The displacement of plasma corticoid peak from morning to evening observed after 30 days from the surgical ablation of olfactory bulbs seems similar to the inversion described by Haus et al. 4 in mice after blindness, calling to mind that in these small mammals normal acrophase is reversed as compared with dogs.

However, regarding the easy adaptation of olfactory receptors, it seems not probable that these stimuli are synchronizers of a circadian rhythm. More probably the removal of the sense of smell, so important for vegetative and environmental life of the dog, or anomalous signals from injured olfactory pathways, could determine a period of functional derangement of neurovegetative system, strictly linked to that of action in macrosmic animals.

In fact after 2 months we have observed again in anosmic dogs a normal circadian rhythm of plasma corticoids with acrophase at morning. Similar pattern has been seen after blindness in rats 3 and mice 4. Moreover a normal circadian rhythm of adrenocortical activity was described in blind men 10.

The temporal normalization of corticoid circadian variations observed 2 months after surgical ablation of olfactory bulbs of the dogs could be a consequence of the reequilibrium between different endogenous and exogenous synchronizers and/or the preminence of rhythmic environmental activities 11.

Résumé. L'extirpation des bulbos olfactifs du chien produit 30 jours après l’intervention chirurgicale une altération des rythmes circadiens des corticoïdes plasmatiques avec un maximum à 20.00 h et un minimum à 08.00 h du matin. Cette altération de l’activité rhythmique adréno-corticales est temporaire, car après 2 mois, les variations circadiennes des corticoïdes plasmatiques sont pareilles à celles qui précédéaient l’intervention chirurgicale.

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Chromosome Secondary Constrictions in Different Stages of Development 1

Karyotype studies are usually done in adult specimens. With relatively few exceptions, the karyotype is constant in the same species, and the chromosome morphology is characterized by its size, centromere position, and secondary constrictions. Presence of satellites is one of these constants, and has been often used in the identification of one or more chromosome pairs.

On the other hand, the secondary constrictions that give rise to the satellites have been frequently identified as nucleolar organizers, containing rDNA cistrons for rRNA synthesis. These cistrons may, in some instances, through amplification, allow a higher rate of ribosomal RNA production, resulting in a more intense protein synthesis 5.

Animal protein metabolism is subject to a wide range of variation during its development, from zygote up to the adult stage. In search of a correlation of these biochemical aspects with the specific morphology of the chromosomes, we decided to make a comparative study on karyotypes of specimens from different species, in the various...
phases of their development. We studied 3 species, representing 3 different animal groups: Odontophrynus americanus (Amphibia), Bothrops alternatus (Reptilia) and Mus musculus (Mammalia).

Karyotypes were obtained from animals both inoculated and not with colchicine. Fragments from liver and intestine were pretreated in distilled water for 15 min, fixed in 50% acetic acid for 15 min and then squashed. After removal of the cover slips on dry ice, the preparations were stained with Giemsa for 10 min and mounted with Permount.

Odontophrynus americanus. This is a tetraploid anuran of the family Ceratophrydidae, and presents 44 chromosomes arranged in 11 groups of 4 homologues each. The karyotype of the adult of this species is characterized by the presence of satellites only at chromosomes of group 11. However, studying the tadpole in the metamorphic stage, from the same population, we found that its karyotype, besides the chromosomes of group 11, further presents satellites at the short arm of chromosome 8 and at the long arm of chromosome 10 (Figure 1).

Bothrops alternatus. This is a snake of the family Viperidae, sub-order Squamata. This reptile presents a diploid number of 36 chromosomes arranged in 16 macrochromosomes and 20 microchromosomes. The karyotype of the adult specimen does not show satellites. However, studying, in this ovoviviparous species, the embryo in its final evolution phase, we found that the karyotype presents satellites at pairs 5 and 6 (Figure 2).

Mus musculus. This rodent of the family Muridae is one of the laboratory animals whose karyotype has been most studied. Its diploid number is of 40 chromosomes. In the adult animal, using different techniques and tissues,

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2 J. G. Gall, Genetics suppl. 61, 121 (1969).
a variable number of secondary constrictions has been reported. In this study, we used specimens of the Swiss Albino strain maintained at the Instituto Butantan since 1956. Employing the same methods and without any special treatment, we compared cytological preparations from adult and newborn animals. We found that there was a greater number of chromosomes with secondary constrictions, mainly of the 'rabbit ear' type, in the metaphases of the young animals (Figure 3).

The 3 species, although phylogenetically distant, and despite their great differences in type of development from zygote to adult, show a common feature of a more intense proteic metabolism in the embryo or the newborn, as compared to the adult.

On the other hand, it is evident that a higher protein synthesis rate would require a higher ribosomal RNA synthesis. We may assume that the rDNA cistrons are mainly located at the nucleolar organizer regions at the chromosome satellites and other secondary constrictions.

Our findings suggest that at the early phases of development, where a higher synthesis rate is needed, there would be an increased number of active cistrons for ribosomal RNA, and that this could be morphologically expressed by a larger number of cytologically detectable secondary constrictions. In the adult, part of the rDNA cistrons would become inactive, thus reducing the number of constrictions visualized at the light microscope.

Furthermore, karyotypic variability as to the number of the secondary constrictions among the different tissues of the same animals, could be due to a different number of active rDNA cistrons, depending on the metabolic activity of the tissue, and on the physiological state of the animal.

**Fig. 3.** Mitotic metaphase of a newborn *Mus musculus*, showing secondary constrictions and 'rabbit ear' chromosomes.