A Further Note on the 'Unmasking' of Lipids in the Cell

Since the author[1] reported on the 'Unmasking' effect of 1% phenol on the lipids in the oocytes of *Chrotogonus*, some more observations[2,3] have become available on the subject which prompt a further comment on the phenomenon of 'masking' in lipids.

BERENBAUM[2] has reported on the use of sudan black *B* in his 'Acetone-Sudan black', 'Burnt Sudan black', and 'Ethanolic Sudan black at 60°C' techniques for colouring the firmly bound lipids of the cell nuclei, reticulin and epithelial brush borders, etc. CLAYTON[3] has investigated the 'unmasking' influence of a number of lipid solvents on the acroblast in *Acheta domesticus*, and has found 90% ethanol and 5% hydroquinone to be the most effective 'unmasking' agents.

Both these papers deal with the techniques which 'unmask' lipids bound firmly with proteins to form lipoproteins. The influence of lipid solvents in breaking the bonds between lipoproteins, and consequently releasing the lipids, has been widely recognized by the biochemists[4]. In this laboratory also, the 'unmasking' effect of acetone and ethanol, especially on the lipoproteins of the mitochondria in oocytes, has very often been observed[5–7].

NATH, GUPTA *et al.*[5–7] have observed in the oocytes of a number of insects three kinds of lipid bodies viz. (1) L_1 bodies having a phospholipid or lipoprotein nature, (2) L_2 bodies having a phospholipid sheath surrounding a triglyceride core, and (3) L_3 bodies of a pure triglyceride nature.

The L_2 bodies show a duplex appearance with a completely sudanophobe core and a sudanophil cortex, in all the variants of sudan black *B*[8–10], even at 60°C. After a treatment in 1% phenol, either of the material or the gelatine sections, these L_2 bodies colour homogeneously in sudan black. Their duplex appearance is again restored after a simple treatment of the 'unmasked' sections in cold acetone for 12–24 h. The cores colour pink in nile blue[11] while the sheaths are blue. Further, the cores of the L_2 bodies are negative to acid hematein[12], Schultz's variants[13], mercuric-bromophenol blue[14] (for proteins), and PAS[15] (for carbohydrates). All these tests establish the presence of triglycerides in them.

Now, the triglycerides are not known to form lipoprotein complexes[16,17]. It is implicit, therefore, that some other phenomenon is involved which keeps the triglycerides in a 'masked' condition in such mixed lipid bodies, widely occurring in oocytes[7].

SCHMIDT[18] had suggested that the phospholipid spheres probably have a series of concentric shells of water in between the bimolecular layers of the phospholipid molecules. He has also drawn a 'water vacuole' surrounded by a bimolecular layer of phospholipids[19]. This observation has been fully confirmed recently by Ross and CHOU[19]. HIRSCH[20] and NATH[21] have often pointed out that such 'osmiophobic' parts of duplex vesicles act as the sites where cell secretions, including fat (triglycerides), are condensed. To the author, it appears that in the L_2 bodies mentioned above the triglyceride cores and the phospholipid sheaths remain separated by a thin layer of water molecules which form an impermeable membrane for the water-insoluble physical lipid colorants like sudan black *B* or sudan III and IV, etc. The ethanol or acetone of sudan solutions should be able to break the water barriers, but they dissolve away the triglycerides also in prolonged treatments and therefore are unable to reveal their 'unmasking' influence. Phenol, on the other hand, might attack the water molecules without destroying any lipids. It is interesting to note that in OsO_4 preparations like Lewitsky-saline[22] (unstained), these L_2 bodies always appear as homogeneously black spheres: but if such sections are bleached in H_2O_2 and then coloured with sudan black[22], the L_2 bodies again appear as rings[7,23].

It is clear, therefore, that in such cases as the L_2 bodies in oocytes, the 'masking' is a purely physical phenomenon involving technical difficulties in colouring. This would add another possibility to CIACCIO's[24] undefined[16] term of 'masked' lipids.

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Résumé

Les triglycérides se trouvant à l'intérieur de quelques corps lipides, dans les oocytes paraissent masqués lorsqu'on utilise des colorants pour lipides. C'est peut-être à cause de la présence d'une couche de molécules d'eau entre les molécules phospholipides de la gaine et les molécules des triglycérides du cœur. Cet écran interposé par l'eau est détruit d'une manière ou d'une autre par une solution d'1% de phénol et ainsi le «masque» disparaît.

