HISTOCHEMICAL STUDIES ON *ARGAS (ARGAS) HERMANNI* (IXODOIDEA: ARGASIDAE) SALIVARY GLANDS

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دراسات كيميائية نسيجية على الغدد اللعابية في أرجاس
(أرجاس) هرماناي (اكسودويديا : أرجاسيدى)

جليلة مـــصطفى خليل   و   عليـــــــة مـــــرزوق
مـحـمـود عـزت مـهلل   و   حصة يوسف جاسم آل ثاني

تمت دراسـة عن مـحـتوى خـلايا الغـدد اللعـابيـة من البروتينات الأسـاسـيـة والتريبتوفـان والتيروسين والمجموعتين الأمينية والهيدروكبريتية الحرتين والفحميات والجليكوجين والمخاطيات متعددة التسكر الحمضية والدهون ، أثناء تطور طوري الحوريات والحيوان البالغ للقراد أرجاس (أرجاس) هيرماناي أودين. وقد لوحظ أن منطقة الحافة المخططة فقط في الحجيرات غير المحببة (النوع الأول) من الغدد قد تفاعلت إيجابياً للبروتينات والدهون بينما تفاعلت الحبيبات الدقيقة في تلك المنطقة إيجابياً للجليكوجين. وقد تفاعل هذه الحجيرات سلبياً للمجـمـوعتين الأمـينية والهيدروكبريتية الحرتين والتريبتوفان والتيروسين والمخاطيات متعددة التسكر الحمضية. وعلى الأرجح أن هذه التفاعلات ذات علاقة بالصفات التركيبية للحجيرات ودورها في عملية التنظيم الأسموزي ونقل السوائل.
أمـا في الحجيرات ذات الحبيبات (النوع الثاني) فلم يتم ملاحظة وجود الجليكوجين أو المخـاطيـات عديدة التسكر الحمـضية في أي من الأنواع وتحت الأنواع الـسـبـعـة من الخـلايا الموجودة بتلك الحجيرات. ويرجح وجود التفـاعـلات المتجـانسـة في عظم الحـالات للأنواع (أ١) (هـ١) من الخـلايا أن كـلا منهـا نوع حقيقي مميز يحتـوي على كريات مكتملة التكوين. أمـا تفـاعـلات كـريات النوع (أ٢) من الخـلايا – والتي اخـتلفت في النوع الواحـد من بعض المواد المختبرة – فترجح أن هذه الكريات في مراحل مخـتلفة من التكوين. كذلك رجحت النتائج أن كريات الخلايا من النوعين (ب) و (ج) ذات تكوين مركب يشمل البروتينات والفحميات والدهون. أمـا تفـاعـلات الكريات في الخـلايا من النوعين (د) و (هـ٢) فقـد تبـاينت كـثـيـراً في جـمـيـع الاختبارات، وكذلك ظهرت حبيبات أو فقاعات بعد التجويع أو بعد الاغتذاء في خلايا بها كريات ذات تفاعلات مختلفة، مما يرجح وجود آليات مختلفة لعملية الإفراز وأن هذه الخلايا قد تنتمي لأنواع مختلفة. وقد تقوم هذه الخلايا بإفراز مواد مانعة للتجلط. كذلك قد تقوم الخـلايا من الأنواع (أ٢) و (د) و (هـ٢) – وكلها تحتوي على التريبتوفان – بتكوين إفرازات محللة وأخرى ذات تأثير كالعقاقير على العائل.

Key Words: Ixodoidea, Argasidae, *Argas hermanni,* Histochemistry, Salivary glands.

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ABSTRACT

Basic proteins, tryptophan, tyrosine, free NH_2 and SH groups, general carbohydrates, glycogen, acid mucopolysaccharides and lipids were investigated during nymphal and adult *Argas (Argas) hermanni* Audouin development. In the agranular type I alveoli, only the striated peripheral part reacted positively for proteins and lipids and the fine granules were glycogen-positive. These alveoli reacted negatively for NH_2 and SH groups, tryptophan, tyrosine and acid mucopolysaccharides. The staining reactions of these alveoli were probably related to their structural characteristics and role in osmoregulation and fluid transport. No acid mucopolysaccharides or glycogen were detected in any of the 7 cell types and subtypes of the granular type II alveoli. The almost uniform reactions, of type a_1 and e_1 cells in all tests suggested that each of them represented a true single cell type probably containing mature globules. The varied reactions of type a_2 cell globules for the same compound suggested that these globules were at different stages of development. Type b and c cell globules were probably of composite nature, containing proteins, carbohydrates and lipids. The globules in type d and e_2 cells varied greatly in their reactions in all tests. Also, granules and vacuoles appearing during starvation or after feeding in cells with different reactions for the same compound suggested different excretion mechanisms and a multiple nature of these cell types. These cells might produce an anticoagulant. Also, these tryptophan-containing cells, as well as type a_2 cells might produce lytic and pharmacologically active secretions.

INTRODUCTION

In a previous study, 2 alveolar types were observed in *Argas (Argas) hermanni* Adouin salivary glands [1]. Type I agranular alveoli consisted of several cells forming a striated peripheral zone around a clear central cell, and their activity was related to osmoregulation and fluid transport. Type II granule-secreting alveoli consisted of 7 cell types and subtypes containing globules of various staining reactions (Zenker-formol fixation and Harris' hematoxylin and eosin), sizes and/or shape. Type a_1 and a_2 cells contained small and large globules, respectively, which were rounded and bright red in color. Type b cells were packed with cup-shaped purple globules and type c cells globules possessed a translucent colorless, purple or bluish purple peripheral zone and a deep purplish blue central core. Type d cell globules were colorless and homogeneous. Type e_1 and e_2 cells contained small and large globules respectively, which were blue or bluish purple. In this work, we investigate certain histochemical properties of the cellular components of *A. hermanni* salivary glands with the aim to understand their functions.

MATERIALS AND METHODS

An *A. hermanni* colony originating from ticks collected from a domestic pigeon *(Columba livia)* house in Suez, Egypt was maintained in an incubator at 28 ± 1°C and 75% RH, and domestic pigeons were used as hosts.

