

**HISTOLOGY AND HISTOCHEMISTRY OF THE VENOM APPARATUS OF THE
BLACK SCORPION *Androctonus crassicauda* (OLIVIER, 1807) (SCORPIONES:
BUTHIDAE)**

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ABSTRACT: The venom apparatus of the black scorpion *Androctonus crassicauda* has been characterized histologically and histochemically in the present study. The results showed that this apparatus consists of paired venom glands, each of which initially presents its own canal and posteriorly both fuse into a single common one. Each gland is covered by a sheath of striated muscle and is lined with extensively folded secretory epithelium (formed of non-secretory and secretory venom-producing cells). The outcomes also revealed that the venom-producing cells of both glands produce neutral mucosubstances, sialomucins, sulfomucins and proteins, but are devoid of glycogen. Cysteine, tyrosine, tryptophan and arginine were also detected along with activities of acid and alkaline phosphatases, mitochondrial adenosine triphosphatase, aminopeptidase, cholinesterase and non-specific esterases. Structure and secretion of scorpion venom glands are discussed within the context of the present results.

KEY WORDS: *Androctonus*, scorpion, venom apparatus, histochemistry, phosphatases, esterases, peptidases.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Androctonus crassicauda has been reported to be present in various localities in Saudi Arabia and in other parts of the Arabian Peninsula (2, 3, 12, 15, 24, 35). This species is considered one of the most dangerous scorpions in Saudi Arabia and neighboring countries (1, 2-7, 12, 28, 30) and its stings may account for mortalities (8, 10, 14, 16).

Although the morphology of scorpion venom apparatus has been described by many investigators, few studies have focused on its histochemistry (19, 20, 25, 27, 34). In this context, the present study was performed to characterize the histology and histochemistry of *Androctonus crassicauda* venom glands.

MATERIALS AND METHODS

The venom apparatus of 16 *Androctonus crassicauda* adult males and females, captured from the central, northeast and Al-Jouf provinces of Saudi Arabia, were utilized. The telson was removed from each scorpion and quickly immersed for five days in one of the following fixatives: 10% neutral buffered formalin (pH 7.4) with 2% calcium acetate (eight telsons); Boui's fluid (four telsons) and Gendre's fluid (four telsons). The cuticles of all fixed telsons were removed with a sharp blade, by scratching, then thoroughly washed in running water, dehydrated, cleared, impregnated and embedded in paraffin wax, sectioned at 4 to 5 μm thickness and stained with hematoxylin-eosin and Masson trichrome stains for histological examination. Eight other telsons were removed from adult scorpions and their cuticles were detached from the intact telson muscular coat after tranquilization with liquid nitrogen for subsequent preparation of fresh, unfixed frozen sections. Paraffin and frozen sections were then to detect mucosubstances, proteins, amino acids and enzymes activities utilized in the following histochemical reactions.

Neutral Mucosubstances

- Techniques: periodic acid-Schiff (PAS), PAS after diastase digestion, PAS after α -amylase digestion and Best's carmine (32).

Neutral mucosubstances are PAS-positive, diastase-resistant, as well as unstainable by cationic dyes. Acetylation produces derivatives of primary and secondary amines

that prevent 1, 2 glycol groups from reacting with PAS, indicating the presence of neutral mucosubstances or sialic acid, separately or simultaneously.

Acid Mucosubstances

- Techniques: alcian blue (AB) at pH 2.5 and 1.0 (13).

Alcian blue is generally considered specific for identifying acid mucosubstances whereas alcianophilia at pH 2.5 and 1.0 is specific for sialomucins and sulformucins, respectively (32).

Distinction between Acidic and Neutral Mucosubstances

- Techniques: AB (pH 2.5)-PAS and AB (pH 1.0)-PAS (32).

Distinction between Sulfomucins and Sialomucins

- Techniques: aldehyde fuchsin (AF) and aldehyde fuchsin-alcian blue at pH 2.5, strong (60°C, 4 h) methylation-saponification-AB, pH 2.5; acid hydrolysis (0.1 N HCl, 60°C, 4 h)-AB (pH 2.5); toluidine blue (TB) buffered at pH 1.7 and 3.4 and critical electrolyte concentration (CEC) technique for quenching of alcianophilia at pH 5.6 in the presence of gradual concentration of Mg^{++} (32).

