The present data from Figures 1 to 4 indicate that the pancreatic α-amylase activity is stimulated by Na^+ ion, as NaCl or Na_2CO_3 in every case is found to be stimulatory, though excess of these ions inhibited the enzyme activity. If Cl^- ion had been the activator as suggested by earlier workers, a similar result would have been obtained when KCl or $CuCl_2$ were used in the incubation mixture. But the results show a completely different picture. The enzymic reaction towards Na_2CO_3 is similar to that obtained when NaCl was added to the incubation mixture in increasing concentrations. To observe whether or not the alteration in pH due to the addition of NaCl or KCl or Na_2CO_3 or $CuCl_2$ could influence the stimulation of α-amylase activity; 2 sets of experiments, one using buffer and another without buffer, were run concurrently. In incubation mixtures with NaCl, KCl or $CuCl_2$ there was no significant change in pH and amylase activity in the non-buffered system. With the addition of Na_2CO_3 a rise in pH occurred in the non-buffered assay medium, inspite of which there was no considerable change in amylase activity when compared to that of the buffered system. The probable reason behind this phenomenon is the ability of sodium ion to activate the α-amylase to a great extent.

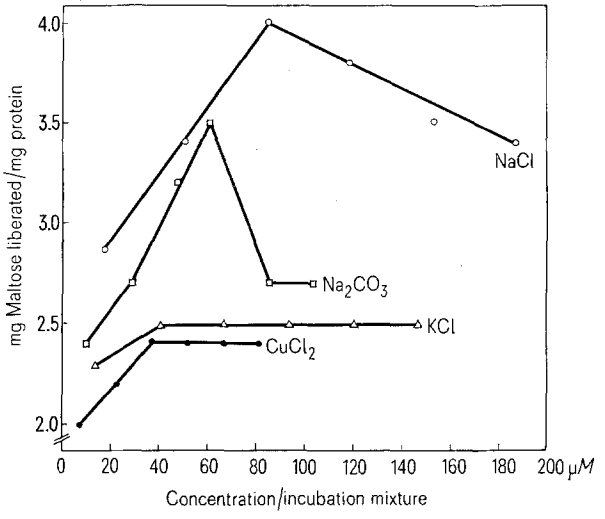

Fig. 4. Effect of NaCl (-○-○-), KCl (-△-△-), Na_2CO_3 (-□-□-) and $CuCl_2$ (-●-●-) on the activity of the pancreatic α-amylase of white rat.

The major function of the sodium ion in the animal body appears to be in connection with osmotic pressure regulation and acid base balance. Although a large amount of sodium is present in the food, it has never received any attention regarding its role in enzymic reactions. HAWK[5] summarizes that a catalytic effect of sodium on enzyme reaction cannot be totally excluded. The involvement of Na^+ ions in the activation of β-galactosidase has recently been demonstrated by WALLENFELS et al.[8]. Previous workers in this field, who supported the chloride dependence of α-amylase, could not avoid the presence of Na^+ in their reaction mixtures in the form of NaCl, assuming that Cl^- was the activator. In contrast the present data clearly shows that Cl^- of KCl or $CuCl_2$ could not stimulate the α-amylase activity. On the other hand, Na^+ without Cl^- (Na_2CO_3) ion stimulated α-amylase activity in all cases. From the above findings it may be suggested that the Na^+ ion has an important role in the α-amylase activation.

Zusammenfassung. Im Gegensatz zu früheren Berichten wird gezeigt, dass die Aktivierung der α-Amylase nicht durch Chloride sondern durch Natrium-Ionen verursacht wird. Weiter wurde gefunden, dass nur NaCl und Na_2CO_3 die Aktivität steigert, nicht aber $CuCl_2$ und KCl. Alle Lösungen, die Na^+ enthalten, haben ebenfalls eine aktivierende Wirkung.

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New Chromosome Numbers in Australian Leptodactylidae (Amphibia, Salientia)

The Australian frogs of the family Leptodactylidae include the living forms phenetically closest to the stem stock that is probably ancestral to all or most of the 'advanced' families of the order[1,2]. This evolutionarily interesting group of Anurans has been subdivided by PARKER[3] into 2 subfamilies (Cycloraninae and Myobatrachinae) whose reciprocal relationships and phyletic roles are still uncertain[4]. The recent discovery in Queensland of a primitive genus *Rheobatrachus*, tentatively included in the Leptodactylidae (but showing other, especially pelobatid, characters)[5], and the anatomical and biological affinities shown by some Cycloraninae (e.g. *Cyclorana*) with the Hylidae[6,4], seem further to complicate the present taxonomic definition of these frogs.

