The hydrosmotic response to medium hypertonicity was not decreased significantly by colchicine but the effect of oxytocin, in agreement with previous results[6,7], was strongly reduced (Table). When copper was added to colchicine-treated bladders, a complete disociation between the response to ADH and hyperosmolarity was observed: while copper inhibits completely the response to ADH, it does not affect significantly the response to hypertonicity (Table).

Hypertonicity potentiates the effect of ADH[2]. To see the action of colchicine on this potentiation, a first stimulation with 10^{-10} *M* oxytocin was followed, after washing, by addition of 50 m*M* sucrose to the serosal bath. 30 min after the increase of serosal osmolarity, a second stimulation with 10^{-10} *M* oxytocin was superimposed to hypertonicity. It can be seen in the Figure that the clear potentiation observed in the control hemibladders is completely prevented in the colchicine-treated ones.

Two hypotheses have been postulated to explain the effects of colchicine[6] and cytochalasine B[11] in toad urinary bladder: 1. These alkaloids affect primarily the permeability to water of the apical membrane of epithelial cells; 2. They disrupt microtubules and/or microfilaments which would play a role in the coupling between the cyclase system and the change in membrane permeability.

Our results indicate that, in contrast to what happens with ADH, the mechanisms involved in the hydrosmotic response to hypertonicity are not altered by colchicine. If, as supported by strong experimental evidence[2], ADH and the elevation of medium tonicity induce similar changes in the mucosal border of epithelial cells, (perhaps envolving an endocytotic process[12], it seems logical to conclude that colchicine is acting at one of the steps previous to the change in membrane permeability. Since colchicine inhibits the action of exogenous cyclic-AMP[6], the effect should be located in the system which couples the nucleotide concentration with the hypothetical change in membrane structure, i.e. a post cyclic-AMP step.

It has recently been reported that in renal epithelial cells colchicine binds mainly to the cytosol fraction[13]. This reinforces the view given here of a non-membrane action of this alkaloid. A direct effect of colchicine on the apical membrane would be conceivable only if there were two permeability barriers, one triggered by ADH and the other by hypertonicity.

Resumen. Los efectos de la ocitocina y la hipertonía serosa sobre la respuesta hidrosmótica de la vejiga urinaria del sapo pueden ser disociados empleando colchicina, y mas evidentemente cuando el alcaloide es colocado junto con Cu^{++}.

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Evolution of the Genome and Cell Sizes in Salamanders

The question of the meaning of the increases in the genome size, which seem to have characterized the phylogeny of Eucaryotes, has given rise to various hypotheses which alternatively place greater weight on the evolutionary[1] or functional aspects of this problem[2]. In some groups of organisms displaying broad variations at the interspecific level, there is some correlation between the genome size and several cytological, physiological or ecological factors subjected to natural selection; hence in various cases the genome size is likely to take on an adaptive meaning[3].

Among vertebrates, the highest interspecific DNA differences are found in the Caudates (Amphibia) in which they range from 30 to over 160 (or 200, according to some authors) picograms per nucleus (pg/N). As in the lungfish, some species in this order possess the highest DNA amounts in the subphylum[4-6]. Hence, these Amphibians are suitable for the study of the pattern of correlation between the DNA amount and cell size, the latter being more manifestly variable according to its adaptive function (some workers maintain that the genome size may depend upon the cell size[7], but there is evidence that the reverse is probably true[8]).

In the present work we have compared the nuclear DNA content with the main morphometric parameters of the cell in 39 species, belonging to all the 8 families of Caudates and possessing DNA amounts which cover practically the whole range of genome variations in this order.

One of us[6] had already measured histophotometrically the erythrocyte nuclear DNA (blocked in G_1[9]). In smears, erythrocytes take on the shape of a cylinder with an ellyptical base[10]. We have measured the diameters of the ellypse by means of a Leitz screw micrometer eyepiece (15 cells for each species) and the cylinder thickness (which was seen to be constant in each smear) by means of a Horn-Gren (Leitz) microinterpherometer in monochromatic light at 546 nm, according to the 'two embedding media' method[11] (10 measurements per cell, 6 cells per species).

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In the erythrocytes from each species, the nuclear volume (Nv, in μm^3), the cell volume (Cv, in μm^3) the cell surface area (Cs, in μm^2) and the cell surface/cell volume ratio (Cs/Cv, indicated here as relative cell surface area), were calculated.

The species under study (listed according to increasing DNA values), their chromosome number (known through other investigations), the nuclear DNA content and the various morphometric parameters for each species are reported in the Table.

The Table shows that no correlation exists between the DNA amount and the chromosome number (the species studied are all diploid, with the possible exception of Sirenids[12]). On the other hand, the fact that all the species from the 4 paedogenetic families of this order (Cryptobranchids, Amphiumids, Proteids and Sirenids), show the maximum DNA levels may be significant.

