que tous les organes embryonnaires du poulet, cultivés in vitro jusqu'ici (milieu standard de WOLFF et HAFFEN[5]), sont capables de se développer et de croître jusqu'à un certain stade, à l'exception des vaisseaux sanguins qui, pourtant, ne sont pas les seuls organes dotés de muscles lisses. Nous pensons qu'à l'origine de cet état de choses, il y a surtout 2 facteurs qui entrent en jeu: un facteur mécanique et un facteur chimique. Dans les vaisseaux explantés, il manque en effet le stimulus mécanique qui, in vivo, est représenté par le flux du liquide traversant la lumière; ce facteur mécanique joue sans doute un rôle morphogénétique important pour la différenciation et l'accroissement des différents composants de la paroi artérielle. Si nous pouvions réaliser un dispositif de perfusion capable d'entretenir, pendant toute la période de culture, le passage d'un liquide dans la lumière du vaisseau, le rôle du facteur mécanique pourrait être précisé davantage. Nous avons déjà fait des essais préliminaires dans ce sens, mais le problème n'est pas facile à résoudre sur le plan technique, compte tenu surtout de la petitesse des vaisseaux explantés. Quant au deuxième facteur pouvant représenter une entrave à la survie des vaisseaux cultivés, il pourrait s'agir sans doute d'une question de milieu qui, tout en étant le milieu idéal pour les autres organes de l'embryon de poulet, ne serait pas le milieu de culture le plus adéquat pour assurer le métabolisme de la paroi vasculaire. Des recherches précédentes[6,7] nous ont confirmé la validité de cette hypothèse. En ajoutant en effet au milieu standard de WOLFF des doses variables de facteur *P*, nous avons constaté pour les artères soumises à ces expériences, (artères mésentérique et chorio-allantoïdienne) une meilleure survie hors de l'organisme: pas de signes de souffrance, la musculature persiste, souvent elle augmente en culture. Nous en avions alors conclu que le facteur *P* est essentiel au développement et au maintien des structures normales des vaisseaux cultivés in vitro.

Summary. The authors have studied the normal embryonal development of chick embryo's vitelline and chorioallantoic vessels and the behaviour of these vessels explanted in vitro. They resumed, afterwards, the results obtained from several series of vessels observed in the same normal and experimental conditions.

G. CONTI et B. CAPPELLI

Institut d'Histologie et d'Embryologie générale de l'Université de Fribourg (Suisse), le 29 mars 1968.

[5] E. WOLFF et K. HAFFEN, Texas Rep. Biol. Med. *10*, 463 (1952).
[6] B. CAPPELLI, G. CONTI, L. LASZT et B. MANDI, Angiologica *5*, 28 (1968).
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Age Dependence of Nuclear DNA Content of Rat Adipose Tissue Cells

Up to now it is an open question whether the enlargement of adipose tissue is due to an actual increase in cell number or simply to an additional storage of fat[1]. To decide this question, biochemical DNA-determinations on adipose tissue have been accomplished by others[2–4]. From the total amount of DNA, estimated by these methods, the number of fat cells can be computed only if the DNA-content of the nuclei is constant and exactly known. Such DNA-determinations can only be made cytophotometrically.

The epididymal fat pads of male wistar A/F Han-Rats (Central Institute for Experimental Animal Breeding, Hannover) have been examined. Adipose tissue cells were isolated according to RODBELL[5]. Smears were fixed in absolute alcohol and Feulgen stained in a modification given by SWIFT[6]. The Feulgen dye content was measured with an integrating microdensitometer[7].

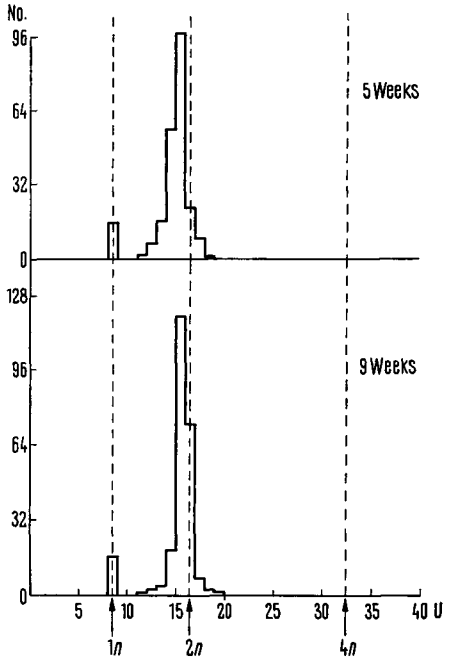

Fig. 1. Nuclear DNA-content of adipose tissue cells of rats aged 5 and 9 weeks. Abscissa: No. of measured nuclei; ordinate: DNA-content in arbitary units (U). The first column represents DNA-content of rat-spermatosomes (reference value). 1*n*, 2*n*, 4*n* = haploid, diploid and tetraploid DNA-value.

[1] F. X. HAUSBERGER, in *Handbook of Physiology, V. Adipose Tissue* (Ed. A. E. RENHOLD and G. CAHILL JR., Williams and Wilkins Co., Baltimore 1965), p. 519.
[2] J. HIRSCH and R. B. GOLDRICK, J. clin. Invest. *43*, 1776 (1964).
[3] S. C. PECKHAM, C. ENTENMAN and H. W. CARROL, J. Nutr. *77*, 187 (1962).
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[6] H. SWIFT, in *The Nucleic Acids* (Academic Press, New York 1955), vol. 2, p. 51.
[7] E. M. DEELEY, J. Scient. Instrum. *32*, 263 (1955).

