Labelling of acrosomes in mouse spermatozoa by L-[1-³H]fucose

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The localization of the synthesis of various macromolecules in the differentiating male germ cell has led to the concept of labelling spermatozoa by introducing specific radioactive precursors during spermatogenesis (Sirlin & Edwards, 1958; for bibliography, see Keneklis, 1975). This approach, in combination with autoradiography, has proved to be fruitful in the study of the cytology of fertilization in the mouse, e.g. in demonstrating steric relationships between paternal and maternal chromatin in the zygote (Odartchenko & Keneklis, 1973) or in tracing the fate of basic nuclear proteins in spermatozoa in fertilization (Ecklund & Levine, 1975; Kopečný & Pavlok, 1975).

This note reports the possibility of similarly labelling another part of the mouse spermatozoon, the acrosomal glycoproteins, by administration of L-[1-³H]fucose.

The mice were F10 crosses of the C57 and A strains, selected continuously for good fertility. The males were 3 months old, with good mating behaviour and were injected intraperitoneally with 1000 μ Ci L-[1-³H]fucose (sp. act. 1.4 Ci/mmol; Radiochemical Centre, Amersham). Semen was collected at various times after the injection by mating or *post mortem* by flushing of ductus deferens or epididymis. Sperm smears were fixed with 4% buffered formalin (pH 7.0) containing 1% (w/v) cetyltrimethylammoniumbromide, rinsed with running tap water and air dried after brief washes with distilled water and 70% ethyl alcohol. The slides were coated with nuclear liquid emulsion Ilford K.5 and exposed for 41 days. The autoradiograms were stained after development with toluidine blue (Gurr) at pH 4.0.

Labelled spermatozoa were detected in smears of spermatozoa from the cauda epididymidis obtained 19 days after injection in 2 males. The labelling was confined to the dorsal and apical part of the sperm head and the most intensively labelled spermatozoa were associated with about 10 grains (Text-figs 1a and b). No similar labelling was detected in spermatozoa isolated at earlier times after treatment. For more concise localization of the labelling on the sperm head, 47 spermatozoa with detectable labelling from both males were photographed and the grains from all autoradiograms (328 grains) were photomechanically concentrated together on a drawing of the sperm head area demarcated after toluidine blue staining (Text-fig. 1c). It was seen that the distribution of grains coincided almost completely with the position of the acrosome on the sperm head (Text-fig. 1d), as described by Stefanini, Oura & Zamboni (1969). The highest concentration of grains on the dorsal surface corresponded to the thickest part of the acrosome.

Labelled fucose is a specific glycoprotein precursor (see review by Bennett, Leblond & Haddad, 1974) which is incorporated as the last residue in the carbohydrate terminal side chains of glycoproteins in the Golgi saccules. Later, the label introduced via radioactive fucose appears on the plasma membrane, in cell secretory material or in lysosomes (Bennett *et al.*, 1974). According to these authors, the lysosome label would be incorporated into lysosomal hydrolases, many of which have been shown to be glycoproteins (Goldstone & Koenig, 1970).

The glycoprotein nature of mammalian sperm acrosomes is well known from histochemical work based on PAS staining. Fucose was shown by Hartree & Srivastava (1965) to be a component of the ram acrosomal glycoproteins. The dynamics of the synthesis of the acrosomal material was studied in the mouse by Sandoz (1972a, b), who also observed incorporation of labelled galactose into spermatids, probably by a process similar to that after fucose administration.

The synthesis (secretion) of the acrosomal material in the spermatid is believed to take place in the early spermatids (stages 1–7), and this accords well with the appearance of labelled spermatozoa

Text-fig. 1. The labelling of the mouse acrosome after injection of L-[1- 3 H]fucose. (a) and (b) Autoradiograms of mouse sperm heads isolated from the cauda epididymidis 19 days after fucose injection. Nuclear liquid emulsion Ilford K.5, exposure 41 days, toluidine blue staining. (c) A schematic demonstration of the distribution of grains overlying the sperm heads. The grains from 47 sperm head autoradiograms were photomechanically concentrated on a drawing of the nuclear area taken from a stained sperm head. The distribution of the grains coincides with the position of the acrosome. (d) Diagram of the parts of the mouse sperm head according to the description of Stefanini *et al.* (1969): A, acrosome; PAC, post-acrosomal cap; PM, nucleus covered only by plasma membrane.

in the cauda epididymidis 19 days after fucose injection. The synthetic process is very slow and takes about 100 hr (Sandoz, 1972a, b). Radioactive fucose disappears rapidly in blood and tissues (Bekesi & Winzler, 1967) and a single injection is probably equivalent to pulse labelling (Bennett *et al.*, 1974). The low level of labelling in our material may have been due to one or both of these reasons. It may, however, be possible to increase the grain yield by repeating injections. Intratesticular application may also result in the improvement of the method which may become useful in studies of acrosome physiology.

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