In the combined aldehyde fuchsin-alcian blue sequential techniques, sulfomucins stain bluish purple and sialomucins, blue (9, 22). Methylation blocks subsequential staining of simple mucosubstances by esterification of carboxyl groups and desulfation of complex sulfated mucosubstances. Subsequent treatment with potassium hydroxide (saponification) after methylation restores the staining of carboxyl groups (13). The mucosubstances that are stained in 0.1 M $MgCl_2$ in the CEC reaction, but not in 0.2 M $MgCl_2$ are believed to contain carboxyl groups and no sulfate. Sulfated mucosubstances, on the other hand, stain strongly and selectively at 0.2 M Mg^{++} , but lose their alcianophilia to different degrees with increasing $MgCl_2$ concentration (32).

Enzyme Digestion Tests

- Techniques: diastase-PAS; neuraminidase (sialidase, *Vibrio cholerae*, type V)-AB at pH 2.5; hyaluronidase (testicular)-AB pH 2.5, neuraminidase-TB (pH 3.7) and hyaluronidase-TB pH 2.0 (32).

Control sections were incubated in the buffer solutions without enzymes. Sialomucins could be identified at pH 2.5 by the presence of alcianophilia, which is partially lost following acid hydrolysis and completely abolished after neuraminidase digestion; however, this property does not affect the staining of sulfated mucosubstances. A loss of alcianophilia after hyaluronidase digestion is due to the removal of hyaluronic acid and chondroitin sulfates.

Proteins

- Techniques: mercuric bromophenol blue; ninhydrin-Schiff and chloramine T-Schiff (32).

Amino Acids

- Techniques: the p-DMAB-nitrate method for tryptophan; the Sakaguchi dichloronaphthol reaction for arginine; Millon's reaction (Baker modification) for tyrosine and the ferric ferrocyanide method for cysteine (32).

Enzyme Activities

Unfixed fresh frozen sections were used for the histochemical characterization of phosphatases according to the following methods: the calcium cobalt method for alkaline phosphatase (23); the lead nitrate method for acid phosphatase (9); the Ogawa and Mayahara method for mitochondrial adenosine triphosphatase (31); the α -naphthol acetate method for non-specific esterases (31); McCabe and Chayen method for aminopeptidase and the acetylthiocholine method for cholinesterases (23). The specificity of each reaction was controlled by incubating a parallel set of sections in the medium without substrate.

RESULTS

Morphology and Histology

The venom apparatus of *Androctonus crassicauda* presents similar completely separated bilateral venom glands covered by cuticle. Each gland has its own canal that fuses into a single common canal in the acleus, the tapered curved end of the telson. The glandular epithelium is folded and consists of a mass of secretory epithelium, covered by a sheath of striated muscles (Figure 1A). The glandular epithelium consists of venom-producing cells and non-secretory supporting cells. The venom-producing cells are apocrine, high columnar in shape, filled with coarse granules in the apical portion and present basally situated small nuclei. The supporting cells are subcuboidal in shape, located between the venom-producing cells and the underlying basement membrane (Figure 1B). The common canal of *Androctonus crassicauda* venom apparatus lacks musculature and is lined with a chitinous internal layer, followed by a non-excretory simple cuboidal epithelium. The extruded venom, within the gland lumen, and within the apical cytoplasm of the venom-producing cells, contains fine and coarse granules, with discrete morphologies, that showed variable coloration patterns when using the same histological stain.

Carbohydrates Histochemistry

As shown in Table 1, the venom-producing cells reacted positively to PAS, but responded negatively to Best's carmine stain (Figure 1C). This reactivity withstood α -amylase digestion, but was completely blocked by both phenylhydrazine and acetylation, and partly restored by deacetylation-PAS sequential techniques. These cells showed alcianophilia at both pH 2.5 (Figure 1D) and 1.0, and this property was not affected by neuraminidase or hyaluronidase digestion, but was partly inhibited by acid hydrolysis, completely blocked by strong methylation, and restored partially with methylation-saponification sequential techniques. The glandular epithelium showed strong reaction with the CEC at 0.2 M and 0.3 M Mg^{++} and a feeble one at 0.4 M Mg^{++} . The venom granules in both the lumen and the apical part of the venom-producing cells were stained positively by aldehyde fuchsin dye, colored purple by AB (pH 2.5)-PAS (Figure 1E) and showed metachromatic reaction with TB at both pH 1.7 and 3.4 (Figure 1F).