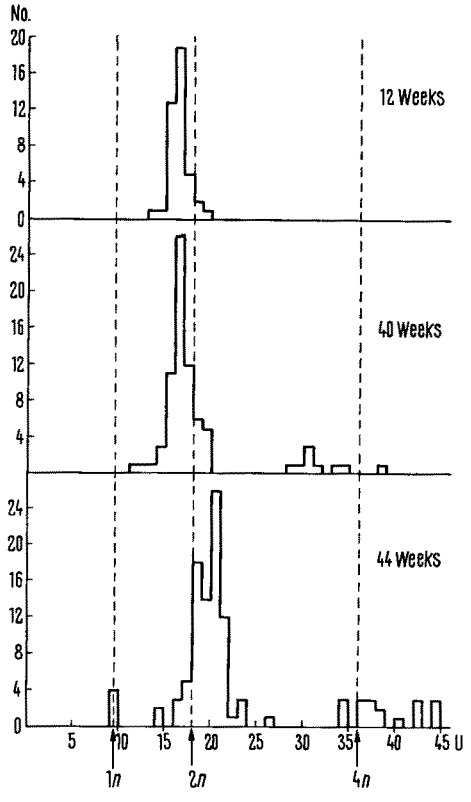

Fig. 2. Nuclear DNA-content of adipose tissue cells of rats aged 12, 40 and 44 weeks. Abscissa: No. of measured nuclei; ordinate: DNA-content in arbitary units (U). The first column represents DNA-content of rat-spermatosomes (reference value). $1n$, $2n$, $4n$ = haploid, diploid and tetraploid DNA-value.

Since an absolute separation of adipose tissue cells from stroma and blood cells proved to be impossible, only those cells were measured that could be clearly identified as adipose tissue cells by means of the nuclear shape (large, mostly oval nuclei, poor in chromatin) and the position of the nucleus in a cytoplasmic structure.

The nuclear DNA-content was found to be close around the diploid DNA-value in animals aged 5–12 weeks (Figures 1 and 2). In older animals (40 weeks and more) tetraploid nuclei appeared besides the diploid ones (Figure 2). The number of tetraploid nuclei amounts to 12–14%. In addition, binucleated adipose tissue cells were noticed in about 5% (Figure 3). The DNA-content of each of these nuclei corresponded with the diploid value.

These findings indicate that biochemical DNA-estimations in the adipose tissue[2–4] do not accurately represent a basis of estimation of the number of cells. Tetraploid adipose tissue cells appear in 12–14% in older animals. In another 5% the appearance of binucleated adipose tissue cells must be expected. These results are in accordance with the findings of LIEBELT[8] but contrast with the observations of HIRSCH and GOLDRICK[2]. Our suggestion is that the cytokinesis is no longer possible in differentiated adipose tissue cells of older animals, while the ability to synthesize DNA may persist[9].

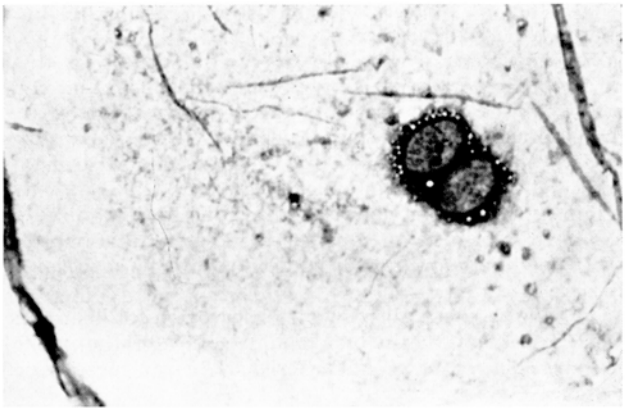

Fig. 3. Binucleated adipose tissue cell, α-Naphthylacetat-esterase reaction. × 1250.

Zusammenfassung. Der DNS-Gehalt isolierter Fettzellen wurde cytophotometrisch bestimmt. Bei älteren Tieren fanden sich 12–14% tetraploide Fettzellen neben 5% zweikernigen Fettzellen. Es wird auf die Folgen für die Bestimmung der Zellzahl aus dem Gesamt-DNS-Gehalt des Fettgewebes hingewiesen.

W. MOHR and G. BENEKE

Department of Pathology, Justus Liebig University, 63 Giessen (Germany), 10 June 1968.

[8] R. LIEBELT, Anat. Rec. *154*, 377 (1966).

[9] This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

Mast Cells and Histamine in a Newt, *Triturus pyrrhogaster* Boié

Although mast cells in the lower vertebrates have been studied by many histologists, it has generally been believed a priori that they contain histamine as mammalian mast cells do. ARVY[1], in his histochemical study, noticed that he failed to demonstrate histamine in mast cells of the frog, but he could not escape the supposition that mast cells are the cells of histamine.

In a previous report[2], we showed the absence of histamine in mast cells of the frog, *Rana catesbiana*, by the combination of histological survey of mast cell population in several organs and bioassay (Code's method) of histamine content in these organs.

We also reported that there is no histamine in mast cells of the vertebrates below the level of reptilia. This agreed with the results of REITE[3], which were obtained by a more indirect method.

[1] L. ARVY, C. r. Ass. Anat. *42*, 217 (1956).

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[3] O. B. REITE, Nature *206*, 1334 (1965).