 8-week-old Swiss OF 1 SPF mice obtained from IFFA Credo, l'Arbresle, France. Infant mice were bred in our laboratory, and immediately after birth, each litter was arranged so that each dam had eight pups; weaning was carried out on day 21. Mice were maintained under controlled lighting conditions, with illumination for 12 of 24 h.

Oral administration of blastospore suspensions. According to the method of Pope et al. (13), 6-day-old mice were separated from dams and fasted for 5 h at 35°C. A 25-μl amount of a blastospore suspension was given to each infant mouse (blastospores were administered with an Eppendorf micropipette equipped with a plastic cone extended by a 0.38-mm-diameter polyethylene thin catheter). After challenge, infant mice were maintained at 35°C for 1 h more before being returned to the dams (without regard for parentage).

Enumeration of colonies in fecal pellets. Enumeration of colonies was carried out on fresh pellets. Since true pellets are only produced after weaning, enumeration was started on day 21 after birth which corresponded to day 15 after ingestion of *C. albicans*. Each mouse was placed in a cage containing absorbent paper. A few fecal pellets were taken between 8 and 9 a.m., weighed, and ground with a Potter homogenizer at a proportion of 0.1 g of feces to 3 ml of sterile deionized water. Tenfold dilutions of this homogenate were prepared, and 1 ml of each was cultured in petri dishes containing 20 ml of Sabouraud glucose agar plus chloramphenicol (500 mg/liter). After a 48-h incubation at 37°C, the number of *C. albicans* colonies was determined. This procedure was carried out every working day; for this initial study we did not consider it necessary to perform continuous determination.

RESULTS

Virulence of the six clones for infant mice. Suspensions of the six clones, ranging from 1.25×10^7 to 2×10^8 blastospores were administered orally to different groups of 10

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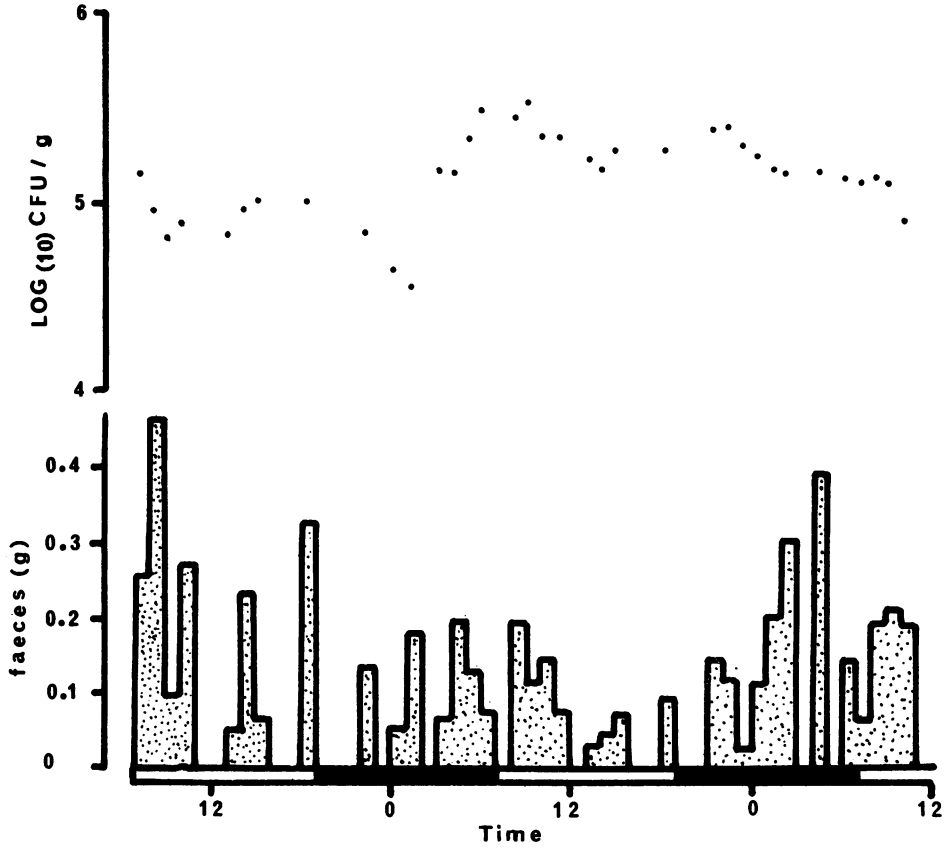


FIG. 1. A 52-h survey of fecal elimination of *C. albicans*. Hourly evaluation of fecal weight, CFU of *C. albicans* per gram of feces. Periods of night are represented by dark lines.

infant mice. The progress of the infection in the animals was followed for 1 month. No mortality was observed with either the clone 6311 or any of the five other clones prepared from strains freshly isolated from human lesions.

