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Free Haemocytes of the Ixodid Ticks, *Hyalomma arabica* and *Hyalomma dromedarii* (Acari: Ixodidae)

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ABSTRACT. Drops of haemolymph were obtained from amputated legs of the various intars of the ixodid ticks *Hyalomma arabica* and *Hyalomma dromedarii* for the identification and classification of their free haemocytes. Haemocytes have also been maintained *in vitro* in Wheaton culture vessels as short-term (24-72h) cultures to facilitate observations on their live shapes and specific characteristics. Six types of haemocytes including prohaemocytes, plasmatocytes, granular haemocytes, spherulocytes, oenocytoids and adipohaemocytes were identified in the haemolymph of each tick species.

Morphologically, the haemocytes of arthropods and other invertebrates are comparable to the vertebrate leucocytes (Jones 1962, 1975, Wigglsworth 1979, Gupta 1979, 1990, 1991, Han and Gupta 1989, and Bardoloi and Hazarika 1992) and constitute an important component of the haemolymph of ticks (Balashov 1983). These cells were discovered and classified into four different categories long time ago (Millara 1947). The phagocytic ability of plasmatocytes and/or granular haemocytes has earned them the name "Immunocytes".

Informations on the haemocytes of ixodid and argasid ticks are meagre. Hence, an attempt is being made in the present study to identify the haemocytes of the ixodid ticks, *Hyalomma arabica* Pegram, Hoogstaal and Wassef and *Hyalomma dromedarii* Koch.

Materials and Methods

Almost fully engorged or semi-engorged females of *H. dromedarii* or *H. arabica* were collected for the establishment of laboratory colonies of either

species. Females of the former were collected from camels in Thumamah, Riyadh Province and those of the latter species from sheep and goats in Taif, Makkah Province. Colonies of both tick species were maintained in the laboratory over saturated sodium solutions into sealed dessicators to provide a relative humidity of 75% (Winston and Bates 1960) at 28°C in an incubator. The various instars of the ticks were fed on New Zealand rabbits in capsules according to the method of Varma (1964).

One leg of either male, female, nymph or larva of either tick species was amputated and drops of haemolymph were smeared onto clean microscope slides. These were air-dried, fixed by absolute methanol and stained with either Leishman or Giemsa stain using an acidic buffer, pH 6.5. The slides were then mounted in neutral D.P.X. medium and examined under the microscope.

Phase contrast microscopy was used to study a hanging drop of haemolymph grown in a short-term culture (24 - 72h) in Eagle's salt medium and sodium bicarbonate. Short-term cultures of haemocytes in Medium 199 supplemented with bovine calf serum supplied material for studying haemocyte live shapes and progressive development. The short-term cultures are made in Wheaton culture vessels (Wheaton Instruments, Millville, New Jersy, USA).

Results

The free haemocytes of *Hyalomma arabica* and *Hyalomm dromedarii* conform to the characteristics of prohaemocytes plasmatocytes, granular haemocytes, spherulocytes, oenocytoids and adipohaemocytes. The main features of each type are listed below:

Prohaemocytes: These are the smallest of the haemocytes. Their cytoplasm is strongly basophilic and forms a thin layer bound by a uniform, clearly visible cell membrane that uniformly surrounds the comparatively large, spherical, compact and strongly basophilic nucleus which Contains scanty nucleoplasm with large, compact chromatin granules. The size of these cells varies from 9 to 11 µm in *H. dromedarii* and 6 - 10 µm in *H. arabica*. Their nuclei range from 6 - 8 µm in the former and 5 - 8 µm in the latter tick species, with a cellular/nuclear ratio (AC/AN) of 1.5 ± 0.45 (Figs. 1a ,2a, 3a and 4a).

Plasmatocytes: These are comparatively large, polymorphic cells with small, spherical to oval nuclei and an AC/AN ratio of 3.2 ± 1.2 (Figs. 1b, 2b, 3b and 4b). They could be grouped into spherical, oval, and irregular plasmatocytes. They form the bulk of free haemocyte population (≥70%) of either tick species. The spherical plasmatocytes are 13 - 19 µm in *H. dromedarii* and 11 - 18 µm in *H. arabica*; their nuclei are generally spherical, 5 - 8 µm in the former and 6 - 9 µm in the latter tick species, with an AC/AN ratio of 3.5 ± 1.5. The cytoplasm of these cells is generally vacuolated .

