

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.

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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.

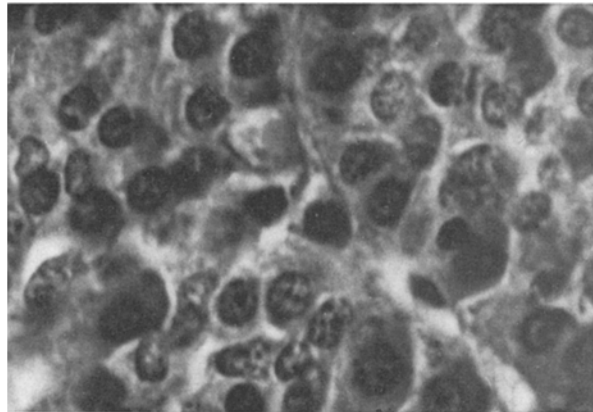


Fig. 1. a) H-E staining of rat anterior pituitary in sham control.

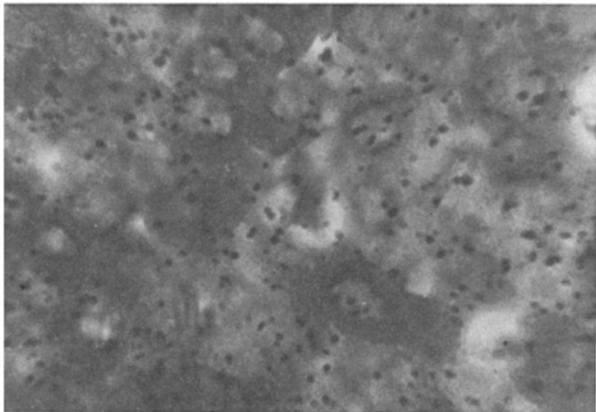


Fig. 2. a) Incorporation of ³H-lysine in sham control.

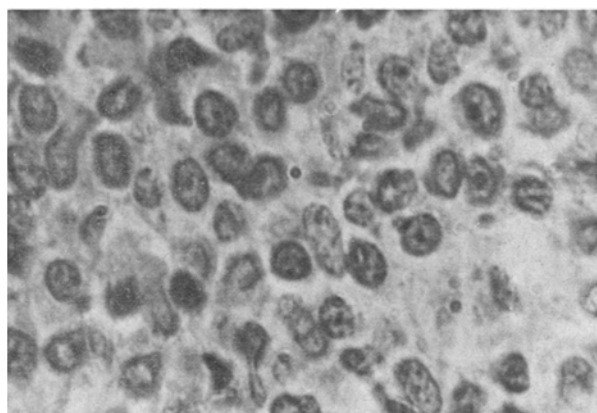


Fig. 1.b) H-E staining of rat anterior pituitary 5 days after adrenalectomy.

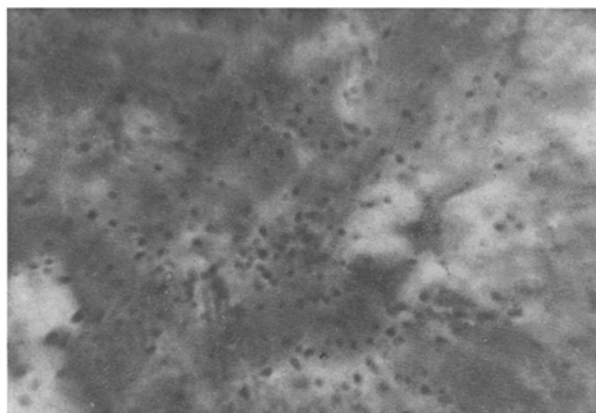


Fig. 2.b) Incorporation of ^3H -lysine in rat anterior pituitaries; 3 days after adrenalectomy.

Table II. Effect of Adrenocorticotropin on pituitary histology of adrenalectomized young male rats (100 g)

	Number			Percentage		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Sham control	190.5	135.2	105.5	44.2	31.3	24.5
Bilateral adrenalectomy 1 day	181.7	137.0	187.7	41.5	31.3	27.1
Bilateral adrenalectomy 3 days	132.0	197.0	150.0	27.5	41.1	31.3
Bilateral adrenalectomy 3 days + ACTH	163.7	154.5	121.0	37.3	35.2	27.5
Partial adrenalectomy 3 days	127.0	174.2	146.0	27.0	39.0	30.0
Partial adrenalectomy 3 days + ACTH	182.5	139.2	114.0	41.9	32.1	26.1

6 IU Cortropin (Organon) was injected i.m. the 2nd and 3rd day post-operation. Results are expressed as average cell number per field and cell percentage per field.

Results. Adrenalectomy caused a gradual decrease in eosinophilia of a cells with a concomitant decrease in cell number. In contrast, b cells became more distinct with a pinkish-blue cytoplasm 1 day after operation; they became very distinct 5 days later. While these chromophilic changes progressed, c cells always remained vaguely stained and lumped together as clusters of 2–3 cells (Figure 1).

In sham controls, a cells represented the predominant cell type with b and c cells followed in decreasing number. Adrenalectomy reversed this proportion between cell types. c cells also tended to decrease in number but their percentage remained fairly constant due to decreasing cell number per field (Table I).

In young male rats, adrenalectomy resulted in cell percentage changes as observed in mature rats. ACTH effectively minimized these changes in partially as well as bilaterally adrenalectomized rats (Table II).

Adrenalectomy caused a preferential incorporation of ^3H -lysine into c cells, and to a less extent, b cells. Silver grain concentration over a cells was indistinguishable from that of background or sinusoid area (Figure 2). Incorporation of ^3H -thymidine was minimal in both control and adrenalectomized rats.

Discussion. Adrenalectomy revealed a consistent pattern of pituitary cell type changes, i.e. decrease in a cells and increase in b cells with c cells relatively unchanged. Since ACTH can minimize these changes, the specific effect of adrenalectomy appears to be significant. The

absence of ^3H -thymidine incorporation indicates that increase in b cells due to mitosis is most unlikely. Changes in cell number is more probably a result of dedifferentiation of a cells and hyperfunction of b cells. Since ACTH carries 4 lysine residues out of 39 amino acids⁵, preferential incorporation of ^3H -lysine into c cells in adrenalectomized rats may either indicate ACTH synthesis, or more likely, a hyperfunctional state of these cells prior to conversion into b cells.

Résumé. Dans l'hypophyse du rat normal, les cellules a occupent 39%. Après l'adrenalectomie bilatérale, les cellules b deviennent le type cellulaire prédominant (47%) au profit des cellules a, tandis que les cellules c restent peu changées. L'ACTH minimise ce changement des types cellulaires. Cela indique que l'adrenalectomie a causé un état hyperfonctionnel des cellules de type b.

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Chung Chi College,
The Chinese University of Hong Kong,
Shatin (N.T., Hong Kong), 4 December 1972.

⁵ C. H. LI, *Scient. Am.* July 1963.