Nycthemeral elimination of feces. Figure 1 shows a kinetic study of fecal elimination in a mouse, hourly, over a 52-h period. For each hour, two values are represented, fecal weight and $\log_{10}$ *C. albicans* CFU/g of feces. A discontinuity in fecal elimination was observed. From this preliminary experiment, we determined the optimal time for rapid and abundant collection of fecal samples.

Daily evolution of colonization. Daily enumeration of viable colonies contained in fresh feces of individual mice was carried out for two groups of mice. The first group of 12 mice was divided into three series of four animals which received 0.62×10^7, 1.25×10^7, and 5×10^7 blastospores, respectively.

Our procedure made it possible to determine variations in the number of colonies isolated from the same animal. From one day to another the number varied by a factor of 10 or more. These variations were very frequent but occurred irregularly. This phenomenon, common to all of our mice, persisted for the duration of colonization, which was estimated only to start from weaning.

The erratic course of persistent digestive tract colonization of mouse I 1 (group I, mouse 1) is shown in Fig. 2 and that of mouse I 3, for which the yeast was finally eliminated from the digestive tract, is shown in Fig. 3. In these figures, the results are expressed as $\log_{10}$ CFU per gram of feces over time. Only results of $>10^3$/g appear since this number is considered to be the limit of sensitivity of the method. Despite the important variations already indicated for mouse I 3, points are distributed around a straight line which is the sign of a trend for elimination according to exponential law.

The schematic representation in straight-line form has been adopted in Fig. 4 to show the development of colonization of the 18 mice studied. Two general trends emerged, either persistence of colonization (mice I 1 and I 4) or an elimination process which, in the first approximation, is of exponential type. Figure 4 shows the duration of half-elimination for each mouse and the level of colonization at the time of weaning assessed by linear regression. The correlation coefficient r^2 expresses points spreading around the straight line and justifies the term "trend" used. Among the mice which eliminated *C. albicans* some showed very similar behaviors. Mice I 7 and I 8 received the same inoculum and had similar levels of colonization at weaning time and similar duration of half-elimination. The same can be said for mice I 3 and I 9 from two series inoculated with 6.2×10^6 and 50×10^6 blastospores, respectively. Conversely, in mice of the same series, e.g., series II, we observed a great diversity in the levels of colonization at weaning time and through the course of colonization. Note again mice I 1 and I 4, which received 6.2×10^6 and 50×10^6 blastospores, respectively, since they remained colonized persistently but at slightly different levels.

Within the limits of our experiments, we can say that the dose initially ingested is unrelated to the level of colonization at weaning time, the duration of half elimination, or the mode of colonization.