The salivary glands of unfed first (N_1) and second-instar (N_2) nymphs, 2 weeks and 6 weeks postmolting, of adult males and females 1 and 2 months postmolting, and of fed N_1, N_2, males and females within 2 hr postfeeding were investigated. The dorsal cuticle was removed while the ticks were flooded with 0.7% saline solution before fixation in buffered neutral 10% formalin. After dehydration in an ascending series of ethyl alcohol and double embedding in celloidin-paraplast, serial sections, 5 μm thick, were prepared and each of 4 consecutive sections was placed on a slide. One section was stained with Harris' hematoxylin and eosin (as a reference for cell types) while the other 3 sections were stained to detect the histochemical composition as described below, and then counter stained with hematoxylin and eosin or hematoxylin only or was not counter stained. The sections were then examined in pairs to match the serial sections of the same cell on consecutive slides using a comparison American Optical (USA) microscope.

General carbohydrates and glycogen were investigated by the periodic acid-Schiff (PAS) technique with and without previous treatment of the sections with human saliva at 37°C for 1 hr [2]. Acid mucopolysaccharides (metachromasia) were tested by the Kramer and Windrum toluidine blue technique with sections in frog intestine being stained simultaneously as a control [3]. Basic proteins were detected by the modified mercuric-bromophenol blue method [3] and tryptophan by the Rosindole reaction with rat pancreas sections being stained simultaneously as a control [2]. Tyrosine was investigated by the Millon's reaction [4], free-SH groups by the ferric ferricyanide method and free-NH_2 groups by the Yasuma Itchikawa ninhydrin-Schiff method [2].

RESULTS

When the different unfed and fed stages were tested, no change was observed in the staining reactions of any of the cellular components of both alveolar types during their development. Figures 1-45 illustrate most of the reactions observed in this study.
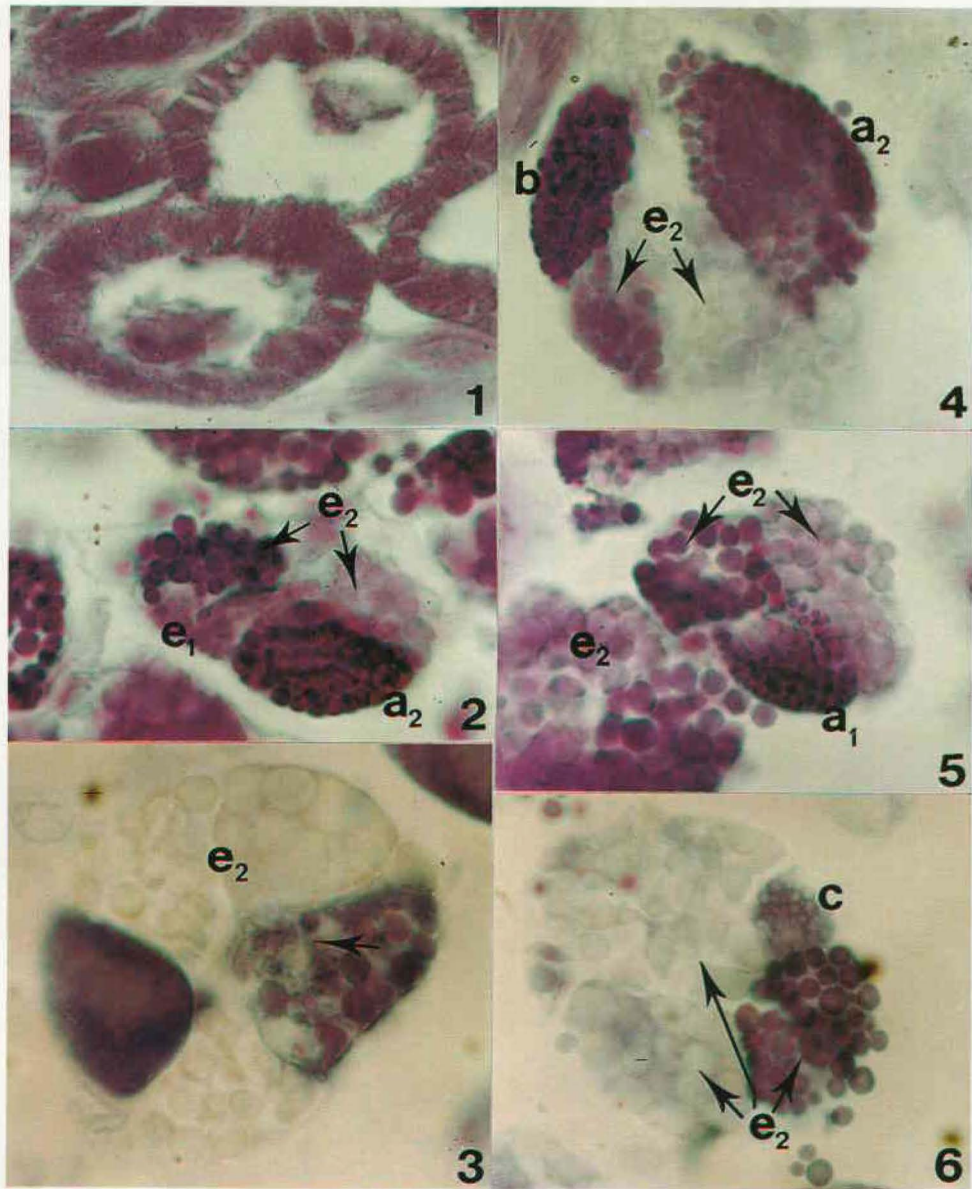
Type I alveoli: (Table 1)

Table 1

Reactions of Type I salivary alveoli in *Argas hermanni* stained for proteins, carbohydrates, lipids and free NH_2 groups*

| Zone or organelle	Proteins	NH_2	Carbohydrates	Lipids
Striated zone:				
Membranes	++**	±	±	+++
granules	++	±	+++***	+++
Central zone:	-	-	-	-

* All alveolar components reacted negatively for tryptophan, tyrosine, free SH groups and acid mucopolysaccharides.

