Structure of Cuticle and Entry of Insecticides through Integument of the Rice Stem Borer¹

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INTRODUCTION

Roughly speaking, the integument of lepidopterous larvae in general consists of sclerotized and membranous parts throughout the entire body. The thorax and the abdomen are differentiated into hard sclerites, alternating with soft intersegmental membranes. The thoracic legs are composed of a number of sclerotized limb segments, each connected with the membranous joint or the articular corium. Even the abdominal legs are considered to be true segmental appendages consisting of rings of sclerotic plates and membranes. All the membranous parts are soft and thrown somewhat into folds in an unstretched state and are commonly regarded as providing flexibility and stretching.

In the present article the results of observations using the larvae of the rice stem borer, *Chilo suppressalis* WALKER, are recorded by which it is demonstrated that the sclerotization is wanting in the cuticles of the membranous parts, that the epicuticle is different in its properties, and in relation to these properties the rate of entry of liquid insecticides is more rapid in or near the membranous parts than in the sclerotized regions.

STRUCTURE OF CUTICLE

Sclerites of trunk. In stained paraffin sections epicuticle, exocuticle and endocuticle are readily recognizable in the cuticle of each sclerite of the thorax and abdomen. The epicuticle is seen as a thin border line stained brown with Heidenhain's iron haematoxylin

and vividly red with Mallory's triple stain. The endocuticle on the other hand, stains blue with the latter but not with the former. The exocuticle resembles the epicuticle in staining reactions but is weaker in strength (Fig. 1).

Fig. 1. Longitudinal section through the abdominal sclerite. cem. cement layer; endoc. endocuticle; exoc. exocuticle.

Fig. 2. Longitudinal section through the arthroal membrane. cem. cement layer; endoc. endocuticle; epic. epicuticle.

Two layers seem to be distinguishable in the epicuticle of sclerites, i.e., outer layer

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which like exocuticle is strongly positive to haematoxylin and to osmic acid and an inner layer where both the reactions are weak. Both layers, however, give equally strong positive reactions with the Millon and xanthoproteic tests. Recently Takahashi (1956) has proved in the silkworm that these two layers of epicuticle represent the paraffin (corresponding to outer or lipid epicuticle of some authors) and the cuticulin layer (corresponding to inner or protein epicuticle) respectively. The cement, wax and protein layers are completely dissolved during various treatments in the preparation of paraffin sections. Judging not only from the staining reactions but also from the structural position, the two layers found in the epicuticle of the present caterpillar will no doubt correspond to the paraffin and cuticulin layer. Like the same layer of *Periplaneta* (DENNELL & MALEK, 1955), the paraffin layer is not affected by the extraction of fat solvents, and even after this treatment it still stains with Sudan black B. These properties suggest the presence of firmly bound lipid.

Intersegmental membranes of trunk. The informations gained here on the structure of cuticle in the intersegmental membranes of the body trunk of the rice stem borer fully agree with those given by Dennell and Malek (1954, '55) in the studies of the cockroach or by Takahashi (1956) in the silkworm. In longitudinal sections, the sharp infolding of the cuticle beneath the posterior margin of each sclerite, marks the beginning of the transition from the sclerite condition to that of the arthrodial membrane. It is lighter in color and more flexible than the sclerite.

The arthrodial membrane consists only of epicuticle and endocuticle. The exocuticle is not seen (Fig. 2). Here the epicuticle is also a thin bounding membrane. The endocuticle, however, differs from that of the sclerite in its greater thickness. Consequently the cuticle of the arthrodial membrane is much greater in total thickness than that of the sclerite. In an example of measurement, the total thickness of the cuticle is 31.2μ (represented only by the thickness of endocuticle) in an arthrodial membrane in

contrast with 17.2μ in a tergite region (exocuticle 3.8μ and endocuticle 13.4μ). In the transitional regions between the sclerites and the arthrodial membrane, the exocuticle appears to be a progressively thinning layer merging ultimately with the epicuticle, as summarized in diagram form in Fig. 3.

Fig. 3. Diagrammatic view illustrating the arrangement of the sclerites and intersegmental membranes in the longitudinal section through the dorsal abdominal cuticle. The regions illustrated in the sections of preceeding figures are indicated by (A-Fig. 1) and (B-Fig. 2).

