

trained animal represents more closely that distribution from the 50 day, immature rat in relation to the low number of 8 and 9 and high percentages of size types 6 and 7. This would be in agreement with the recent heart studies in our laboratory where a training induced shift to the size types corresponding to 6 and 7³.

In the heart the smallest size mitochondria are of size 7 and maturation and training resulted in a shift towards this size. The present data clearly show that for all ages the predominate size mitochondria in the soleus muscle is of size types 6 and 7. Due to the non-existence of mitochondrial size types 8 and 9 in the heart and the results of our previous studies we have hypothesized that:

1. there exists an 'optimal' mitochondrial size type in the

soleus and 2. the mitochondria of size types 8 and 9 are precursor mitochondria.

The older soleus muscle samples (720 day) tend to have a high percent number and area of size type 8 and 9 mitochondria, similar to the mature non-trained animals. If the aging muscle cell is in a state of non-growth, low energy requirement, this could account for the increase in these size types with age.

It appears that the training either resulted in the formation of less smaller mitochondria, or that the smaller mitochondria are precursor mitochondria to the predominate size types in the muscle cell. This could account for the higher percent mitochondria in the medium and larger size types, as less smaller mitochondria are found in trained muscle. It is a possibility that these smaller mitochondria are non-functional and are only precursors to the larger size mitochondria within the muscle cell.

We can hypothesize that in the soleus muscle cell an 'optimal' mitochondrion size is existent, and there exists pre-functional mitochondrial. These optimal mitochondrial sizes could be influenced by both cell function and by subcellular localization. Further analysis of soleus mitochondrial data with regard to specific subcellular localization is now in progress.

Résumé. Analyse par microscopie électronique du muscle jambier postérieur chez des rats âgés de 50 jours, adultes, avec ou sans entraînement physique et âgés de 720 jours. Les résultats laissent supposer l'existence d'une taille optimum pour les mitochondries de même que la présence de mitochondries avant-courrières.

D.W. EDINGTON and W.B. McCAFFERTY

The Commonwealth of Massachusetts, University of Massachusetts, Dept. of Exercise Science, Muscle Biochemistry Laboratory, Amherst (Massachusetts 01002, USA), 24 November 1972.

Percent number and percent area distributions of mitochondria by size grouping

Group (N)	Size categories*			
	1,2	3,4,5	6,7	8,9
Percent of total mitochondrial number				
50 (2)	1.1	17.0	66.1	15.9
Mature non-trained (6)	1.7	18.6	56.0	23.7
Mature trained (6)	1.7	19.8	61.4	17.1
720 (1)	0.7	13.6	60.4	25.3
Percent of total mitochondrial area				
50 (2)	4.8	34.9	56.1	4.2
Mature non-trained (6)	7.4	38.0	48.5	6.2
Mature trained (6)	5.9	37.7	51.5	4.8
720 (1)	3.1	28.4	60.3	8.2

* Size categories correspond to absolute sizes ranging from $1.7 \times 10^{-2} \mu\text{m}^2$ to $108.6 \times 10^{-2} \mu\text{m}^2$.

A Comparative Study of the Correlations Existing in the Ratio Between Nucleolar Dimensions and Number of Nucleolini in the Oocytes of Amphibia

Introduction and methods. As early as 1910, CAJAL¹, had been able to show that the nucleolus contained argentophil spherules, which he called 'intranucleolar spherules'. In addition to these structures, the author had been able to ascertain the presence of nucleolar vacuoles, isolated granules and the nucleolini noted by many authors². The nucleolini, which are found in relation to particular functional stages of the nucleolus^{3,4}, can be made to demonstrate well with the method of platinum chloride impregnation⁵. They are also present in the cells in vivo and have the characteristic of being highly refractive^{3,6,7}.

In the oocytes of *Bufo vulgaris*, ALBANESE and BOLOGNARI⁸ had been able to ascertain, by statistical examination, that there is a correspondence between the nucleolar granules and the diameter of the nucleoli.

Studies carried out also along these lines by BOLOGNARI and CAMINITI⁹ on mononucleolate oocytes of *Echinus melo* confirmed that these nucleolini, which are absent in the smallest nucleoli, become increasingly numerous as the volume of the nucleoli increases. In order to see whether this behaviour is to be found in other species, we turned our attention to the oocytes of 2 Anouuran Amphi-

bians, *Rana esculenta* and *Discoglossus pictus*, and those of a Urodelan Amphibian, *Triturus cristatus*. We carried out a statistical examination, adopting the same criteria that were used previously⁸, so that we could also make a comparison between the 3 species of Amphibians. For the statistical examination, carried out on preparations treated by the method of impregnation with platinum chloride, 400 nucleoli were considered for each species, the number of nucleolini present in each nucleolus being counted and their maximum diameter measured.