Table 1. Histochemical reactions revealed by the glandular epithelium in *Androctonus crassicauda* venom apparatus

Histochemical method*	Result
Periodic acid-Schiff (PAS)	++, P
Diastase digestion-PAS	Nb
Best's carmine	-
Phenylhydrazine-PAS	Cb
Acetylation-PAS	Cb
Acetylation-deacetylation-PAS	+, P
Alcian blue (pH 1.0)	+
Alcian blue (pH 2.5)	++
Alcian blue (pH 2.5)-PAS	++, Bp
Aldehyde fuchsin	+, P
Aldehyde fuchsin-alcian blue (pH 1.0)	+, Bp
Aldehyde fuchsin-alcian blue (pH 2.5)	+, Bp
Acid hydrolysis-alcian blue (pH 2.5)	+, B
Strong methylation-alcian blue (pH 2.5)	-, Cb
Strong methylation-saponification-alcian blue (pH 2.5)	+, B
Critical electrolyte concentration (AB, 0.1 M)	++
Critical electrolyte concentration (AB, 0.2 M)	+±
Critical electrolyte concentration (AB, 0.4 M)	±
Neuraminidase-AB (pH 2.5)	Nb
Hyaluronidase-AB (pH 2.5)	Nb
Toluidine blue (pH 1.7)	+, M
Toluidine blue (pH 3.4)	+, M
Ninhydrin-Schiff	+
Hg-bromophenol blue	+
Chloramine T-Schiff	+
Ferric ferrocyanide method	+
p-DMAB-nitrate method	+
Sakaguchi reaction	+
Millon's reaction	+

Alkaline phosphatase activity	±
Acid phosphatase activity	+
Mitochondrial ATPase activity	+
Non-specific esterase activity	+
Aminopeptidase activity	++
Cholinesterase activity	+
Lipase	-

*Histochemical reactions intensity: – (negative); ± (feeble); + (moderate); ++ (strong); M (metachromasia – indication of acidic groups).

Degree of histochemical reaction blockade: Cb (complete blockade); Pb (partial blockade); Nb (no blockade). Colors: B (blue); Bp (bluish purple); P (pink).

