Nuclear Morphology in the Nutritive Cells of *Drosophila* Testes

The behaviour of the nutritive cells in relation to the germinal cells during spermatogenesis in *Drosophila* has been summarized by COOPER[1]. However, the characteristics of the nuclear organization in nutritive cells seems to have been overlooked. The present report deals with the morphology of the nutritive cell nuclei which have been studied in vivo under the light microscope.

Testes of normal larvae from *Drosophila melanogaster*, *D. testacea*, *D. hydei*, *D. inmigrans*, *D. repleta*, *D. buzzatii* and *D. phalerata* were isolated in *Drosophila* Ringer's solution. Testes from *XO* and *XYY* larvae of *D. melanogaster* were also used. After a gradual flattening of the testes[2], the germinal and nutritive cells form a monolayer, which allows observation in vivo for 10–15 min. The pictures were obtained in a Zeiss Photomicroscope with Planapo X 100 phase contrast objective.

The nutritive cell nuclei show a species-specific morphology, as in the case of the *Y*-chromosome loops in the spermatocyte nuclei[3]. The former nuclei show a small size in comparison with those of spermatocytes but they are slightly greater than spermatogonia. In the species studied, the nucleolus appears as a globular and prominent body, 3–5 μm in diameter, which in *D. testacea*, *D. hydei*, *D. inmigrans* and *D. phalerata* generally shows a peripheral dark region and a lighter central one.

The nuclei in *D. melanogaster* and *D. testacea* show two dense and free knobs and another dense knob which is attached to the nucleolus (Figure 1). Each free knob appears embedded in a diffuse material which forms irregular-shaped masses. Often the nucleolar knob also presents a short diffuse arm. The same morphology was observed in the nutritive cell nuclei of *XO* and *XYY* testes of *D. melanogaster*.

A prominent spherical nucleolus appears bound to a granular dense body which is surrounded by granular material in *D. hydei* (Figure 2). In *D. immigrans* there is a round body, with or without central vacuole, in close relation with the nucleolus and the masses of granular material. Generally this latter material also presents a dense knob (Figure 3). *D. repleta* only shows a nucleolus and some small areas of low density (Figure 4). *D. buzzatii* presents a vacuolated dense body near the nucleolus and a mass of diffuse material. The nucleolus in nutritive cell nuclei of *D. phalerata* is attached to the nuclear envelope. In this species 2 free knobs, each surrounded by a small area of granular material was observed.

The different morphology of the nutritive cell nuclei in the testes of several *Drosophila* species, seems to indicate certain similarities with the species-specific loops of the *Y*-chromosome in spermatocytes from this genus[3,4]. However, the general organization of nutritive cell nuclei in the *XO* and *XYY* male larvae of *D. melanogaster*, did not show any appreciable difference to those from *XY* normal larvae, each having the nucleolar knob and the 2 knobs embedded in diffuse material. It can be assumed that the nuclear structures we have described do not correspond to any *Y*-chromosome loop.

At present the optical features of the nutritive cell nuclei in *Drosophila* testes are not yet clear. We hope that the cytochemistry and ultrastructural analysis, which are now in progress, may explain the peculiarities of this nuclear organization[5].

Resumen. Se describe en microscopía óptica una serie de estructuras características dentro del núcleo de celulas nutricias viventes en testículos de varias especies de *Drosophila*. Estas estructuras consisten en corpúsculos densos, homogeneos o vaculados, y en masas de material difuso o granular.

J. C. STOCKERT[6] and P. ESPONDA

Centro de Investigaciones sobre Reproducción, Facultad de Medicina, Paraguay 2155, Buenos Aires (Argentina), and Departamento de Citología, Instituto de Biología Celular, Velazquez 144, Madrid-6 (Spain), 22 April 1971.

