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Comparison of the Protein Content in Selected Tissues at Different Feeding Stages in the American Dog Tick *Dermacentor variabilis* (Say)

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COMPARISON OF THE PROTEIN CONTENT IN SELECTED TISSUES AT DIFFERENT FEEDING STAGES IN THE AMERICAN DOG TICK *Dermacentor variabilis* (SAY)

by

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ABSTRACT

COMPARISON OF THE PROTEIN CONTENT IN SELECTED TISSUES AT DIFFERENT FEEDING STAGES IN THE AMERICAN DOG TICK *Dermacentor variabilis* (SAY)

Mark John Beveridge
Old Dominion University, 1990
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Selected tissues from *Dermacentor variabilis* (Say) were collected at different feeding stages (unfed, part-fed, and replete) and examined by several different protein analyses to determine soluble protein content, protein or polypeptide molecular weights, and pI values. Results showed a relationship between the salivary gland, ovary and hemolymph and evidence indicates the synganglion produces and secretes substances that may control oocyte development.

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Introduction

The control of ticks and tick-borne diseases depend upon an understanding of the chemical events which occur in the cells and tissues of the vector (Dolp, 1970). Soluble protein content, in the tick, has been examined previously but the majority of the work has been done on body fluids (i.e. hemolymph, salivary, and coxal secretions). The hemolymph and its cellular components are especially important because of the many functions they control or assist such as enzyme and protein transportation, phagocytosis, coagulation, and immunologic response. It has been noted for ixodids such as Hyalomma dromaderii Koch and Hyalomma anatolicum excavatum Koch, and for argasid ticks such as Argas (Persicargas) persicus (Oken), and Argas (Persicargas) arboreus Kaiser, Hoogstraal & Kohls, that feeding correlates with hemolymph protein content and concentration (Dolp, 1970; Dolp and Hamdy, 1971). It has also been observed that egg yolk proteins of the cattle tick Boophilus microplus (Canestrini) are incorporated into the ovum unchanged from the hemolymph (Tatchell, 1971). In female Ornithodoros moubata (Murray), two proteins (hemoglyco-lipoproteins) appear in high concentrations in the hemolymph. Similar proteins are also found in the egg yolk where they constitute a major

portion of the yolk proteins (Diehl, 1969). Aeschlimann and Hecker (1967) hypothesized that these hemoglyco-lipoproteins are incorporated into the egg through micro-pinocytosis. Ben-Yakir and Barker (1985) studied hemolymph proteins of Amblyomma americanum (L.) and Dermacentor variabilis (Say) in various stages of their life cycle to determine possible points of enzymatic inhibition or antigenic responses for possible immunological or biological control of these animals. They found that there are 21 and 19 bands in D. variabilis and A. americanum hemolymph respectively, and most of the bands are present throughout feeding in both species. They hypothesized that the hemolymph proteins that occur in low concentrations are the most likely candidates for an antitick vaccine, but they did not establish a basis for this in their abstracted work.

Several studies on the proteins of tick salivary glands have been reported. Coons and Lamoreaux (1986) compared several different cell types in male and female D. variabilis during feeding and reported that the cellular components of the salivary gland in each sex are similar. However, there are differences in the rate of change these cells undergo during feeding and the acceleration of this rate caused by mating. The effects of antigens on host resistance in the salivary gland of

A. americanum are reported by Needham et al. (1986). The immediate immune response causes a near doubling in the length of time the ticks fed from 12 days to more than 20 days. There is also a reduction in engorgement weight and egg viability (Brown, 1986). Kaufman (1986) reported that the degeneration of the salivary glands in the female tick, Amblyomma hebraeum is controlled by a humoral agent in the hemolymph, they labeled the "tick salivary gland degeneration factor".

In the current study, various tissues and cell types from D. variabilis were examined, in several ways, to compare soluble protein content. Selected tissues were removed at different stages in the adult life cycle (i.e. unfed females, part-fed females, replete females, and partially fed males) and examined using isoelectric focusing (IEF) to obtain pI values and SDS polyacrylamide gel electrophoresis (SDS PAGE) for molecular weights. Comparisons were made between different tissues in the same stage of the life cycle, and between the same tissues in different stages of the life cycle. Embryonic cells (EC) and D. variabilis cells in culture (CC), i.e. an established cell line, were also examined and compared to each other using two dimensional electrophoresis, with isoelectric focusing in the first dimension and SDS polyacrylamide gel electrophoresis in the second.

One objective of this study is to expand the data base in the biochemistry and physiology of D. variabilis with the hope of identifying proteins important to cell division and organ development in this medically and veterinarily important ectoparasite. A second objective is to compare the protein content of embryonic cells to cells in culture with the hope of identifying some bands of proteins important to cellular differentiation and embryonic development. This study could provide the basis for continuation of the biochemical and molecular analyses necessary for identifying enzymes, hormones, and eventually genes essential to the development and maturation of D. variabilis.