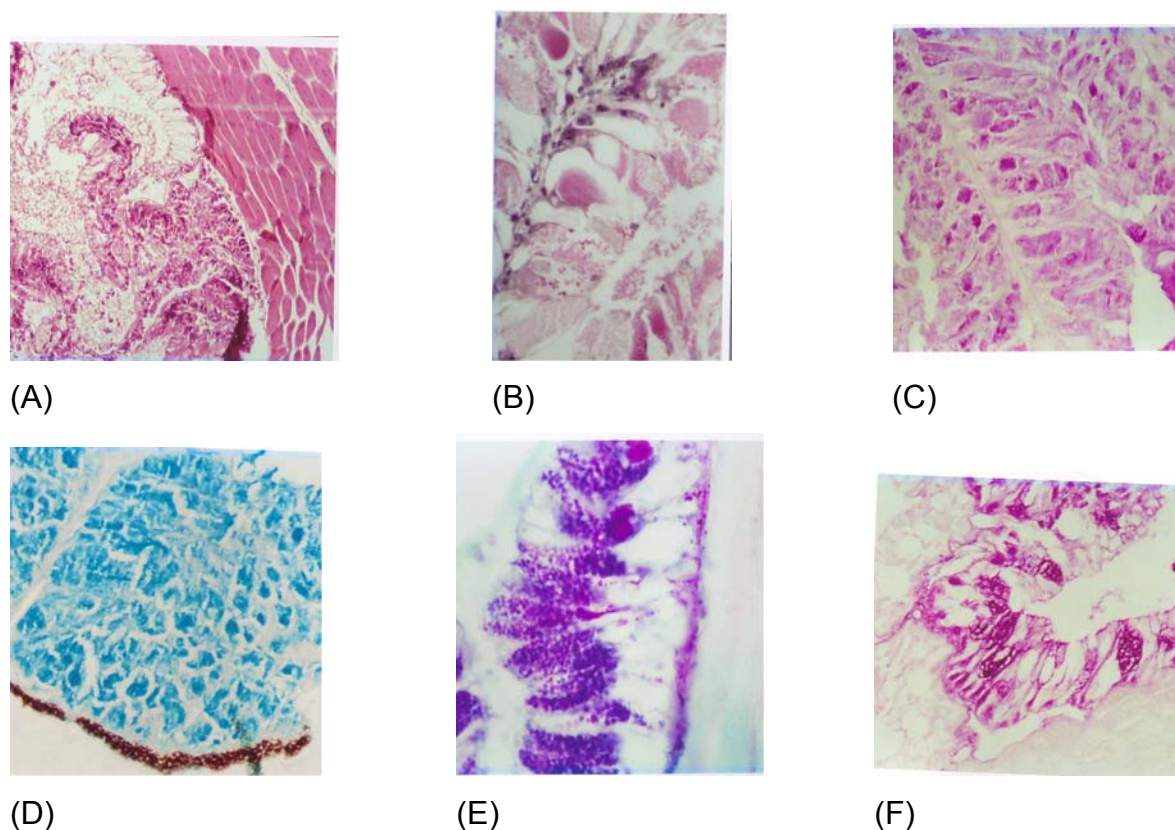


Figure 1. Cross-section of *A. crassicauda* venom glands stained with: (A) hematoxylin-eosin, secretory epithelium covered by striated muscle; (B) hematoxylin-eosin, with venom-producing cells and non-secretory supporting cells; (C) periodic acid-Schiff stain; (D) alcian blue (pH 2.5) stain; (E) periodic acid-Schiff-alcian blue (pH 2.5) stain; (F) toluidine blue (pH 3.4).

Protein Histochemistry

The venom-producing cells reacted positively to ninhydrin-Schiff, chloramine-T Schiff and mercuric bromophenol blue techniques (Figure 2A).

Amino Acid Histochemistry

As shown in Table 1, positive histochemical reactions for cysteine, tyrosine, tryptophan and arginine were seen in both venom-producing cells and luminal-released venom granules (Figure 2).