Granular haemocytes: These are few, mostly spherical cells of moderate size, 13 - 16 µm in *H. dromedarii* and 11 - 19 µm *in H. arabica* with comparatively small, round eosinophilic nuclei measuring 5 - 9 µm in both species. Their cytoplasm is mildly basophilic with few large, round eosinophilic granules and their AC/AN ratio is 2.5 ± 1.0 (Figs. 1c, 2c, 3c and 4c).

Spherulocytes: These are also few and are similar to the granular haemocytes are 12 - 17 µm in *H. dromedarii* and 12 - 20 µm in *H. arabica*, but have one or more basophilic inclusions. The nucleus is round (6 - 10 µm in both species) to oval with compactly arranged chromatin granules.The AC/AN ratio is 2.0 ± 0.5 (Figs. 1d, 2d, 3d and 4d).

Oenocytoids: These are round or oval in shape with granular and strongly basophilic cytoplasm that has one or two vacuoles. The nucleus is more basophilic than the cytoplasm, and is round and eccentric in position. These cells are 15 - 18 µm in both tick species, but there are some oval ones which are 17 - 21 µm in length and 14 - 18 µm in diameter. The AC/AN ratio is 2.5 ± 1.2. (Figs. 1e, 2e, 3e, and 4e).

Adipohaemocytes: These constitute over 30% in the haemolymph of both tick species and are moderate in size, 15 - 21 µm in *H. dromedarii* and 14 - 19 µm in *H. arabica*. They are round with very large cytoplasmic fat inclusions (fat globules), their nuclei are 6 - 8 µm and the ratio AC/AN is 3.5 ± 1.5.(Figs. 1f, 2f, 3f and 4f).

Discussion

Similar to the observations of Gupta (1991) in insects, the prohaemocytes, plasmatocytes and granular haemocytes are the fundamental and most common types of haemocytes in both *H. arabia* and *H. dromedarii*. The prohaemocytes are the germinal blood cells that divide to generate similar and other types of haemocytes (El-Shoura 1986). On the other hand, the plasmatocytes described in the present study are similar to those reported by Brinton and Burgdorfer (1971) from *Dermacentor andersoni* and by El-Shoura (1986) from *Argas (Persicargas) arboreus*. Together with granular haemocytes, they are also considered to be the immediate derivatives of prohaemocytes (Crossley 1975) an event explicitly augmented by the *in vitro* observations in the present study. Also the inverse relationship of the population of the prohaemocytes and plasmatocytes might predict the interconversion of these two cell types as observed by Al-Khalifa and Siddiqui (1985). However, similar to the observations of Farkas and Zethan (1989) in *Galleria mellonella*, the plasmatocytes and granular haemocytes were treated in the present study as two separate identities. Furthermore, the plasmatocytes and/or granular haemocytes have also been cumulatively called the *"Immunocytes"* because they are actively involved in phagocytosis, encapsulation and nodule formation as a defence mechanism (Han and Gupta 1989). The spherulocytes are the rarest type of haemocytes and are characterised by having large inclusions in their cytoplasm. Similar observations were also made in insects by Gupta (1979). Similar to the observations of Jones (1975) and Gupta (1985) in insects, haemocytes with larger vacuoles and eccentric nuclei were observed in both tick species studied. The sixth type of haemocytes in both tick species is the adipohaemocytes which are sometimes called the nephrocytes by Balashov (1983). These cells are known to engulf macromolecules or fat globules from the haemolymph, degrade them and retain them in their cytoplasm (Arnold and Sohi 1974).