The few fata collected on the chromosomes of the Myobatrachinae (4 species belonging to the genera *Ranidella*[7], *Uperoleia* and *Pseudophryne*) and of the Cycloraninae (3 species of *Limnodynastes*) show that these frogs are karyologically very homogeneous (all have $2n = 24$ and similar karyotype morphology)[8]. They also appear to be generally more differentiated than some groups of Neotropical and African Leptodactylidae with $2n = 26$ (this higher chromosome number seems to be basic for this and for most of the advanced families[8]).

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We have found new chromosome numbers in the subfamily Cycloraninae and a peculiar karyotype in *Rheobatrachus silus*; since we believe that these data may be of some aid in solving the taxonomic problems of these frogs, we present here some preliminary results from our work. The species studied (including for comparison some Australian Hylidae, subfamily Nyctimystinae) are listed in the Table and most of their karyotypes are shown in the Figure.

Among the Leptodactylidae here studied, *Cyclorana alboguttatus* is the only species having $2n = 26$. In chromosome number and morphology, its karyotype is very similar to that of some sympatric Hylidae (for example, to that of *Litoria nasuta*, see Figure). Besides constituting further proof of some relationship between *Cyclorana* (or at least some species of this genus) and the Nyctimystinae, these data also suggest that *Cyclorana* is karyologically less differentiated than many other Australian leptodactylid genera, and that it approaches some primitive Leptodactylidae from other geographical areas (i.e. *Heleophryne*, the Ceratophryinae, many Telmatobiinae etc., all with $2n = 26$)[8].

Among the Cycloraninae, *Mixophyes fasciolatus* and *Adelotus brevis* have $2n = 24$, a karyotype similar to that shown by some species of *Limnodynastes* of the *peroni* group (*L. fletcheri*, *peroni* and *tasmaniensis*), all with $2n = 24$[9]. However, the 4 species of *Limodynastes* here studied (*dumerili*, *terraereginae*, *salmini* and *ornatus*) have a lower chromosome number ($2n = 22$), thus seeming karyologically more advanced than the species of the *peroni* group.

The 2 species of Myobatrachinae here studied have $2n = 24$; *Ranidella (Crinia) parinsignifera* is similar in the chromosome morphology to other species of the same genus, while the primitive *Taudactylus diurnus* has larger chromosomes, rich in heterochromatic areas, thus approaching *Pseudophryne bibroni* in these characters[9]. On morphological grounds, the karyological differences between certain Cycloraninae (e.g. *Mixophyes*) and certain Myobatrachinae (e.g. *Taudactylus*) are not larger than those occuring at interspecific level within each subfamily; this conclusion is in agreement with the 'classic' hypothesis of PARKER[3] on a close relationship between some of the Myobatrachinae and some Cycloraninae.

The newly described *Rheobatrachus silus* has the same diploid number as many Australian Leptodactylidae ($2n = 24$) but shows 2 large acrocentrics (the 6th pair), and 4 very small acrocentrics (the 11th and 12th pairs) which are very similar to the microchromosomes typical of the 'primitive' frog families (Ascaphidae, Discoglossidae and Pipidae) but not typical of the remaining families of the order (which, like the Australian Leptodactylidae, generally show bi-armed chromosomes)[8]. Up to this point, the karyotype of *Rheobatrachus* is somewhat different from that of the studied sympatric Leptodactylidae, while it approaches that of some Ascaphidae (especially *Leiopelma hochstetteri*)[10,11] and, to a lesser extent, that of other primitive frogs. However, in the spermatocyte line this species shows precociously

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The karyotypes of : *Litoria nasuta* (Ln); *Cyclorana alboguttatus* (Ca); *Mixophyes fasciolatus* (Mf); *Adelotus brevis* (Ab); *Limnodynastes dumerili* (Ld); *Ranidella (Crinia) parinsignifera* (Cp); *Taudactylus diurnus* (Td); *Rheobatrachus silus* (Rs). The scale is 10 μm long.