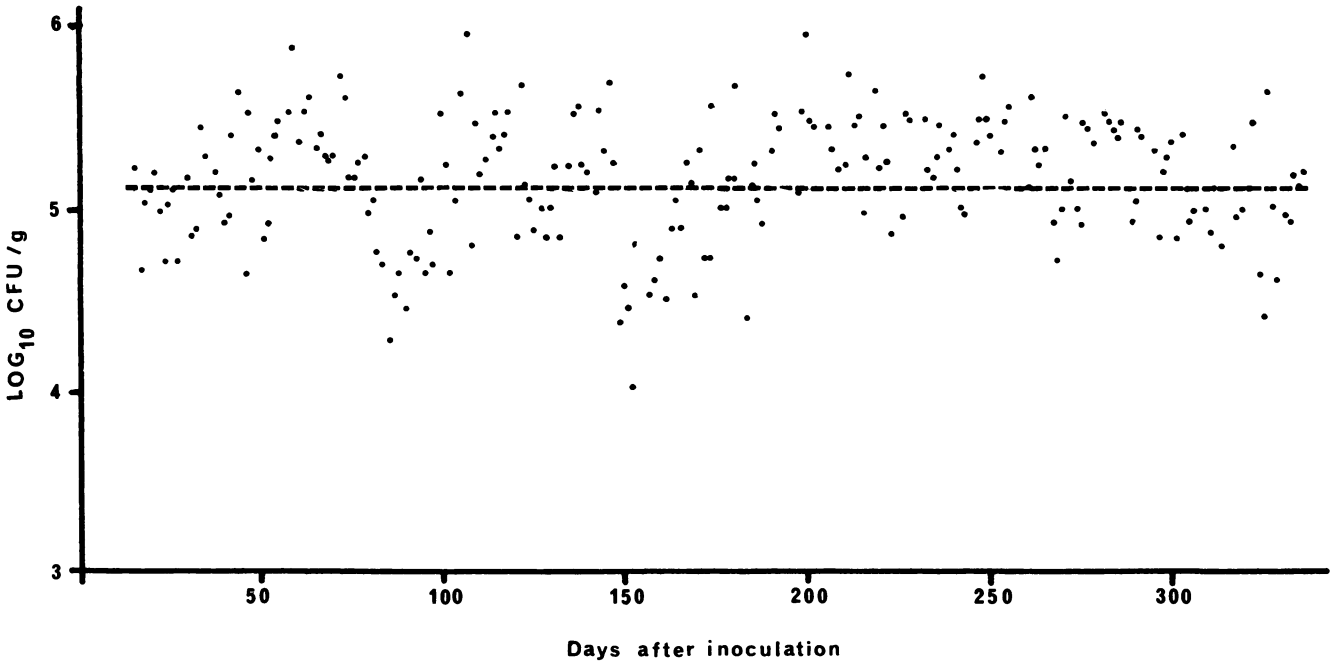


FIG. 2. Course of the colonization of the digestive tract of mouse I 1 evaluated from fecal pellets.

The presentation of Fig. 4 in the form of general evolutive trends is a good means of comparing the mice, but it has the disadvantage of masking the details concerning the dynamics of colonization. We considered it desirable to use a mode of expression intermediate between primary results (Fig. 2), which were difficult to compare directly, and that shown in Fig. 4 which is a too schematic representation. To counter the variations occurring rapidly for periods of time less than 7 days, we smoothed the curve by noting for day (D) the arithmetic mean of the results obtain between $D - 3$ and $D + 3$ (6). The smoothed curves for mouse II 6, which eliminated yeasts, and mouse I 1, whose colonization was persistent, are shown in Fig. 5 and 6. We stress the fact that the scale is arithmetic on these figures and not logarithmic as used previously. In fact, we did not have to show an evolution of exponential type, and each point of the smoothed curve is an arithmetic mean.

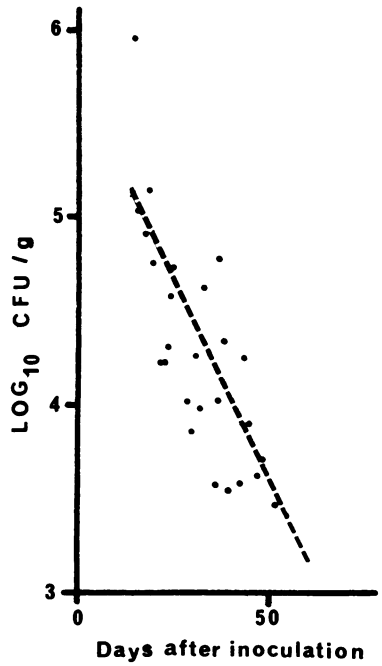


FIG. 3. Course of the colonization of the digestive tract of mouse I 3 evaluated from fecal pellets. Dashed line represents the trend of the evolution.

Figure 5 indicates information which can be drawn from the comparison of the three modes of expression, real values shown as points, an exponential curve revealing the evolutive trend, and a smoothed curve. The smoothed curve for mouse I 1 presents fluctuations organized in periods of high colonization interrupted by periods of crisis, already visible in Fig. 2. However, these variations do not seem to be linked to a precise rhythm.