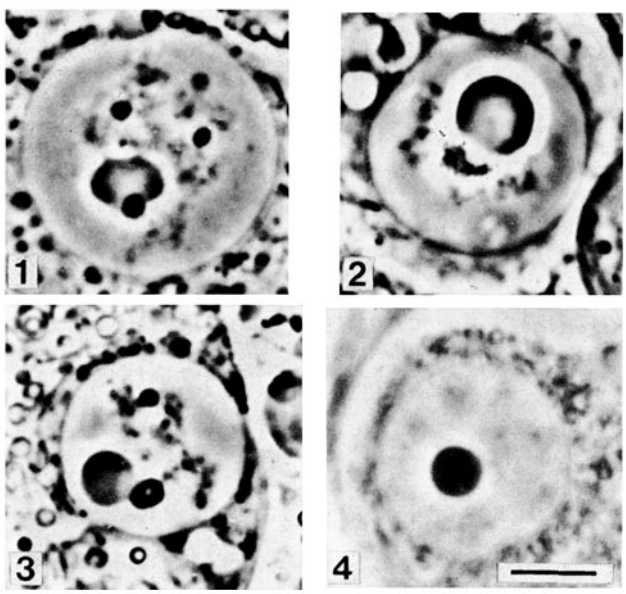

Fig. 1–4. 1. Nutritive cell nuclei from testes of *Drosophila testacea*. 2. *D. hydei*. 3. *D. inmigrans*. 4. *D. repleta*. Phase contrast, ×2200. Scale mark, 5 μm.

[1] K. W. COOPER, in *Biology of Drosophila* (Ed. M. DEMEREC; John Wiley and Sons, Inc., New York 1950), p. 1.
[2] G. F. MEYER, Drosoph. Inf. Serv. *40*, 80 (1965).
[3] O. HESS and G. F. MEYER, Adv. Genetics *14*, 171 (1968).
[4] O. HESS, Ann. Embr. Morph., Suppl. *1*, 165 (1969).
[5] We wish to thank Dr. E. TORROJA, Instituto de Genetica y Antropología, Madrid (Spain), for providing the *Drosophila* species and the *XO* and *XYY* larvae of *D. melanogaster*.
[6] Supported by The Population Council, Inc., New York.

Repopulation of Thymus, Spleen and Bone-Marrow of Irradiated AKR Mice Injected with Normal or Irradiated Bone-Marrow Cells

The inflow of marrow cells into the bone-marrow and into the lymphoid tissues has been studied in mice by several authors using transfusions of syngeneic normal bone-marrow cells carrying marker chromosomes. Experiments on non-[1], part-body[2,3] or whole-body irradiated[4–8] male CBA recipients receiving CBA-T6T6 normal bone-marrow cells, indicated that cells may be transferred from the marrow to the thymus, although dividing donor cells were observed in the thymus at a considerably later time than in the other tissues studied. The present experiments have been

designed in order to compare the repopulation of irradiated AKR/T1Ald mice injected with irradiated or normal AKR bone-marrow cells at short time intervals after treatment.

Material and methods. 12-week-old AKR and AKR/T1Ald male mice were used for these experiments. AKR/T1Ald mice have only 38 chromosomes: 36 acrocentric chromosomes and 2 metacentric chromosomes[9,10]. The incidence of leukemia is very high (90%) in both strains[11]. The inbred AKR and AKR/T1Ald strains are antigenically compatible as tested by survival of reciprocal skin grafts and by survival of supralethally irradiated mice after reciprocal bone-marrow transplantation[12].

Irradiated AKR/T1Ald mice were injected i.v. within 1 h after irradiation with 10^7 bone-marrow cells from either non-irradiated or irradiated AKR mice. When irradiated AKR mice served as donor, bone-marrow cells were taken immediately after irradiation. All the AKR/T1Ald recipients and the irradiated AKR donors were given a whole-body dose of 650 R of X-rays (Maxitron, 260 kV, 20 mA, HVL 2 mm Cu, exposure rate 100 R/min). The mice were exposed in groups of 11 in partitioned lucite cages. The X-ray dose was measured with an integrating dosimeter (Philips 37470/100) during each X-ray exposure.

Irradiated recipients given normal or irradiated bone-marrow cells were killed 6 or 12 days after treatment, 1 h before sacrifice they received an i.p. injection of 0.5 ml of a 0.025% solution of colchicine. Thymus and spleen or thymus and femoral bone-marrow were examined cytologically.

Results (see Table I). *Non-irradiated AKR donor cells* injected into irradiated AKR/T1Ald recipients are able to proliferate and represent a large percentage of the total cell population in thymus, spleen and bone-marrow 6 days after injection. In bone-marrow and spleen, this percentage remains about constant during the following 6 days period, but in thymus it decreases by a factor of about 4.

Irradiated AKR donor cells, proliferating in an irradiated recipient 6 days after exposure, constitute a much smaller percentage of the cell population, particularly in the bone-marrow and the thymus, when compared with non-irradiated donor cells. Under these conditions, cells in metaphase are scarce and this rendered the scoring at 6 days rather difficult. Nevertheless, it is evident that even irradiated donor cells can enter the thymus and there proliferate. During the subsequent period, from 6 to 12 days after exposure, the percentage of donor cells in the thymus decreases by a factor close to that observed when non-irradiated donor cells were given. Meanwhile, the distribution between donor and host cells does not change significantly in bone-marrow and spleen.