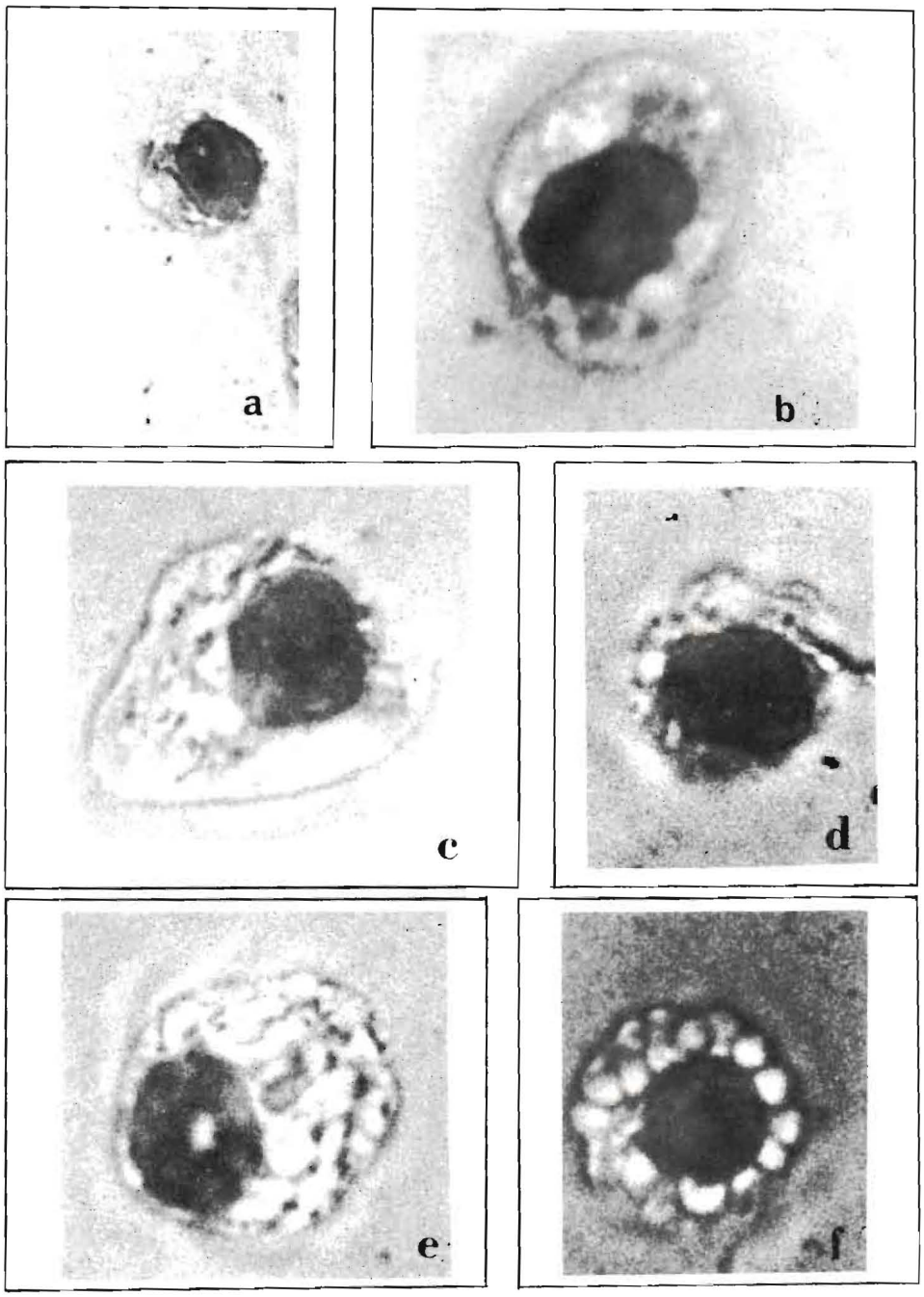

Fig. 1. Photomicrographs of free haemocytes in the haemolymph of *H. arabica*. a, prohaemocyte; b, plasmatocyte; c, granular haemocyte ; d, spherulocyte ; e, oenocytoid ; f, adipohaemocyte .