DISCUSSION

After intragastric challenge of infant mice, Pope et al. (13) observed early mortality occurring between 1 and 3 weeks. Some strains that they considered to be more virulent than others gave a mortality of 50% or more. However, these authors did not explain why the same strain demonstrated to be virulent in an initial experiment (13) proved to be less virulent when retested (6).

We noted the absence of mortality in infant mice administered doses ranging from 1.25×10^7 to 2×10^8 blastospores of clone 6311 orally on day 6. This could have been explained by avirulence of the strain chosen, so the experiment was repeated with the clone 6311 and the five other clones recently isolated from human lesions. When administered orally at a dose of 2×10^8 blastospores, none of the clones were observed to cause mortality.

Several hypotheses can be proposed to explain this difference in behavior and they are summarized below. (i) The possibility that our mice were more resistant cannot be excluded. (ii) All six strains were avirulent. This is not probable since five of the six strains were recently isolated

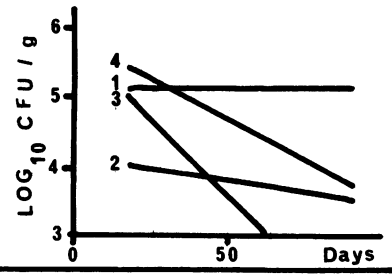
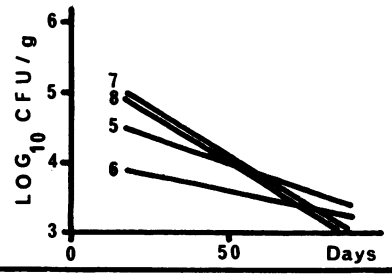
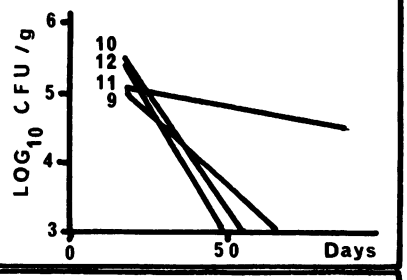
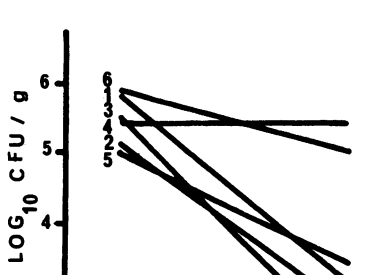
Mouse	Inoculum ($\times 10^6$ yeasts /mouse)	Half-elimination Time (Days)	Colonization at weaning ($\times$1000 CFU/g)	Correlation coefficient (r^2)	
I 1	6.2		137		
I 2		63.0	10,2	0.17	
I 3		7.1	113	0.77	
I 4		13.9	242	0.70	
I 5	12.5	22.4	24.7	0.54	
I 6		38.5	6.7	0.02	
I 7		10.7	136	0.69	
I 8		11.6	103	0.77	
I 9	50	7.2	117	0.58	
I 10		5.0	368	0.75	
I 11		38.5	122	0.27	
I 12		4.3	241	0.74	
II 1	50	8.8	609	0.73	
II 2		9.5	143	0.55	
II 3		6.9	318	0.74	
II 4			273		
II 5		13.3	137	0.61	
II 6		19.8	762	0.62	

FIG. 4. Individual trend of the evolution of candidiasis in 18 mice. The colonization of digestive tract was evaluated from fecal pellets.

from human lesions. Preliminary experiments on the virulence of strains administered intravenously would not have been relevant as Field et al. (6) pointed out the lack of correlation between virulence measured by intravenous inoculation and the ability of the same strain to colonize or invade the digestive tract. For this reason, oral administration was the only method used to determine the virulence of the six strains. (iii) Intragastric inoculation of infant mice causing traumatism as noted by Hector and Domer (10) and may be the cause of the mortality reported by Pope et al. (13). In fact Hector and Domer used infant mice challenged by using mothers whose nipples had been coated with a culture of *C. albicans*, a procedure which often causes a mammary infection in the mother. The absence of mortality in their infant mice study may be explained two ways, either the absence of traumatism during ingestion or protection of infant mice fed by mothers sensitized during *C. albicans* infection of the mammary gland. Our technique of inoculation has the advantage, over that of Hector and Domer of making it possible to administer definite quantities of yeasts, avoiding trauma of the digestive tract as well as any *Candida* infection in suckling mothers.