** - : negative, ± : weakly positive, ++ : strongly positive, +++ : very strongly positive.

*** After treatment with human saliva, only the granules reacted negatively for carbohydrates, i.e., very strongly positive for glycogen.
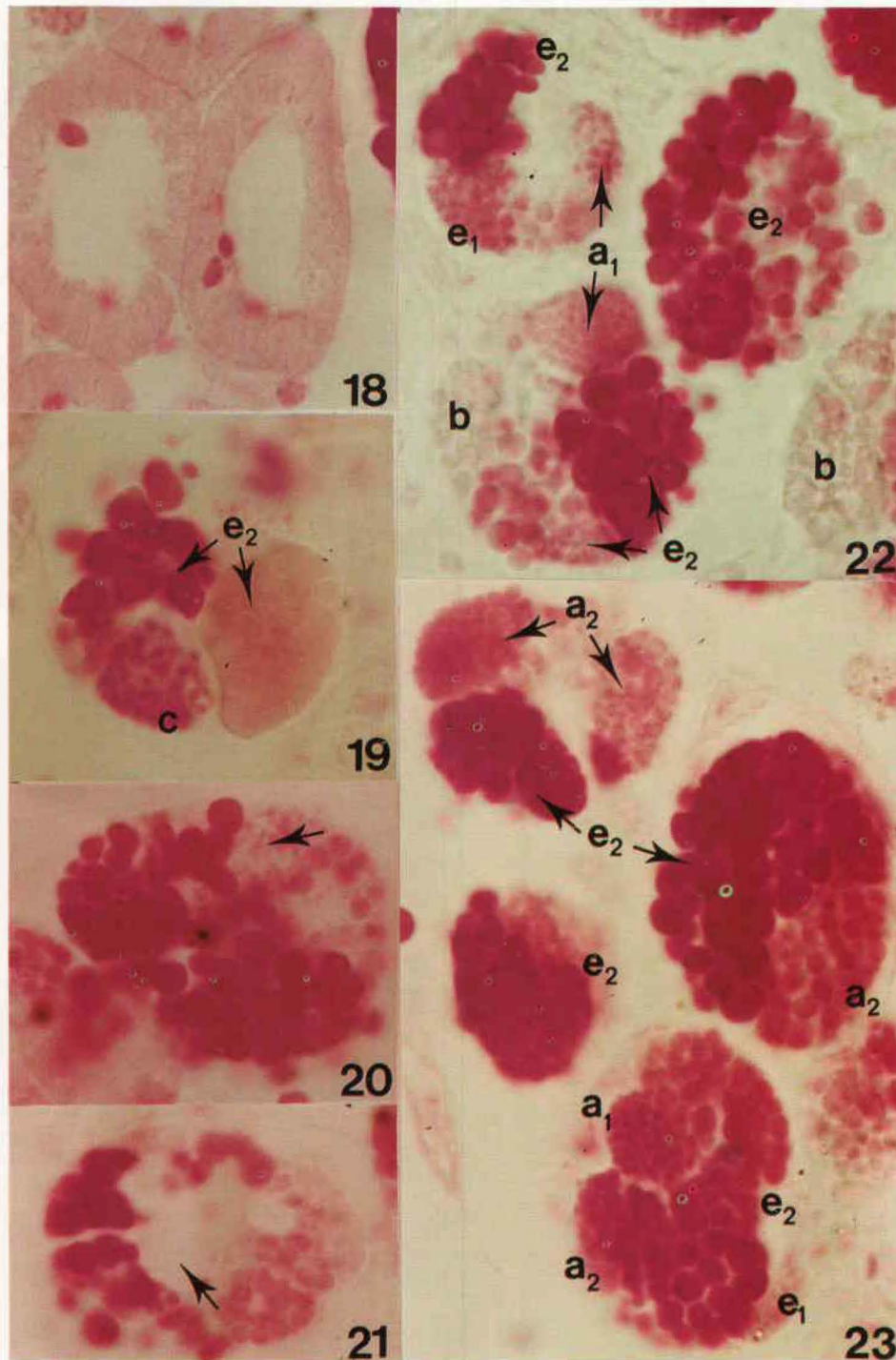
Figs. 1-6: Sections in *A. hermanni* salivary glands stained with mercuric-bromophenol blue for basic proteins: 1. Type