Discussion. Observations in bone-marrow and spleen. The spleen colony technique[13,14] has provided a basis for a quantitative assessment of the proliferative capacity of normal and irradiated bone-marrow stem cells in an irradiated host. In this way, a dosis of 95 rad and an extrapolation number of 1.5 was observed for stem cells irradiated in vivo. Under our experimental conditions (650 R), the surviving fraction of stem cells capable of forming a colony would, therefore, be in the range of 0.002 to 0.004. An estimate of total viable bone-marrow cells and of CFU in recipient and transplant based on data published in the literature[13–16] is compiled in Table II). When non-irradiated donor cells are given to an irradiated recipient the ratio, donor to host cells, is in the order of

[1] H. S. Micklem, C. M. Clarke, E. P. Evans and C. E. Ford, Transplantation *6*, 299 (1968).
[2] C. E. Ford, H. S. Micklem, E. P. Evans, J. G. Gray and D. A. Ogden, Ann. N.Y. Acad. Sci. *129*, 283 (1966).
[3] H. S. Micklem and D. A. Ogden, IVth Int. Congr. Radiation Res., Abstr. 565 (1970).
[4] D. W. H. Barnes, G. Breckon, C. E. Ford, H. S. Micklem and D. A. Ogden, *The Lymphocyte in Immunology and Haemopoiesis* (Ed. J. M. Yoffrey; Arnold, London 1967), p. 207.
[5] C. E. Ford, J. L. Hamerton, D. W. H. Barnes and J. F. Loutit, Nature, Lond. *177*, 452 (1956).
[6] H. S. Micklem and C. E. Ford, Transplant. Bull. *26*, 436 (1960).
[7] H. S. Micklem, C. E. Ford, E. P. Evans and J. G. Gray, Proc. R. Soc. B. *165*, 78 (1966).
[8] A. Takada, Y. Takada, C. C. Huang and J. L. Ambrus, J. exp. Med. *129*, 445 (1969).
[9] A. Léonard and Gh. Deknudt, Experientia *22*, 715 (1966).
[10] A. Léonard and Gh. Deknudt, Nature, Lond. *214*, 504 (1967).
[11] A. Léonard and Gh. Dekundt, IAEA, SM-*118/27*, 305 (1969).
[12] A. Sassen, personal communication.
[13] J. E. Till and E. A. McCulloch, Radiation Res. *14*, 213 (1961).
[14] J. E. Till and E. A. McCulloch, Radiation *18*, 96 (1963).
[15] L. G. Lajtha, *Current Topics in Radiation Research* p. 139 (Ed. M. Ebert and A. Howard; North Holland Publisher Co., Amsterdam 1965, vol. 1, p. 139.
[16] S. Hellman and H. E. Grate, IAEA, SM-*103/4*, 187 (1968).

Table I. Incidence of donor type mitotic cells in recipients AKR/T_1Ald mice

Treatment of the donor	Days after injection	Mitotic cells scored								
		Thymus			Femoral bone-marrow			Spleen		
		No. of recipients	Total cells	Donor cells	No. of recipients	Total cells	Donor cells	No. of recipients	Total cells	Donor cells
0 R	6	18	145	110 75.9% (68.0–82,5)	9	900	816 90.7% (88.5–92.5)	9	900	893 99.2% (98.5–99.7)
	12	18	576	112 19.4% (16.4–23.0)	9	821	766 93.3% (91.3–94.9)	9	840	807 96.1% (94.5–97.2)
650 R	6	18	49	9 18.4% (8.7–32.0)	9	359	25 7.0% (4.9–10.8)	9	43	33 76.7% (61.3–88.2)
	12	15	1088	35 3.2% (2.4–4.8)	8	439	20 4.6% (2.8–7.0)	7	310	194 62.6% (57.4–68.3)

Confidence intervals ($P \leq 0.05$) are indicated in parenthesis.

Table II. Estimated distribution of donor and host CFU for the different experimental treatment

Treatment	Donor		Host
0 R	Marrow cells inoculum	10^7	Cells in total marrow 4×10^8
	CFU	12×10^2	CFU in total marrow 50×10^3
			CFU in circulating pool 110–150
650 R[a]	Marrow cells inoculum	10^7	Surviving CFU in total marrow 100–200
	Surviving CFU	2–5	Surviving endogenous spleen CFU 4–8
			Surviving CFU in circulating pool <0.3

[a] Surviving fraction: 0.002–0.004.

10:1; consequently, bone-marrow as well as spleen are rapidly and permanently colonized by the donor cells. This is in agreement with previous observations by other authors[7,8].