It is worthy to note that the double layers of epicuticle clearly distinguishable in the sclerite, can not be detected in the arthrodial membrane, and that the reactions indicating the presence of lipid and protein by means of osmic acid or of the Millon and xanthoproteic tests are less intense than those in the sclerite.

Thoracic and abdominal legs. In the lepidopterous larvae the free part of the thoracic legs comprises in general four movable segments (femur, tibia, tarsus and claw-like pretarsus). All the joints between the segments are membranous rings of the leg wall called articular corium (or membrane). The abdominal legs of lepidopterous larvae are said by embryologists to be developed from limb rudiments in the embryo that correspond to those of the thoracic legs. They appear, therefore, not to be mere muscular outgrowths but true segmental appendages. Each leg consists of four parts. Proximally there is a ring of flexible membranous integument; beyond this there is a long cylindrical sclerotized section; distally the leg ends by way of a small area of membranous ring in a refractile lobe called planta which bears the crochets.

Fig. 4. Longitudinal section through the cuticle of a thoracic leg. Posterior view. In this and succeeding figures regions consisting of epicuticle and exocuticle are indicated by a thick border line, while regions where exocuticle is absent are written with a slender line.

Fig. 5. Longitudinal section through the cuticle of an abdominal leg. Lateral view. a-m. tergite; b-l. dorsopleural line; c-k. pleurite; d-j. proximal membranous part; e-i. sclerotized cylindrical part; f-h. distal membranous part; g. terminal end of planta.

Fig. 6. Longitudinal section through the cuticle of a caudal leg. Lateral view. Abbreviations as in Fig. 5.

The structural relations of cuticles between the sclerotic and the membranous parts of both thoracic and abdominal legs are quite in accordance with the foregoing observations between the sclerite and the intersegmental membrane of the body trunk. The cuticles of all the segments of thoracic legs² and of the cylindrical sclerotic sections of abdominal legs are composed of epicuticle, exocuticle and endocuticle, while in the articular membranes of thoracic legs and in the membranous parts of abdominal legs, the exocuticle is absent (Fig. 4 and Fig. 5). Besides this, there are indeed marked similarities between both regions in the construction of epicuticle, in the thickness of endocuticle, in the progressive disappearance of exocuticle in the membranous parts towards the surface in passing away from the sclerotized parts and in the strength of reaction proving the deposits of lipids and proteins.

It is evident that the cuticle of the distal end of the planta of the abdominal legs, from the first to the fourth, is composed of three layers, but that in the terminal end of the fifth or caudal leg the exocuticle appears to be wanting (Fig. 6).

ENTRANCE OF INSECTICIDES

Experiments were conducted with the assumption that the entrance of insecticides or the penetration of liquids in general might have some connection with the structural differences of the larval cuticle thus far mentioned. Droplets of 5 μ l commercial oil emulsion of BHC (containing 10% gamma-hexachlorocyclohexane) and of 1 μ l Folidol (containing 46.6% 0, 0-diethyl-0-para-nitrophenylthiophosphate) were applied topically to the following areas of integument to find the difference in the speed of penetration as judged by the induced paralysis or death of the test larvae: —

Dorsal, subspiracular and ventral lines in the middle portion of the second thoracic and third abdominal body segments; dorsal

² Very thin amber-colored exocuticle which shows little affinity to haematoxylin and acid fuchsin seems to be distinguished from the underlying colorless exocuticle in the sclerotic region of each thoracic leg (Fig. 4).

lines of the intersegmental membranes from the second thoracic (between the second and third thoracic segments) to the fourth abdominal segments; openings of the first thoracic and the fourth abdominal spiracles; central regions of outer side of the second thoracic, the third and the fifth (caudal) abdominal legs.

