part of the chromosome in figure 4a is 3-stranded and one of the strands corresponds to the region translocated to the Y-chromosome. Since the Y-chromosome is not polytenic in the salivary glands but heterochromatic and incorporated into the centromeric heterochromatin, this strand is frequently associated with heterochromatin or with the nucleolus (as in fig. 4a). Balanced males have polytene chromosomes in which 1 of the 2 strands is interrupted at the position of the translocation break point (indicated by arrows in fig. 4b). Knowing which part of the chromosome is translocated and having the information from the allozyme pattern, a decision can be made on which part of the chromosome the gene must be situated. With enough different translocations a rather precise localization can be achieved.

To make things clear, the existence of autosome-Y translocations was deduced only from the way of inheritance of the marker genes and the enzyme loci. Further evidence comes from the salivary gland chromosomes of male larvae (e.g. partial triploidy in fig. 4) and the allozyme pattern of male larvae. Yet, no direct cytological proof from mitotic metaphase chromosomes exists. Further studies in this direction are planned.

Discussion. In our experience the method works quite well. Exceptional or unexpected results did, however, appear in a few cases. Autosome-autosome-Y translocations or insertion of an intermediate section of an autosome into the Y-chromosome or X-ray-induced inversions proved to be responsible for them. However, the normal, unambiguous situation described above is frequent enough to allow the exclusion of the exceptions from the experiments. Up to now we have examined about 50 different translocations involving all 4 autosomes of *D. subobscura*. We have never observed the repression of any of the different enzyme genes (Adh, Aph, Est, α-Gpdh, Hk, Idh, Mdh, Me, Pgm) due to the altered position in the heterochromatic Y-chromosome. Thus we are rather confident that this translocation technique can be applied not only to species of the genus *Drosophila* (we have unpublished data for *D. melanogaster*, *D. pseudoobscura*, *D. ambigua*, and *D. paulistorum*) but also to other genera of Diptera with a similar organisation of sex-chromosomes.

1 Acknowledgments. The work was supported by the Deutsche Forschungsgemeinschaft (Projekt Sp 146-6/1). We are very much obliged to Miss I. Kaipf and to Mrs. K. Stögerer for their excellent technical help.

2 Nevo, E., Theor. Pop. Biol. *13* (1978) 121.
3 Pinsker, W., and Sperlich, D., Z. zool. Syst. Evolut.-forsch. *20* (1982) 161.
4 McKusick, V.A., and Ruddle, F.H., Science *196* (1977) 390.
5 Voelker, R.A. Ohnishi, S., and Langley, C.H., Biochem. Genet. *17* (1979) 769.
6 Steinemann, M., Chromosoma *87* (1982) 77.
7 Ayala, F.J., Powell, J.R., Tracey, M.L., Mourao, C.A., and Pérez-Salas, S., Genetics *70* (1972) 113.

0014-4754/84/020203-04$1.50 + 0.20/0

Intramitochondrial inclusions in maturing and senescent muscle cells of rat myocardium[1]

J. Cano and A. Machado

Departamento de Morfologia, Facultad de Medicina, Universidad Autonoma, Madrid 34 (Spain), and Departamento de Bioquimica, Facultad de Farmacia, Sevilla (Spain), 28 March 1983

Summary. Intramitochondrial myelin-like structures were found in ventricular myocardial cells of the rat heart. These inclusions were found in both the first 2 postnatal weeks and in senescent stages. The origin of such myelin-like structures could be related to an age-dependent metabolic change, specifically to an inability to oxidize long-chain fatty acids described in both periods.