I alveoli; 2, 4-6. Type II alveoli showing different cell-types; 3. granules among positive globules in a type e_2 cell (arrow). (X 1000).
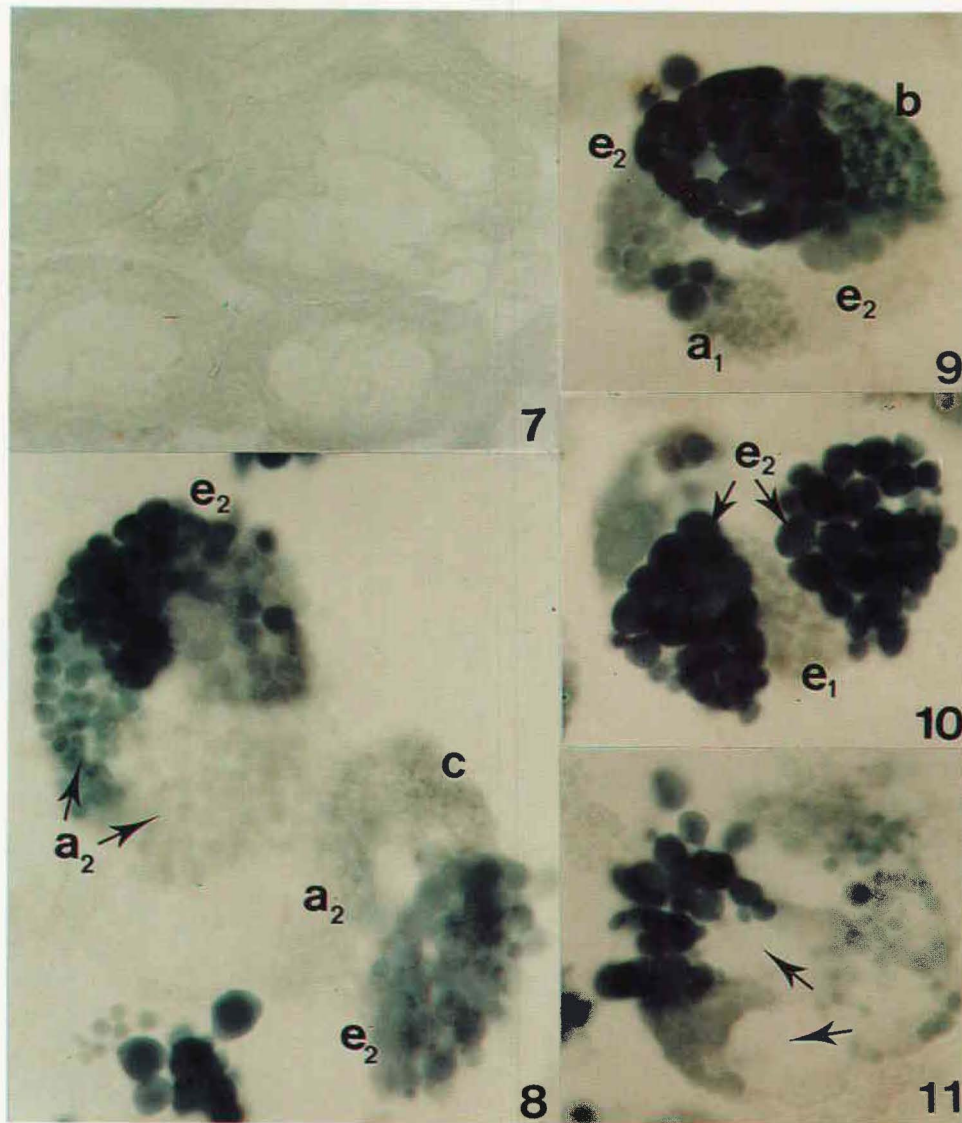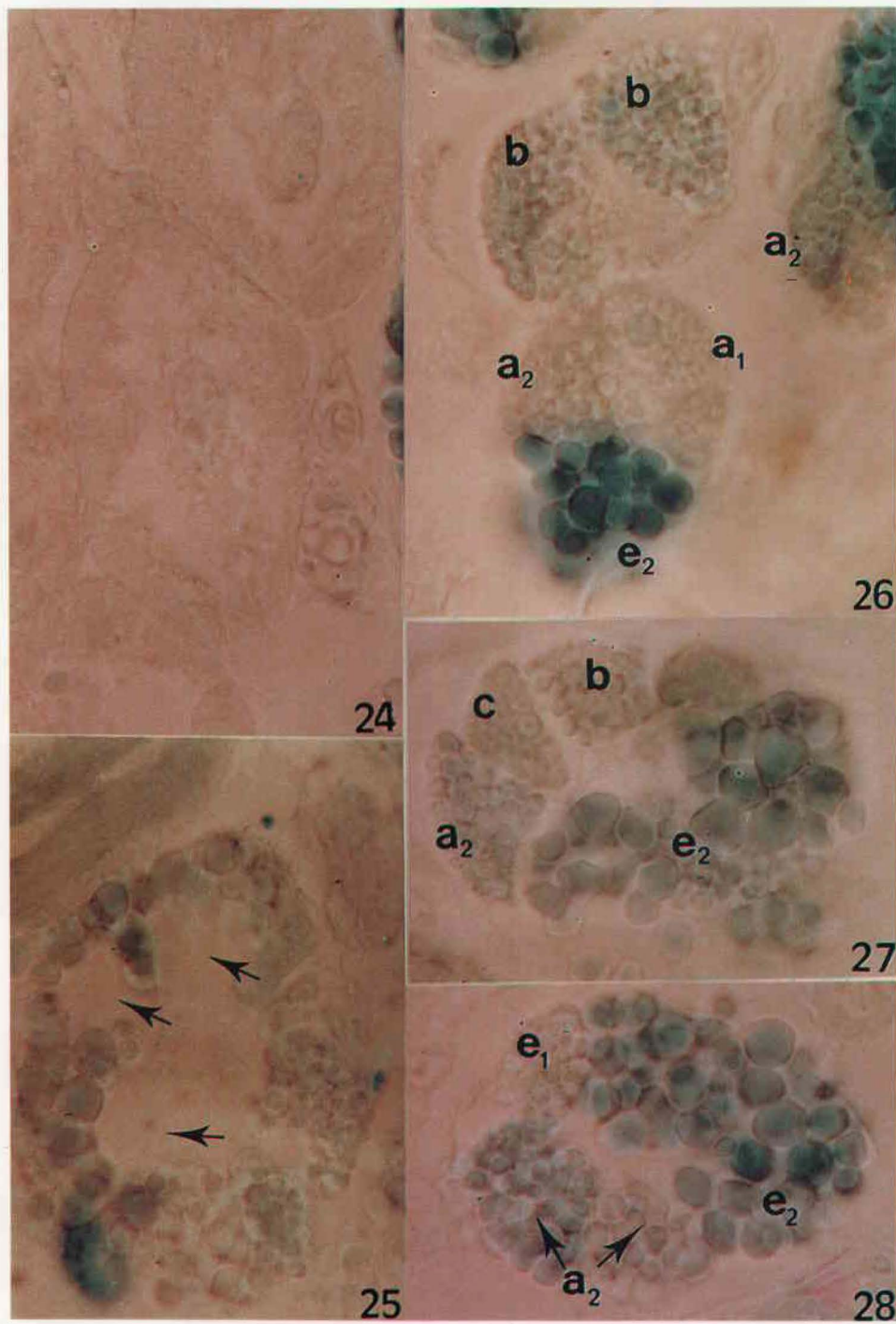
Figs. 7-11: Sections in *A. hermanni* salivary glands stained by the Rosindole method for tryptophan: 7. Type I alveoli; 8-10. Type II alveoli showing different cell