

## The Early Post-moult Cuticle in *Buthus*

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### SUMMARY

The cuticle of the scorpion, *Buthus tamulus*, has been studied at the early post-moult stage. There is a distinct two-layered epicuticle, which is non-chitinous, and a procuticle consisting of a chitin/protein complex. Polyphenols, phenolase, and sulphur are present at this stage. Quinones and groups containing sulphur seem to play a part in the subsequent hardening of the cuticle.

### INTRODUCTION

SINCE Krishnan (1953) reported the occurrence of —S—S— bonds in the cuticle of *Palamnaeus swammerdami*, a keen interest has been evinced in the cuticle of arachnids in general. Even earlier, Lafon (1943) noted the presence of over 2.85% of sulphur in the cuticle of *Limulus* and suggested that the cuticular proteins were similar in this respect to the keratinous type of proteins of the vertebrates. On another count, the cuticle of arachnids attracted the attention of workers in the field. Browning (1942) reported that what was considered to be the epicuticle in the spider, *Tegenaria atrica*, was only an optical artifact. Although later work on the same genus by Sewell (1955) showed the presence of an epicuticle, the homology of the arachnid epicuticle was once again a bone of contention; for Krishnan, Ramachandran, and Santanam (1955), studying the X-ray diffraction patterns of the epicuticle in *Palamnaeus*, observed the presence of chitin in this layer. Until then the epicuticle was considered to be a non-chitinous lipoprotein layer, distinct from a lamellated chitin/protein complex constituting a procuticle. Kennaugh (1959), investigating the cuticles of *Pandinus imperator* and *Scorpiops hardwickii*, reported that the epicuticle in these forms is free from chitin. What Krishnan described as the inner epicuticle corresponds to what Kennaugh called hyaline exocuticle. Shrivastava (1954) did not find any sulphur in the inter-moult cuticles of *Palamnaeus bengalensis* and *Buthus*. And again, on the basis of the amino-acid constitution of the cuticular proteins, Hughes (1959) suggested the possibility of the occurrence of quinone/sulphur links in an acarine.

Thus the questions whether there was a chitin-free epicuticle and whether sulphur played a part in hardening the cuticle remained unsolved. Hence it was considered desirable to study the histochemistry of the newly exposed, soft, early post-moult cuticle of a scorpion.

### MATERIALS AND METHODS

The colourless soft cuticle from the freshly moulted scorpion, *B. tamulus*, has been used for the present study. Fresh hand-sections, as well as frozen [Quarterly Journal of Microscopical Science, Vol. 101, part 4, pp. 433-8, 1960.]

and paraffin sections, were made and studied in both unstained and stained preparations. Mallory's triple, Masson's trichrome, and Lower's iodophil stains have been used to colour the cuticle.

Xanthoproteic, Millon's, Baker's, Sullivan's, Jackson's, Schulze's, Nadi, azide/iodine, and chitosan tests were amongst the few histochemical tests employed.

#### OBSERVATIONS

##### *Structure and staining reactions*

In the newly moulted scorpion the cuticle appears opaque white when it is in contact with the underlying tissues, while on separation and cleaning it is seen to be colourless and transparent. In surface view it presents a granulated appearance except where the tonofibrillae are attached to it. In the latter places the surface presents a honeycomb pattern. The ducts of the tegumental glands appear as rounded openings, distributed all over the body. The area around the opening of the ducts does not appear different from the rest of the surface.

In cross-section the cuticle appears as a thin layer  $12\ \mu$  in width. This thin strip is composed of two distinct divisions: an outer non-lamellated, homogeneous layer,  $3.5\ \mu$  thick, and an inner lamellated layer,  $8.5\ \mu$  thick. In regions where the cuticular surface is granulated, the outer layer appears sinuous because of the thickening. When sections are stained by Masson's or Mallory's method, the two layers are more prominently distinguishable because of their differential staining. The outer layer stained red with both the stains while the inner (lamellated) layer stained blue with Mallory's and green with Masson's stain. In accordance with the nomenclature suggested by Richards (1951), these two layers may be termed the epicuticle and procuticle. A very thin layer at the outer border of the epicuticle stains differently from the rest of the outer layer. This thin border stains blue with Mallory and green with Masson. These two portions of the epicuticle may be termed the inner and outer epicuticle, corresponding to those of the larva of *Sarcophaga* (Dennell, 1946). No subdivisions of the procuticle are seen at this stage, although in the inter-moult cuticle there may be as many as three distinct subdivisions.