For topical application a microsyringe was built from a hypodermic one, the plunger of which can be depressed by a micrometer screw-head. Though the oil emulsions applied were too viscous to be delivered accurately from the fine glass capillary which was attached to the needle of the syringe, the desired amount could always be obtained by selecting the length and width of the capillary and by learning the knack of handling the screw-head. Immediately after treatment the animals were transferred individually to a small Perti-dish kept at 13~15°C to observe the speed of intoxication. The droplets sometimes fell off from the site of application by contact with the wall of the dish. Such results were omitted in the treatment of the data by the stochastic method of Smirnoff. The symptoms of poi-

soning, such as a latent period, excitation, ataxia, convulsion, paralysis and death which are generally recognizable clearly in active insects, are difficult to distinguish accurately in this sluggish caterpillar. In the experiment of BHC the time necessary for death was observed by determining the loss of response to tactile stimulation. But as variations among individuals were great in this case, the timelapse of onset of the first violent convulsion of the entire body was observed with the use of Folidol.

The experiments were conducted from November to December of 1953 (BHC) and 1954 (Folidol), using the larvae in the beginning of the hibernation period. Table 1 gives the average time of death (BHC) or of convulsion (Folidol) occurring in 15 to 30 individuals for each experiment.

Relations between the site of application and the rate of poisoning in both insecticides run parallel to each other. In BHC no differences were found in 99% level of confidence in the intoxication of the three areas of the thoracic sclerite, six intersegmental membranes, spiracles and legs of thorax and abdomen, except in the ventral and lateral

Table 1. Toxicities (time in minutes) of commercial oil emulsions of BHC (5 μ l) and Folidol (1 μ l) applied topically to the larvae of *Chilo suppressalis*.

Site of application		BHC		Folidol		Average	
		$\bar{x}$	S	$\bar{x}$	S	BHC	Folidol
Thoracic sclerite	II. segm., dorsal	128.4	25.4	139.8	6.9	129.3	134.3
	" ventral	116.0	22.9	129.4	8.8		
	" lateral	143.4	14.8	133.7	7.3		
Abdominal sclerite	III. segm., dorsal	134.1	16.2	144.8	6.1	131.9	141.5
	" ventral	140.4	17.8	153.4	5.7		
	" lateral	121.1	14.6	126.2	7.1		
Intersegmental membrane	II. thoracic	108.5	18.1	115.7	8.9	112.4	118.2
	III. "	118.0	11.2	118.2	7.0		
	I. abdominal	107.8	11.7	117.2	7.4		
	II. "	113.3	11.8	—	—		
	III. "	112.5	15.3	121.8	10.7		
	IV. "	114.3	21.9	—	—		
Spiracle	I. thoracic	87.6	10.3	65.6	6.5	92.5	69.6
	IV. abdominal	97.3	7.4	73.7	5.4		
Leg	II. thoracic	97.3	18.8	78.3	6.7	95.0 158.7	77.5 114.0
	III. abdominal	92.6	17.2	76.7	8.0		
	V. "	158.7	19.2	114.0	4.7		

$\bar{x}$ =Mean value S=Standard deviation

areas of the abdominal sclerite and of the caudal and the other legs. In Folidol no discernible differences could be found either in intersegmental membranes or in spiracles and the legs. But differences seem to occur in the thorax, abdomen and between caudal and the other legs.

The entire integument of the larval body may be divided roughly into five parts with respect to the speed of intoxication. These are body sclerites, intersegmental membranes, spiracles, thoracic and abdominal legs and caudal legs. The results of the stochastic examinations on the differences of the average rate of poisoning between these five parts are indicated in Table 2. From this we obtain the order of the regional susceptibility to insecticides in the larvae.

Spiracles=thoracic and abdominal legs>intersegmental membranes>body sclerites>caudal legs for BHC;

Spiracles=thoracic and abdominal legs>intersegmental membranes=caudal legs>body sclerites for Folidol.

Table 2. Differences of significance in the average rate of intoxication caused by topical application of BHC and folidol to the five principal areas of larval body of *Chilo suppressalis*.

Liquid Area	BHC				Folidol			
	B	C	D	E	B	C	D	E
A	+(1)	+(1)	+(1)	-(1) +(5)	+(1)	+(1)	+(1)	+(1)
B		-(5)	-(5)	+(1)		+(1)	+(1)	-(5)
C			-(5)	+(1)			-(5)	+(1)
D				+(1)				+(1)

A=Body sclerite B=Intersegmental membrane
C=Spiracle D=Thoracic and abdominal legs
E=Caudal leg. Numbers in parenthesis denote the level of significance.