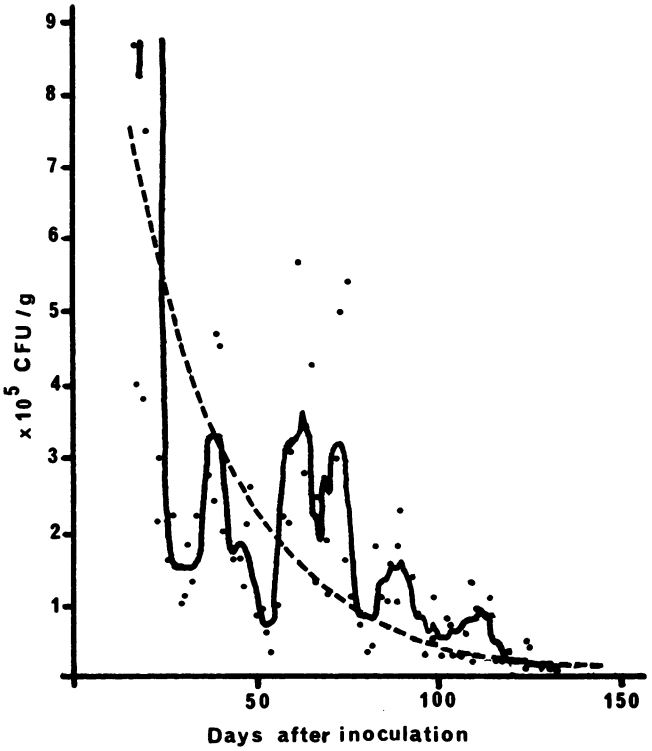


FIG. 5. Course of the colonization of the digestive tract of mouse II 6 evaluated from fecal pellets. Dots represent the measures, the dashed line shows the trend of evolution, and the solid line shows a smoothed curve with a moving average 7 days in length.

The technique of CFU enumeration in feces was used by Field et al. (6) and Hector and Domer (10) to assess the duration of colonization of the digestive tract of their mice, although their methods differed from those used in this study. Field et al. performed counts every week, from week 3 to week 10 postinoculation, on different batches of mice colonized by four strains of *C. albicans*. Each week, a different batch of mice was used, and the percentage of carrier mice to inoculated mice and the mean CFU per fecal pellet were calculated. The authors observed that colonization of the digestive tract sometimes had a duration of 10 weeks or more, although elimination often commenced after week 5 or 6. Comparing several strains, they stressed differences in the ability of the strains to cause colonization. Hector and Domer assessed the percentage of animals from a batch of mice which had eliminated *C. albicans* by the age of 6 weeks. This was found to be 6%, although the rate of colonization was not assessed for positive mice.

Our method of daily and individual evaluation revealed two interesting features concerning the evolution of digestive tract colonization which had not been observed in the sequential analysis of previous authors. These included the fluctuating aspect of this colonization, which often varied markedly from day to day, and the existence of two modes of colonization, either transitory colonization which was the most frequent, usually persisting for more than 12 weeks after inoculation (72% of mice still had colonization of $>10^3$ CFU/g at this date), or colonization which persisted for more than 44–46 weeks (mice I 1 and II 4, respectively).

As modes of colonization and elimination, individual variations, and variations between animals are known, it did not seem desirable to express the results as the mean