Literature Review

A considerable amount of research has been done on insect proteins. A summary of these studies will be limited to vitellogenins, which are a group of proteins similar in composition in all animals with yolky eggs (Hagedorn and Kunkel, 1979). The definition of vitellogenins includes several characteristics: (a) they comprise 60 - 90% of the soluble egg yolk proteins (Bell, 1970; Hagedorn and Judson, 1972), (b) they are not made in the oocyte but synthesized in the fat body (Brookes, 1976; Pan et al., 1969), (c) they are present in large amounts (but not exclusively) in the female (Bell, 1969;

Pan et al., 1969), and (d) they are selectively taken up by the oocyte during vitellogenesis (Telfer, 1960).

In the grasshopper Locusta migratoria, vitellogenin appears in the female's hemolymph 7 days after the animal molts into the adult form (Chen et al., 1979). The rate of vitellogenin production in the fat body increases up to 60% by day 13, after which, levels in the hemolymph decrease due to oocyte uptake (Chen et al., 1976). In the desert locust Schistocerea gregaria and the american cockroach Periplaneta americana, a specific hemolymph protein is necessary for ovarian development (Hill, 1962; Menon, 1963). Bodnaryk and Morrison (1966) found that in the house fly Musca domestica, extracts of mature eggs contain protein fractions with electrophoretic mobilities identical to hemolymph fractions. In the american cockroach, Neilsen and Mills (1968) reported 15 bands in the hemolymph, and in the soluble protein fraction of late stage oocytes, 6 bands appeared which matched hemolymph protein bands 7 through 12.

For comparison of ticks with other mammalian ectoparasites, the best insect model for blood meal digestion is the female mosquito. The protein content from the blood meal is necessary for egg development, and early work in mosquito reproduction suggests that protein is the only essential requirement for this process (Woke,

1937; Yeoli and Mer, 1938). In the mosquito Aedes aegypti ecdysterone production and secretion occur only after a blood meal (Hagedorn et al., 1975) and is transported to the fat body where it induces vitellogenin production (Fallon et al., 1974). Flanagan and Hagedorn (1977) reported that before the fat body can respond to ecdysteroids it must first be exposed to juvenile hormone which either stimulates the appearance of ecdysterone receptors or causes synthesis of vitellogenin mRNA that is translated only in response to ecdysterone. This hormonal control of oocyte development and vitellogenesis, specifically the role of juvenile hormone, has not been demonstrated in ticks (D.E. Sonenshine, personal communication).

Feeding, digestion and reproduction in the ixodid tick is a complex physiological cycle that includes bloodmeal concentration and absorption, host protein degradation, synthesis of proteins essential for tick reproduction, transportation of these proteins to the tick ovary and finally incorporation into the yolk of the tick egg. The feeding process can be divided into three periods. The first feeding period is preparatory, which begins after the female attaches to a host and lasts approximately one day. During this period the ticks body weight and size do not change significantly, even though

the digestive system matures and becomes active (Coons et al., 1986).

The second feeding period, the growth phase, begins one day after attachment and lasts until approximately one day before detachment. In this period the ticks body weight and size slowly increase. The midgut hemoglobin concentration also increases slowly which indicates a high rate of feeding and digestion. In the midgut two types of cells are associated with the digestive process. One group, the digestive cells, is involved in uptake and digestion of the bloodmeal which results in the synthesis of usable products and unusable byproducts (which are stored in residual bodies, an aggregate of several phagolysosomes, in the cytoplasm). When the cytoplasm is filled to capacity with residual bodies, digestive cell fragments or the entire cell may detach and float free in the lumen of the midgut. The digestive cell is replaced by an inactive, relatively undifferentiated replacement cell (Holtzman and Novikoff, 1984). These replacement cells differentiate into mature digestive cells and a new cycle of uptake and digestion begins. This growth phase is associated with the uptake, concentration, digestion, and use of host blood for organ and integument growth (Araman, 1979). Similar results were reported by Kitaoka and Yajima (1958) and Baloshov (1972).

While in this growth phase the salivary glands become very important to feeding. As the bloodmeal is concentrated the salivary glands move water and ions from the midgut back to the host. They also secrete a substance that helps cement the mouth parts of the tick to the host (Balashov, 1972; Chinery, 1973). It is in this phase that pathogenic agents and viruses are transmitted to the host. During this feeding period females are also stimulated to produce new salivary gland proteins. These proteins seem to be related to the development of two groups of alveolar cells which are thought to increase the ticks ability to secrete fluids (McSwain et al., 1982).

