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SOME TOXIC, PHYSICAL AND CHEMICAL PROPERTIES OF THE ORAL SECRETION OF THE SAND TAMPAN, *ORNITHODOROS SAVIGNYI* AUDOUIN (1827)

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ABSTRACT

HOWELL, C. J., NEITZ, A. W. H. and POTGIETER, D. J. J., 1975. Some toxic, physical and chemical properties of the oral secretion of the sand tampan, *Ornithodoros savignyi* Audouin (1827). *Onderstepoort J. vet. Res.*, 42 (3), 99–102 (1975).

Mice, exposed to the effects of the oral secretion of *Ornithodorus savignyi* by injection, revealed that a potent toxin is present in the secretion. Electrophoretic and chromatographic separations of the secretion showed a heterogeneous pattern of protein-like substances. Toxicity trials on these fractions showed that the toxic principle is protein in nature and fairly heat stable.

INTRODUCTION

The role played by ticks in the transmission of a variety of diseases in man and his domestic stock is well known. The disease conditions which arise from tick bites may result from the introduction of pathogenic micro-organisms into the tissues of the host or they may be due to the oral secretions of the arthropods injected into the host during the process of feeding. These oral secretions have been shown to be responsible for paralysis in man and animals, as well as for a variety of "tick toxicoses", where micro-organisms are not involved (Ross, 1926, Theiler, 1950; Neitz, 1956).

The salivary glands from which these secretions arise have been studied in several different species (Arthur, 1962) and outstanding contributions in obtaining and measuring the quantity of secretions are those of Gregson (1957). It has been shown by Howell (1966) that the use of a parasympathetic stimulant provides fairly large quantities of material for further examination.

The sand tampan *Ornithodoros savignyi*, a sand-dwelling argasid confined to the arid North Western Cape Kalahari and South-West Africa, is prevalent wherever suitable sandy soil is present. Numerous reports from, and investigations in these areas have shown that the ticks are frequently associated with mortality in stock. Young calves and sheep appear to be particularly susceptible to their bites and have to be kept away from heavily infested corrals or resting places.

This tick is not known to transmit any infectious disease. Hence it is apparently free from pathogenic micro-organisms and ideally suited to studies on the biological and chemical properties of the oral secretion.

A method for the isolation of the toxin present in the salivary secretion has been described (Neitz, Howell & Potgieter, 1969). However, no data on the toxicity or on the physical and chemical properties of the secretion have been reported. In this paper some of these data are presented.

MATERIALS AND METHODS

Collection of ticks and oral secretion

The ticks used were collected in the Bray area of the North Western Cape Province by means of traps baited with solidified CO_2 (dry ice) (Nevill, 1964). Visits to the area were undertaken every 3 months for the purpose of trapping fresh specimens, of which only adult females were used for the collection of oral secretion. These were kept in a room controlled at 26 °C with a relative humidity of 85%.

The collection of oral secretion is based on the stimulation of the nervous centre in the tick by the introduction of pilocarpine hydrochloride* into the haemolymph (Howell, 1966).

Determination of LD_{50}

The method described by Weil (1952) was used. To determine the end-point of activity serial two-fold dilutions of fresh oral secretion, which ranged from 1:2 to 1:256 were prepared in distilled water. Three-week-old mice were injected subcutaneously at a dosage rate of 0,05 ml per 10 g body mass, using 5 mice for each dilution. The highest dilution which caused death was 1:16. A geometric dilution series which ranged from 1:8 to 1:64 was selected for determining the LD_{50} and 10 mice were used per dilution.

The dosage rate and route were identical to the above. Deaths were recorded over a period of 24 hours. Oral secretion stored at 0 °C in a refrigerator for 3 months was compared with fresh material in a similar experiment.

Paper and thin layer chromatography

Whatman No. 1 filter paper and silica gel G** were used for ascending chromatography.

Isopropanol: acetic acid: water 8:1:1 was used for fresh material, and n-butanol: acetic acid: water 4:1:5 for samples hydrolyzed at 100 °C with 6 N HCl for 18 hours. For descending chromatography, phenol: water 3:1 was used. Staining was achieved with 0,1% ninhydrin in butanol or 0,2% ninhydrin with 10% (v/v) glacial acetic acid in butanol.