types; 11. Vacuoles among positive type e_2 globules (arrows) from a fed nymph. (X 1000).
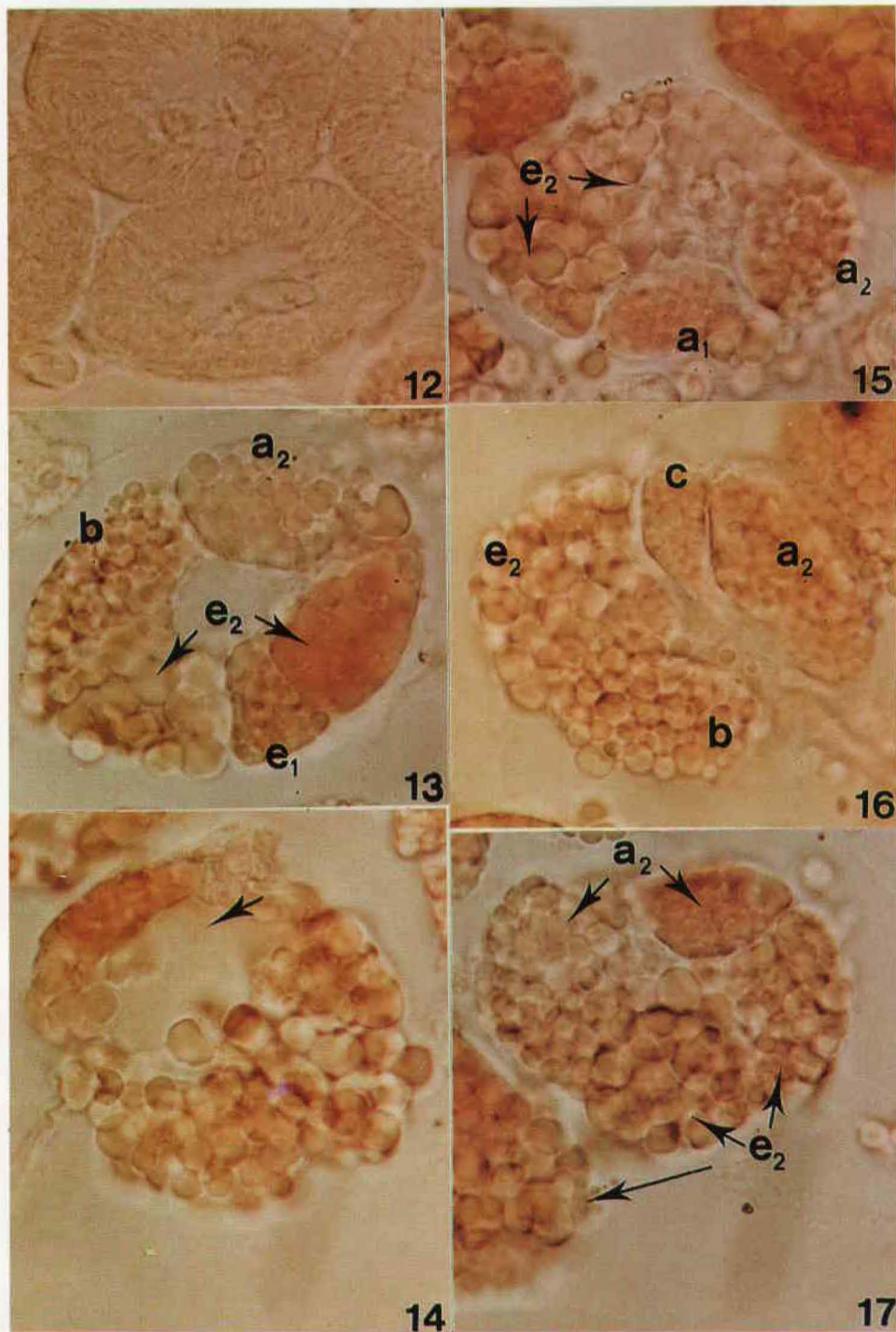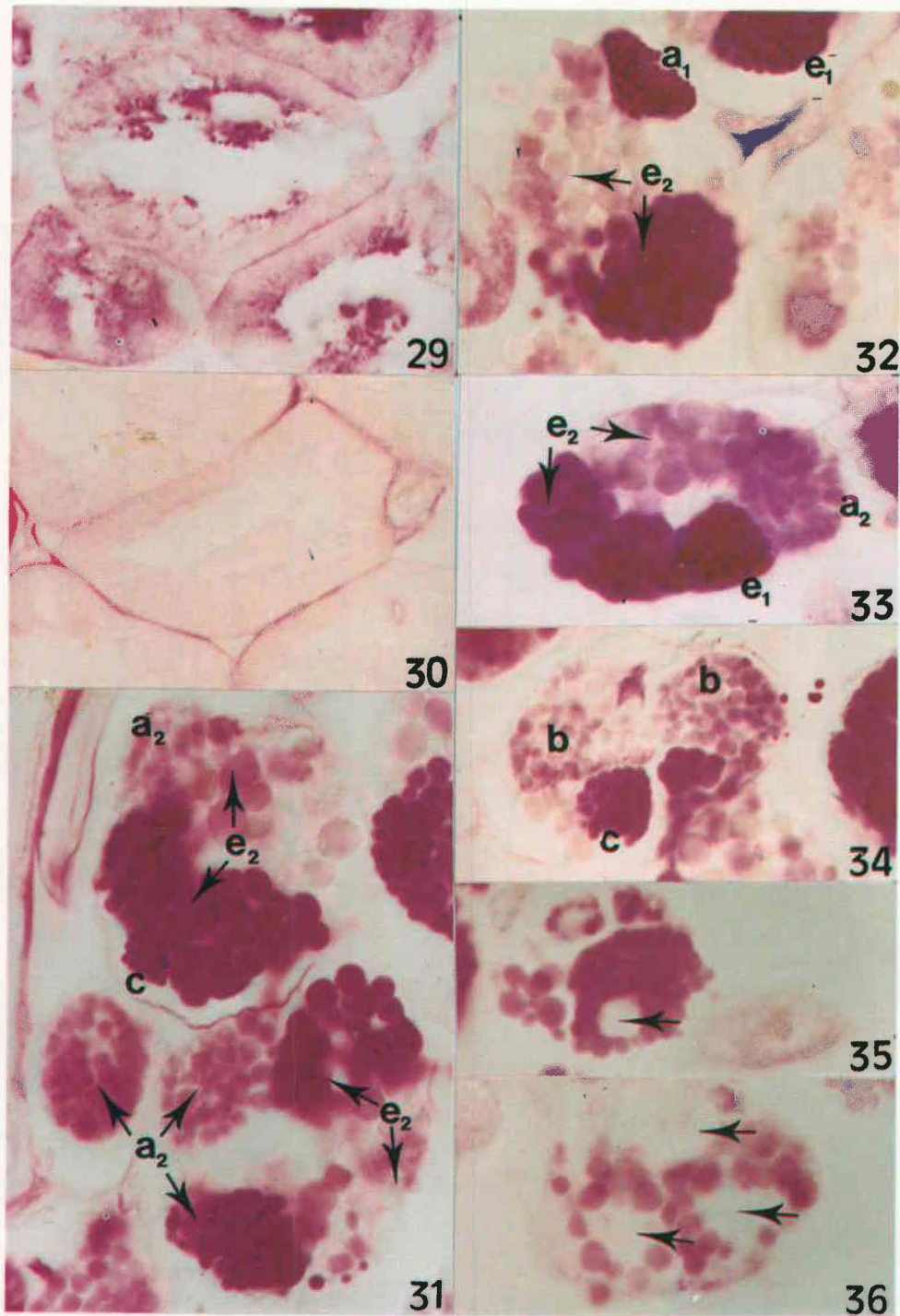
Figs. 12-17: Sections in *A. hermanni* salivary glands stained by the Millon's method for tyrosine: 12. Type I alveoli; 13, 15-17. Type II alveoli showing different cell types; 14. A vacuole in type e_2 cell reacting positively (arrow). (X 1187).