When cells from an irradiated donor are injected into an irradiated recipient, the ratio donor to host cells is in order of 1:40. Accordingly, very few of the dividing cells in the bone-marrow are of donor origin. On the other hand, the proportion of cells from donor origin in the spleen is larger (76%) than expected from Table II (30 to 50%). The bone-marrow appears to be repopulated with the same efficiency by exogenous and endogenous cells whereas the spleen is repopulated with a higher efficiency by exogenous cells. One must, however, consider that in an irradiated recipient, the circulating pool of CFU is very small ($<$ 0.3 CFU), and that colony seeding from host bone-marrow would proceed over a longer period of time than occurs when donor cells are injected directly into the blood.

Observations in thymus. From experiments based on morphological observations[8,17,18], as well as from those involving injected bone-marrow cells (with chromosome markers)[2,7,8], it has been assumed that the thymus of sublethaly or supralethaly irradiated animals repopulates in two phases. The first phase (from day 4 to day 12) is characterized by the proliferation of surviving precursor cells in the thymus. The second and final phase starts 2–3 weeks after irradiation and features proliferation of small lymphocytes derived from the bone-marrow.

Our data indicate, however, that injected bone-marrow cells can directly enter the thymus of AKR/T1Ald mice and there proliferate at early times after exposure (6 days). This occurs regardless of whether AKR donors have been previously irradiated or not. The early period of thymus repopulation by donor cells is followed by a second one during which the proportion of donor cells in the thymus decreases. Evidently, the host cells crowding out the donor cells cannot come from the bone-marrow which at this time is already populated by cells of donor origin. Several explanations are possible for this observations but no definite explanation can be proposed since the cells involved in regeneration of the thymus and their cellular parameters are still insufficiently known.

a) The donor cells which have been proliferating and maturing in the thymus during the initial 6-day-period migrate out from the thymus afterwards. A more primitive type of precursor cells not present in the bone-marrow transplant but which survives in the thymus after irradiation starts to divide and mature. Most likely the latter cells are incapable of bringing on full regeneration of the gland, since finally repopulation of the thymus by cells originating in bone-marrow takes over. Such a pattern would correspond to the biphasic type of regeneration found for thymus by certain authors[8–17].

b). The bone-marrow donor cells rest in the thymus and divide more slowly than precursor cells endogenous to the thymus. Again in this case, thymus would have to contain cell types not present in the bone-marrow transplant and our results therefore suggest that thymocytes can stem from 2 different cell types, as has already been proposed[7]. It should finally be pointed out that the strains of mice utilized in our experiment (AKR, AKR/T1Ald) display certain particularities such as have been reported with respect to isogenic restauration in irradiated AKR mice[19] and leukemogenesis in AKR/T1Ald isogeneic radiochimaera[20].

Résumé. L'utilisation d'une race de souris (AKR/T1Ald) ne possédant que 38 chromosomes a permis de montrer que les cellules de moelle que l'on injecte à un individu irradié repeuplent directement la moelle, le thymus et la rate du receveur. Si le donneur est irradié, la pénétration des cellules n'est importante que dans la rate du receveur.

A. LÉONARD, A. DECLÈVE and J. R. MAISIN[21]

Department of Radiobiology, Centre d'Etude de l'Energie Nucléaire, C.E.N./S.C.K., B-2400 Mol (Belgium), 19 March 1971.

[17] H. BLOMGREN, Expl. Cell Res. *58*, 353 (1969).
[18] D. B. THOMAS and C. C. CONGDON, J. Physiol., Lond. *188*, 28P (1967).
[19] J. F. DUPLAN, C. r. Soc. Biol., Paris *159*, 1277 (1965).
[20] E. LEGRAND and J. F. DUPLAN, C. r. Acad. Sci., Paris *268*, 2990 (1969).
[21] Acknowledgements. This work was supported by grants from the 'Fonds de la Recherche scientifique fondamentale collective'. The helpful assistance of Mr. G. LINDEN and G. MATTELIN is gratefully acknowledged.

Effect of Metabolic Stress on Activation of Glutathione Reductase by FAD in Human Red Cells

Glutathione reductase (GR) plays an important role in the protection of protein in red cells against oxidation. Increased activity of GR has been reported in red cells in patients with severe metabolic disorders, e.g., severe cases of uremia[1] and of cirrhosis of liver[2]. Increased GR activity has also been observed in red cells with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency[3]. The reason for these increases in GR activity is unknown.

Recently, it has been reported that GR is present in at least 2 forms, the active form which is associated with fla-