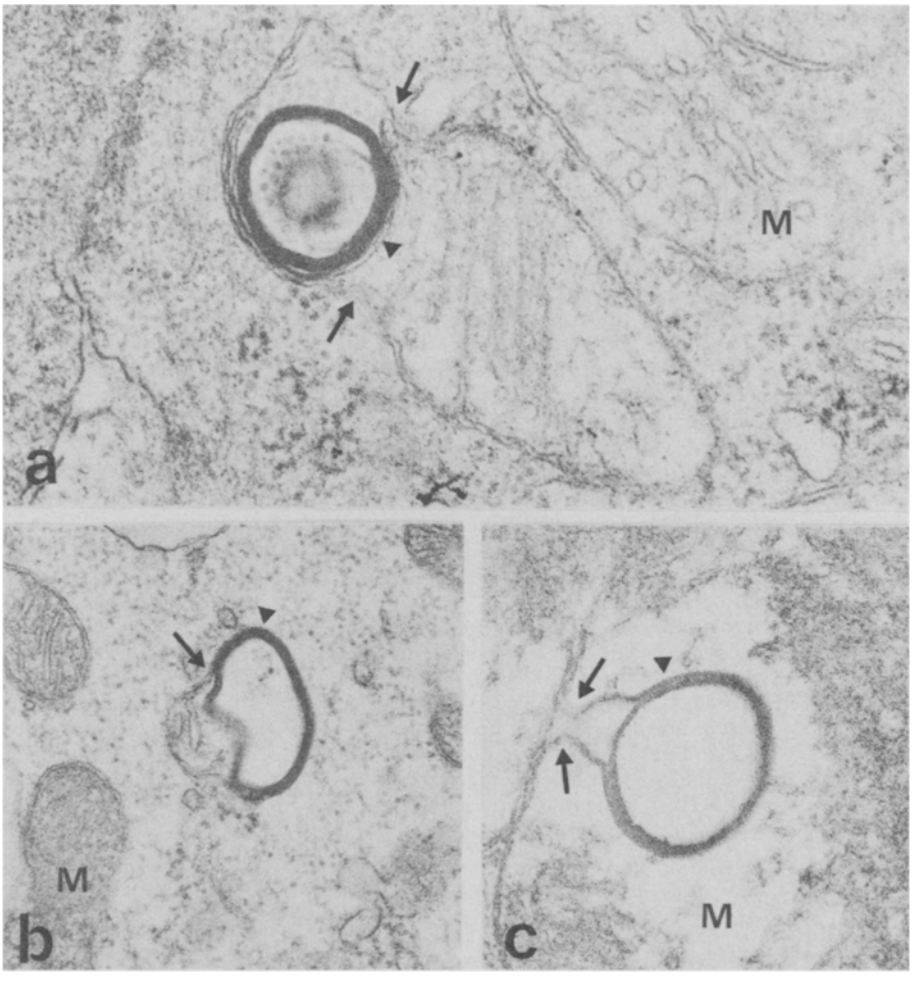
The major constituents of the cardiac muscle cell have been the subject of numerous electron microscopic investigations. However, less conspicuous components have received little attention. Recent investigations showed the appearance of cytoplasmic myelin structures during the first 2 postnatal weeks and in the senescent periods of the rat's life[2]. Also, intramitochondrial inclusions have been found in different tissues, and these have been related to the aging process[3] to pathological conditions[4], or to metabolic activity[5]. We describe here an intramitochondrial inclusion that could be age-dependent and related to metabolic changes.

36 male and female Wistar rats aged 4–15 days and 1, 3, 12 and 26–30 months, were used. Anesthesia was induced with ether and the hearts were perfused with Karnovsky's mixture[6], buffered with 0.2 M collidine. The material was postfixed in OsO_4, dehydrated and embedded in Epon 812. Ultrathin sections for electron microscopy were stained with uranyl acetate-methanol and lead citrate and studied in a Philips EM-301 electron microscope.

Myelin-like structures were found in the mitochondrial during the first 2 postnatal weeks (young animals). After that stage they were not seen in adult animals, but appeared once again in 2-year-old animals (senescent period). These myelin-like structures consist of highly osmiophilic material arranged in lamellae. The lamellae are composed of multilayers of osmiophilic bands regularly spaced at a distance of 40–50 Å (figs a and c).

These figures are similar in both young and senescent animals, and are also similar in form and in time of appearance to those previously reported in the cytoplasm[2]. When they are present within mitochondria there is a continuity between the myelin structures and the 2 mitochondrial membranes (fig. b). At high magnification the myelin-like structures seem to be formed from the inner membrane of the mitochondria (fig. c).

