Cell population kinetics in the small intestine of the Mongolian gerbil[1]

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Summary. The cell population kinetics studies reveal differences when compared to other rodents. Proliferative activity is slower in the gerbil than the mouse and the rat. Further study is indicated since the use of the gerbil for research is expanding.

The Monogolian gerbil, *Meriones unguiculatus* is a common laboratory animal currently being used in research. Billingham and Silvers[3] previously investigated histocompatability factors and Magalini et al.[4] reported that the gerbil exhibited unique anatomical and physiological features which make it suitable for homologous and heterologous tumor grafts.

Since interest in the gerbil is expanding, a thorough analysis of its population kinetics is needed. The purpose of this study is to examine specifically its ileal cell population and to compare those findings with previously published findings for the rat and mouse. The scope of our experiments falls into 2 categories: the establishment of labeling index (LI), mitotic index (MI) and the number of cells per crypt and the analysis of the cell cycle and its respective phases.

Materials and methods. Adult male gerbils (*Meriones unguiculatus;* 12–18 weeks old and weighing 52–66 g) were used. They were raised from 12 original pairs obtained from Tumblebrook Farm. The colony was inbred for 2 years before animals were used for analysis.

The squash technique[5] was used to determine the LI (percent cells labeled per crypt), MI (percent of cells dividing per crypt) and the number of cells per crypt. 4 or more animals were used for determining the LI, MI, and the number of cells per crypt with at least 15 crypts per animal counted. A minimum of 2 animals per h were used, with an average of over 50 mitoses per point counted for cycle times. Animals were injected i.p. with tritiated thymidine (^{3}HTdr 0.8 µCi/g.b.wt, sp.act. 7.0 Ci/mM) 45 min before sacrifice (for ML, LI, and cells/crypt) and at various times prior to sacrifice (for cell cycle) by cervical dislocation. A small piece of the ileum approximately 2.5 cm from the ileocecal junction was removed for analysis. Samples were fixed in cold Carnoy's, stained by the Feulgen reaction, crypts dissected free and squashes prepared. Autoradiographs, were then made using Kodak NTB2 emulsion after which slides were exposed for 7.5 days at 0 °C. A control series of slides was developed to determine optimal exposure time.

Statistics and modeling. LI, MI and the number of cells per crypt were calculated and expressed as means and the SEM. Analysis of cell cycle time employed graphic analysis and the automatic analysis of FLM Curves[6].

Results and discussion. Labeling index was found to be 22.4 ± 6.5, mitotic index 0.65 ± 0.13 and number of cells per crypt 181 ± 11. Graphic analysis of the FLM curve (fig.) gave the time of the S phase as 9.5 h and T_c as 19 h. The duration of $T_{g2} + \frac{1}{2}$ Tm and T_{g2} was determined to be approximately 1.5 and 0.75 h respectively. Tm was calculated to be 1.5 h and compared well with 1.2 h using the technique of Fry et al.[7] T_{g1} phase was 6.5 h.

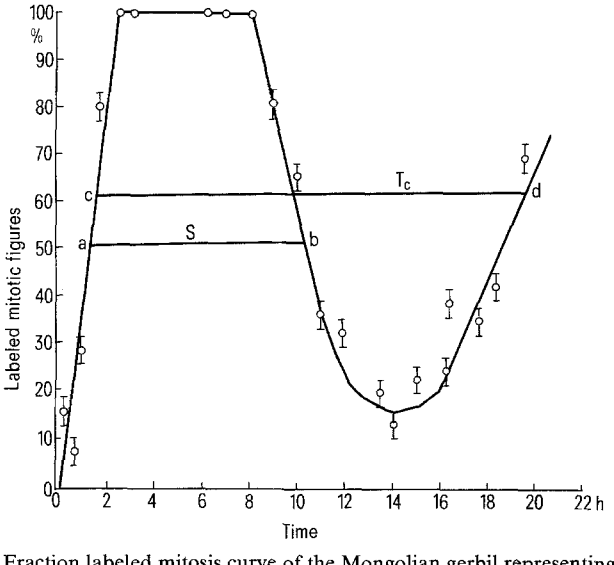
Data analyzed by the method of Takahashi et al.[6], gave statistically significant results for the 1st wave, however, the variability in the 2nd wave precluded using this method for determining Tc. Preliminary analysis resulted in times of $T_{G1} = 7.6$ h; $T_S = 9.4$ h; $T_{G2} = 1.4$ h; $Tm = 6.1$ h and $Tc = 19.0$ h. Optimal values with CVs below 0.5 resulted in $T_{G2} = 1.0$ h; $T_S = 9.4$ h and $Tm = 0.6$ h.

The Mongolian gerbil is a useful addition to cell kinetic modeling. The gerbil population in the United States is thought to have arisen from 8 original mating pairs. As a result, it has been useful in grafting experiments, and may provide a base for the establishment of a number of new transplantable tumor lines. The gerbil has been shown to have high radioresistance[8,9] and an extremely slow intestinal transit time[10–12] indicating perhaps a lower rate of proliferative activity than seen in the same tissue of more radiosensitive species. Therefore, the kinetic profile of the gerbil may provide different information not available from other rodents such as the mouse and rat.