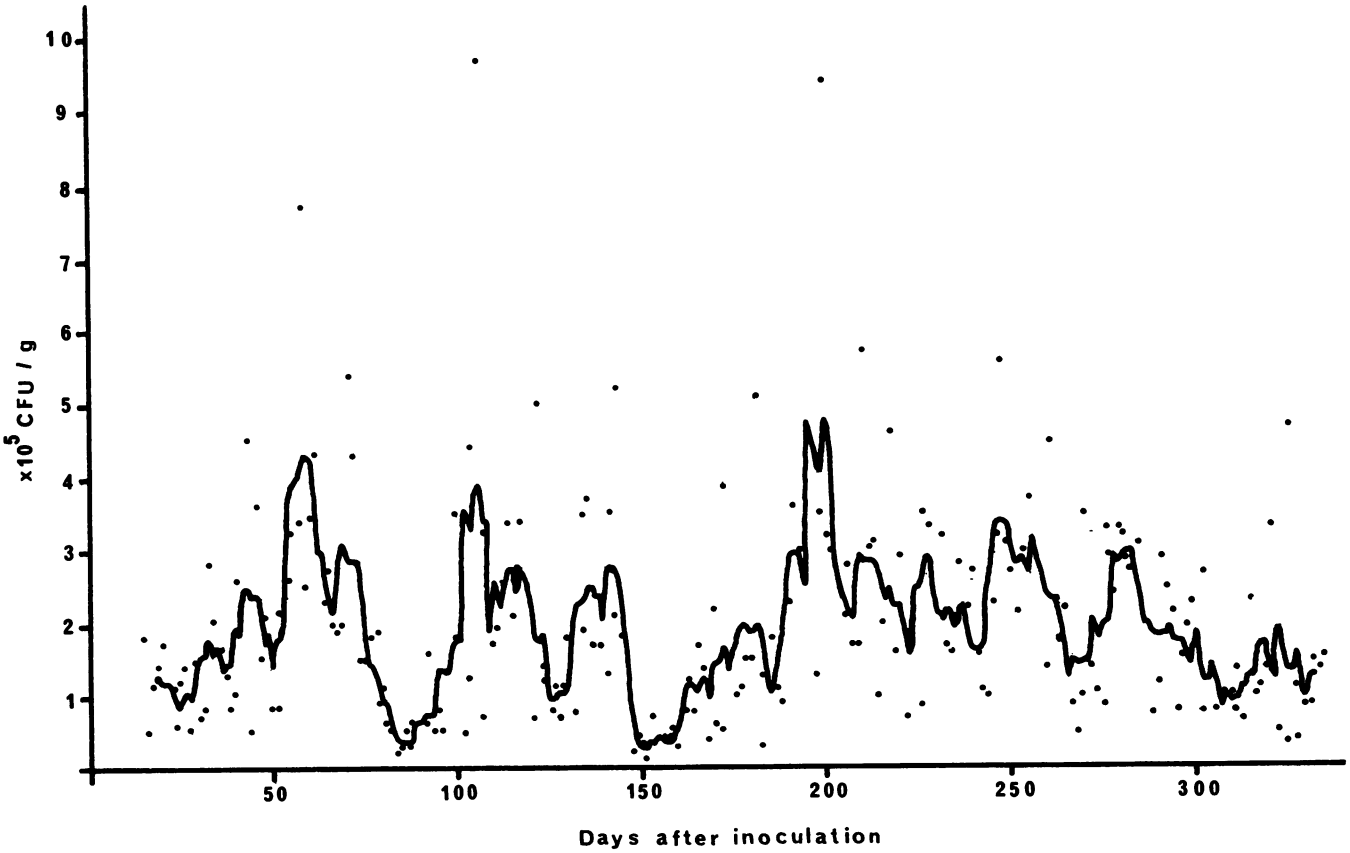


FIG. 6. Course of the colonization of the digestive tract of mouse I 1 evaluated from fecal pellets. Dots represent the measures, and the solid line is a smoothed curve with a moving average 7 days in length.

carriage rate for a large number of animals at a given time of their colonization. It seemed more interesting to try and interpret the results in terms of dynamics. Asymptotically steady states were considered. For our results, one of these steady states was that of the noncarriers. Most mice reached this state. It is more difficult to characterize the steady state reached by mice I 1 and II 4. An attracting closed orbit is not in question, smoothed curves did not show "periodicity," and a deep study, Whittaker's test and autocorrelation (11), confirmed that time series were not cyclic.

A stable equilibrium associated with noise could be considered, but a phenomenon of turbulence associated with a "strange attractor" (14) could not be discounted. Guckenheimer (7) has suggested a method for distinguishing noise (randomness) from chaos in which a sensitive dependence to initial conditions prevents long-term prediction. Unfortunately, our time series were not complete and were too short (see above) to find close situations and the development from these situations. However, this way of approaching the results raises the question of attractor bifurcation and the problem of the different behavior of the mice (elimination or colonization) not in terms of individual variations of mice (genotype) but in terms of experimental conditions and sensitivity to initial conditions (14).

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