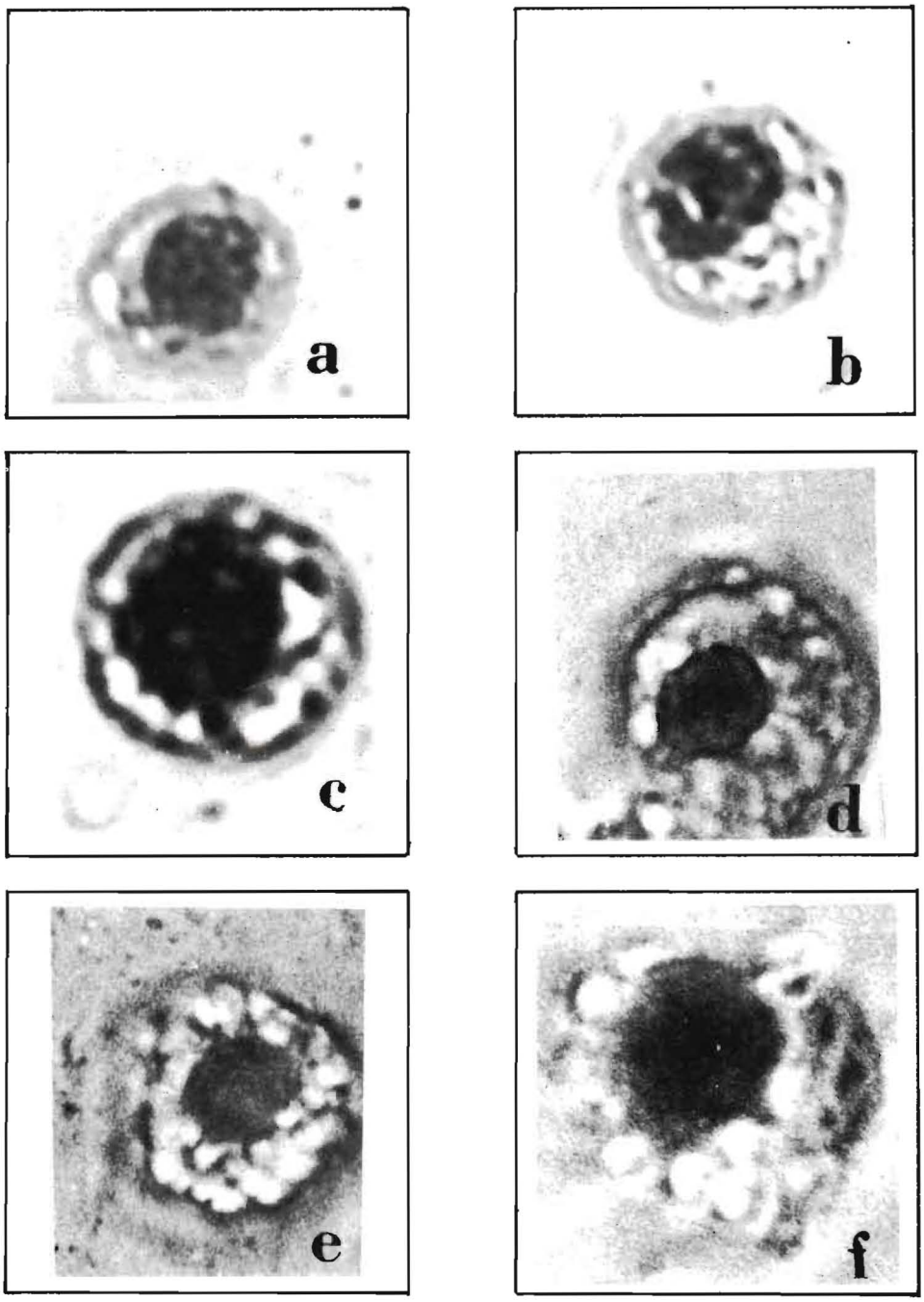

Fig. 2. Photomicrographs of free haemocytes in the haemolymph of *H. dromedarii*. a, prohaemocyte; b, plasmatocyte, granular haemocyte ; d, spherulocyte ; e, oenocytoid ; f, adipohaemocyte .