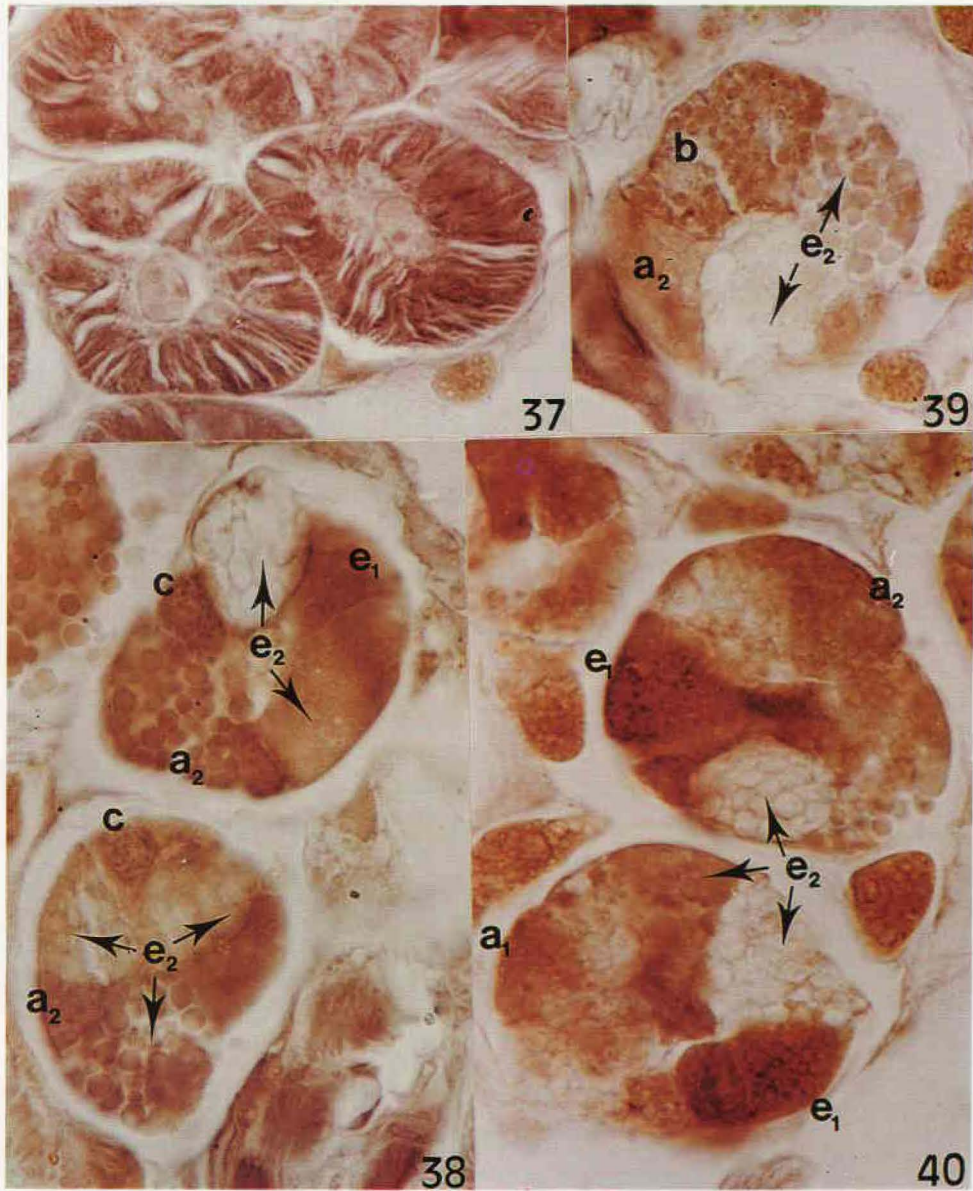
Figs. 18-23: Sections in *A. hermanni* salivary glands stained by the ninhydrin-Schiff method for free NH_2 groups: 18. Type I alveoli; 19, 22-23. Type II alveoli showing different cell types; 20. Granules in type e_2 cells reacting positively (arrow). 21. Vacuoles in type e_2 cells reacting positively. (X 1000).