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Intraspecific Sexual Preferences in *Drosophila prosaltans* Duda and in *Drosophila equinoxialis* Dobzhansky

By direct observation[1,2] of courtship and mating behaviour of neotropical strains of *Drosophila prosaltans* Duda

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and of *Drosophila equinoxialis* Dobzhansky (Table I), using the 'male choice method'[3], it has been possible to detect indications of incipient sexual isolation among the different geographic strains studied. Courtship information was gathered from a 30 min observation period. Further 30 min were allowed for copulation. Within these 60 min, only the first copulations were recorded.

Since, in *Drosophila*, the male is the agressor in courtship and the female the determining acceptor[4], the male choice method (one male with two females in a 7×2 cm empty vial) allows the analysis of the preference which the males exert towards either type of female. The male is, therefore, always choosing between his own and the alien female.

Table I

Geographic strains used in the present work. The number after the name of the country constitutes a mark given to every collection

Species	Locality	Combinations	Observations
D. prosaltans	Trididad Mexico Brazil	6	120
D. equinoxialis	Cuba 25 Cuba 101 Costa Rica 9A Costa Rica 9B Colombia 26F Colombia 26G Brazil	42	429

Some investigators[5] have discovered that, through the stimuli response threshold in recognition of like mates, sensory divergence precedes gross courtship divergence between different species. Others have found that within *D. melanogaster* different mating systems (inbreeding and outbreeding) may give rise to courtship preferences, which in turn are indicative of incipient isolation[6]. Moreover, in geographic populations of certain species, some authors[7] have asserted some sexual isolation.

In Table II we mean by 'Orientations' and 'Tappings' what other authors have described[1,2]. 'Orientation', therefore, would constitute the distant perception of a male to a passing female. The male acknowledges the female by moving towards it. Some degree of distant stimulus (as yet unknown) excites the male and invites it to courtship. However, the interaction of the individual excitation with its surroundings (the courtship threshold[1]) is low when its excitement is aroused by a simple stimulus. It is high when the same stimulus fails to secure response. 'Tappings', on the other hand, represents a proximal stimulus. It is also described[1,2] as the male touching with his foretarsi or proboscis the female body. These organs are already known to convey gustatory stimuli[8]. Thus species recognition is easily accomplished; likewise, the female may exert discrimination against the alien male by discouraging him[5].

In Table II we present in a summarized manner courtship preference within each of the two species here considered. Furthermore, a mating preference was also observed. It is worthy of note that the courtship display of a geographical strain shows significant preference in both distant and proximal stimuli for their own strain within each species. Greater X^2 in 'Tappings' have been interpreted as due to weakness of distant stimulation ('Orientation'), while proximal stimuli ('Tappings') reinforce recognition.

As briefly indicated in these Tables, incipient isolation within *Drosophila* species may occur at the courtship level and corresponds to mating preferences. The stimulus response recognition is necessarily only preferential and not isolational, since the strains are members of an interbreeding unit, the species in which common genotypic combinations are shared.

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Riassunto

Mediante il metodo della «scelta da parte del maschio» si è osservato il corteggiamento nelle combinazioni fra 7 linee di *D. equinoxialis* e 3 di *D. prosaltans* provenienti da località diverse.

E stata messa in evidenza una preferenza dei maschi verso le femmine della loro stessa linea, per quanto concerne «l'orientamento», «il picchiettamento con zampe e proboscide» e «l'accoppiamento». Si discute sul possibile significato di questo incipiente isolamento.

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Table II

Pooled courship behaviour (Orientations, Tappings and Matings) of all combinations of the mentioned strains within each species, to indicate preferences. Own and other refer to females with which the male is confronted

Species	Orientations				Tappings				Matings			
♂♂	own ♀♀	other	X^2	P	own ♀♀	other	X^2	P	own ♀♀	other	X^2	P
D. prosaltans	388	272	10·2	1%	1210	501	146·6	1%	53	15	10·6	1%
D. equinoxialis	301	183	14·4	1%	649	396	32·6	1%	55	33	2·7	10%

Mobilization of Leukocytes into the Peritoneal Fluid[1]