Monitoring for toxicity of the spots on the filter paper was done by staining only the side of the paper for identification of the spots. The unstained portions were then cut out and ground to a pulp in small quantities of distilled water. The thin layer spots were similarly identified and then scraped off in water. The samples thus obtained were injected subcutaneously into mice to establish the toxicity of the different spots and the influence of hydrolysis on the oral secretion.

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* British Drug Houses

** Kiesel gel G Merck

Nitrogen determination

Nitrogen was determined by the micro-Kjeldahl method (McKenzie & Wallace, 1954). The non-protein nitrogen of the secretion was determined on the supernatant liquid after precipitation of the protein with 1% picric acid (Stein & Moore, 1954).

Quantitative determination of the total and free amino acids

For the quantitative determination of the total amino acids, 0,25 ml oral secretion was introduced into a 15×0,75 cm glass tube and freeze-dried. The lyophilisate was suspended in 1 ml glass-distilled 6N HCl. The tube was then inserted into a bath of solid carbon dioxide and acetone. After the sample had frozen, the tube was evacuated with a water-jet pump, and sealed under vacuum.

Hydrolysis was performed at 110 °C for 18, 30 and 70 h on 3 samples. After the hydrolysis period, the tube was cooled to room temperature, opened and the pH adjusted to 2,0 with 10N NaOH. The sample was then transferred to a 10 ml volumetric flask with 0,2N sodium citrate pH 2,2 and made up to volume. One-tenth aliquots were analysed on a Beckman Model 120B amino acid analyzer. Tryptophan was determined spectrophotometrically on the original oral secretion (Beaven & Holiday, 1952). Methionine, determined as methionine sulphone, and cysteine, together with cystine as cysteic acid, were determined in an oxidised hydrolysate prepared in the following way: a 1 ml oral secretion sample was oxidised by the method described by Weindner & Eggum (1966) and hydrolyzed with 150 ml 6N HCl. The free amino acids were determined on 1 ml samples of the protein-free supernatant prepared as described for non-protein nitrogen. Synthetic mixtures of amino acids were analyzed before and after analysis of the samples.

Ultra-violet spectrum

A Beckman DK2A ratio recording spectrophotometer was used with 1 cm light path length silica cells.

Microzone electrophoresis

Microzone electrophoresis of freshly collected oral secretion was carried out at pH 8,6 (sodium barbital buffer, ionic strength 0,075) in a Beckman microzone electrophoresis apparatus on cellulose acetate membranes. A constant voltage of 250 V for 30 min. was employed. The strips were fixed and stained in Ponceau S solution and scanned with a Beckman Densitometer.

Results

General characteristics of the oral secretion

The fresh oral secretion has a colourless appearance, is somewhat viscous and tends to form a persistent froth when shaken in a container. It is highly fluorescent, and the fluorescence appears to increase with age. The secretion has an average specific gravity of 1,0236 and an average pH of 7,9. The water content is 95,7%. The toxin is heat-stable to approximately 80 °C as shown in Table 1.

Toxicity

The LD_{50} for mice of the fresh oral secretion was found to be 0,206 ml/kg body mass while that of the 3 months old material was 0,220 ml/kg. The difference could be due to either a slight loss of toxicity of the oral secretion with ageing, or to experimental error.

TABLE 1 Residual toxicity of the oral secretion after exposure to elevated temperatures for 15 minutes

Temperature	Average survival time of mice
55 °C	8 min
60 °C	7 min
65 °C	8 min
70 °C	8 min
75 °C	14 min
80 °C	19 min
85 °C	Alive after 24 h