The third period, the expansion phase, takes place during the last day of feeding and is initiated by mating. During this period there is a rapid increase in the tick's weight and size. Although feeding increases at this time and enters the rapid-engorgement feeding stage, the digestive rate declines temporarily, then accelerates (Coons and Lamoreaux, 1986). This temporary decline in digestion allows the bloodmeal to accumulate so that it can be used in the vitellogenic process for egg yolk protein production. Chinzei et al. (1983) purified vitellogenin and vitellin from the hemolymph and eggs of the soft tick, O. moubata and showed that

vitellogenin is comprised of two components Vg-1 and Vg-2. Vitellin and Vg-2 have a molecular weight of 600 Kd and Vg-1 has a molecular weight of 300 Kd. These data, along with electron micrographs, indicate that vitellin and Vg-2 may be dimers of Vg-1. Using SDS-polyacrylamide gel electrophoresis Chinzei et al. (1983) found vitellogenin has six polypeptides (molecular weights from 100 to 215 Kd), and that vitellin also has six polypeptides (molecular weights from 50 to 160 Kd). Four of the six polypeptides are common to both. These data suggest an enzymatic degradation of large polypeptides in vitellogenin to smaller polypeptides in vitellin. Chinzei et al. (1983) also found, through isoelectric focusing, that both vitellin and vitellogenin have a common band at pH 6.9.

The rate of bloodmeal breakdown again increases during preoviposition. Some of this digestion is due to an increase in growth of vitellogenic cells. Although there are vitellogenic cells in the midgut this is not the only location of vitellogenin synthesis. Synthesis also occurs in the fat body (Coons et al., 1982; Tarnowski, 1983). The vitellogenin from these two sources are immunologically identical (Araman, 1979). Vitellogenin is transported from the midgut and fat body to the ovary via the hemolymph, where it is converted to

vitellin in the oocytes. In Rhipocephalus sanguineus, immunoelectrophoresis shows that vitellin from the eggs and vitellogenin from the midgut and hemolymph have similar electrophoretic mobilities (Coons et al., 1982).

As mentioned above, once inside the oocytes vitellogenin proteins are known as vitellin. In some cases molecular changes occur during uptake into the oocyte (Coons et al., 1982). These vitellogenins usually comprise 60 to 90 percent of the soluble yolk proteins. They are present in large amounts only in females and are selectively taken up by the oocytes during vitellogensis. The uptake mechanism allows all hemolymph proteins to be absorbed, but only vitellogenin is selectively removed and concentrated in the oocyte such that its concentration is twenty to one hundred times (depending on the organism) the level found in the hemolymph (Bell, 1970; Telfer, 1954). In insects this uptake of vitellogenin is controlled by juvenile hormone and/or ecdysone (Wyatt and Pan, 1978; Hagedorn and Kunkel, 1979; Engelman, 1979). As the tick oocytes develop, yolk granules appear; first in a wide zone around the nucleus, then later throughout the cytoplasm. These granules enlarge and become spherical while the cytoplasm is gradually reduced to a network between yolk sphericals. Finally, the cytoplasm is squeezed into a thin layer

around the oocyte periphery (Khalil, 1969). In Ornithodoros and Ixodes ticks, the yolk of these eggs has a brown color which is derived from host hemoglobin (Wigglesworth, 1943; Bremner, 1959). Proteins similar to the yolk proteins are found in the hemolymph of reproducing females, and Tatchell (1971) reported that in B. microplus they comigrate electrophoretically, but he could not prove that they are the same. Similar results were reported for D. andersoni by Bocton and Kamel (1976). The evidence, therefore, indicates that the two female specific hemolymph proteins are probably the same as the proteins found in the egg yolk.

Materials And Methods

Cell Cultures

Continuous cell line cultures (CC) were obtained from Dr. Conrad Yunker, then of the Rocky Mountain Laboratories, National Institutes of Health, Hamilton, Montana (RML) and were derived at RML from embryonic cells of laboratory-reared D. variabilis. These cultures were maintained in Yunker-Meibos medium (Yunker and Meibos, 1979) at room temperature.

Embryonic Cells

Embryonic cells (EC) were obtained from eggs of laboratory-reared D. variabilis. Eggs were collected 8 to 10 days post initiation of oviposition and processed according to techniques described by Yunker and Meibos (1979). Eggs were gently broken open with a sterile, flattened glass rod and the cells filtered through sterile gauze. The cells were washed several times in the Yunker-Meibos medium and incubated for two days to allow for proliferation. The cells were then homogenized and centrifuged at 15,000 RPM for 15 min in an Eppendorf microcentrifuge 5412, at 3 C. The supernatant was removed and frozen until needed.