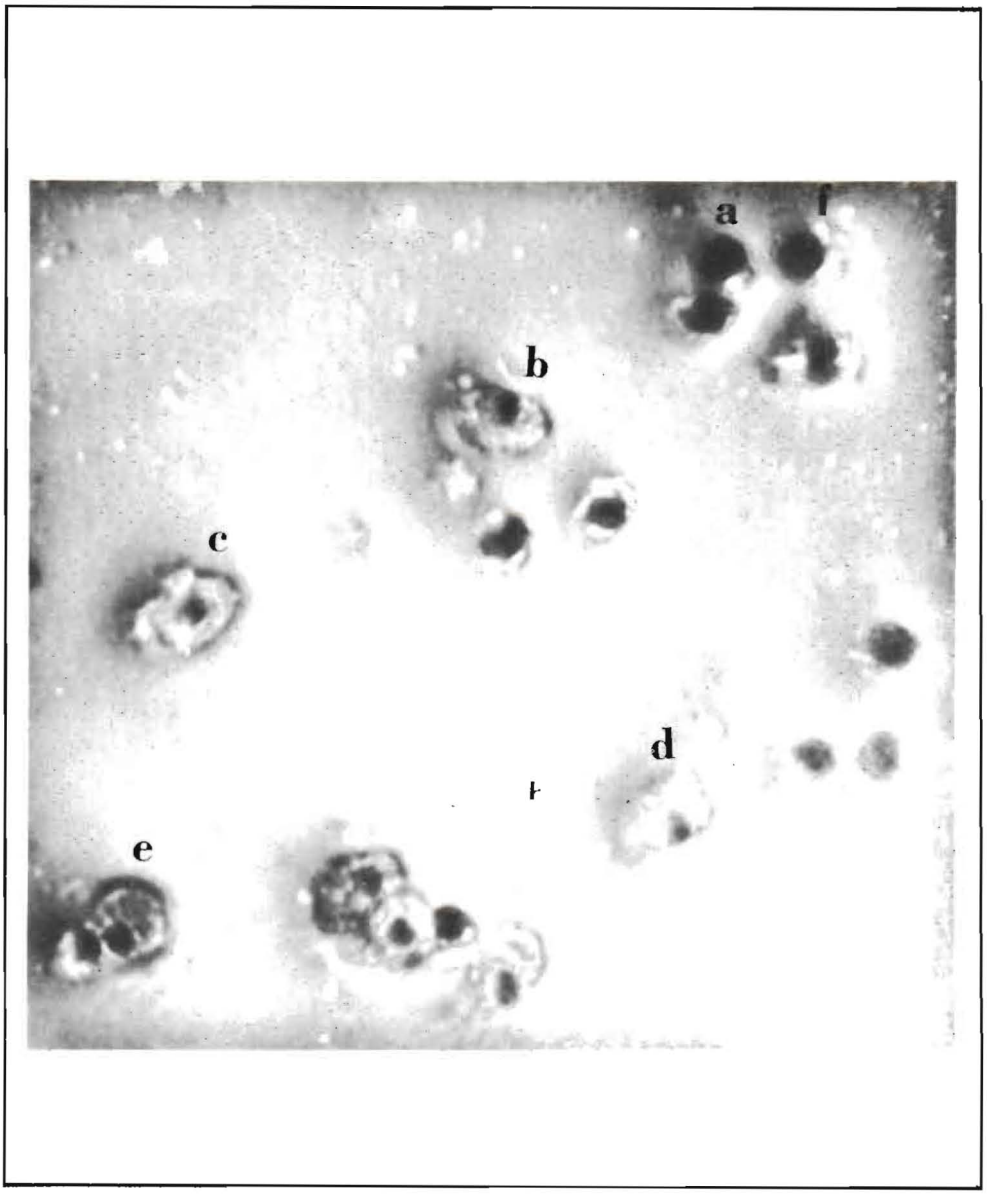

Fig. 3. Phase contrast photomicrographs of free haemocytes in a short term *in vitro* culture of the haermolymph of *H. arabica*. a, prohaemocyte , b, plasmatocyte ; c, granular haemocyte ; d, spherulocyte ; e, oenocytoid ; f, adipohaemocyte .

