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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.

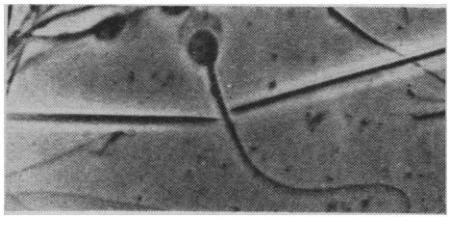

Fig. 1. Living guinea-pig spermatozoon with two microneedles showing diameter and position for rotation with relation to tail. Phase contrast. (×1150)

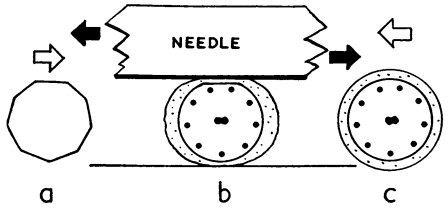

Fig. 2. (*a*) Polygonal structure effective for rotation by movement of needle across surface. (*b*) Tail of spermatozoon on section with suggested deformation of surface protoplasm on pressure of needle; resistance of peripheral fibrils and central protoplasm. (*c*) Normal structure and relative disposition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

(yeast extract powder, 0.2 percent; peptone, 0.18 percent; ammonium sulfate, 0.15 percent; monopotassium acid phosphate, 0.1 percent; magnesium sulfate, 0.05 percent) supplemented with the dye to be tested. Fifty milliliters of the medium in 100-ml erlenmeyer flasks were inoculated with about 1000 cells of the original culture per milliliter and incubated at 30°C for 48 hours. A few drops of these cultures were diluted in sterilized water, spread on a normal nutrient agar medium, and scored for *petite* mutants after 48 hours of incubation at 30°C.

Table 1 summarizes the results of the experiments with various dyes in varied concentrations. The effects of acriflavine and of caffeine in the collateral tests with the same basal medium are shown for comparison. Most of the inducers are toxic with respect to the yeast and suppress growth at fairly low concentrations. The increase in the frequency of occurrence of the *petite* mutants is, however, greater in the fully grown cultures (up to 3×10^7 cells per milliliter) than in the inhibited cultures. In other words, maximum induction in terms of increased frequency of the *petite* mutants in the final population appears at concentrations far below the lethal level. Comparison based upon molar concentrations of the dyes instead of on milligrams per liter reveals greater differences in efficacy in both the induction of *petite* mutants and the suppression of growth.

With the exception of acriflavine, the dyes fall into two major groups—namely, triphenylmethane dyes and xanthene dyes. The general trends in the relationship between chemical nature and efficacy may be summarized as follows: (i) all the effective dyes are basic dyes; (ii) efficacy tends to be greater when the number of methyl groups in the molecule is greater; (iii) the aminotriphenylmethane derivatives which have methyl groups introduced into their amino groups are more effective than those which have methyl groups introduced into the benzene nuclei; (iv) the dyes which have ethyl groups instead of methyl groups are as effective as, or sometimes more effective than, the dyes which have methyl groups in the corresponding positions. The Victoria blue dyes B and 4R show rather low efficacy as compared with other triphenylmethane dyes in spite of high affinity (absorption) to the yeast cells. Such characteristics are perhaps due to the presence of a phenyl-α-naphthylamine moiety in the molecule.

An improved modification of the triphenyltetrazolium chloride (TTC) overlay technique was employed for scoring the *petite* mutants in the cultures. The molten agar (1 percent) containing 0.05 percent TTC and 0.5 percent glucose was gently poured onto colonies (200 to 300 per standard petri dish) of the sample cultures growing on nutrient agar of the same composition as the basal medium. The cultures, thus covered 3 to 4 mm deep, were incubated again at 30°C. Color differentiation between normal (red) and mutant (white) colonies appeared more clear-cut and occurred more quickly than color differentiation produced by the procedures hitherto followed (*3*).

The dyes were examined by paper chromatography. The ascending development with acetone, water (4:6) and *n*-butanol, acetic acid, water (4:1:5), respectively, gave fairly good results. No serious admixture or decomposition product has been detected so far (*4*).

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Acid Mucopolysaccharide of the Crustacean Cuticle

Abstract. The acid mucopolysaccharide found in the crab *Hemigrapsus nudus* and containing glucose, galactose, and fucose residues is found both in the cuticle and in the digestive gland of the crab. The concentration of mucopolysaccharide is somewhat higher in the cuticle, where it it the only soluble polysaccharide, than in the digestive gland, where it makes up 10 to 25 percent of the total polysaccharide content.

The occurrence of acid mucopolysaccharides in integumentary and skeletal tissues has been reported frequently in recent years. The present report provides direct chemical evidence of the occurrence of such a substance in the integument and digestive gland of the crab *Hemigrapsus nudus* (Dana). Trim (*1*) reported the occurrence of a nonchitin carbohydrate in the arthropod cuticle. Travis (*2*), using histochemical methods, demonstrated the presence of a mucopolysaccharide in the integument and digestive gland of lobsters. Hu (*3*), in his recent study, observed that a polysaccharide other than glycogen is present in *H. nudus*. The polysaccharide fraction separated by Hu yielded, on hydrolysis, fucose, galactose, and glucose. Nothing was known as to whether this polysaccharide is similar in distribution to that observed by Trim or Travis. Hence, it was thought worth while to study the distribution of this polysaccharide in the crab, with special reference to the cuticle.

The animals were collected from Coos Bay in the vicinity of Charleston, Ore. All the animals used in the experiment were in the C_4 stage of the intermoult cycle (*4*).

The cuticle was prepared according to the method used by Frankel and Rudall (*5*) for insects. The polysaccharides were precipitated with alcohol from alkali extracts of the powdered cuticle and of the fresh digestive gland. The polysaccharides were separated by paper electrophoresis at *p*H 5, as suggested by Bera *et al.* (*6*). The Karler-Kirk curtain-electrophoresis apparatus was used (*7*). The polysaccharide was stained on the paper by the method of Köiw and Gronwall (*8*) and of Hammerman (*9*). Quantitative estimation of the polysaccharides after electrophoretic separation was made by the anthrone method of Roe (*10*). For the hydrolysis of the polysaccharide and analysis of the sugars, the methods adopted by Hu (*3*) were followed.

The digestive gland contains, besides glycogen, an acid mucopolysaccharide which gives a positive test with toluidine blue staining on the paper. The acid mucopolysaccharide moves to the cathode and is readily separated from glycogen on paper during electrophoresis. Hydrolysis of the polysaccharide yielded fucose, galactose, and glucose, glucose being more abundant than the other two sugars, as observed by Hu (*3*). The cuticle also showed the presence of acid mucopolysaccharide which

Table 1. Polysaccharide content of digestive gland and cuticle of *Hemigrapsus nudus*.

Tissue	Polysaccharide			
	Glycogen (mg/100 gm of dry tissues)		Acid* (mg/100 gm of dry tissues)	
	Mean	Extremes	Mean	Extremes
Digestive gland	5073	2506–7392	671	285–2460
Cuticle			939	842–1085

* Expressed in terms of glucose equivalents by the anthrone method.