G- and C- band patterns and nucleolus organizer regions in somatic chromosomes of *Clyomys laticeps laticeps* (Rodentia, Echimyidae)[1]

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Summary. 7 specimens of *Clyomys laticeps laticeps* (Rodentia, Echimyidae) collected in Itapetininga, State of São Paulo, Brasil, were analyzed and a diploid number of 2n = 34 was confirmed. G- and C-banding patterns and silver staining data are presented.

The family Echimyidae comprises about 46 species[3]. Karyological studies have been performed in some species of the genus *Proechimys*[4,5] and species from other genera; *Echimys sp.* (2n = 90), *Euryzygomatomys guiara* (2n = 46) and *Clyomys laticeps laticeps* (2n = 34)[6], most of them based on conventional methods of chromosome analysis.

Chromosomal variability of sex chromosomes and nucleolus organizer regions (NORs) in *Trichomys apereoides* (2n = 30) were reported[7]. All species of the family Echimyidae present only 1 chromosome pair with a secondary constriction and this pair is very useful as a marker. 7 specimens of *Clyomys laticeps laticeps*, 3 males and 4 females, were studied cytogenetically. The skins and

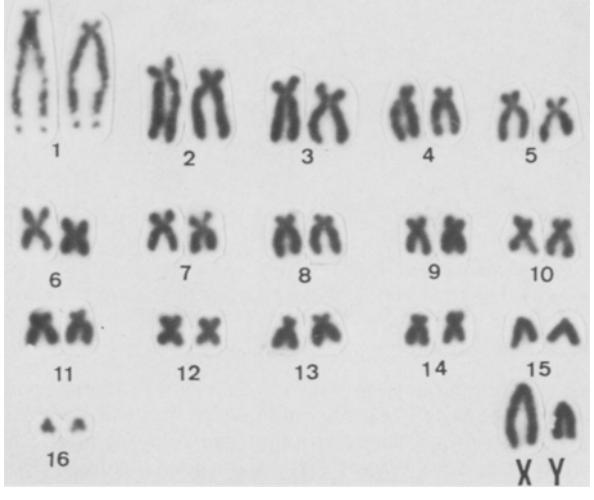

Figure 1. Karyotype of a male *Clyomys laticeps laticeps* (2n = 34), conventionally stained chromosomes.

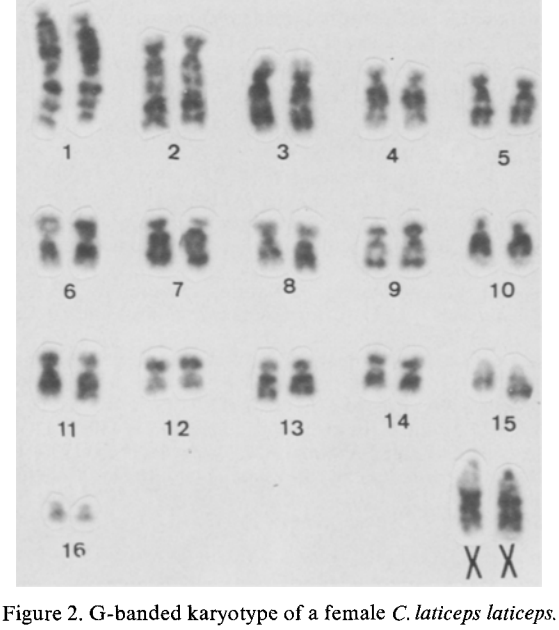

Figure 2. G-banded karyotype of a female *C. laticeps laticeps.*

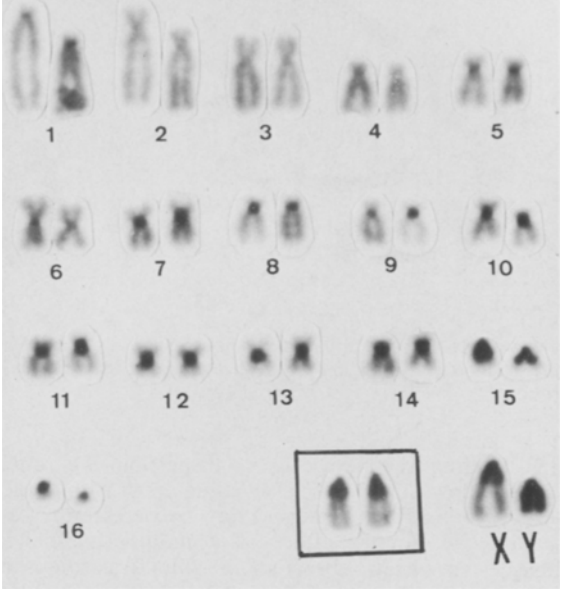

Figure 3. C-banded karyotype of a male *C. laticeps laticeps.* In the inset, sex chromosomes of a female.