Figs. 24-28: Sections in *A. hermanni* salivary glands stained by the ferric ferricyanide method for free SH groups: 24. Type I alveoli; 25. Vacuoles in type e_2 cells containing globules reacting negatively to moderately positive (arrows), from a fed adult; 26-28. Type II alveoli showing different cell types. (X 1187).

Figs. 29-36: Sections in *A. hermanni* salivary glands stained by the periodic acid-Schiff method for general carbohydrates: 29. Type I alveoli; 30. Type I alveoli stained after treatment with human saliva for 1 hr at 37°C; 31-34. Type II alveoli showing different cell types; 35, 36. Vacuoles in type e_2 cells containing globules reacting weakly to strongly positive. (X 1000).

Figs. 37-40: Sections in *A. hermanni* salivary glands stained with osmic acid for lipids: 37. Type I alveoli; 38-40. Type II alveoli showing different cell types. (X 1000).
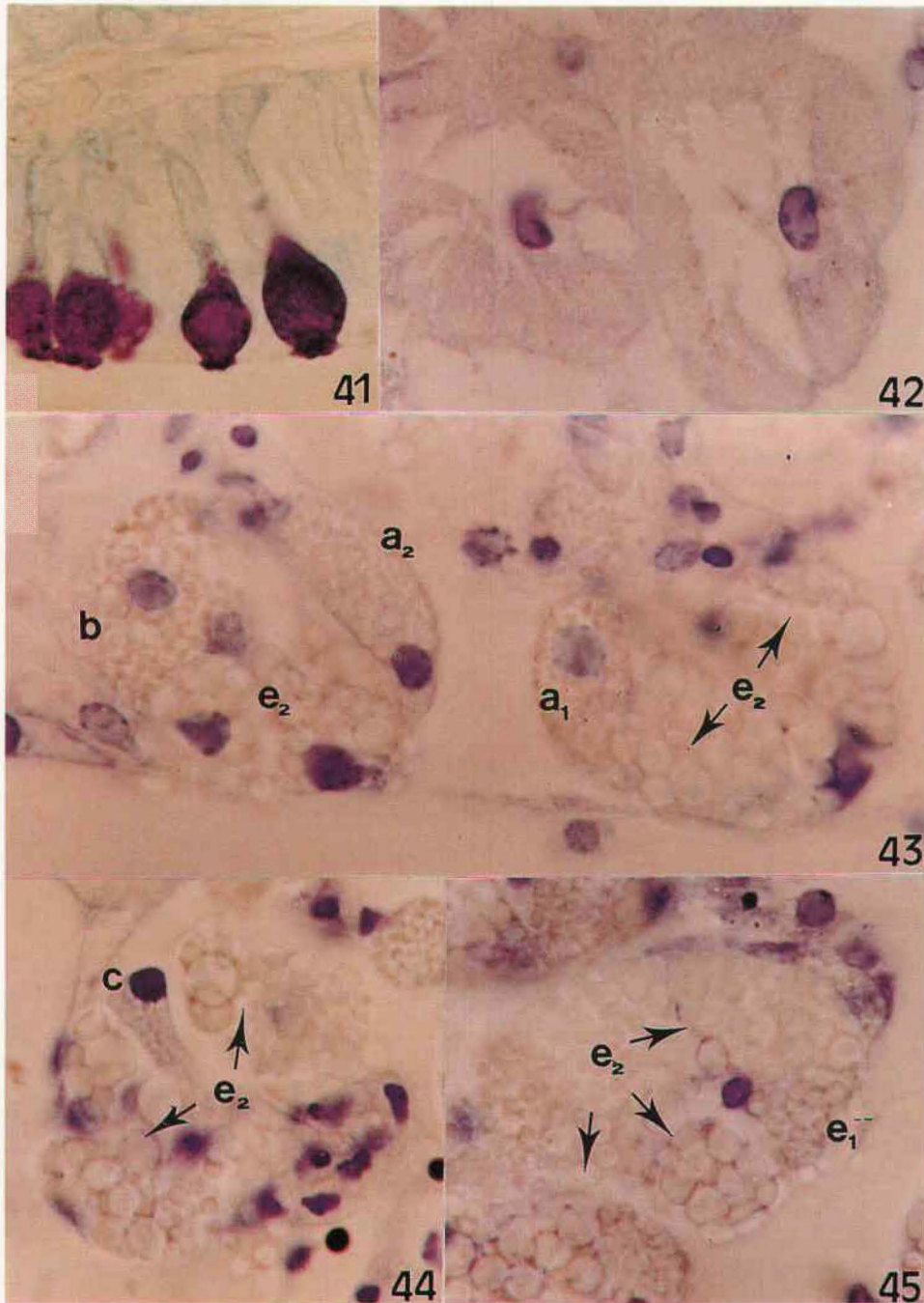
Fig. 41: Section in frog intestine stained with toluidine blue indicating positive metachromasia in the goblet cells (control).

Figs. 42-45: Sections in *A. hermanni* salivary glands stained with toluidine blue for acid mycopolysaccharides: 42. Type I alveoli; 43-45. Type II alveoli showing different cell types. (X 1187).

The membranes forming the striations and the granules in the peripheral zone reacted strongly positive for proteins and very strongly positive for lipids (Figs. 1, 37). Only the granules reacted positively for glycogen (Figs. 29, 30). Very little free NH_2 groups (Fig. 18) and no tryptophan, tyrosine, free SH groups or acid mucopolysaccharides were detected in this zone (Figs. 7, 12, 24, 42). The central zone reacted negatively in all tests (Figs. 1, 7, 12, 18, 24, 29, 37).

Type II alveoli: (Table 2)

positive for tryptophan (Fig. 8), free NH_2 groups (Fig. 23) and lipids (Figs. 38, 39), from negatively to moderately positive for tyrosine (Figs. 13, 15-17) and free SH (Figs. 26, 28) and from weakly to very strongly positive for carbohydrates (Figs. 31, 33).

Type b: These cells reacted very strongly for basic proteins (Fig. 4). However, the reaction ranged from being negative to moderately positive for tyrosine (Figs. 13, 16) and to strongly positive for carbohydrates (Fig. 34), and was moderately to

Table 2

Reactions of Type II salivary alveoli in *Argas hermanni* stained for proteins, tryptophan, tyrosine free NH_2 and SH groups, carbohydrates and lipids*

Cell type	Proteins	Tyrosine	Tryptophan	NH_2	SH	Carbohydrates**	Lipids
a_1	+++***	+	±	± to ++	-	+++	+
a_2	++ to +++	- to +	- to ++	- to ++	- to +	± to +++	± to ++
b	+++	- to +	+ to ++	-	±	- to ++	± and ++ ****
c							
periph	++	- or ±	±	±	-	+++	±
core	-	±	±	++	±	+++	++
e_1	+	+	±	- to +	±	+++	++ to +++
$d + e_2$	- to +++	- to +	- to +++	- to +++	- to ++	- to +++	- to +

*   All cell types reacted negatively for acid mucopolysaccharides.

**   After treatment with human saliva no change occurred in the staining reactions of any of the cell types to carbohydrates, i.e. glycogen was not detected.

***   - : negative, ± : weakly positive, + : moderately positive, ++ : strongly positive, +++ : very strongly positive.

****   The basal part of the cup-shaped globules was strongly positive (++) while the cup wall was weakly positive (±).

After fixation in buffered 10% formalin, some cell types exhibited a staining reaction with hematoxylin-eosin different from that observed after fixation in the Zenker-formol fixative [1]. The globules in some type a_2 cells appeared bright red while others stained purple. Also, type d and e_2 cell globules, which have nearly similar dimensions, stained purple or gray and, therefore, these cell types were indistinguishable from each other in the present study. Therefore, these cell types will be termed here-in-after as type e_2 cells. All cell types reacted negatively for acid mucopolysaccharides (Figs. 43-45) and glycogen.

Type a_1: The globules in these cell types reacted very strongly for basic proteins (Fig. 5) and carbohydrates (Fig. 32) and weakly to strongly for free NH_2 groups (Figs. 22, 23). The reaction was weak for tryptophan (Fig. 9) and moderate for tyrosine (Fig. 15) while no free SH groups were detected (Fig. 26).

Type a_2: These cells reacted strongly or very strongly for basic proteins (Figs. 2, 4). The globules in the same or different cells exhibited varied reactions in the other tests. They reacted from negatively or weakly positive to strongly

positive for tryptophan (Fig. 9), negative for free NH_2 groups (Fig. 22) and weakly positive for free SH groups (Figs. 26, 27). The basal part of the cup-shaped globules reacted strongly positive while the wall part reacted weakly positive for lipids (Fig. 39).

Type c: The globules in these cells reacted very strongly for carbohydrates (Figs. 31, 34), while for lipids (Fig. 38) and free NH_2 groups (Fig. 19) only the core reacted strongly but the peripheral part reacted weakly positive. For proteins, the peripheral part reacted strongly positive while the core reacted negatively (Fig. 6). Tryptophan (Fig. 8), tyrosine (Fig. 16) and free SH groups (Fig. 27) were absent or nearly absent from the globules.