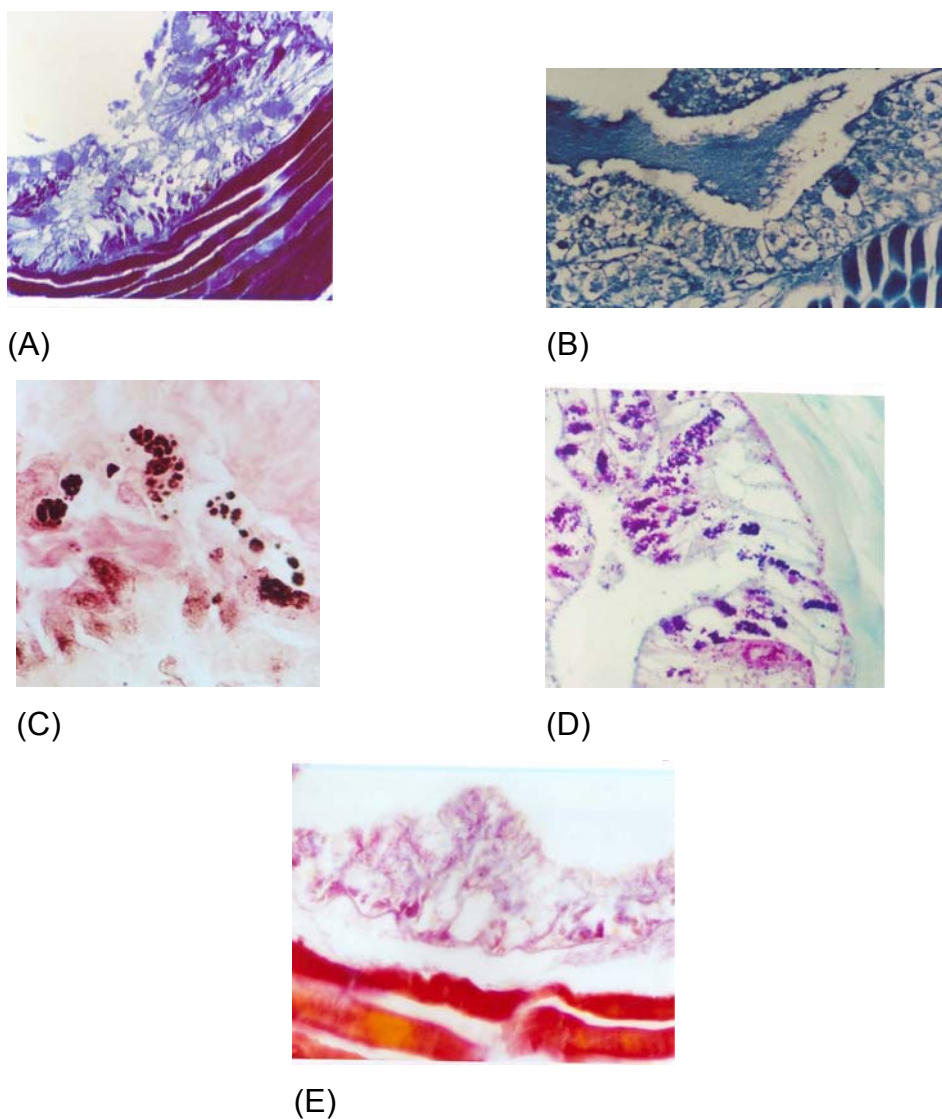


Figure 2. Cross-section of *A. crassicauda* venom glands showing: (A) proteins; (B) cysteine; (C) tyrosine; (D) tryptophan; (E) arginine.

Enzyme Histochemistry

Considerable activity of amino peptidase (Figure 3A) was detected in both venom-producing cells and luminal-released venom granules. Acid phosphatase, mitochondrial ATPase, non-specific esterases and cholinesterase (Figures 3B-3E) were also demonstrated while the alkaline phosphatase activity was feeble while lipase activity was absent.