Adult Tissues

Adult tissues (AT) were collected from laboratory-reared D. variabilis ticks, which were maintained in an Aminco Climate Lab environmental chamber (American Instrument Co., Silver Springs, MD) at 27 C (+/-0.5 C) and 90% RH (+/-2.0%). The tissues collected were from part-fed adult males (testes only), unfed adult females (synganglion, ovary, salivary gland, and hemolymph), part-fed adult females (synganglion, ovary, salivary gland, and hemolymph), and replete females (synganglion, ovary, salivary gland, and hemolymph). The ticks were fed and mated on albino rabbits (Oryctolagus cuniculus)

and the tissue samples were collected on the day of parturition. The hemolymph was collected first by removing one of the ticks limbs and using a micro-capillary tube to draw up the hemolymph that had accumulated. The ticks were then cut open dorso-ventrally in sterile saline, the organs were removed, separated, and pooled until enough were collected to process. The tissue samples were macerated with a 0.1 ml micro-tissue grinder (Wheaton), to release cellular content. This material was centrifuged at 15,000 RPM for 15 min in an Eppendorf microcentrifuge 5412. The supernatant was then removed and frozen immediately. The tissues were kept at 3 C from the time they were removed from the tick's body until they were frozen.

Protein Assay

For tissue extracts in each of the three adult feeding stages, total soluble protein concentrations were determined by protein assay using bovine gamma globulin standards (Bio-rad, Rockville Centre, N.Y.). For comparison, the protein content was calculated in ug/organ, as well as ug/mg tissue. This second analysis was used to evaluate and compare protein concentrations from tissues of different sizes.

Protein Separation Experiments

Molecular Weight Separation

Electrophoretic separation of the proteins collected from the tick cells and tissues were preformed on a 10% gel. The gel was prepared using 13.4 ml of 30% acrylamide/bis stock solution, 10 ml TRIS-HCl (1.5M, pH 8.8), 16.1 ml distilled water, 0.4 ml SDS (10%), and degassed for 15 min. After degassing, 10 ul of N,N,N,N-tetramethylethylenediamine (TEMED) and 130 ul of 10% ammonium persulfate were added. This solution was quickly pipetted into 125 mm glass tubes, an overlay of distilled water was added to form a flat surface and the gels were allowed to polymerize (ca. 30 min). A 5% stacking gel was added to each tube and polymerized before the samples were added. A SDS tris-glycine solution (0.04 M, pH 8.3) was used in both the top and bottom buffer chambers.

The samples were prepared by adding 50 ul of sample buffer (1% SDS, 10% glycerol, 5% 2-mercaptoethanol, 5% bromophenol blue, and 70% 0.0625 M TRIS-HCl, pH 6.7) to 50 ul of protein concentrate, and the proteins in sample buffer were then heated to 95 C for 4 min. Predetermined amounts (200 ug protein/tube) were then added to the tops of the tube gels. Two tubes of molecular weight markers were run along with the sample tubes. All samples and

markers were carefully applied to form a single concentrated layer. The unit was then attached to a Buchler 3-1500 constant power supply, anode (+) to the bottom chamber and the cathode (-) to the top chamber. This unit was operated at 3 mA/tube (54 mA for 18 tubes). The current was turned off when the tracking dye reached the bottom of the glass tubes. The gels were then removed from the tubes and stained for 1 h in a solution of Coomassie brillant blue, R-250 (0.125%), methanol (50%), and acetic acid (10%). The gels were destained in a 50% methanol and 10% acetic acid solution and stored in 5% acetic acid for future study.

Isoelectric Focusing Separation

Cell and tissue samples were also separated by isoelectric focusing (IEF). IEF gels contained 1 ml of Biolyte 3/10 (BIO-RAD), 4 ml of monomer concentrate (24.25% acrylamide:0.75% Bis-acrylamide), 4 ml glycerol (25% w/v), and 11 ml glass distilled water. This solution was degassed under vacuum for 15 minutes and 0.1 ml of ammonium persulfate (2%) and 0.1 ml of Riboflavin-5-phosphate (0.1%) were added. This mixture was pipetted under a 125 X 100 mm glass plate on a Capillary Gel Caster (BIO-RAD) and light polymerized for 1 h. The corners of the polymerized gel and any other portion which reached over the edge of the glass were

removed to prevent "arcing" to the platform, which could cause current uniformity problems. Felt wicks (BIO-RAD) were then placed across the top (cathode) and bottom (anode). The cathode was saturated with 1 N sodium hydroxide and the anode was saturated with 1 N phosphoric acid. Sample application pads were punched out of the felt wicks and placed approximately 3 cm from the cathode. The samples were added to the pads and the gel was placed in the electrofocusing unit (Model 1415 chamber, BIO-RAD). The unit was then attached to a Buchler 3-1500 constant power supply and run in the constant power mode at 6 W. The gel was started with low voltage and high amperage, generally about 200 V and 12 mA, and by the end of the run, the voltage was high and the amperage was low, stabilizing around 950 V and 2-4 mA. The elapsed time was usually 2.5 to 3.0 h. The gel was removed from the IEF cell, a pH gradient was recorded using a surface pH probe (Fisher Scientific, Pittsburg, Pa.), and the gel was placed into a fixative containing sulfosalicylic acid (4%) and trichloroacetic acid (12.5%) for 1 h. The gel was removed from the glass plate and stained for 2 h. in a 0.04% Coomassie blue, 27% ethanol, 10% acetic acid, 0.5% cupric sulfide solution. It was then destained in a 12% ethanol, 7% acetic acid, 0.5% cupric sulfide solution for 2-3 changes (ca. 30-45 minutes) until the background of the gel was clear.