##### *Chemical nature*

Gross studies of the chemical nature of the newly moulted cuticle indicate the presence of fundamental differences in the chemical composition of the two layers (epicuticle and procuticle). Treatment with hot saturated alkali dissolves the epicuticle, while the procuticle is resistant to the treatment. The procuticle after this treatment colours violet with iodine / sulphuric acid, and dissolves in 3% acetic acid; this suggests the presence of chitosan. Treatment with concentrated sulphuric acid dissolved both the layers, but the epicuticle was more resistant than the procuticle.

The proteins of the cuticle, as indicated by the xanthoproteic and biuret

reactions, are distributed throughout the cuticle, although there seem to be differences in the intensity of the reaction in the two layers. With the xanthoproteic test the epicuticle was more intensely coloured than the procuticle, while with Millon's reagent the cuticle was only faintly positive (see table 1).

TABLE I  
Summary of the reactions of the early post-moult cuticle

Reagent	Epicuticle	Procuticle
Xanthoproteic	+++	++
Millon's	+	+
Millon's (Baker's modification)	++	++
Chitosan	—	++
Jackson's	+	—
Sudan black B	+	—
Argentaffin	+	+
Nadi	+	—*
Nadi with KCN	—	—
Guaiacol and phenolphthalein	blue	colourless
Mallory's stain	blue† red‡	blue
Masson's stain	green† red‡	green

\* The procuticle is also coloured royal blue, but this was considered to be due to diffusion as it is faint and appears after a lapse of time.

† Outer epicuticle.

‡ Inner epicuticle.

The table shows that there are sudanophil substances in the epicuticle but not in the procuticle. However, the surface of the cuticle does not show any colouring of lipids, either immediately after moulting or within the next 12 h. This fact presumably indicates the absence of a separate paraffin layer similar to the one described in *Rhodnius* (Wigglesworth, 1947).

Aromatic substances in the cuticle are of considerable importance because of their role in the tanning of the cuticle. Their importance in the insect cuticles has been demonstrated by several workers (Pryor, 1940; Dennell, 1947a). It was shown that the cuticle in an arachnid *Thelyphonus* is hardened by phenols (Krishnakumaran, 1959). The role of phenolic substances in sulphur-hardened cuticle is obscure.

In the early post-moult cuticle of *Buthus* Millon's test is only faintly positive. However, Baker's test (mercuric sulphate and sodium nitrite) and the argentaffin reaction are strongly positive in the cuticle and also in the ducts of the tegumental glands. The formaldehyde / sulphuric acid test and the phosphomolybdic acid test for polyphenols (Feigl, 1954) are positive. Another significant feature of the cuticle at this stage is the strong Nadi reaction, which is cyanide-sensitive and thermolabile. The colour reaction with guaiacol and phenolphthalein indicates that the enzyme involved is a phenolase.

In addition to the phenolic substances and also the phenolase, there are some sulphur-containing compounds in the cuticle. The azide/iodine reaction, by immediate ebullition, suggests that the sulphur is in the form of sulphhydryl

or thiosulphate or sulphide. Since pretreatment of the cuticle with dilute mineral acids does not reduce the reaction with the azide/iodine, it is presumable that thiosulphates and sulphides are not involved. Pretreatment with iodine solution, however, renders the cuticle less sensitive to the action of the azide/iodine reagent. This is presumably due to the oxidation of —SH groups to —S—S— groups, and the latter do not react easily with the azide/iodine reagent (see Feigl, 1954). The nitroprusside and ferricyanide reactions are positive in fresh material. The distribution of sulphur-containing compounds was not clear, as the cuticle is very thin and the region from which nitrogen evolved could not be determined accurately. For the other two reactions hand-sections were used, and these were too thick to show definitely whether the positive reaction was in the epicuticle or in the procuticle. However, it is clear that the sulphur is present in the procuticle.

#### *Changes after moulting*

The changes that the cuticle undergoes soon after the moult involve the argentaffin and the sulphur-containing substances. Changes in the colour and the staining properties are also noticeable. Twelve hours after the moult the cuticle is reddish yellow, and in sections the lamellation previously noted in the procuticle is to a large extent masked. When these are stained the entire procuticle becomes red with both Mallory and Masson. Thus the procuticle is impregnated with some acidophil substances, presumably the tanning precursors. There is thus a correspondence with the stage described in the cuticle of *Schistocerca* (Schatz, 1952). The layer concerned may be termed the mesocuticle (see Lower, 1956).