Type e_1: These cells reacted very strongly for carbohydrates (Figs. 32, 33), strongly or very strongly for lipids (Figs. 38, 40) and moderately for basic proteins (Fig. 2). The reaction was negative to moderately positive for free NH_2 groups (Figs. 22, 23), moderately positive for tyrosine (Fig. 13) and was weakly positive for tryptophan (Fig. 10) and free SH groups (Fig. 28).

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Type e_2: The globules in the same or different cells of this type exhibited a gradient of reactions ranging from negative to very strongly positive for proteins (Figs. 2-6), tryptophan (Figs. 8-10), carbohydrates (Figs. 31-33) and free NH_2 groups (Figs. 19, 22, 23) and to strongly positive for free SH groups (Figs. 25-28). Their reaction also ranged from being negative to being moderately positive for tyrosine (Figs. 13, 15-17) and lipids (Figs. 38-40).

In unfed nymphs 6 weeks postmolting and adults 2 months postmolting, granules were observed among and sometimes replacing some of the globules in type e_2 cells as was observed in the histological study [1]. These granules reacted positively and appeared in cells with globules also reacting positively for proteins (Fig. 3), free NH_2 groups (Fig. 20) and carbohydrates, and in cells reacting negatively or weakly for tyrosine. Sometimes vacuoles were observed in cells with globules reacting positively for tyrosine (Fig. 14) and negatively or weakly positive for carbohydrates (Fig. 36). After feeding, the vacuoles appearing in these cell types were observed in those containing globules reacting positively for proteins, free NH_2 groups (Fig. 21) and tryptophan (Fig. 11), in cells with globules reacting negatively to moderately positive for free SH groups (Fig. 25) and in cells with all grades of reactions for carbohydrates (Figs. 35, 36). In these ticks, granules appeared in cells with a negative reaction for tryptophan. In cells containing only granules, which replaced all the globules, the granules reacted weakly positive for free NH_2 groups and lipids and negatively for tyrosine. The few globules remaining in cells which have lost most of their secretory content reacted positively for basic proteins, tryptophan, free NH_2 groups, weakly to moderately positive for free SH groups, negatively to weakly positive and strongly positive for tyrosine and carbohydrates, respectively.

DISCUSSION

In *A. hermanni* Type I alveoli, the lipids and proteins demonstrated in the present study were probably associated with the structural characteristics of the membranous infoldings and mitochondria described in the peripheral zone of these alveoli in argasids [5-8]. Proteins and lipids reported in Type I alveoli in *A. persicus* [9, 10] *Ornithodoros moubata* [8, 11] and *Hyalomma asiaticum* [11] were considered to be in the form of lipoproteins or phospholipoproteins [10].

Similar to *A. arboreus* [12] and *A. persicus* [9], the granules in the striated zone in *A. hermanni* Type I alveoli were rich in proteins. However, Balashov [5] reported them as protein-negative in *O. papillipes*. While the granules in *A. hermanni* were rich in glycogen, Khalil [12] described them as diastase-fast in *A.arboreus*. Also, while Chinery [10] reported that they reacted positively for glycogen Roshdy [9] described them as diastase - fast in *A. persicus*. Roshdy and Coons [7] and El Shoura [8] suggested that energy supply for water transfer might be provided by the lipid- and carbohydrate-rich granules in these alveoli.

Similar to the negative reaction in *A. hermanni*, very little free NH_2 and SH groups [10] and no tryptophan or tyrosine were found in *A. persicus* Type I alveoli [9]. However, after using different techniques, Chinery [10] observed a positive reaction for both amino acids in the latter species. In *A.*

arboreus, Khalil [12] reported uptake of relatively small quantities of H^3-tyrosine ingested with the blood meal by Type I alveoli. The negative reactions of the central zone in Type I alveoli in all tests might support the assumption that this central cell stored fluid before secretion as a participation in osmoregulation and fluid transport [13].

Type a_1, and e_1 cell globules in Type II alveoli exhibited a more or less uniform reaction for each of the tested compounds. Therefore, each of these cell types probably represented a true single cell type containing mature secretory globules.

The different staining reactions of Type a_2 cell globules with hematoxylin and eosin (after fixation in buffered neutral 10% formalin) and the great variation in their reactions in the other tests, except for basic proteins, suggested that their contents were at different stages of development; those exhibiting strongly positive reactions for the complex inclusions were probably the mature ones.

The uniform reaction of Type b cell globules for some compounds, including proteins, but not for others, including carbohydrates, suggested that the globule contents were of a composite nature, the protein and lipid components probably being formed first while the carbohydrate one being formed later.

The composite nature of Type c cell globules observed histologically [1] was further demonstrated by the difference in the reactions of the core and peripheral zone for basic proteins, free NH_2 groups and lipids. The components of the 2 regions might be in an inactive state, becoming active only as they were released during feeding.

The globules in Types d and e_2 cells exhibited great variations in their reactions in all tests which might suggest that these cells represented a single type containing globules at different stages of development. On the other hand, other aspects did not support such assumption. These aspects included the appearance of vacuoles in cells with reactions different from those containing granules among the globules and in cells with difference reactions for the same compound. Also, these observations suggested that degradation and release of their contents might occur by different mechanisms. More detailed studies are required to verify these assumptions.

In *A. hermanni*, the tests used demonstrated no glycogen or acid mucopolysaccharides in any of the cell types in Type II alveoli. The proteins and carbohydrates in these cells might be in the form of complexes such as mucoproteins or neutral mucopolysaccharides. Acid mucopolysaccharides might be present in low concentrations not demonstrable by the technique used. Highly sulphated acid mucopolysaccharides, such as heparin, with strong anticoagulant activity were strongly metachromatic [14, 15] while less sulphated forms, such as heparin monosulphate, were orthochromatic [15, 16]. Certain globules in *A. hermanni* reacting positively for proteins, carbohydrates and SH groups (i.e., in type d and e_2 cells) might contain a precursor of the anticoagulant component of *A. hermanni* salivary glands.

Tryptophan-containing compounds were known to occur in cells with zymogenic activity and might include

pharmacologically active indole derivatives [2]. Howell [17] showed that *O. savignyi* saliva contained a proteolytic enzyme. In *A. hermanni*, tryptophan-containing Type a_2, d and e_2 cells might produce lytic as well as pharmacologically active salivary secretions. These assumptions require further investigation.

ACKNOWLEDGMENTS

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