ARGENTAFFIN REACTION

Comparing the histological structures of the cuticle, relying only upon the paraffin section, appears to be insufficient to inquire into the cause of its local difference in the penetration of insecticides, since the wax layer of epicuticle which is believed to be the most responsible for the impermeability

of cuticle to liquids is completely washed away during the preparation of paraffin section. Accordingly, the speed of argentaffin reaction was compared in various parts of cuticle. The intact insects which had been washed thoroughly in distilled water to be freed from dirt was immersed in 0.5% solution of silver nitrate for varying lengths of time, and was transferred to tap water under the sunshine after the removal from the solution in distilled water. Prominent granules of deep brown believed to be induced by the reduction of silver are visible microscopically in a layer or layers beneath the transparent surface of the cuticle. It is not known to us exactly where these granules are formed, but it is highly probable that the site is a layer or layers which lie under the layer of wax. So the speed of the reaction will represent roughly that of the silver solution passing through the wax layer, although it may depend to some extent on the amount of polyphenols deposited or on the substances responsible for the production of the brown stain. Table 3 gives the result.

Table 3. Formation of brown granules in the cuticle of *Chilo suppressalis* larvae immersed in 0.5% solution of AgNO_3 for various durations.

Part	Duration of immersion, min.				
	10	15	20	30	60
Head	-	+	+	++	++
Body sclerite	-	-	-	+	+
Interseg. memb.	+	++	++	++	++
Leg	+	++	++	++	++

- No granules formed, + Rarely, ++ Somewhat densely, +++ Very densely.

The whole integument is stained completely black if the insect is soaked more than 3~4 hours, while such region as body sclerite will not produced any granules if immersed less than 20 minutes. This fact will indicate that the result shown in Table 3 represents the difference in the speed of penetration of the silver solution through the wax layer.

No recognizable difference, regional or segmental, could be detected in the body sclerites and intersegmental membranes. This

was also the case with all legs. The formation of the granules takes place very rapidly in the intersegmental membranes and the legs, but very slowly in the body sclerites.

DISCUSSION

It may be generalized from the foregoing statement that, with an exception of spiracles, the drops of insecticides applied directly or nearly so to the membranous parts of the integument kill the larvae with greater rapidity than those applied to the sclerotized parts. When applied to the intersegmental membrane or to the leg, the drop either runs quickly into fold and readily reaches the arthrodial membrane or spreads over articular membrane or membranous ring (of abdominal leg).

This may be due to the difference of permeability of the liquids to these two regions. The rapid passage through the membranous parts is perhaps due to the absence of exocuticle as well as to the properties of the epicuticle in those regions. Denser structure of exocuticle brought about by the deposition of the hardening substances that form the sclerites will affect the rate of the entry of the liquids. Though some authors say that impermeability to water is independent of sclerotization in the cuticle (see for example WIGGLESWORTH, 1948), the relationship has not yet been satisfactorily developed in literature (see RICHARDS, 1951).

The result of the experiment on the argenta-ffin reaction leads us to conclude that the wax layer in the membranous parts are not identical, either in thickness or in physico-chemical property, with that in the sclerotic parts. This layer constitutes the most effective barrier to the passage of liquids in this insect (KOIDSUMI, 1951, '52). The reactions proving the presence of lipids and proteins, especially of the former, in the epicuticular layers visible in the paraffin section are measurably weaker in all membranous parts as compared with those in sclerotic regions. The differential properties of the cuticulin layer of both parts were fully discussed by Dennell and Malek (1955) in *Periplaneta*, according to whom a basal protein matrix

of the layer remains unmodified in the arthrodial membrane, but becomes densely impregnated in the sclerotized regions with lipid and a further protein possessing an aromatic group. Such a difference will also explain the differential permeability of both regions. Though any definite restrictive functions to the passage of liquids have not hitherto been assigned to sublayers other than that of wax, but in recent years it was clearly proved by Richards *et al* (1953, '57) that the water barrier is located in the inner epicuticle (=cuticulin layer) in fly larvae possessing no wax layer.

As for the special slow rate of permeability we saw in the integument of caudal legs, we can not find any specific mechanism at present, though it is not impossible to make some speculations.

SUMMARY

1. The cuticle of the membranous parts such as arthrodial membrane of body trunk, articular corium of thoracic legs and the membranous ring of abdominal legs of the larvae of the rice stem borer, *Chilo suppressalis* WALKER, consists of epicuticle and endocuticle, but sclerotized exocuticle is absent.

