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A simple one-step procedure for staining the nucleolus organizer regions

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Summary. A simple silver staining technique for routine use is described by which the nucleolus organizer regions of mammalian chromosomes, including those of mouse chromosomes, are stained selectively.

Using the Ag-As staining technique developed by Bloom and Goodpasture[2], it is possible preferentially to stain rRNA genes in animal and plant cells. Miller et al.[3] have shown that a positive result of the staining is at the same time an indication of the activity of these genes.

For staining purposes, the two-step procedure (Ag-As) of Bloom and Goodpasture[2] is now used predominantly, in which first silver nitrate solution and subsequently ammoniacal silver solution and formalin are used as developers. One difficulty of this method is the control of the short developing time, whereby an overstaining often occurs.

An easier technique to stain the nucleolus organizer regions, the single application of silver nitrate solution, has already been mentioned by the authors[2]. The staining quality is excellent, the reproducibility, however, is very poor. The cause of this appears to be that the storage of the $AgNO_3$-solution (either in the dark or in the light, at room temperature or cooled, as well as the age of the solution) is decisive for the routine use of this technique.

When these parameters are controlled, the staining time can be accurately predicted after a single testing of the solution. The $AgNO_3$-solution cannot be used for an unlimited time. Storage in a dark-brown bottle at +4 °C in a refrigerator has proved best. It appears that the developing time decreases with increasing age of the silver nitrate solution. When using a freshly prepared Ag-solution (1–2 days old), the Ag-bands occur after 24–48 h. This period is reduced to 4–5 h, when a 3–5-week-old silver nitrate solution is used (figure 1). After more than 6–8 weeks, the solution no longer stains the NORs preferentially. If the silver nitrate solution is stored in clear bottles in the light at room temperature, a premature ageing process sets in which leads to a shorter developing time.

The age of the preparations (some days or months) and the storage temperature (+4 °C or +20 °C) are virtually of no significance for the staining process.

The individual steps are as follows:

1. Preparation of air-dried chromosomal preparations as usual.
2. Application of 50% w/v silver nitrate solution ($AgNO_3$ in aqua bidist.) and mounting. An air-tight closing of the cover-glass with cover-glass cement, the melting point of

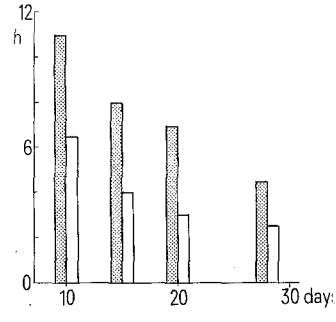

Fig. 1. Dependence of the staining time on the age of the silver nitrate solution when the solution is stored in a dark-brown bottle (hatched columns) and in a clear glass bottle.

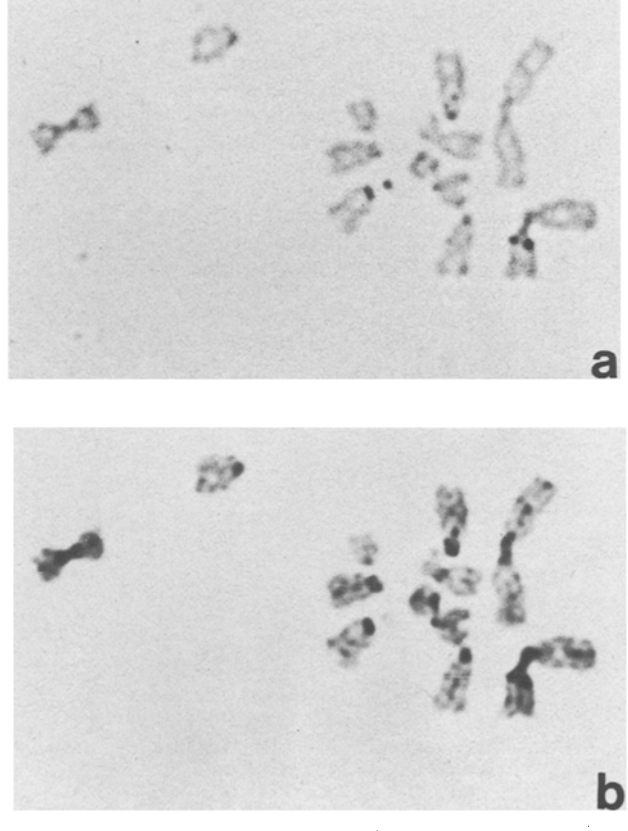

Fig. 2. Silver stained metaphase chromosomes of the mouse A-9 cell line. At first the NORs are selectively stained (a), after prolonged staining the C-band regions also became visible (b).

which is higher than + 60 °C, has proved most suitable (e.g. cover-glass cement according to Kroenig; Merck, Darmstadt, FRG). Thus, not only is drying of the $AgNO_3$-solution prevented, but also contamination of the laboratory equipment. Moreover, the differentially stained preparations can be stored at + 4 °C in the dark for more than 6 months without any loss in quality.

