

# CHEMICAL NATURE OF THE CHORIONIC PAD OF THE EGG OF *Aedes aegypti*

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**ABSTRACT.** A histochemical investigation was conducted on the nature of the substance constituting the chorionic pad of the egg of *Aedes aegypti*. The pad material gives evidences

for the presence of hyaluronic acid which is known for its swelling and adhesive properties in water.

## INTRODUCTION

It is known that the chorion of the eggs of mosquitoes is composed of 2 layers, the exo- and endochorions (Nicholson, 1921, DeBuck 1938, Christophers 1945). The exochorion swells in water and forms a very thick gelatinous chorionic pad, which may play an important role of fastening the egg ventral-side up to an object (see Christophers 1960).

Hermes and Freeborn (1921) and Christophers (1945) note that the endochorion in the mosquito eggs is made of a tanned protein similar to that of the hardened shell of the *Calliphora* pupa (Pryor 1940). Although the exochorion may contain an untanned protein (see Christophers 1960), the gelatinous material on the ventral surface probably has additional different chemical structures. The present investigation has been conducted to throw some light on these.

## MATERIALS AND METHODS

The materials used were the eggs of a laboratory colony of *Aedes aegypti* (Linnaeus). Freshly laid eggs were kept for one hour submerged in water to enable the chorionic pad to swell. Such eggs were immediately sectioned by means of an electronically operated freezing microtome at 3 to 5  $\mu$  and the sections were subjected to histochemical studies.

The details of the histochemical tests employed, together with the references, are presented in Table 1.

## RESULTS AND DISCUSSION

The chorionic pad was positive to PAS

and digestion with diastase had no effect on the PAS reaction (Table 1). Among the diastase-stable, PAS-positive compounds which are likely to be present in animal tissues, one may include such carbohydrate substances as acid mucopolysaccharides, mucoproteins or neutral mucopolysaccharides and such lipid compounds as glyco- or phospholipids and lipoproteins (Pearse 1968). That the chorionic pad continued to be positive to PAS even after pyridine extraction suggests that the PAS-positive nature of the pad is not due to lipid compounds.

Acid mucopolysaccharides may be distinguished from mucoproteins and neutral mucopolysaccharides by the ability of the former to exhibit metachromasia (Pearse 1968). The chorionic pad, when treated with Toluidine blue-O, demonstrated gamma metachromasia (reddish purple) suggesting the presence of acid mucopolysaccharides. Positive reactions shown by the chorionic pad to aldehyde fuchsin (Table 1) and alcian blue corroborate the above inference.

Acid mucopolysaccharides with sulphate groups can be detected by means of the positive Bracco-Curti method (Table 1). The chorionic pad yielded a negative reaction to this test, denoting the absence of sulphate groups. This is supported by the observation that the material of the pad continued to show a metachromatic reaction to Toluidine blue-O even after treatment with 95% alcohol. Pearse (1968) recorded that alcohols at higher concentration block the sulphate groups, if present.

Application of methylene blue- extinction technique yielded very interesting in-

Table 1. Histochemical reactions shown by the chorionic pad of the egg of *Aedes aegypti*.

Tests	References	Reaction
PAS	Gomori 1952	+
PAS—after diastase	Pearse 1968	+
PAS—after pyridine extraction	Lillie 1965	+
Toluidine blue-O	Pearse 1968	Metachromasia Gamma
Aldehyde fuchsin	Gomori 1950	+
Alcian blue	Steedman 1950	+
Bracco-Curti method	Bracco and Curti 1953	-
Toluidine blue-O after 95% alcohol	Pearse 1968	Metachromasia Gamma
Methylene blue	Dempsey et al. 1947	
a) at pH 4.5		-
b) at pH 4.6		+
c) at pH 4.8		+
d) at pH 5.0		+
e) at pH 5.2		+
f) at pH 5.3		-
Azure-A	Kryvi 1971	
a) at pH 4.5		-
b) at pH 4.6		+
c) at pH 4.8		+
d) at pH 5.0		+
e) at pH 5.2		+
f) at pH 5.3		-

formation on the nature of the acid mucopolysaccharides. The first noticeable staining of the chorionic pad, with methylene blue, occurred at pH 4.6, and no staining was evident at pH 5.3. The methylene blue-extinction point therefore, lies between pH 4.6 and 5.2. Similar results are noted with Azure-A also (Table 1). These observations would indicate the presence of an acid mucopolysaccharide having weak acidic groups. In the light of the observations of Wolman (1956) this would imply the presence of hyaluronic acid in the chorionic pad because hyaluronic acid is the only acid mucopolysaccharide containing weak acidic groups and without sulphate groups. To verify this with controls, paraffin sections of the domestic cock's comb, which is well known to contain hyaluronic acid, were tested for the dye-binding capacity with methylene blue and Azure-A. The first noticeable staining was evident at pH 4.3 and there was no staining at pH 5.3. This would indicate that the methylene blue extinction point for the hyaluronic acid is between pH 4.3 and

5.2, which is very much similar to that of the carbohydrate fraction of the chorionic pad of the egg of *Ae. aegypti*.

Blumberg and Ogston (1958) observed that the hyaluronic acid has the property of swelling in water and becoming jelly-like with high adhesive properties. It is therefore reasonable to infer that the occurrence of weak acid mucopolysaccharides similar to hyaluronic acid in the chorionic pad of the egg of *Ae. aegypti* may be responsible for its swelling and adhesivity.

#### ACKNOWLEDGMENTS

One of us (KP) is grateful to the University Grants Commission for the award of a Fellowship during the tenure of which the present investigation was conducted. She is indebted to the authorities of the University of Madras for provision of facilities.

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