The labeling index in rats has been reported as 12.7%[13], 24.8%[14] and 29.2%[15] and in C57 mice as 40%[16]. The mitotic index in rats has been reported as 1.43%[13] and 1.54%[14]. Our values of 22.4% for labeling index and 0.65% for mitotic index indicate a slower rate of proliferation in the gerbil than in the mouse or rat.

This slower rate of proliferation is also revealed in comparison of total cell cycle time and time of the S phase. Total cell cycle times of 11.6 h[17] and 10.4[15] in the rate have been reported, lower than our value of 19 h, however, Quastler and Sherman[16] reported an almost identical value of 18.8 h in the mouse. Reports of S phase durations of 7.5 h[16] in the mouse, 8.2 h[17] and 7.3[15] in the rat are again shorter than our value of 9.4 h.

The present paper reports that labeling index and mitotic index indicate a slower proliferative activity in the intestine of the gerbil when compared to the mouse and the rat. Total cell cycle time was longer than in the rat but not appreciably longer than the mouse. This is not unexpected since various rodents show the same type of differences.



Fraction labeled mitosis curve of the Mongolian gerbil representing the cell cycle and its respective phases. Points a and b represent 50% labeled of the 1st ascending slope and descending slopes. Point c represents 60% labeled of the 1st ascending slope and point d 60% of the 2nd ascending slope. S represents the time of DNA synthesis and T_c the total cell cycle time. Bars indicate SE.

Crypt size may also affect results since the number of cells/crypt in rats is approximately 450[14] and 500 in mice[18] as compared to 181 in the gerbil. 2 factors which may affect the various parameters were analyzed; age and diurnal variation. Both variables were carefully monitored.

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2 The authors wish to acknowledge the assistance of Dr W.E. Strawderman, Associate Professor of Statistics, Rutgers University. Present address of H.S.: Merck, Sharp & Dohme. Res. Laboratories West Point, Pennsylvania 19486, USA.

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Morphology of a non-occluded virus isolated from citrus red mite, *Panonychus citri*

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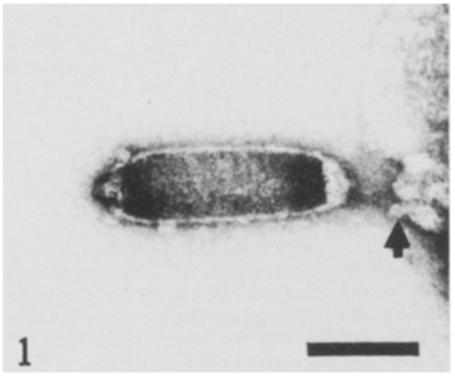
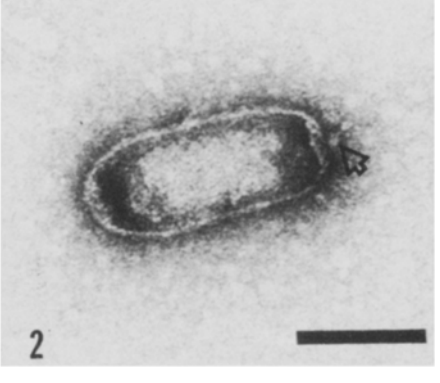
Summary. The size and morphology of virus particles isolated from citrus red mite *(Panonychus citri)* are similar to those observed in thin sections. The similarity to the virus particles isolated from *Oryctes rhinoceros* suggests affinity to the *Baculovirus* group.

The noninclusion virus of the citrus red mite, *Panonychus citri* is rodshaped and located in midgut epithelial cells[2]. Similar rod-shaped virus particles were reported in nuclei of fat cells of *Panonychus ulmi*[3]. We report here attempts to isolate and describe the morphology of the citrus red mite virus.

Laboratory colonies of citrus red mites were infected with virus disease by spraying them with an aqueous suspension of triturated diseased mites. Mites were incubated for disease development symptoms ordinarily appearing within 6-7 days, collected and stored at −5 °C until used. For testing, 1-2 g of mites were frozen in liquid nitrogen in a mortar, then ground to a fine powder with a pestle[4]. The frozen powder was suspended in 0.1 M potassium phosphate buffer at pH 6.6 and homogenized in a Virtis homogenizer[5] cooled in an ice bath. After clarification with 2 cycles of high (25,000 × g) and low (10,000 × g) speed centrifugation, particles were further concentrated either by high speed centrifugation (36,000-50,000 × g) or by dialysis of the supernatent from low speed centrifugation against a 40% solution of polethylene glycol (PEG) 6000. The pellet obtained from the high speed centrifugation was resuspended in 0.1 M potassium phosphate buffer at pH 6.6 and centrifuged again at low speed. Concentrated materials were layered over linear (0.15-1.5 M) sucrose gradients and subjected to rate-zonal centrifugation in preformed density gradients at 80,000 × g for 2.5-3.0 h (SW 25.1 rotor in a Spinco Model L ultracentrifuge). Fractions were collected with an ISCO Model D density gradient fractionator and flow densitometer equipped with a 254-nm UV-light source. Negatively stained samples were prepared with 3.0% uranyl acetate which were examined with a Hitachi HU 12 electron microscope.

Discrete banding of virus particles in gradients was never





Figures 1 and 2. Nucleocapsids isolated from citrus red mite and negatively stained with 3% uranyl acetate. Solid arrow indicates possible remnants of virion envelope; open arrow indicates the knob or projection sometimes observed at one end of the virion. Bar × 100 nm.