3. Simultaneous exposure of the preparations to + 56–60 °C and to light from a 60-W bulb. A thermostat, which is heated and illuminated by two 60-W bulbs, has proved especially well-suited for this purpose.

This uncomplicated technique for visualizing NORs has been successfully applied to various kinds of bird and mammalian chromosomes, such as those of humans, numerous cervidae and different rodents. With the latter, it appeared that with this one-step procedure – quite in contrast to the original procedure – staining of the C-band regions can be avoided, thus enabling us to see clearly the NORs of the mouse (figure 2).

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Persistence of body weight cycles in dormice maintained with a limited food supply

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Summary. Dormice kept under food restriction for several months continued to exhibit body weight cycles with characteristic periods of about 2 months, despite an impaired ability to attain the high body weight levels observed in ad libitum conditions. These results imply that the cycle is controlled by a mechanism whose properties are more consistent with an endogenous oscillator than with a sequence of stages, in which the relevant stages are defined by upper and lower body weight limits.

Food intake and body weight cycles of approximately 2 months duration can be observed in dormice, *Glis glis*, kept in constant conditions of lighting and high ambient temperature[2,3]. It has been suggested that these cycles represent accelerated hibernation rhythms[2]. The short dormouse cycles are in many ways similar to the circannual rhythms of other hibernating mammals. Although a circannual oscillator is generally thought to be the controlling mechanism underlying the long term rhythms[4], an alternative hypothesis, which proposes a sequence of linked stages, each taking a given amount of time to complete, must be considered[5–8]. The stages might be based on hormonal, nutritional, neural or other internal states, and it should be possible to alter the periodicity of the cycle by slowing down, speeding up or reducing certain stages[6]. Thus, high ambient temperatures leading to an elevated energy expenditure in the weight loss phase of the body weight cycle in *Glis glis* can be thought to cause a shortening of that stage[5]. In this sense, the dormouse cycle seems to fit the sequence of stages hypothesis. This paper, however, reports findings which suggest that the cycle is controlled by an internal oscillator.

A version of the sequence of stages hypothesis – which proposes that the dormouse cycle represents oscillations between upper and lower body weight limits[9] – predicts that dormice maintained on restricted food intake will exhibit cycles in which period is lengthened due to the greater amount of time required to achieve 'programmed' levels of fattening during the weight gain phase. On the other hand, an oscillator hypothesis predicts a preservation of periodicity during food restriction. The following experiment was carried out to test these hypotheses.

Methods. 9 dormice, purchased from a dealer in France (Stacel), were individually housed in cages 19 × 22 × 37 cm, and initially provided with food (ground Purina laboratory chow, 4.25 kcal/g) and water ad libitum. The animals were kept in light/dark 12/12 (fluorescent lighting on between 08.00 and 20.00 h), and the room temperature remained 23 ± 1 °C. From the end of September 1976, the dormice were maintained on various levels of food restriction, the particulars of which are shown in the figure. Data from 2 animals which died and from 2 which did not cycle at any time during the experiment are not included. The daily food allowances were selected to be less than one-half the maximum daily ad lib food intake of dormice during a body weight gain phase (27 ± 1 g, n = 27), previously determined for a group of animals kept in similar conditions. At the conclusion of the food restriction phase of the experiment, the animals were again offered food ad libitum. Throughout the restricted and subsequent ad lib period, food intakes were recorded daily and body weight was measured twice a week.

Cycle periods were estimated by periodogram analysis, using the root-mean-square excursion from mean value of each form estimate as a measure of 'amplitude'[10]. This method permits more objective treatment of the data than is possible with visual criteria, and greatly facilitates the analysis of 'noisy' data (e.g. animals 676 and 681). Since data from cycle phases coinciding with both the final change in food restriction level and return to ad lib were likely affected by these manipulations, they were excluded from the analysis.

Results and discussion. The body weight and food intake data, as well as cycle period estimates, are presented in the figure. The food restricted dormice continued to show body weight cycles. Further, the mean period of these cycles did not differ statistically from that obtained when food was available ad libitum (8.6 ± 0.5 [SEM] weeks and 8.8 ± 1.6 weeks, $t = 0.11$, 4 df, $p > 0.93$). During food restriction, periods during which the animals consumed their entire daily food ration were followed by periods of reduced food intake. It is clear that the hyperphagia which characterizes the weight gain phase of dormice in ad lib conditions is not a prerequisite to a subsequent normal weight loss phase.