A histochemical study of succinate dehydrogenase in mouse oocytes and early embryos

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Ishida & Chang (1965) found that hamster and rabbit embryos show a gradual increase of the activity of the mitochondrial enzyme succinate dehydrogenase (SDH: E.C. 1.3.99.1) between the 1- and 8-cell stages, followed by a steeper rise at blastulation. Baranska, Dorywalski & Kujawa (1973) reported that SDH activity increases from the 1- to 4-celled stage and decreases in the morula and blastocyst in the mouse. In both these studies SDH was demonstrated by the Nachlas method (Nachlas, Tsou, Souza, Cheng & Seligman, 1957), a histochemical reaction based on the reduction of nitro blue tetrazolium (NBT), in which no intermediate electron acceptor between SDH and the tetrazolium dye is used. However, Horwitz, Benitez & Bray (1967) have shown that SDH cannot transfer electrons directly to NBT, but only by way of coenzyme Q or another exogenous electron carrier, such as phenazine methosulphate. Therefore, if no excess carrier is added, the demonstration of the enzyme may be limited by the availability of coenzyme Q. In the system used in the present study, phenazine methosulphate was used as the intermediate electron acceptor between the reduced flavoenzyme and NBT.

Oocytes and embryos were obtained from Swiss mice (CD-1 COBS, from Charles River Italia). Isolated oocytes at several stages of development were obtained as previously described (Mangia & Epstein, 1975). Females were induced to superovulate and eggs were collected using standard procedures (Biggers, Whitten & Whittingham, 1971) in phosphate-buffered saline supplemented with 4 mg bovine serum albumin (BSA)/ml. After four washings in the same medium, oocytes and eggs were transferred in a small volume to 0.8 ml of a solution of 12.5 mM-tris-HCl (pH 7.4); 1.25 mg BSA/ml; 1.25 mM-CaCl₂; 1 mM-NaCN (pH 7.4); 62.5 mM-disodium succinate (Boehringer), pH 7.4. In control groups succinate was replaced by 90 mM-NaCl. The watch glass and its contents were then frozen on solid CO₂ and thawed at 37°C; 0.1 ml each of 10 mg nitro blue tetrazolium (B.D.H.)/ml and 5 mg phenazine methosulphate (B.D.H.)/ml were added, and the watch glass was then incubated at 37°C for 15 min. The histochemical reaction was stopped by the addition of buffered formalin, and the eggs transferred to pure buffered formalin. After fixation the eggs were transferred to a drop of aqueous mounting medium on a slide, gently squashed under a coverslip, and examined at ×200 magnification. The thickness of the specimen, evaluated with the use of the graduated fine-focusing knob of the microscope, was found to be uniform among the different preparations. The intensity of the purple formazan deposit was classified as absent, weak, moderate or strong.

A positive succinate dehydrogenase reaction was seen in oocytes and embryos of all stages examined (Table 1; Pl. 1, Figs 1–9). There was no reaction in the absence of succinate. Succinate dehydrogenase concentration progressively increased during follicular growth, reaching a maximum in preovulatory oocytes; no further change was detected in the tubal unfertilized egg. There were no large variations in SDH activity during early cleavage, but an increase occurred at the blastocyst stage.

The volume of the dictyate oocyte increases about sevenfold during the growth period we have examined; therefore the increase in enzyme activity/oocyte appears to be even greater. This may be due to an increase in the absolute number of mitochondria per oocyte and/or to an increased enzyme activity per mitochondrion. No quantitative data are presently available on the proliferation rate of mitochondria in growing mouse oocytes, but since oocyte mitochondrial configuration does not change during oogenesis (see review by Szollosi, 1972), it seems reasonable to assume that the observed SDH increase is related to mitochondrial proliferation.

Table 1. Intensity of succinate dehydrogenase activity in mouse oocytes and eggs at various developmental stages

Stages of development	No. of eggs examined	Eggs showing SDH reaction (%)			Significance*
		Weak	Moderate	Strong	
Small oocytes (45–60 µm)	57	37	63	0	Small versus large oocytes, $P < 0.005$
Medium oocytes (60–70 µm)	56	7	93	0	
Large oocytes (75–85 µm)	101	0	16	84	
Unfertilized eggs	118	0	9	91	Large oocytes versus unfertilized eggs, $P > 0.25$
1-cell embryos	61	0	18	82	
2-cell embryos	88	0	10	90	1-cell versus 8-cell embryos, $P > 0.1$
8-cell embryos	62	5	22	73	
Early blastocyst	91	0	3	97	8-cell embryos versus early blastocyst, $P < 0.005$
Late blastocyst	87	0	6	94	

* Calculated on 2×3 contingency tables.

The augmented SDH activity that occurs when the blastocyst is formed is, however, due to an increase in the concentration of the enzyme/mitochondrion, since mitochondria do not proliferate during preimplantation development in the mouse (Pikò, 1970).

The increase of SDH activity which characterizes the blastocyst stage accords with the structural changes (formation of well developed transverse cristae) observed in the mitochondria at this stage (Stern, Biggers & Anderson, 1971) and with the increased metabolic activity of the embryo at this time (Mills & Brinster, 1967). The apparent lack of variation in enzymatic activity in the embryo before the blastocyst stage, however, does not exclude the possibility that minor changes may occur, since variations in SDH activity of less than 35% cannot be detected with certainty by histochemical methods (Riecken, Goebell & Bode, 1969).

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PLATE 1

Succinate dehydrogenase activity in mouse oocytes and embryos. After the histochemical reaction the eggs were fixed in formalin and mounted whole in aqueous mounting medium. $\times 400$. **Fig. 1.** Small oocyte. **Fig. 2.** Medium oocyte. **Fig. 3.** Large oocyte. **Fig. 4.** Unfertilized tubal egg. **Fig. 5.** One-cell embryo. **Fig. 6.** Two-cell embryo. **Fig. 7.** Eight-cell embryo. **Fig. 8.** Early blastocyst. **Fig. 9.** Late blastocyst.