Species	2n
Hylidae	
Nyctimystinae	
Litoria nasuta	26
Litoria chloris	26
Leptodactylidae	
Cycloraninae	
Cyclorana alboguttatus	26
Mixophyes fasciolatus	24
Adelotus brevis	24
Limnodynastes dumerili	22
Limnodynastes terraereginae	22
Limnodynastes salmini	22
Limnodynastes ornatus	22
Myobatrachinae	
Ranidella parinsignifera	24
Taudactylus diurnus	24
(incertae sedis)	
Rheobatrachus silus	24

terminalized chiasmata (usually 12 'ring' bivalents) as in the advanced families of the order[8]; since we have found a small number of spermatocyte metaphase plates showing a few dot-like univalents, it is possible that the microchromosomes of *Rheobatrachus*, like those of *L. hochstetteri*[10,11], are supernumerary chromosomes.

Karyological research on the Australian Leptodactylidae is still in a preliminary stage and, as we have seen, it is capable of providing cytotaxonomically interesting results. In particular, if new research confirms the peculiarity of the karyotype of *Rheobatrachus* within the Australian Anurans, then the presence of this primitive frog in an old Queensland refuge belt would be of relevance in the zoogeographical problems regarding the origin and radiation of the leptodactylid frogs, given that it could represent a relict from some stock karyologically intermediate between an ascaphoid and a leptodactyloid (or pelobatoid) condition[12].

Summary. Fra i Leptodactylidi Australiani, *Cyclorana alboguttatus* ha $2n = 26$ come le forme più generalizzate della famiglia, mentre alcune specie di *Limnodynastes* hanno $2n = 22$; specie di altri 5 generi hanno $2n = 24$. Fra questi ultimi, il problematico *Rheobatrachus*, completamente acquatico, possiede alcuni microcromosomi come i membri più primitivi dell'ordine.

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Incorporation of Thymidine into the Chromosomes of Aphid (*Myzus persicae*) Embryos

In parthenogenetic female aphids, embryogenesis starts before the mother is born[1]. Only a few days later, at the final moult, the maternal haemocoel contains 60–70 embryos, the oldest of which are usually fully-formed and ready for independent existence. Apart from the classical work on aphid embryology (reviewed by HAGAN[2]), and some more recent descriptive work[3,4], relatively little is known of this extremely rapid reproductive and developmental process which is a principal reason for the considerable importance of aphids as pests.

Regular ovulations take place from the germaria of the ovarioles, until each ovariole comes to contain a sequence of embryos at different development stages. The newly extruded oocyte is apparently supplied with a stream of nutrients during its initial growth phase by nurse cells in the anterior part of the germarium, but as the young embryo moves down the ovariole and further ovulations occur, this nutrient supply is presumably cut off and the later growth of the embryo, still continuing rapidly, must depend on provision of nutrients directly from the maternal haemocoel, through the follicular epithelium and the embryonic membranes. An indication of the probable nutritive function of the follicular epithelium is the fact that it persists until after the cuticula is formed in late embryonic development. However, the physiological mechanisms involved in what must be an extremely efficient provision of food materials to the developing embryo have not been investigated.

During the course of cytological studies on the chromosomes of *Myzus persicae* (Sulzer) attempts were made to label the chromosomes autoradiographically with tritiated thymidine so that they could be separately identified. The principal method used provided information on the uptake of thymidine from the maternal haemocoel by young embryos.

Material and method. Preparturition apterous adults of *M. persicae* were anaesthetized by brief exposure to ether vapour and placed with the ventral surface uppermost in a small drop of water on the stage of a binocular microscope. About 0.5 μCi of tritiated thymidine (Amersham, specific activity 2000 mCi/m*M*) was injected with a fine glass needle through a thoracic intersegmental membrane into the haemocoel of each aphid. Care was taken to insert the tip of the needle only just below the cuticle to avoid damaging any embryos.

Aphids recovered from injection after 15–20 min and mortality was negligible. They were left on a potato leaf at 20 °C for an interval ranging from 30 min to 3 h after injection. They were then dissected in 22% acetic acid and chromosome preparations made of the youngest embryos by a rapid Feulgen squash technique[5]. The embryos used were estimated to be between the 3rd and 6th day of their development. After freezing off the coverslip the cells on the slide were either covered with Kodak AR-10 stripping film or dipped in diluted Ilford K2

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