TABLE 2 Total amino acid composition of the oral secretion

Amino acids	Hydrolysis time (hours)			Corrected values	Contribution of amino acids to total nitrogen
	18	30	70		
	mg/ml	mg/ml	mg/ml	mg/ml	mg
Lysine	2,006	1,887	2,064	1,982	0,380
Histidine	0,458	0,454	0,478	0,463	0,125
Ammonia	0,647	0,673	0,842	0,647	0,533
Arginine	0,888	0,878	0,808	0,858	0,276
Cysteic acid	1,006	—	—	1,006	0,083
Aspartic acid	2,401	2,225	2,257	2,294	0,241
Methionine sulphone	0,435	—	—	0,435	0,034
Threonine	0,857	0,591	0,067	1,496	0,176
Serine	0,420	0,224	completely destroyed	1,078	0,144
Glutamic acid	2,777	2,512	2,671	2,653	0,253
Proline	0,087	0,103	0,105	0,105	0,013
Glycine	1,322	1,205	1,304	1,277	0,238
Alanine	0,813	0,756	0,816	0,795	0,125
Valine	1,307	1,237	1,316	1,287	0,154
Isoleucine	0,520	0,488	0,439	0,571	0,061
Leucine	1,225	1,197	1,286	1,236	0,132
Tyrozine	1,283	1,131	0,681	1,551	0,120
Phenylalanine	0,773	0,760	0,767	0,767	0,065
Tryptophan	—	—	—	0,692	0,095
					3,248

Paper and thin layer chromatography

Paper and thin layer chromatography of both fresh and hydrolyzed oral secretion gave rise to 7 coloured spots which stained either purple or various tones of purple, pink and brown. An unstained fluorescent spot was always present above the highest spot. Saliva 90 days old gave rise to an 8th spot which developed about midway along the run. Elution of the different components proved that the purple staining spot at the origin of the run was associated with the toxicity, Hydrolysis destroyed the toxic effect.

Nitrogen content

The total nitrogen content was found to be 3,64 mg/ml. Assuming that all this nitrogen is of protein origin containing 16% N, the total protein in the oral secretion is 22,77 mg/ml. The non-protein nitrogen was found to be 0,488 mg/ml.

Total and free amino acid content

The total and free amino acid content is summarized in Tables 2 and 3 respectively. Corrections were made for the loss of threonine, serine, isoleucine and tyrosine (Moore & Stein, 1963). Proline continued to increase through 70h of hydrolysis and this value was assumed to represent essentially complete liberation. The increase of the ammonia is adequately accounted for by destruction of threonine, serine, isoleucine and tyrosine. The contribution of the free amino acids to the non-protein nitrogen (0,488 mg/ml) is 0,117 mg/ml, indicating that 0,371 mg/ml of the total nitrogen in the oral secretion (3,643 mg/ml) is contributed to by components other than protein, amino acids or ammonia. Taking this into account the nitrogen recovery of the total amino acid analysis is 99,3%.

TABLE 3 Free amino acids present in the oral secretion

Amino acids	μg/ml	Contribution of amino acids to non-protein nitrogen μg
Lysine	98,904	18,950
Histidine	19,632	5,313
Ammonia	65,263	53,744
Threonine	42,578	5,007
Serine	4,624	00,616
Glycine	4,544	0,848
Alanine	17,641	2,773
Valine	68,269	8,165
Isoleucine	34,637	3,700
Leucine	77,933	8,323
Tyrosine	63,782	4,930
Phenylalanine	55,425	4,700
		117,074

Ultra-violet absorption spectrum

The ultra-violet absorption spectrum shows an absorption peak at 275 nm (Fig. 1) indicating the possible presence of proteins.

Microzone electrophoresis

Four major bands were detected on cellulose acetate strips. Fig. 2 shows a scanned strip of fresh oral secretion compared with that of bovine serum.

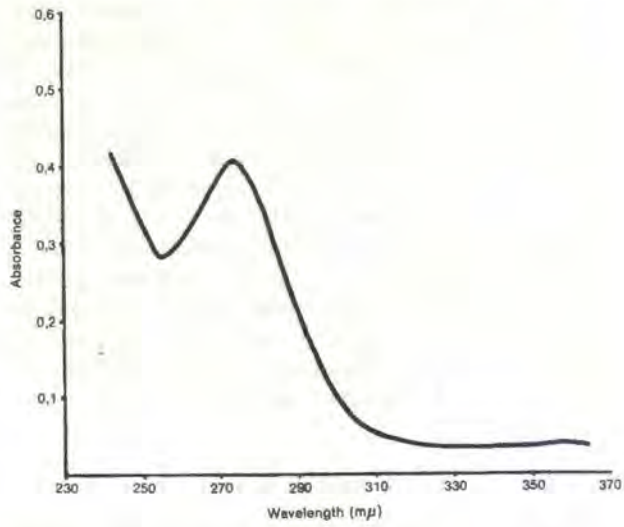

FIG. 1 Ultra-violet absorption spectrum of 0,05 ml oral secretion, diluted to 3 ml with 0,02 M Tris, 0,08 M NaCl buffer, pH 7,9.

