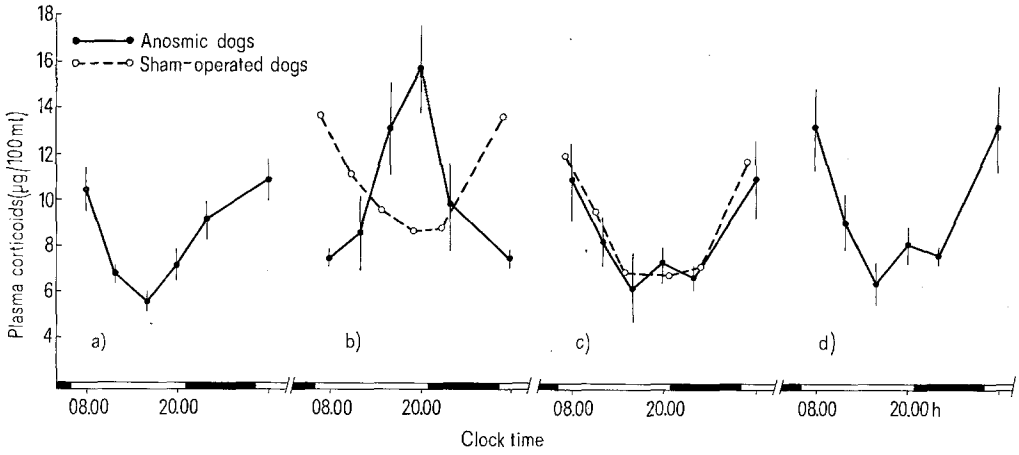

Diurnal variation of plasma corticoid levels in the dog before (a) and at 30 (b), 60 (c) and 120 days (d) after surgical ablation of olfactory bulbs (●—●: mean ± E.S.) and 'sham' operation (○---○).

2 months, circadian variations similar to those observed before surgery reappeared and this pattern was still observed at 4 months. In 'sham-operated' dogs, surgical operation has never modified the circadian pattern of plasma corticoids during the period of our study.

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.

The loss of an external synchronizer as olfactory stimuli in our experiments, and visual stimuli in other researchs[3-5], could explain the inversion of plasma corticoid peak. The new rhythm could indicate a new equilibrium between endogenous and exogenous synchronizers or the expression of primordial endogenous rhythm, because in humans during early life corticoid peak was observed at evening[9].

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 determinate a period of functional derangement of neurovegetative system, strictly linked to of laction 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 enviromental activities[11].

Résumé. L'extirpation des bulbes olfactifs du chien produit 30 jours après l'intervention chirurgicale une altération des rhythmes 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 adrénocorticale est temporaire, car après 2 mois, les variations circadiennes des corticoïdes plasmatiques sont pareilles à celles qui précédaient l'intervention chirurgicale.

P. ARCANGELI, V. DIGIESI, G. MADEDDU[12] and R. TOCCAFONDI

Istituto di Patologia Medica (R), Università di Firenze, via Pieraccini 18, I–50139 Firenze (Italy), 11 August 1972.

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[12] Present adress: Clinica Medica Universitaria, Via San Pietro 8, Sassari, Sardinia, Italy.

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[2].

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

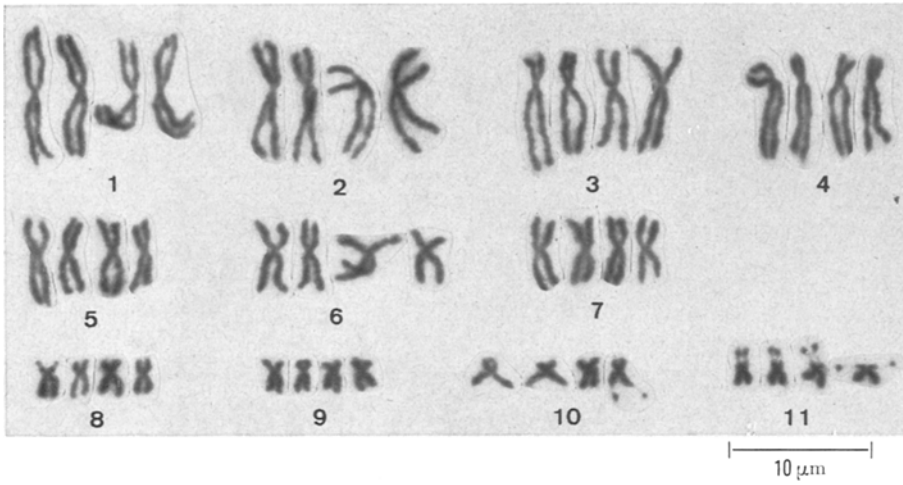

Fig. 1. Karyotype of a tetraploid tadpole *Odontophrynus americanus*, showing satellites at chromosomes 8, 10, and 11.