Two-Dimensional Electrophoresis

The protein patterns of the embryonic cells (EC) were further compared to cultured cells (CC) by running a second dimension electrophoresis pattern on unfixed IEF gels. Individual sample lanes of the IEF gels were cut into 1 cm strips and equilibrated for 30 min in SDS sample buffer (0.002 M tris at pH 6.7, 2.3% SDS, and 5% 2-mercaptoethanol) and heated to 95 C for 4 min. This was done to separate the proteins from the carrier ampholytes and to allow the 2-mercaptoethanol to react with sulfhydryl groups so the proteins could move freely in the second dimension.

For the second dimension, a 10% SDS-PAGE gel was prepared using the same procedure described in the section on molecular weight separation. The solution was injected slowly between glass plates in the electrophoresis unit (BIO-RAD Model 220) to 2 cm below the top of the inner glass plate and overlayed with glass distilled water to make a flat surface. The gel was allowed to polymerize for 1 h, and then the water was removed and the stacking gel added. The stacking gel consisted of 1 ml acrylamide stock (4.75%), 1 ml Tris-HCl buffer (0.5M) at pH 6.7, 0.04 ml SDS (10%), 2 ml ammonium persulfate (0.145%) and 9 ul TEMED (BIO-RAD). This solution was layered over the separating gel 1 cm from

the top of the inner glass plate and covered with glass distilled water until polymerized. The water was removed and the IEF gel was inserted between the plates and covered with hot agarose. When the agarose solidified, small amounts were removed from each side of the IEF gel to form wells for molecular weight markers and bromophenol blue tracking dye. Both top and bottom electrode chambers used the same buffer solution (12.1 g tris, 57.6 g glycine, 4 g SDS; filled to 4 L with glass distilled water). After carefully adding the molecular weight markers and dye, the unit was attached to a Buchler 3-1500 power supply, and electrophoresed (using constant power) at 5 mA/gel for approximately 17 h until the tracking dye reached the bottom of the gel (O'Farrell, 1975). When the run was completed, the gels were removed from the glass plates and stained. Both Coomassie blue and silver staining techniques were used.

Silver Staining

The second dimension gels were developed in silver stain (BIO-RAD) which is more sensitive than Coomassie blue. First the gel was fixed with a solution of 40% methanol and 10% acetic acid for 1 h and washed twice with a 10% ethanol, 5% acetic acid solution for 30 min each wash. The gel was then oxidized with a potassium dichromate and nitric acid solution for 10 min, rinsed

three times with deionized water (10 min each wash) and placed in the silver reagent for 30 min. The gel was removed, washed once with deionized water for 2 min and placed in developer (2.5 ml of 1% sodium carbonate and 250 ul of 38% formaldehyde) for 1 min. Fresh developer was added for approximately 5 min and this procedure was repeated until protein spots became visible. At this point the gel was placed into a 5% acetic acid stop solution to prevent over-development. All flat gels were stored between two sheets of electrophoresis film (#E-138 0, Sigma Chemical), for future study.

Statistical Methods of Analysis

For statistical comparison of similarities of band patterns between samples (i.e. the presence or absence of bands), the Coefficient of Jaccard (CJ) (Sneath, 1957) was used. This formula was determined to be the best statistical method to examine the band similarities between tissues since it gave more weight to matches than to mismatches by omitting the consideration of negative matches. This formula only analyzes the presence of bands not their concentrations.

$$CJ = \frac{A}{A + B + C}$$

where A = bands in common, B = bands unique to sample lane B, and C = bands unique to sample lane C. To describe the applied degree of similarity, cluster analyses were done to divide these data into three categories, coefficients of 30% or more were considered very similar, 15% to 29% randomly similar, and below 15% very dissimilar. Mean molecular weights and pI values also were chosen for analysis to determine overall changes in tissue protein composition (i.e., a decrease in the mean molecular weight and/or mean pI value would indicate a loss of polypeptides, an increased production of smaller polypeptides, or an increased volume of already existing proteins).