2. The epicuticles in all membranous parts are different in properties from those in sclerotized regions, namely, the wax layer is believed to have such a property and a thickness as to make the passage of liquids easier and the reactions to prove the presence of lipids and proteins in the outer (paraffin) and inner epicuticle (cuticulin) are both weaker compared with those of sclerotized regions.

3. The rate of entry of insecticides is generally faster through the cuticle of membranous parts than that of sclerotic regions. Topical applications of oil emulsions of BHC and Folidol made on various areas of integument resulted in the development of toxic symptoms of the larvae in the order of the rate of permeation, spiracles=thoracic and abdominal legs<intersegmental membranes<caudal legs>body sclerites.

4. Such differential penetrability of in-

secticides may be due to the differences in the structures and the properties of the cuticle of the body regions as described above.

LITERATURE CITED

- DENNELL, R. and S. R. A. MALEK (1954) Proc. Roy. Ent. Soc. London [B] **143**: 126~136.
 DENNELL R. and S. R. A. MALEK (1955) *ibid.* **143**: 239~257.
 KOIDSUMI, K. (1951) Jap. Jour. Appl. Zool. **16**: 1~6.
 KOIDSUMI, K. (1952) Ann. Zool. Jap. **25**: 388~393.
 RICHARDS, A. G. (1951) The Integument of Arthropods, London.
 RICHARDS, A. G., M. B. CLAUSEN and M. N. SMITH (1953) J. cell. comp. Physiol. **42**: 395~414.
 RICHARDS, A. G. (1957) J. ins. Physiol. **1**: 23~39.
 TAKAHASHI, Y. (1956) Ann. Zool. Jap. **29**: 187~195.
 WIGGLESWORTH, V. B. (1948) Biol. Rev. **23**: 408~451.

摘 要

ニカメイチュウの皮膚構造と薬剤の透過性

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1) 物質の透過性に関して、ニカメイチュウ（おそらく多くの鱗翅目幼虫）の皮膚は、総括的にみて、硬皮部（sclerotized part）と膜状部（membranous part）に分けられる。頭部・胸腹部・胸肢などの各環節の皮膚は前者に属するが、これらの環節と環節とをつなぐ関節部（joint）は淡色で屈伸性に富み、いわゆる膜状部を形成する。幼虫の腹肢もこのような硬皮部と膜状部からできている。

2) 皮膚のパラフィン切片を、ハイデンハインのヘマトキシリンやマロリーの方法で染めてみると、硬皮部の角皮には表角皮（epicuticle）・外角皮（exocuticle）・内角皮（endocuticle）の3部分が区別されるが、膜状部ではこのうち外角皮と称する部分が存在しない。しかしこの部分の内角皮は硬皮部よりもかえって厚い。

3) 表角皮の性質にも硬皮部と膜状部に差異がみられる。すなわち蠟層の直下の層における硝酸銀還元顆粒の出現速度を比較すると、皮膚の物質通過制限の最大責任部位と考えられる蠟層（wax layer）には、膜状部では硬皮部よりも液体を容易に通過させるような性質が存在

するし、またパラフィン切片に出現するパラフィン層（paraffin layer）とクチクリン層（cuticulin layer）のリピッドならびに蛋白に対する反応は硬皮部では膜状部より明らかに強い。

4) 皮膚のいろいろな部分に BHC やホリドールの微滴を塗布し、幼虫の中毒死あるいはけいれん発現に至る時間をはかることによって、皮膚の部位による薬剤透過性の速度を比較して、次の順位のあることがわかった。

気門＝胸腹肢≥胴部節間膜>尾肢>胴部環節本体

すなわち気門を別にすれば、一般に膜状部またはそれに近接した部分では（肢の皮膚に塗ると薬剤は直ちに膜状部に拡散する）、硬皮部に比べて、薬剤の透入はいちじるしく速いことがわかる（尾脚は他の脚に比べておそいがこの原因はよくわからない）。

5) このように膜状部における薬剤透過性の大きいことは、その部分の角皮に外角皮を欠くこと、表角皮の性質に差異のあることが大きな原因になっているのではないかと思われる。