The Table has been set out on a system of Cartesian axes, the DNA values being represented along the abscissa and the various cellular parameters up the ordinate. Statistical elaboration of the correlations between these values was performed with the aid of a computer, to which the calculation of the integration patterns of the various graphs was also entrusted (Figures 1 and 2).

A direct, linear and significant correlation exists between the DNA amount on the one hand, and the nuclear volume (A), the cell volume (B) and the cell surface (C) on the other (Figure 1). It may be inferred from the Table that the increase in cell volume is proportionally smaller than that in nuclear volume (as already seen in less homogeneous sample[13,14]).

In our view, the relationships between the relative cell surface (Cs/Cv) and nuclear DNA are of particular interest. In fact, the first parameter is directly correlated with the permeability coefficient[15], which represents the critical factor involved in the control of the oxydative metabolism of the cell[16]; hence the variations in the relative cell surface yield an exact evaluation of the variations in cell metabolism.

In relation to the DNA increase, the relative cell surface exhibits a behaviour which can significantly be identified by a function of exponential type (Figure 2): it rapidly drops until DNA values range around 70 pg/N, beyond which value it tends to decrease only imperceptibly

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	2n	DNA	Nv	Cv	Cs	Cs/Cv
Desmognathus fuscus (P)	28	30.2	122	765	897	1.17
Leurognathus marmoratus (P)	28	33.1	77	417	830	1.99
Hynobius dunni (H)	56	33.8	111	437	799	1.83
Hynobius retardatus (H)	40	38.3	83	413	714	1.73
Hynobius nebulosus (H)	56	38.4	49	380	865	2.28
Hynobius naevius (H)	56	40.9	123	681	1085	1.59
Eurycea bislineata (P)	28	41.5	141	1138	1141	1.00
Eurycea lucifuga (P)	28	42.1	129	557	1003	1.80
Triturus cristatus (S)	24	43.6	91	731	1182	1.62
Gyrinophilus danielsi (P)	28	44.5	182	1079	1351	1.25
Plethodon cinereus (P)	28	46.1	248	1299	1263	0.97
Ambystoma opacum (A)	28	47.7	212	1611	1299	0.81
Ambystoma texanum (A)	28	48.3	287	1462	1250	0.85
Triturus vulgaris (S)	24	48.5	77	449	833	1.85
Pseudotriton ruber (P)	28	48.7	171	1157	1273	1.10
Tylototriton verrucosus (S)	24	49.0	97	524	1157	2.21
Gyrinophilus porphyriticus (P)	28	49.6	359	1664	1171	0.70
Eurycea longicaudo (P)	28	52.2	132	704	1061	1.51
Ambystoma macrodactylum (A)	28	52.3	247	1192	883	0.74
Ambystoma maculatum (A)	28	52.4	103	559	1054	1.89
Plethodon glutinosus (P)	28	54.2	326	1791	1287	0.72
Taricha torosa (S)	22	56.0	241	1518	1350	0.89
Taricha granulosa (S)	22	59.1	490	1795	1161	0.65
Taricha rivularis (S)	22	59.8	119	1828	2002	1.09
Ambystoma talpoideum (A)	28	62.2	90	538	1088	2.02
Salamandra atra (S)	24	65.1	407	2649	2308	0.87
Paramesotriton hongkongensis (S)	24	68.4	213	1575	1548	0.98
Notophthalmus viridescens (S)	22	69.6	198	1255	1092	0.87
Batrachoseps attenuatus (P)	26	84.0	228	1233	1092	0.88
Ensatina eschscholtzii (P)	28	84.3	281	1523	1595	1.05
Aneides lugubris (P)	28	85.7	435	1995	1765	0.88
Pseudobranchus striatus (Si)	64	90.8	364	2021	1377	0.68
Megalobatrachus japonicus (C)	60	92.9	502	2105	1819	0.86
Proteus anguinus (Pr)	38	96.8	327	1472	1324	0.90
Megalobatrachus davidianus (C)	60	100.1	412	1831	1547	0.84
Siren intermedia (Si)	46	107.7	517	1902	1660	0.87
Siren lacertina (Si)	52	114.4	831	2712	1468	0.54
Amphiuma means (Ap)	28	149.9	640	3520	2605	0.74
Necturus maculosus (Pr)	38	165.1	946	3348	2377	0.71

A, Ambystomatids; Ap, Amphiumids; C, Cryptobranchids; H, Hynobiids; P, Plethodontids; Pr, Proteids; S, Salamandrids; Si, Sirenids.

bly, even despite large nuclear DNA variations. Thus the Caudates possessing a greater DNA amount generally have a lower metabolism (in keeping with other observations[17]); nevertheless, beyond certain DNA values – which are in any case overstepped by all the paedogenetic species – large differences in genome (and cell) size are paralleled by slight metabolic variations.