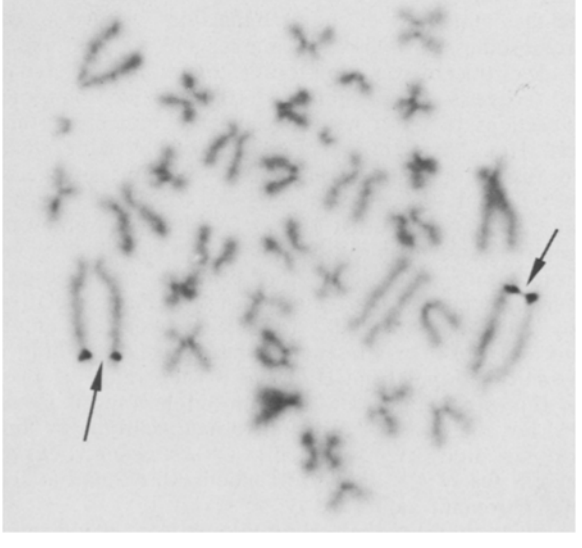

Figure 4. Silver stained metaphase of a male *C. laticeps laticeps.* Arrows indicate the NORs in the pair 1.

skulls were deposited in the collection of the Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo. Air-dried preparations of bone marrow and testis were made after in vivo colchicine treatment. G- and C-banding were performed according to routine techniques[8,9]. Silver staining followed the method described by Lau et al.[10].

All specimens had a diploid number of 2n = 34, with an autosomal complement of 14 metacentric or submetacentric pairs and 2 small acrocentric pairs. The pair 1 presents a large distal secondary constriction in the long arm. The X is a medium sized acrocentric and the Y a small one, both perfectly identifiable morphologically (fig. 1). This karyotype has previously been described by Yonenaga[6].

About 360 metaphases were analyzed using banding techniques. G-band patterns of a female karyotype are shown in figure 2. The C-banded karyotype presents heterochromatic blocks at the centromeres of the autosomes, with the exception of pairs 2, 3 and 4 which show no heterochromatin. The X chromosome, corresponding to about 8% of the haploid set, has heterochromatin in the centromere region and in the proximal segment corresponding to one third of its long arm. The Y chromosome, whose relative size is about 4.5% of the haploid set, is entirely heterochromatic (fig. 3). The relatively large size of the sexual pair appears to be due to the presence of large amounts of constitutive heterochromatin. In *Clyomys laticeps laticeps*, NORs are restricted to the secondary constriction of pair 1 (fig. 4). In all 62 cells analyzed, 2 pairs of Ag-NORs per methaphase were always found and these homologues were never seen in association. The presence of a large secondary constriction in only 1 pair of autosomes is characteristic of the family Echimyidae; the morphology and size of this chromosome pair is, however, variable. In the species where silver staining was used, *Trichomys apereoides, Proechimys iheringi iheringi* and *Clyomys laticeps laticeps*, the chromosome pair with secondary constriction is indeed the NOR chromosome.

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Age-related response of citrate synthase to hydrocortisone in the liver and brain of male rats[1]

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Summary. The activity of citrate synthase of the liver and brain of rats shows a gradual increase as a function of age. Adrenalectomy causes no significant change in the activity of citrate synthase in either of these tissues in young, adult or old rats. Administration of hydrocortisone to adrenalectomized rats depresses the activity of this enzyme maximally in the liver and brain of young rats. Administration of actinomycin D tends to normalize the depressed level of this enzyme.

Citrate synthase (citrate oxaloacetate lyase (CoA-acetylating) EC 4.1.3.7) is the first enzyme of the Krebs cycle, which catalyzes the conversion of oxaloacetic acid and acetyl-CoA to citric acid, a step which is considered to be the major regulatory site of Krebs cycle activity[4]. There are only a few conditions known to change the total activity of citrate synthase in rat tissues. Prolonged exercise causes a 2-fold increase in the activity of muscle citrate synthase[5]. Kirsten and Kirsten[6] have demonstrated that aldosterone injections cause a transient 30% increase in the level of citrate synthase. It has also been reported that administration of dexamethasone increases and decreases, respectively, the activity of PEPCK and citrate synthase in rat liver[7]. Mukherjee et al.[8] reported that the activity of hepatic citrate synthase increases by 2-3-fold in vitamin B_{12} deficiency. In the present investigation, citrate synthase was selected as a model enzyme, since it is the key regulatory enzyme of the Krebs cycle, and the rate of oxidation through the Krebs cycle might be controlled by the rate of citrate synthase activity as limited by oxaloacetate concentration. There has been no report so far on any enzyme that is depressed and repressed by hydrocortisone and actinomycin D, respectively, in any organism during different phases of its life-span.

Materials and methods. Male albino rats of the Wistar strain of 3 different age groups (6-, 30- and 90-week-old), maintained under standard laboratory conditions, were used.

Pilot experiments were undertaken to investigate the time and dose dependence of citrate synthase in rats of various ages given hydrocortisone. The rats of each age group were divided into 4 sets with 4–5 rats each. The set-1 rats served as the control. The rats of sets 2, 3 and 4 were bilaterally adrenalectomized. These rats were given 0.9% NaCl ad libitum instead of water for 10 days following adrenalectomy. On the 11th day, the set-2 rats received 1.0 ml of 0.9% NaCl i.p. and these rats served as the control for the induction studies. The rats belonging to sets 3 and 4 were given an i.p. dose of hydrocortisone (5.0 mg/100 g b.wt, suspended in 1.0 ml of 0.9% NaCl) at a fixed time of day for 3 days. The set-4 rats were also given actinomycin D (10.0 µg/100 g b.wt, suspended in 1.0 ml of 0.9% NaCl), 1 h prior to the hydrocortisone administration, for 3 days. All the rats were killed 3 h after the final injection.

The rats were killed by cervical dislocation, and their livers and brain tissues were taken out. The mitochondria were separated[9] and the activity of citrate synthase was determined by measuring the initial rate of the reaction at 412 nm by the DTNB method[10]. One unit of this enzyme is the amount that catalyzes the liberation of 1 µmole of CoA-SH/min under the standard conditions. The activity