Table 1. ACTIVITY OF *E. coli* β-GALACTOSIDASE TOWARDS LACTSUCROSE

Sugar in growth medium*	Inducer†	β-Galactosidase activity‡		Hydrolysis of the β-galactosidic linkage‖	
		Washed cells	Cell extract§	Lactose	Lact-sucrose
Glucose	—	0·0	0·5	−	−
Glucose	lactsucrose	0·1	1·1	−	−
Glucose	lactose	9·8	182·5	+	+
Lactose	—	12·4	240·0	+	+

* Growth was conducted for 24 hr. in '56' synthetic medium containing 2 per cent sugar (ref. 1).

† Washed cells (in a concentration equivalent to 5 mgm. dry weight/ml.) were incubated 5 hr. at 37° C. in an induction medium (ref. 4) containing 0·02 *M* inducer.

‡ Expressed in terms of units of hydrolysis of *o*-nitrophenyl-β-galactoside (ref. 5) per mgm. dry weight.

§ Cells were mechanically disintegrated with glass beads in a Mickle vibrator. The supernatant solution given by centrifuging for 15 min. at 8,000*g* served as the enzyme solution.

‖ (+) indicates > 50 per cent and (−) < 2 per cent hydrolysis of substrate, both by cells and by their extract. The reaction mixture contained 0·1 *M* sugar, 0·05 *M* phosphate buffer *p*H 7·0 and fresh cells (5 mgm. dry weight/ml.) or enzyme solution (1 mgm. dry weight/ml.). After incubation at 37° C. for 6 hr. products of hydrolysis and decrease in the initial amount of substrate were observed by methods of paper chromatography and reducing sugar determination (ref. 3).

attributed to inability of the trisaccharide to reach the site of β-galactosidase induction, and/or a lack of inductive capacity proper. The permeation step is known to be controlled by a specific inductive system[7-9]. It is conceivable that raffinose and lactsucrose are incapable of inducing the permeation system, whereas cells induced by lactose possess it and hence are also penetrated by trisaccharides.

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Demonstration of Some 'Masked' Lipids in the Oocytes of *Chrotogonus trachypterus* with Sudan Black *B* and Phenol 'Unmasking' Techniques

SUDAN black *B*, in spite of being the most specific and vigorous dye for the demonstration of lipids in tissue sections[1,2], has its limitations[3,4]. The cell inclusion believed to be lipid may not be coloured with sudan black *B*, because: (1) some lipids (like phospholipids) may escape from the tissue into the fixative by the process of emulsification[2,3]; (2) the lipids present have a high melting point and thus remain 'solid' in tissue sections[3]; or (3) the lipids present are 'masked' by proteins or other substances.

All these difficulties have been experienced in this laboratory during the histochemical investigation of the lipids in the oocytes of a number of animals[5]. The first one can always be eliminated by fixing the tissue in fluids containing free calcium ions[3] (like formaldehyde–calcium of Baker[2]), and by post-chroming; while the second difficulty can be overcome to a great extent by using the dye at 60° C. for longer periods (say 30 min.). But the lipids still remaining uncoloured have either a melting point much higher than 60° C. (for example, cholesterols and cholesteryl esters), or they may be 'masked'. The cholesterols and cholesteryl esters can always be detected by using specific histochemical tests[1,4,6]. It is the presence of 'masked' lipids which has troubled many previous workers.

Although the 'masked' lipids may be 'unmasked' and thus coloured with sudan black *B* by a simple treatment of the tissue sections with the various fat-solvents like acetone, ether, etc., which 'unmask' the lipids not removed by these solvents[5,7,8], yet such treatments are inadequate to 'unmask' the lipids in larger lipid bodies for which some special unmasking techniques recommended by Ciaccio[9] must be employed.

In the oocytes of *Chrotogonus* it has been found that a large number of sudanophil lipid spheres constantly give a 'ringed' or 'crescentic' appearance (that is, a dark sudanophil sheath enclosing a completely non-sudanophil core of various sizes) even when the sections of the material fixed according to the formaldehyde–calcium/postchroming technique[2] are coloured with sudan black *B* in propylene glycol[10] at 60° C. for 1 hr. These bodies give a completely negative reaction to all the tests for cholesterol and its esters[1,4,6], in spite of repeated attempts. This shows that if lipids are present in the core they must be 'masked'.

Ciaccio's[9] various techniques were tried to 'unmask' them. The methods, which include an 'unmasking' fixative, namely, Da Fano, Ramon y Cajal with or without potassium dichromate, did not prove successful. Of the two special 'unmasking' agents recommended, trypsin also was not so successful, but phenol gave excellent results. The material fixed in formaldehyde–calcium for 6 hr. was treated with 1 per cent phenol solution at 37° C. for 24 hr. The gelatin sections of this material when coloured with sudan black *B* did not reveal any 'ringed' or 'crescentic' appearance of the lipid bodies, which now coloured uniformly blue-black. This shows clearly that the lipids present in the uncoloured cores of the 'crescentic' and 'ringed' lipid spheres in the oocytes of *Chrotogonus* were 'masked' ordinarily by proteins; the presence of the latter is shown by the stainability of these lipid bodies with mercuric–bromophenol blue[11].

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