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[3]. 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[4]. 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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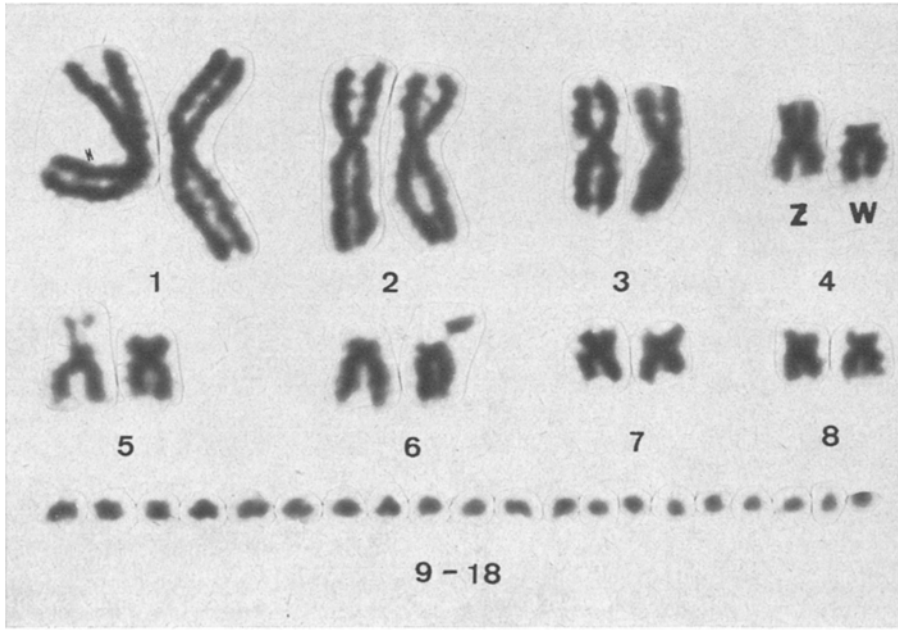

Fig. 2. Karyotype of an embryo snake *Bothrops alternatus*, showing satellites at macrochromosomes 5 and 6.

a variable number of secondary constrictions has been reported[5]. In this study, we used specimens of the Swiss Albino strain maintained at the Instituto Butantan since 1956[6]. 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).

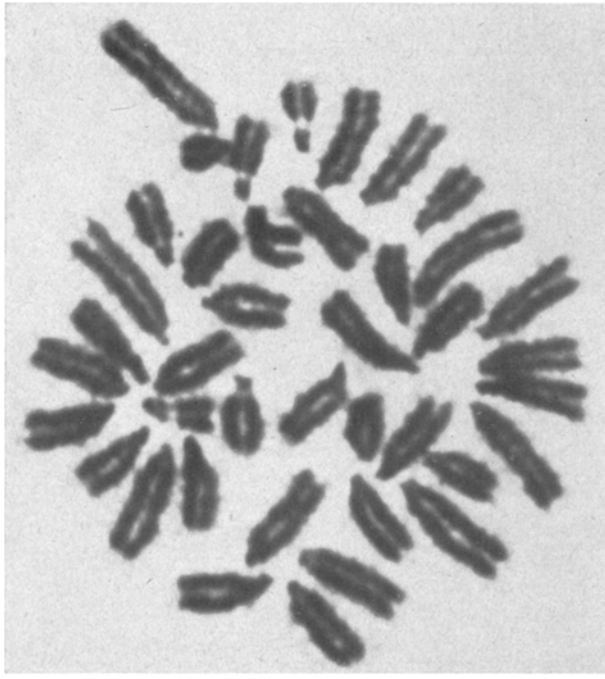

Fig. 3. Mitotic metaphase of a newborn *Mus musculus*, showing secondary constrictions and 'rabbit ear' chromosomes.

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, karyotypical 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.

Zusammenfassung. In den Metaphasen von embryonalen und neugeborenen Amphibien, Reptilien und Säugern wurde eine grössere Anzahl von Satelliten und anderen sekundären Strukturen beobachtet als in solchen von ausgewachsenen Tieren. Dies kann bedingt sein durch eine grössere Anzahl von Cistronen der rDNS, die sich aktiv in den Zellen junger Tiere befinden.

MARIA LUIZA BEÇAK and W. BEÇAK

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Die Bedeutung des Serumeiweissbildes zur Diagnose von *Bufo calamita* Laur., *Bufo viridis* Laur. und deren Bastarden (Amphibia, Anura, Bufonidae)

Zur spezifischen Diagnose der beiden europäischen Krötenarten *Bufo calamita* (Kreuzkröte) und *Bufo viridis* (Wechselkröte) wurden bislang Merkmale des Körperbaus und der Färbung und Zeichnung herangezogen, so die relative Länge der Hinterbeine, die relative Länge des 1. und 2. Fingers, die Grösse der Schwimmhaut am Hinterfuss, die Form der Ohrdrüsen, die Zahl der Zehengelenkhöckerchen, die Fleckenzeichnung und das Vorhandensein eines Rückenbandes[1–3]. Hiervon haben die beiden letzteren allerdings nur für mitteleuropäische Vertreter beider Arten volle Gültigkeit, nachdem diese Zeichnungsmerkmale bei den Kreuzkröten der iberischen Halbinsel[4] und bei mittelmeerländischen, insbesonders östlichen Wechselkröten[5] stärkere Variation jeweils in den Bereich der anderen Art aufweisen. Unter Einbeziehung auch dieser südlichen Formen erscheinen von den Körperbaumerkmalen die relative Hinterbeinlänge, die relative Fingerlänge, und mit Einschränkung die Zahl der Zehengelenkhöckerchen als am besten zur Arttrennung geeignet. Auch mit ihnen kann jedoch im Randbereich der Variation nicht immer eine eindeutige Aussage erzielt werden.

Eine eindeutige Differenz zeigt sich im Serumeiweissbild, in dem sich beide Arten durch die Lage ihrer jeweiligen Albuminfraktion unterschieden. Bei elektrophoretischer Auftrennung der Serumeiweisse laufen die Albumine von *Bufo viridis* etwas weiter als diejenigen von *Bufo calamita*, was bei Auftragung einer Mischung von Seren beider Arten zur Verdoppelung der Albuminfraktion führt (Figur 1). Diese Situation findet sich bei allen bislang daraufhin untersuchten Formen und ist damit als artspezifisch zu werten. Während sie sich für vor dem Erkennen dieses Sachverhaltes untersuchte Wechselkröten aus der Türkei[5] nur durch vergleichende Messung der Laufstrecke ebenfalls bestätigen liess, konnte sie über Mischserumbildung mit jeweils morphologisch eindeutig

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