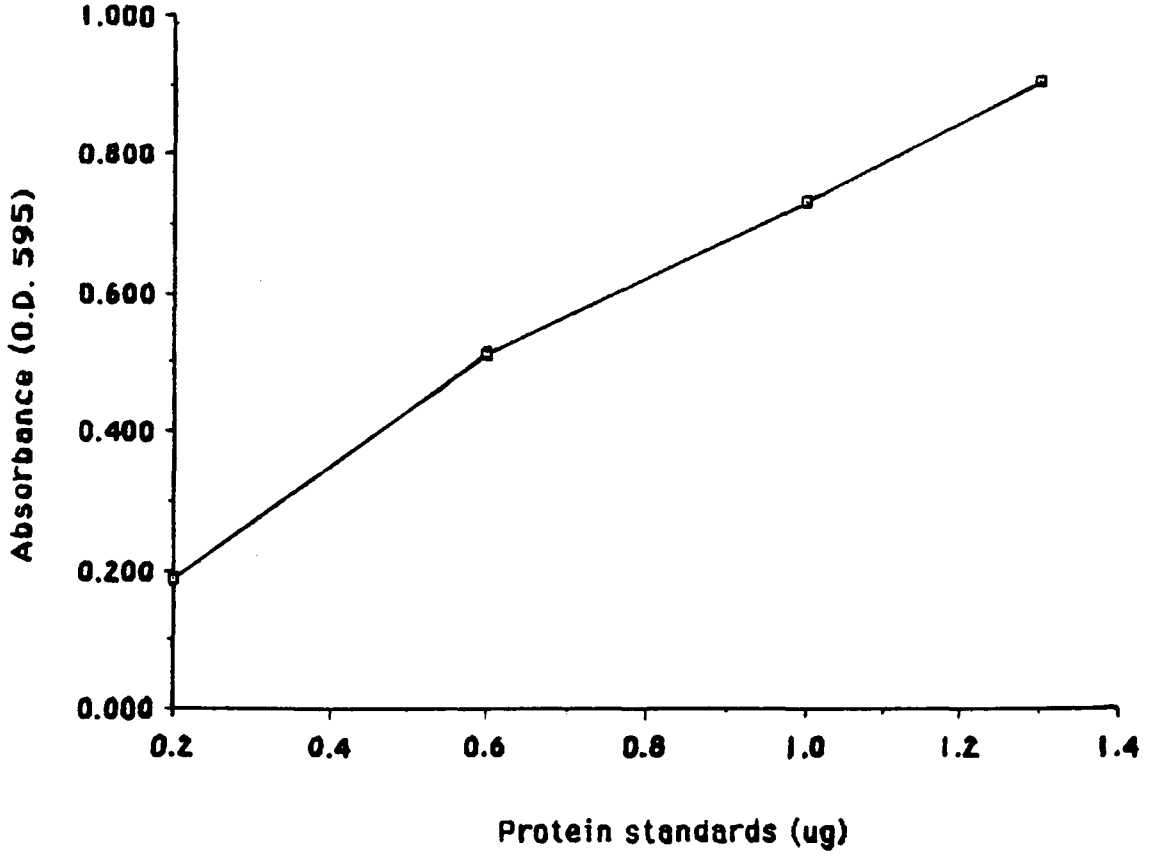
Results

The results are divided into the following sections: cellular comparisons, adult tissue comparisons, and cross tissues comparisons. Table 1 shows the total soluble protein content of all the tissues examined over all the feeding stages, using known concentrations of bovine gamma globulin for a standard curve (Fig. 1). When median molecular weights and pI values are substituted for mean molecular weights and pI values, the actual numbers may be slightly different but the results are the same.

TABLE 1. Total soluble protein content of selected *D. variabilis* tissues in various feeding stages of the adult (unfed,part-fed, replete female tissues, and part-fed male testes). All values are the averages of pooled tissue samples.

Tissue	Stage of life cycle	Tissue weight, mg/tick	ug protein/ organ	ug protein/ mg tissue
Synganglion	unfed	0.026	0.002	0.096
	part-fed	0.021	0.011	0.543
	replete	0.065	0.013	0.206
Hemolymph	part-fed	1.0 ul	0.115	114.60
	replete	1.0 ul	0.143	142.40
Salivary gland	unfed	0.469	0.095	0.203
	part-fed	0.898	0.777	0.865
	replete	4.035	0.647	0.160
Ovary	unfed	0.131	0.017	0.128
	part-fed	0.429	0.133	0.311
	replete	4.643	2.223	0.479
Testes	part-fed	0.221	0.160	0.724

Fig. 1. Standard curve of bovine gamma globulin protein standards.


