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Resistance to Deformation of Axial Structures in Living Guinea-Pig Spermatozoa

Abstract. Rotation of living guinea-pig spermatozoa can be effected by drawing the shaft of a long microneedle of diameter 0.5 to 1.0 μ across the tail; the special characteristics of this rotation demonstrated in the tail a polygonal structure and high resistance of the peripheral fibrils to deformation by pressure of the microneedle.

In the course of research on fragility (in immune reactions) of guinea-pig spermatozoa (*1*) during which use was made of micromanipulation to effect dissection of living spermatozoa, it was observed that it was easily possible to rotate an immobilized spermatozoon by drawing a long glass rod (or long shank of a microneedle) at right angles to the length of the spermatozoon across the tail, approximately halfway between the midpoint and the body. This was effected by making the diameter of the needle close to the diameter of the tail (Fig. 1) (in general about 0.5 to 1.0 μ) and about 200 μ or more long.

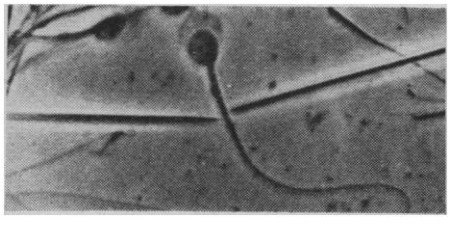

Fig. 1. Living guinea-pig spermatozoon with two microneedles showing diameter and position for rotation with relation to tail. Phase contrast. (×1150)

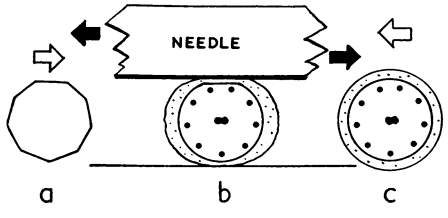

Fig. 2. (*a*) Polygonal structure effective for rotation by movement of needle across surface. (*b*) Tail of spermatozoon on section with suggested deformation of surface protoplasm on pressure of needle; resistance of peripheral fibrils and central protoplasm. (*c*) Normal structure and relative disposition of fibrils in tail (schematic).

The special feature of the rotation of the spermatozoon (shown by rotation of the head) was its positive nature (in that the needle did not slip and rotation was directly proportional to the speed of movement of the needle). This indicated, first, that the tail cannot be round or flat but must possess a basic polygonal structure on section (Fig. 2*a*) in order that the tail may be engaged between the needle and the slide surface (Fig. 2*b*); this polygonal structure is shown by electron microscopy of the tail where a set of nine peripheral fibrils surrounds a double axial thread (Fig. 2*c*). Second, micromanipulation demonstrated that the basic fibrillar structure must be highly resistant to deformation; here electron microscopy shows a thin outer protoplasmic sheath (which contains microsomes) smoothly rounded in outline with a spiral layer outside the peripheral fibrils; a light structureless protoplasm occupies the central region around the axial fibril (Fig. 2*c*).

This second conclusion on the physical properties of the spermatozoa shown by micromanipulation appears to confirm an early observation on micromanipulation of spermatozoa by Terni (*2*); in addition, observations by Duryee (*3*) on microdissection of *Triturus pyrrhogaster* spermatozoa revealed similar properties in this species.

Deformation of the thin surface protoplasmic layer must take place in order that the needle may be brought in close contact with the fibrils. The question then arises as to why the internal protoplasm, greater in amount than that between and around the peripheral fibrils, does not also deform; if any displacement of fibrils takes place it must be slight since rotation requires persistence of the polygonal structure; these observations also concern the forces which maintain the fibrils in position; the forces not only resist pressure from the surface but resist tension on their long axes without evidence of elasticity; such lack of elasticity is to be expected from the characteristics of the surface resistance.

It would be desirable, and it should be possible, to obtain quantitative data on these forces. The observations presented indicate that the forces will be found to be high among biological materials of fibrillar structure.

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13 May 1959

Induction of the Respiration-Deficient Mutation in Yeast by Various Synthetic Dyes

Abstract. Series of triphenylmethane and xanthene dyes were found to be effective as inducers of respiration-deficient mutation in yeast. The quantitative difference in the mutagenic effect appeared to be in close relationship to the chemical structure of the respective dyes. This survey may provide a useful clue for elucidation of the mechanism of mutagenic induction.

Among various inducers of the respiration-deficient (*petite colonie*) mutation in yeast, several synthetic dyes show pronounced effects (*1*). The work under discussion deals with a comparative survey of the *petite-colonie*-inducing effects of various dyes in an attempt to seek the relationship, if any, between the chemical structure and the efficacy of the dye as an inducer.

A strain of baker's yeast (Fleischmann) mentioned in an earlier report (*2*) was cultured in a liquid medium

Table 1. Production of mutants by various dyes. Minimal concentration is required to produce more than 3 percent mutants, optimal to produce more than 90 percent.

Dye (Manufacturer)	Concentration (mg/lit)		
	Minimal	Optimal	For entire suppression
Crystal violet* (Merck)	0.2	0.6	2.4
Ethyl violet* (Bayer)	0.2	0.4	2.0
Methyl violet* (Merck)	2.0	4.0	8.0
Malachite green* (Japan)	1.0	3.0	10.0
Pararosaniline* (Japan)	2.0	8.0	50.0
Rosaniline* (Japan)	3.0	8.0	60.0
Victoria blue B* (Japan)	6.0	20.0 (<40%)	30.0
Victoria blue 4R* (Japan)	6.0	10.0 (<60%)	30.0
Pyronine Y† (Bayer)	2.0	6.0	50.0
Pyronine B† (Bayer)	2.0	6.0	50.0
Acridine red 3B† (Japan)	12.0	40.0	100.0
Acriflavine (Japan)	0.4	1.5	12.5
Caffeine (Japan)	1500	2500	3500

* Triphenylmethane dye; † xanthene dye. Acridine red 3B is a xanthene dye in spite of the name.