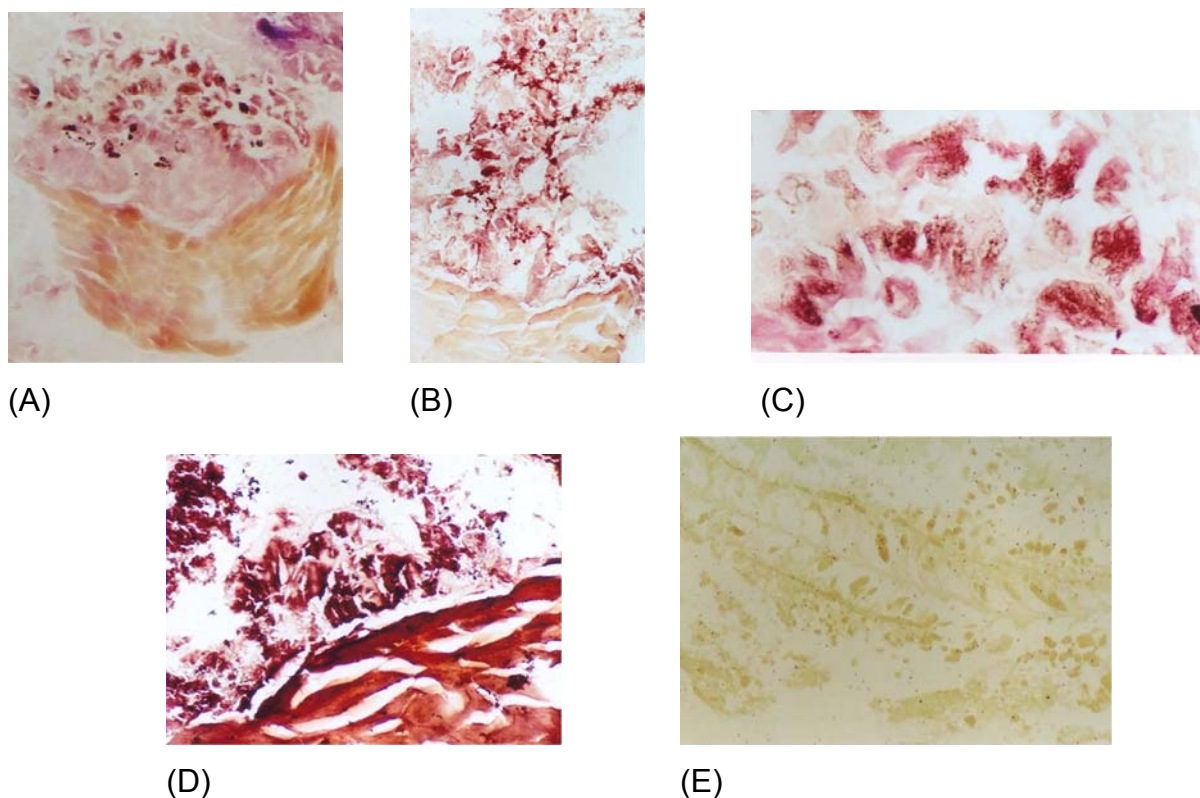


Figure 3. Cross-section of *A. crassicauda* venom glands presenting: (A) aminopeptidase activity; (B) acid phosphatase activity; (C) mitochondrial ATPase activity; (D) non-specific esterase activity; (E) cholinesterase activity.

DISCUSSION

A tentative interpretation of mucosubstance type secreted by the *A. crassicauda* venom gland could be made depending on both the present results and classification basis of mucosubstances, as proposed by various histochemists (9, 18, 22, 32). Thus, in the present study the venom-producing cells of *A. crassicauda* were found to be PAS-positive and diastase-resistant, which indicates that this scorpion species

venom contains neutral mucosubstances and are devoid of glycogen. On the other hand, its glands were positive by the specific techniques of alcian blue and aldehyde fuchsin, and therefore considered organs that secrete acidic mucosubstances. According to Pearse's interpretation (32), *A. crassicauda* venom has been shown to contain neutral mucosubstances, sialidase-labile sialomucins and hyaluronidase-resistant sulphomucins. Neutral mucosubstances have been found in the venom of some other scorpion species and are reported to be abundant in the most dangerous ones (19, 20, 34). The nature of neutral mucosubstances, which are polymers of glucosamine, may indicate a possible role in osmoregulation and in the transfer of venom protein fragments to the victim's tissue. The present investigation's finding that contradicts those from previous studies (19, 27, 34) is the presence of both sialomucins and sulfomucins in the venom of *A. crassicauda*. In this respect, *Leiurus quinquestriatus* venom contains sialomucins but no sulfomucins (34), while the venoms of *Heterometrus scaber* and *Pandinus imperator* include sulfomucins rather than sialomucins (19, 27). Sialomucins and sulfomucins present in the *A. crassicauda* venom, as seen in the present work, may indicate a role in accelerating venom absorption upon injection into the victim.

The histochemical outcomes of the current investigation indicate the presence of cysteine, arginine, tyrosine and tryptophan in *A. crassicauda* venom. Cysteine may provide sulfhydryl groups that can interact to stabilize the venom fractions, while arginine suggests a basic aspect of protein. It is believed that basic proteins are responsible for venom neurotoxic activities that may block sodium channels of excitable cell membranes (11, 17). Tryptophan in *Tityus serrulatus* venom was found to contribute to the binding of the toxin to victim neuronal membranes (21).

The results of the present research demonstrate that *A. crassicauda* venom possesses considerable activity of phosphatase, aminopeptidase, cholinesterase and non-specific esterase. Alkaline phosphatase activity was also revealed in the venoms of *Leiurus quinquestriatus*, *Palamneus gravimanus*, *Hardrurus arizonensis* and *Paroroctonus mesaensis* (27, 29, 30), while proteinase activity was recognized in toxins from *L. quinquestriatus* and *Buthus quinquestriatus* (26, 34). Aminopeptidase may facilitate distribution of other venom components at the injection site. Phosphatases and esterases in scorpion venom may play a part in the victim's shock through a cardiac reflex while cholinesterases inhibit muscle control by affecting the neuron end-plates, thereby immobilizing small prey (2). The activity of some other

enzymes such as hyaluronidase and phospholipase was detected from the venom of other scorpion species (33). Hyaluronidase aids in separating acidic mucosubstances of connective tissues, which quickens the venom absorption in the victim while phospholipase decomposes membrane phospholipids of the target cells. However, the real role of these venom enzymes remains unknown and requires more clarification.

Additional histochemical investigations are needed on the venom of scorpions to identify their toxic and non-toxic antigenic components.

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