The chemical nature of the procuticle at this stage shows that the procuticle is also positive to Sudan colouring agents and that the xanthoproteic reaction is equally strong in the epicuticle and the subjacent procuticle. The Nadi reaction is not thermolabile and is insensitive to cyanide. The argentaffin reaction and also the azide/iodine reaction are not as strong as in the early post-moult stage. The azide/iodine reaction is hastened by treatment of the cuticle with alkaline ferrous hydroxide solution. Corresponding with these changes in the chemical constitution, the cuticle becomes more resistant to the action of acids and alkalis. The surface of the cuticle is no longer wetttable and appears to be stretched taut.

#### DISCUSSION

From the foregoing observations it is evident that the cuticle of *Buthus* is fundamentally similar to that of the insects and other arthropods that have been investigated. As in the other arthropods, it is broadly distinguishable into a non-chitinous, lipoproteic, structureless, homogeneous, thin epicuticle and an underlying, broader, lamellated procuticle, formed of a chitin/protein complex. The latter seems to differentiate into subdivisions which have been described in the inter-moult cuticle of this and other scorpions. Evidence in favour of the view that the epicuticle is a double-layered, non-chitinous region

has been obtained in the present investigation of the unhardened cuticle of *Buthus*. At this stage, the epicuticle is not resistant to the action of hot saturated sodium hydroxide solution, and this suggests that it is not chitinous. It is also suggested that the great resistance of the inter-moult epicuticle is due to the hardening of this layer, rather than to the presence of chitin inextricably bound with protein (see Krishnan, 1956). Any chitin found in the epicuticle of the inter-moult cuticle should have passed into this layer after it is deposited; but such a deposition of chitin is considered unlikely.

The positive Nadi reaction in the newly formed cuticle, its sensitivity to heat and to cyanide, and the colour reaction with guaiacol and phenolphthalein indicate the occurrence of a phenolase in the cuticle at this stage. It has been shown that such an enzyme as this, in the cuticle of insects and Crustacea, is responsible for the conversion of polyphenols into quinones (Dennell, 1947 *a, b*). The positive reaction to Baker's modification of Millon's test, to the formaldehyde / sulphuric acid test, and to the phosphomolybdic acid test indicates the presence of phenolic substances, presumably the precursors of tanning agents.

The presence of sulphhydryl groups in the early post-moult cuticle is a noteworthy feature. Their absence later, owing presumably to their conversion to —S—S— groups, is significant. (The slow azide/iodine reaction in freshly-formed cuticle, together with the hastening of the reaction by treatment with alkaline ferrous hydroxide solution, suggests the presence of disulphide or similar groups (Feigl, 1954).) As no specific enzyme is involved in the oxidation of —SH into —S—S— groups, it is here suggested that quinones may play a significant part in this conversion. Whatever may be the mode of formation, the quinones and the disulphide groups appear at about the same time. (The cyanide-insensitive Nadi reaction and the faint colour of the cuticle suggest the presence of quinones.) By about the same time the changes in the staining properties and the resistance to the action of acids and alkalis are noticeable. All these indicate that the cuticle is preparing for the tanning and is probably already tanned to some extent.

Hughes (1959), in his study of the cuticle of a tick, suggested that the quinones and sulphur are linked together. The basis for this suggestion was a high percentage of lysine in the cuticular protein, which rendered quinone tanning unlikely. Krishnakumaran (1959) suggested the presence of similar bonds in the cuticle of *Galeodes*. On the basis of the circumstantial evidence, it is here suggested that the sulphhydryl groups are converted into disulphide groups by the wave of quinones formed by the oxidation of phenols. This simultaneous formation of quinones and disulphide groups aids the condensation of both these groups with the protein to form the resistant material that seems to be characteristic of the cuticles of arachnids.

The absence of any paraffins (i.e. sudanophil material) on the surface of the cuticle indicates that there is no wax layer comparable to that of *Rhodnius*; and the loss of 'wettability' of the surface in cuticles at the 12-h stage is to be correlated with the hardening of the epicuticle.

Thus, while the cuticle of *B. tamulus* resembles that of other arthropods in having a chitin-free, double-layered epicuticle and a procuticle composed of a chitin/protein complex, hardened in part by phenols, it differs from them in the occurrence of sulphur-containing compounds which also seem to play some part in the hardening.

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