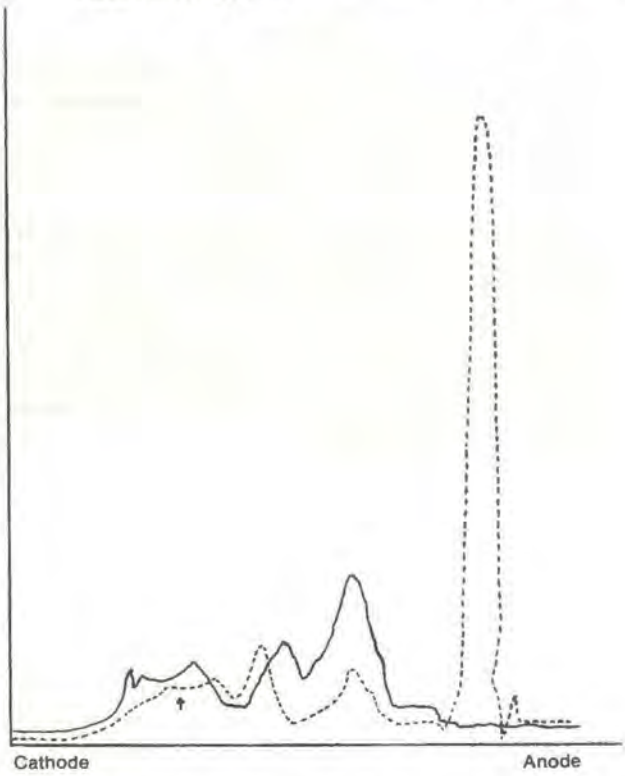

FIG. 2 Electrophoretic comparison of the oral secretion with blood serum. A densitometer trace of the electrophoresis strip stained with Ponceau S. The arrow indicates the origin. The dashed line represents the blood serum.

Discussion

The consensus of opinion hitherto was that sand tampans caused death by exsanguination of their hosts (Hoogstraal, 1956; Du Toit & Theiler, 1964). The first author stated that "Camels and cattle suffer greatly and may even be killed by the volume of blood lost to numbers of eyed tampans in their pens". The development of a technique to obtain oral secretions from this argasid tick, however, provided the opportunity to prove that this material is highly toxic. Parenteral inoculation of the crude oral secretion into test animals clearly illustrated its lethal effects under conditions where exsanguination played no role.

The oral secretion has a high nitrogen content, most of which is protein nitrogen. Electrophoresis and chromatography show the presence of at least 10 different protein fractions, only 1 of which is toxic. Investigations so far reveal that the toxic principle is of protein nature and heat-stable to approximately 80 °C. The remaining fractions, although not toxic as such, may play an important role in the syndrome produced by the secretion. It is possible that some of these fractions, the toxic fraction included, possess enzymatic activities, as in snake venoms. Preliminary investigations have indeed shown the presence of proteolytic and cholinesterase activities in the oral secretion (A. W. H. Neitz, unpublished data).

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Résumé

HOWELL, C. J., NEITZ, A. W. H. & POTGIETER, D. J. J., 1975. *Quelques propriétés physiques et chimiques de la sécrétion orale de la tique Ornithodoros savignyi Audouin* (1827). *Onderstepoort J. vet. Res.* 42 (3), 99–102 (1975)

Injectant des souris avec la sécrétion orale de O. savignyi, *les auteurs ont pu démontrer la présence d'une toxine puissante. La séparation en électrophorèse et sur chromatographie de cette sécrétion a permis de mettre en évidence certains aspects hétérogènes des substances protéiques. Soumettant ces fractions aux essais de toxicité, le propriété toxique s'est révélé être de nature protéique et thermostable.*

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