Much work has been done on the mechanisms involved in the diapedesis, chemotaxis, and phagocytosis by leukocytes with the use of saline solutions of undetermined purity. DeHaan[2] was perhaps the first to report that the intraperitoneal injection of a 0·9% NaCl solution causes large numbers of neutrophils to enter the peritoneal fluid in rabbits. Hamburger[3] described this method in detail, and it has become a popular one for harvesting neutrophils for morphological, physiological, and biochemical studies[4]. In these investigations it has been assumed or implied that the salt solution itself has the power to evoke a local neutrophilia. However, there remains a measure of uncertainty, because the water used as a solvent in such experiments has been of unknown or of unspecified purity. Distilled water of the sort ordinarily used in laboratories often contains pyrogenic substances, and even nonpyrogenic water will become pyrogenic if it is left exposed to the air[5], probably through bacterial contamination. It is noteworthy that Harris, Menkin, and Yoffey[6] found 'virtually no exudate or cells' in the peritoneal fluid of guinea pigs that were injected intraperitoneally with a substance extracted from an inflammatory exudate and suspended in sterile, pyrogen-free saline. These investigators conjectured that the absence of an exudate might be the result of (a) the small amount of material injected, (b) the pyrogen-free nature of the solvent, or (c) the type of animal used. The experimental results which follow offer evidence that a 0·9% NaCl solution injected intraperitoneally into rats has little or no neutrophil-mobilizing power provided that the solutions used are free from certain trace contaminants. Moreover, minute amounts of a bacterial extract, when added to such solutions, produce results which mimic the effects seen after the injection of ordinary laboratory saline.

Male rats of the Sprague-Dawley strain (Holtzman) weighing 180–250 g were used. All rats were given an initial peritoneal lavage with 30 ml of saline. Sterile, nonpyrogenic 0·9% NaCl[7] was used for Groups I and III. Group II received 0·9% NaCl made with water which had been distilled a single time in the laboratory. The rats were challenged immediately by an intraperitoneal injection of one of the following solutions: (a) 10 ml of 0·9% sterile, nonpyrogenic saline (Group I), (b) 10 ml of 0·9% NaCl prepared with ordinary single-distilled water (Group II), or (c) 10 ml of sterile, nonpyrogenic saline to which was added 0·1 μg of a bacterial polysaccharide preparation derived from Pseudomonas[8] (Group III). The animals were sacrificed 5 h later and a second peritoneal lavage was performed. The numbers and types of cells removed by each washing were determined, and the total influx of new cells was calculated. This is especially easy to do for the neutrophils since the peritoneal fluid of rats contains few or no neutrophils in the unstimulated state[9].

The Table summarizes the results. It is evident that sterile, nonpyrogenic saline is not effective in evoking a neutrophilia of the peritoneal fluid. This is in sharp contrast to either ordinary saline or a solution of nonpyrogenic saline to which 0·1 μg of a bacterial extract has been added. These solutions cause millions of neutrophils to pour into the peritoneal fluid. Another interesting fact is that a significant influx of mononuclear cells also occurs. Such cells are primarily macrophages, but some cells resembling lymphocytes are present. We have chosen to group macrophages and lymphocytes together, because numerous transitional forms are seen, making it difficult to distinguish clearly between these cells.

The observation that a nonpyrogenic salt solution fails to evoke a neutrophil response renders untenable the idea that almost any substance introduced intraperitoneally will cause the appearance of large numbers of neutrophils. Indeed, glucose which often is described as a powerful chemotactic agent for neutrophils, is also impotent in mobilizing these cells in the peritoneal fluid. The relevant data pertaining to this finding will be published elsewhere. It may suffice here to point out that

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Net Influx of Leukocytes into the Peritoneal Fluid Measured after 5 h

Group	Treatment	No. of Rats	Neutrophils, Millions	Mononuclear Cells, Millions
I	10 ml nonpyrogenic saline	15	0·10 ± 0·05*	3·0 ± 0·5
II	10 ml 0·9% NaCl in laboratory distilled water	15	38·3 ± 2·8***	14·5 ± 1·7***
III	10 ml nonpyrogenic saline + 0·1 μg bacterial extract	6	48·5 ± 7·3***	8·5 ± 2.3**

* Means ± Standard error of the mean.

** $P < 0.05$
*** $P > 0.01$
} compared with Group I.