Mitochondrial structure undergoes drastic changes in relation to changing physiological activity; examples are the changes during the hibernation cycle of *Cylellus lateralis*[7], or those in cardiac muscle during experimentally induces hyperthyroidism[5]. In addition, different kinds of intramitochondrial bodies or inclusions have been reported in many different metabolic conditions; for example, intramitochondrial bodies have been found in bovine adrenocortical cells[8]. The number of inclusion bodies in the zona glomerulosa was increased by an uncompensated loss of body sodium[9] and by sodium restriction[10], and similar bodies appeared more frequently in the corpus luteum during early gestation[11]. These findings may indicate a relationship



a Myelin-like intramitochondrial structures. Observe the outline of the mitochondria (arrows). Mitochondria (M) Section of muscle cell from 4-day-old animals. ×75,000. *b* Myelin-like inclusions in continuity with both membranes of the mitochondria (arrows). Section of muscle cell from 4-day-old animal. ×23,000. *c* The myelin-like structure seems to be formed the inner membrane of the mitochondria. Section of muscle cell from a 26-month-old animal. ×27,000.

between these inclusion bodies and the functional activity of the adrenal gland.

In other studies the formation of mitochondrial inclusions has been related to aging[3,12] rather than to a decline of mitochondrial function (respiratory control). Myelin-like inclusions found by us in mitochondria in young animals between 4 and 15 days and in senescent periods (2-years-old) could be related to age. However, this relationship to the aging process is not easy to establish, because the inclusions are also found in very early stages (4–15 days) which appear to be the same as those found in two-year-old animals (fig.c). They could be related to age-dependent metabolic change, since they are composed principally of lipids, and they have been described as coinciding with a reduction in lipid metabolism. Fetal and newborn mammalian hearts have an inability to oxidize long-chain fatty acids[13] due to a lack of major fatty-acid metabolizing enzymes[14]. These enzymes are induced in developmental periods[15] and attain the level of adult activity during the 1st postnatal month. Also, during aging there is a decrease in respiratory capacity[16,17] and a reduction in fatty-acid oxidation and in several tricarboxylic acid enzymes[18,19]. We looked for these myelin-like structures in the pre-lactation period and we could not find any either in mitochondria or in the cytoplasm: they appear only after lactation. This agrees with the appearance of myelin-like structures when there is a drug-induced reduction in fatty acid oxidation[20]. Therefore, all these facts suggest that occurrence of such inclusions is related with physiologic change (reduced lipid metabolism) present at different stages of the rat's life.

1 Acknowledgment. This work was supported by grant No. 801/82 of CAICYT.

2 Cano, J., Hervas, J.P., and Machado, A., Mech. Aging Dev. *17* (1981) 132.
3 Cheah, K.S., and Cheah, A.M., J. Bioenerg. Biomembranes *9* (1977) 105.
4 Baskerville, A., Acta anat. *95* (1976) 218.
5 Callas, G., and Hayes, J.R., Anat. Rec. *178* (1974) 539.
6 Karnovsky, M.J., J. Cell Biol. *27* (1965) 137A.
7 Grodums, E.I., Cell. Tissue Res. *185* (1977) 231.
8 Kai, O., Fujoka, T., and Yasuda, M., Cell Tissue Res. *185* (1977) 69.
9 Weber, A.F., Usenik, E.A., and Whipp, S.C., 5th Int. Congr. Electron Microscopy 1962 vol.2; p.1.
10 Long, J.A., and Jones, A.L., Anat. Rec. *166* (1970) 1.
11 Crisp, T.M., Dessouky, D.A., and Denys, F.R., Am. J. Anat. *127* (1970) 37.
12 Saito, A., Smigel, M., and Fleischer, S., J. Cell Biol. *60* (1974) 653.
13 Wittels, B., and Bressler, R., J. clin. Invest. *44* (1965) 1639.
14 Warshaw, J.B., Biochim. biophys. Acta *223* (1970) 409.
15 Warshaw, J.B., Devl. Biol. *28* (1972) 537.
16 Gold, P.H., Gee, M.V., and Strehler, B.L., J. Geront. *23* (1968) 509.
17 Chen, J.C., Warshaw, J.B., and Sanadi, D.R., J. Cell Physiol. *80* (1972) 141.
18 Rodis, S.L., and Wohouny, G.V., Biochim. biophys. Acta *208* (1970) 153.
19 Victorica, J., Cano, J., Satrustegui, J., and Machado, A., Mech. Aging Dev. *16* (1981) 105.
20 Zbinden, G., Pharmac. Rev. *30* (1979) 292.

0014-4754/84/020206-02$1.50 + 0.20/0