¹ S. RAMON CAJAL, Trab. Lab. Invest. biol. Univ. Madr. 8, 27 (1910).

² T. H. MONTGOMERY, J. Morph. 15, 265 (1898).

³ A. BOLOGNARI, Atti Soc. pelorit. Sci. fis. mat. nat. 8, 1 (1961).

⁴ M. P. ALBANESE and A. BOLOGNARI, Caryologia 14, 329 (1961).

⁵ A. BOLOGNARI, M. P. ALBANESE and A. DONATO, Boll. Soc. ital. Biol. sper. 35, 764 (1959).

⁶ A. BOLOGNARI, Arch. Zool. ital. 44, 53 (1959).

⁷ A. BOLOGNARI, Experientia 16, 307 (1960).

⁸ M. P. ALBANESE and A. BOLOGNARI, Z. mikrosk.-anat. Forsch. 66, 423 (1960).

⁹ A. BOLOGNARI and M. CAMINITI, Experientia 28, 554 (1972).

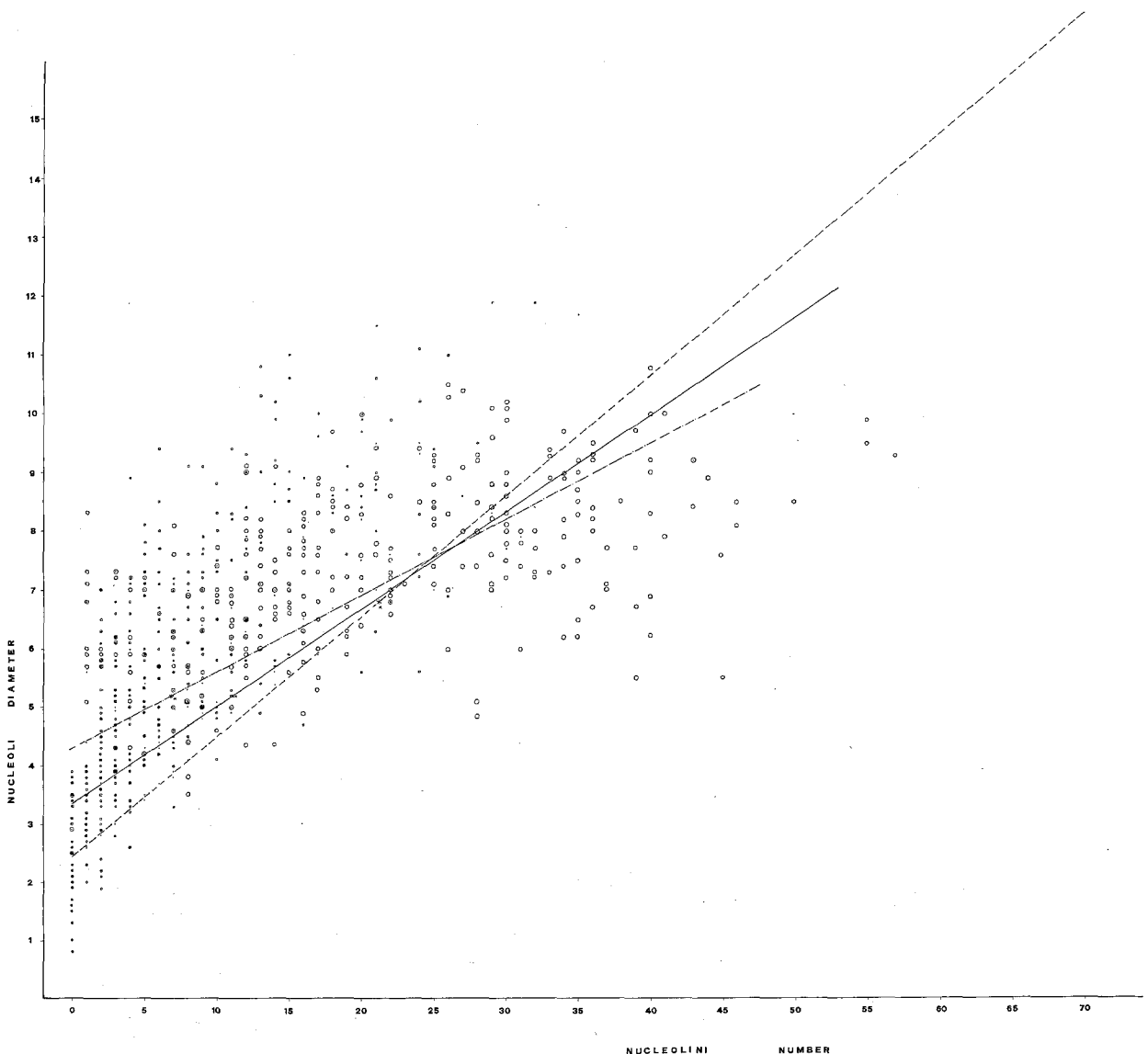
Results and discussion. In the oocytes of *Rana esculenta*, *Discoglossus pictus* and *Triturus cristatus* there are a considerable number of nucleoli, in which nucleolini are nearly always present. Though lacking in the relatively small nucleoli, they become more numerous as the size of the latter increases and are generally spheroidal in shape.