1.000
0.800
0.600
0.400
0.200
0.000
Absorbance (O.D. 595)
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Protein standards (ug)

Cellular Comparisons

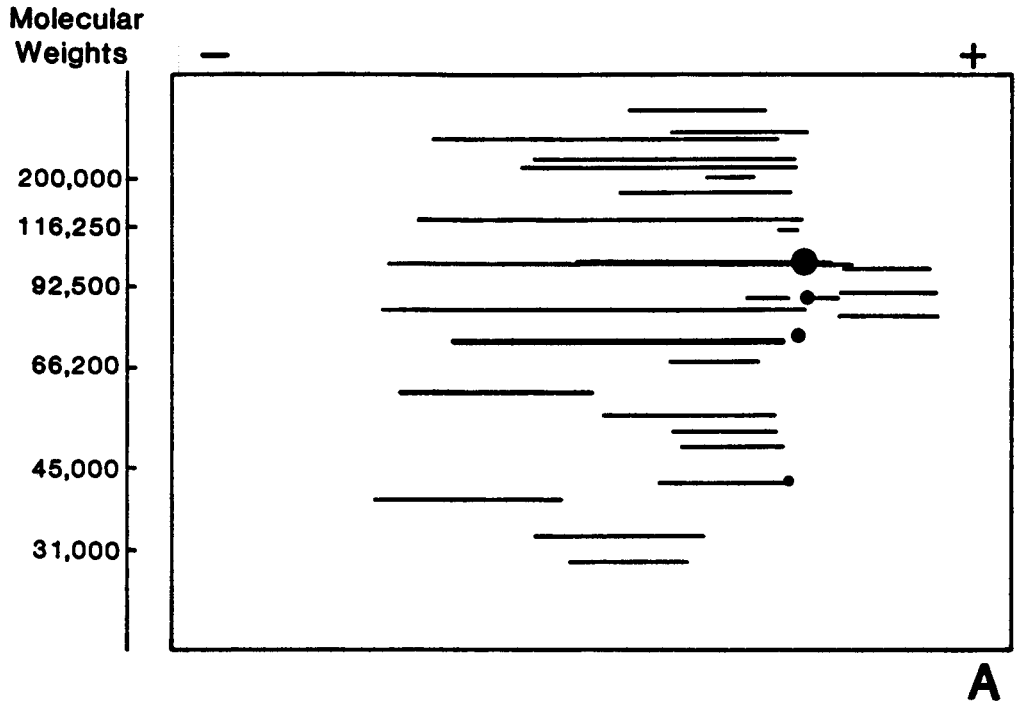
When comparing cultured cells and embryonic cells by two dimensional polyacrylamide gel electrophoresis (Fig. 2), there were six major spots in the cultured cells and four major spots in the embryonic cells. In the cultured cells all but two of the spots had a neutral pH and spread down the gel evenly in the molecular weight dimension, while in the embryonic cells all four spots had a positive pH and three of the four had molecular weights between 95 and 65 Kd. In the single dimension polyacrylamide gels (Fig. 5A), the cultured cells had nine bands and a mean molecular weight of 112 Kd, whereas the embryonic cells had thirteen bands and a mean molecular weight of 126.8 Kd (Table 2). In isoelectric focusing gels (Fig. 4), the cultured cells had twenty six bands and a mean pI value of 5.72 while the embryonic cells had eight bands and a mean pI value of 5.51 (Table 3). The percent similarity between these two samples was 37.5% in polyacrylamide gels and 13.3% in isoelectric focusing gels (Tables 4A and 5A).

Adult Tissues Comparisons

Synganglion

In the synganglia, when comparing samples from unfed to part-fed ticks, several differences occured (Fig. 3A and 4A). In the early stages of feeding, the total