According to a hypothesis developed elsewhere by one of us[18], furtherance of the correlations described above

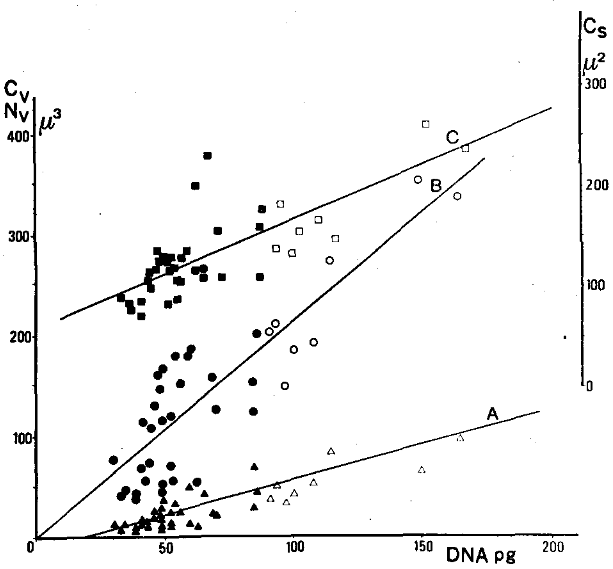

Fig. 1. The nuclear volume (Nv: triangles), cell volume (Cv: circles), and cell surface (Cs: squares) on the ordinate, plotted against the nuclear DNA content, on the abscissa, in 39 species of Caudates (Table). White symbols for the paedogenetic species. The resulting straight lines (A is relative to Nv, B to Cv, and C to Cs) are highly significant.

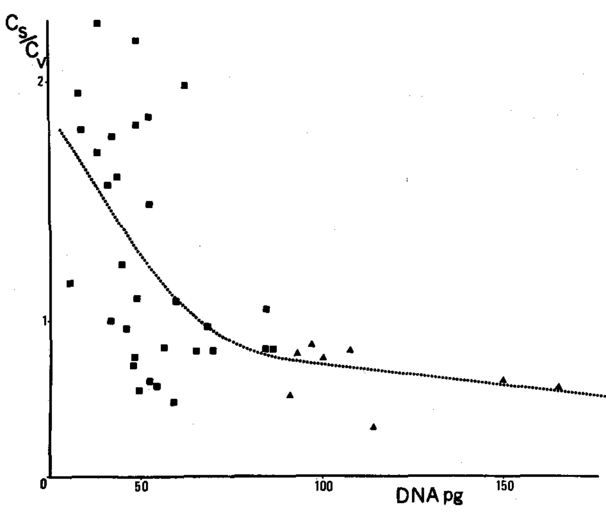

Fig. 2. The relative cell surface (Cs/Cv), on the ordinate, plotted against the nuclear DNA content, on the abscissa, in the same sample of Caudates. Triangles indicate the paedogenetic species. A well fitting regression line (F 'Snedecor' test significant at a level of p 0.01) is the hyperbolic curve presented in the figure, calculated by a computer.

might provide a plausible interpretation of the fact that in the Caudates, despite their not being more evolved than the other tetrapods, nor possessing peculiar mechanisms relative to nuclear or chromosome functions (according to the hypotheses forwarded[1,2]), the genome and cell sizes are on an average considerably larger than those of the other land Vertebrates. Although this is at odds with some workers' opinion[5], the Caudates may be looked upon as having differentiated (like the lungfish[19]) from organisms with smaller genomes, comparable to those of most fishes (*Latimeria* itself possesses only 13 pg/N of DNA)[20]. In the habitats proper to the Caudates, progressive metabolic decay may have proved selectively advantageous[21]: these phenomena are associated with the increase in the genome and cell size. In the Caudates which are more specialized for given environments (notably the water habitat, in which some paedogenetic species are adapted to conditions which are adverse to otherwise competitive forms, e.g. the fish[22]), oxydative metabolism has fallen to very low levels. Further variations, however slight, can be acquired only in parallel to a huge increase in DNA amount. This accounts for the fact that, among paedogenetic forms, large differences in nuclear DNA are shown by species exhibiting an analogous evolutionary or physiological level (such is the case for *Proteus* and *Necturus* among the perennibranchiate, or *Amphiuma* and *Megalobatrachus* among the semilarval species).

To conclude, that sort of nuclear hypertrophy which characterizes the paedogenetic Caudates (having given rise to a variety of speculations on the role of such a large DNA bulk[5]) may be the goal of adaptive processes at the cell level in those organisms which are also phenotypically specialized, though permanently retaining a larval habitus[23].

Riassunto. Sono state studiate le relazioni fra alcuni parametri morfometrici della cellula e la quantità di DNA nucleare in 39 specie di tutte le famiglie di Caudati. Volume nucleare e cellulare e superficie cellulare aumentano in parallelo al DNA in maniera lineare; il rapporto superficie/volume della cellula (funzione del suo metabolismo) decresce rapidamente con l'aumento del DNA, tendendo però a stabilizzarsi a valori di DNA superiori ai 70 pg. Poichè questi ultimi sono raggiunti da specie pedogenetiche di varie famiglie, viene fatta l'ipotesi di un loro ruolo adattativo connesso con le variazioni nel metabolismo cellulare.

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