In *Discoglossus* and in *Triturus*, the nucleoli with a fairly large number of nucleolini mostly reveal vacuoles of varying size, which differ from the nucleolini in that their content is less refractive; it is found that these vacuoles are absent in *Rana*. The statistical examination on the polynucleolate oocytes of *Rana esculenta*, *Discoglossus pictus* and *Triturus cristatus* showed that the extreme values for the diameters were respectively: 2.5 μm and 10.6 μm ; 0.8 μm and 15.8 μm ; 0.8 μm and 11.9 μm ; for the numbers of nucleolini the values observed were respectively: 0 and 70; 0 and 52; 0 and 32.

In *Rana* up to a maximum value of diameter of 3.5 μm no nucleolini were found. The mean values obtained were: diameter of nucleoli 6.8 μm ; number of nucleolini 21.2.

In *Discoglossus* up to a maximum value of diameter of 2 μm no nucleolini were found. The mean values obtained were: diameter of nucleoli 5.2 μm ; number of nucleolini 11.1. In *Triturus* too up to a maximum value of 1.9 μm no nucleolini were found. The mean values obtained were: diameter of nucleoli 5.2 μm ; number of nucleolini 6.9. The values found in all 3 species were used to compile the dispersion diagram (Figure) relative to nucleolar diameter/number of nucleolini, and also the correlation tables.

From these were obtained a regression coefficient (by/x) of 0.10 for *Rana*, 0.16 for *Discoglossus* and 0.26 for *Triturus* and a correlation coefficient (r) of 0.62 for *Rana*, 0.64 for *Discoglossus* and 0.71 for *Triturus*. As a general rule, as may be seen from the diagram, the number of nucleolini increases for all 3 species in correspondence with the increase in the diameters of the nucleoli. The distribution along the line of regression in the 3 species is seen to be non-uniform, which is related to the fact that frequently the maximum values of one dimension do not correspond to the maximum values of the other. However, if we



Dispersion diagram relative to nucleolar diameter/number of nucleolini of *Triturus cristatus*, *Discoglossus pictus* and *Rana esculenta* oocytes. O = *Rana esculenta*, \square = *Triturus cristatus*, \bullet = *Discoglossus pictus*, — = *Discoglossus pictus*, ---- = *Rana esculenta*, - · - · - = *Triturus cristatus*

consider that the numerical value of the correlation coefficient (r) is equal to 0.62 in *Rana*, 0.64 in *Discoglossus* and 0.71 in *Triturus*, we can conclude that, between the 2 quantities relative to the data in question, there exists a statistically significant correlation, in the nucleoli of the oocytes of the three species considered.

The statistical analysis carried out on the oocytes of *Rana esculenta*, *Discoglossus pictus* and *Triturus cristatus* has confirmed once more that the nucleolini, though absent in the smallest nucleoli, become increasingly numerous as the size of the nucleoli increases. However, there were seen to be differences as regards the regression coefficient (by/x), which was found to be 0.10 in *Rana*, 0.16 in *Discoglossus* and 0.26 in *Triturus*. The value of the regression coefficient found in *Discoglossus* is comparable with that of *Echinus*⁹, which is found to be equal to 0.15; that relative to *Triturus* is very close to that of *Bufo*⁸, equal to 0.28; but that found in *Rana* differs from all the species considered. As regards the correlation coefficient (r), the values were found to be fairly close in the 3 species

studied, being 0.62 in *Rana*, 0.64 in *Discoglossus* and 0.71 *Triturus*. The correlation coefficient of *Triturus* is somewhat comparable with that of *Bufo*, which is equal to 0.75. Consequently, the most significant correlation is to be found in *Triturus*.