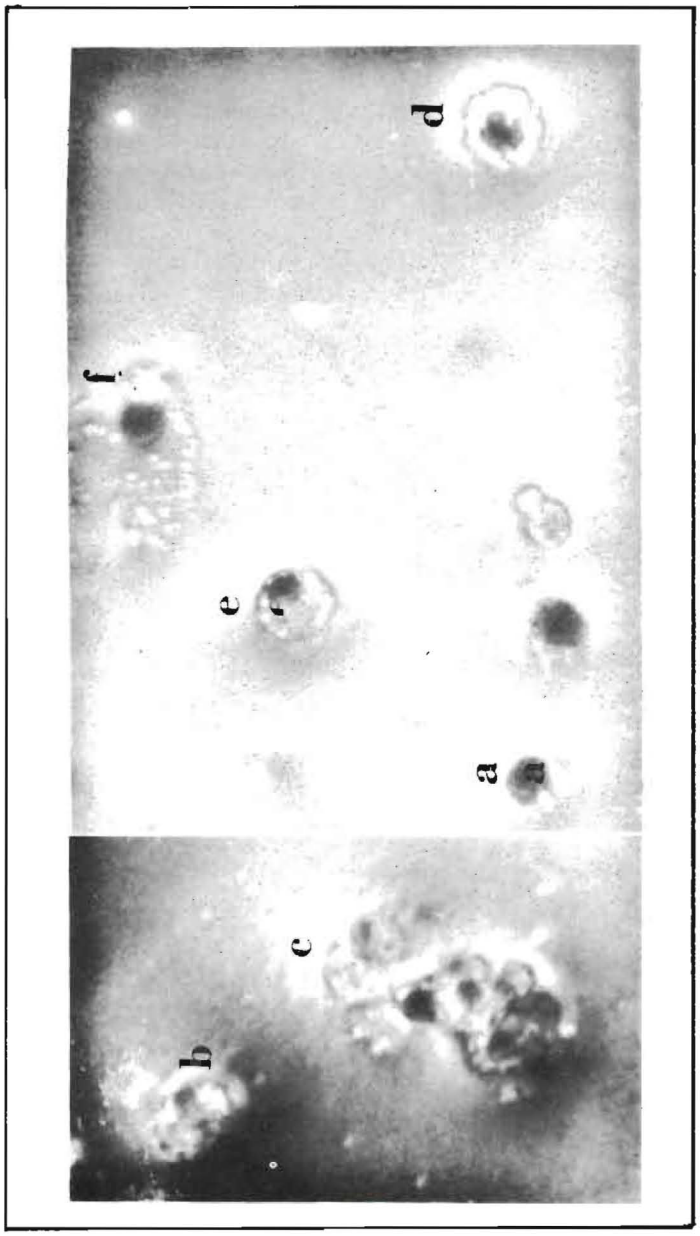

Fig. 4. Phase contrast photomicrographs of free haemocytes in a short term *in vitro* culture of the haemolymph of *H. dromedarii*. a, prohaemocyte ; b, plasmatocyte ; c, granular haemocyte ; d, spherulocyte ; e, oenocytoid ; f, adipohaemocyte .

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دراسة خلايا الدم الحرة في القراد الجامد نوعَي *Hyalomma dromedarii* و *Hyalomma arabica*

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لقد تم جمع قراد الماعز والضأن العربي *Hyalomma arabica* من الطائف في منطقة مكة المكرمة وقراد الابل *Hyalomma dromedarii* من الثمامة في منطقة الرياض والتي استعملت لاستحداث مستعمرتين حيوانيتين لكل من النوعين المدروسين بقسم علم الحيوان - كلية العلوم - جامعة الملك سعود - الرياض. ولقد تم تغذية أفراد هاتين المستعمرتين على الأرانب النيوزيلندية في كبسولات معدنية حسب طريقة فارما (١٩٦٤) كما وتم حفظها عند درجة حرارة ٢٨°م ورطوبة نسبة ٧٥٪.

لقد تم جمع قطرات دم من كل من الذكور والاناث والحوريات واليرقات لكل من قراد الماعز والضأن العربي *H. arabica* وقراد الابل *H. dromedarii* عن طريق بتر رجل كل منها، ومن ثم عُمِلَت سحبات على شرائح زجاجية نظيفة، ثبتت بواسطة كحول ميثلي وبعد ذلك صُبِغَت بأي من صبغتَي ليشمن أو جمزأ (في وسط حمضي) وطُمِرَت في وسط طمر متعادل (D.P.X.) وفُحِصَت باستعمال المجهر الضوئي للتعرف عليها ولتصنيف خلايا الدم المتواجدة في كل منها. اضافة إلى ذلك، تمت زراعة بعض خلايا الدم في مزرعة قصيرة الأمد (٢٤ - ٧٢ ساعة) باستعمال أوعية ويتن لزراعة الخلايا (Wheaton culture vessels) مما مكننا من رؤية أشكال هذه الخلايا الدموية حية ودراسة بعض خصائصها وصفاتها باستعمال مجهر الطور المتباين.

لقد وجد في كل من نوعَي القراد المدروسين ستة أنواع من خلايا الدم متميزة عن بعضها البعض وهي: خلايا الدم الأولية (prohaemocytes) وخلايا الدم البلازمية (plasmatocytes) وخلايا الدم المحببة (granular haemocytes) وخلايا الدم الكروية (spherulocytes) وخلايا الدم الاينوسيتويد (oenocytoids) وخلايا الدم الدهنية (adipohaemocytes).