Riassunto. Le indagini statistiche effettuate sui nucleoli degli ovociti di *Rana esculenta*, *Discoglossus pictus* e *Triturus cristatus* hanno consentito di confermare che i nucleolini, assenti nei nucleoli più piccoli, diventano sempre più numerosi coll'aumentare delle dimensioni dei nucleoli. Nelle tre specie in esame sono stati altresì ottenuti coefficienti di correlazione approssimativamente simili.

M. P. ALBANESE CARMIGNANI and F. MINNITI

*Istituto di Zoologia e di Anatomia comparata,
Università di Messina, Messina (Italy),
13 November 1972.*

Adrenalectomy Effect on Pituitary Cell Types

The cellular origin of each pituitary hormone can be ascribed to one particular cell type, but ACTH is ascribed to both β -1 and β -3 (γ or c) cells¹. Avoiding elaborate staining techniques, we propose to study the dynamic aspect of pituitary histology in adrenalectomized rats by a simple and functional approach. Without the inhibitory

effect of circulating glucocorticoids², the adenohipophysis is subjected to prolonged action of the cortical releasing factor³. Thus, hyperfunction of the ACTH secreting cells may render it detectable by H-E staining and autoradiography.

Material. Male Wistar rats (200–250 g) were bilaterally adrenalectomized by mid-ventral laparotomy. In partial adrenalectomy, only the adrenal content was squeezed out. Control rats were sham-operated. Pituitaries were fixed in alcoholic Bouin, sectioned at 5 μ m and stained with H-E. Cells were counted with a grid at $\times 450$; 4 to 8 fields (about 0.1 mm²) were chosen from each pituitary. Each cell type was counted separately. High specificity ³H-lysine and ³H-thymidine was given i.v. at 40 μ Ci/100 g for 1 h. Slides were coated with Ilford K₂ emulsion at 1 : 1 dilution and developed with Kodak Microdol-X⁴.

Table I. Effect of bilateral adrenalectomy on pituitary histology in mature male rats.

	Number			Percentage		
	a	b	c	a	b	c
Sham control	192.1	161.0	143.3	39.0	32.4	28.6
1 day	139.6	163.1	101.3	34.5	40.4	25.1
2 days	159.5	187.5	104.0	35.4	41.6	25.1
3 days	154.6	231.3	135.3	29.6	44.4	25.0
5 days	138.0	212.2	101.7	30.5	47.0	22.5

Results are pooled from 2 experiments and expressed as average cell number per field and cell percentage per field.

¹ F. H. NETTER, *Endocrine system and Selected Metabolic Diseases* (Ciba Collection of Medical Illustrations; Ciba 1970), vol. 4, pp. 10 and 11.

² L. MARTINI, *Archs int. Pharmacodyn.* 140, 156 (1962).

³ R. BURGUS and R. GUILLEMIN, *A. Rev. Biochem.* 39, 499 (1970).

⁴ A. FICQ, *Monograph, Inst. Interuniv. Sci. Nucl., Bruxelles* 1961, p. 18.

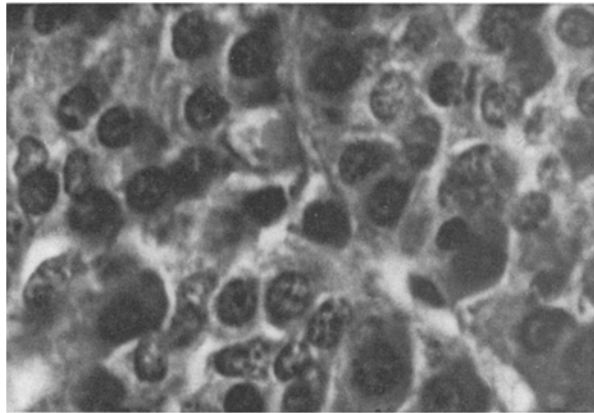


Fig. 1. a) H-E staining of rat anterior pituitary in sham control.

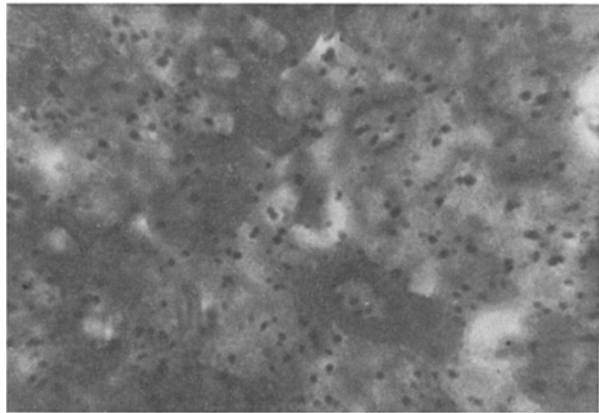


Fig. 2. a) Incorporation of ³H-lysine in sham control.