Fig. 2. Two dimensional electrophoretic protein patterns of D. variabilis. A) Embryonic cells (200 ug protein); B) Cultured cells (200 ug protein). Isoelectric focusing was used in the first dimension (pI values) and SDS-polyacrylamide electrophoresis was used for the second dimension (molecular weights).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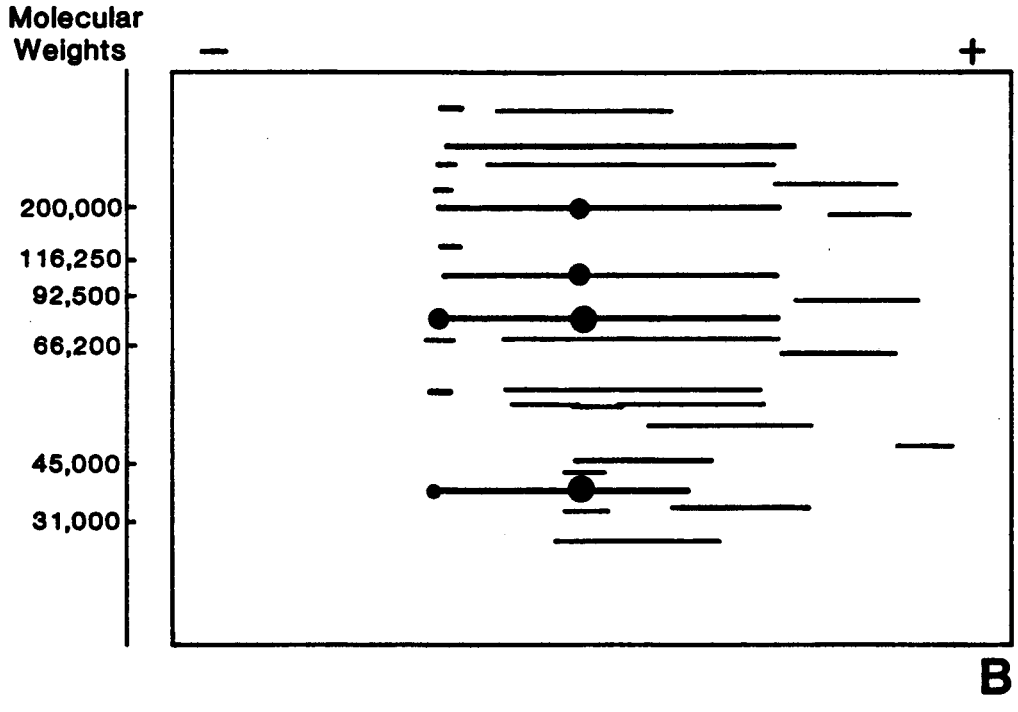
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TABLE 2. Polyacrylamide gel electrophoresis band protein analysis of selected tissues from adult *D. variabilis* ticks, cultured cells and embryonic cells.

Tissue	Stage of lifecycle	Number of bands	Mean molecular weight (Kd)
Cell	cultured	9	112.0 +/- 53.6
	embryonic	13	126.8 +/- 65.1
Synganglion	unfed	16	143.1 +/- 56.5
	part-fed	14	107.4 +/- 57.3
	replete	6	127.0 +/- 51.9
Hemolymph	part-fed	23	131.2 +/- 66.0
	replete	19	104.5 +/- 57.7
Salivary gland	unfed	17	150.8 +/- 53.2
	part-fed	14	148.2 +/- 60.3
	replete	13	129.2 +/- 44.3
Ovary	unfed	13	165.5 +/- 47.1
	part-fed	17	138.6 +/- 54.6
	replete	15	111.7 +/- 63.6
Testes	part-fed	10	104.2 +/- 51.2

TABLE 3. Isoelectric focusing band protein analysis of selected tissues from adult *D. variabilis* ticks, cultured cells and embryonic cells.

Tissues	Stage of lifecycle	Number of bands	Mean pI value (pH)
Cell	cultured	26	5.72 +/- 1.16
	embryonic	8	5.51 +/- 1.13
Synganglion	unfed	18	6.67 +/- 1.17
	part-fed	21	6.17 +/- 1.31
	replete	13	6.46 +/- 1.11
Hemolymph	unfed	36	6.59 +/- 1.44
	part-fed	23	5.84 +/- 1.66
	replete	16	6.13 +/- 1.11
Salivary gland	unfed	37	6.87 +/- 1.39
	part-fed	26	6.31 +/- 1.56
	replete	18	6.40 +/- 1.17
Ovary	unfed	25	6.61 +/- 1.25
	part-fed	33	6.24 +/- 1.56
	replete	22	6.79 +/- 0.99
Testes	part-fed	18	6.39 +/- 1.22

TABLE 4. Polyacrylamide gel electrophoresis protein similarity comparisons of selected adult *D. variabilis* tissues, cultured cells, and embryonic cells using the Coefficient of Jaccard.

A. Same tissue, different feeding stages

Sample	Comparison	% similar
Cell	culture/embryo	37.5
Synganglion	unfed/ part-fed	20.0
	unfed/ replete	22.2
	part-fed/ replete	11.1
Hemolymph	part-fed/ replete	44.8
Salivary	unfed/ part-fed	40.9
gland	unfed/ replete	30.4
	part-fed/ replete	35.0
Ovary and	unfed/ part-fed	25.0
Testes	unfed/ replete	12.0
	unfed/ testes	4.5
	part-fed/ replete	23.1
	part-fed/ testes	22.7
	replete/ testes	47.1

B. Different tissues, same feeding stage

Sample	Comparison	% similar
Unfed	synganglion/ salivary gl.	26.9
	synganglion/ ovary	26.1
	salivary gl./ ovary	20.0
Part-fed	synganglion/ hemolymph	40.9
	synganglion/ salivary gl.	16.7
	synganglion/ ovary	34.8
	synganglion/ testes	14.3
	hemolymph/ salivary gl.	23.3
	hemolymph/ ovary	17.9
	hemolymph/ testes	17.9
	salivary gl./ ovary	24.0
	salivary gl./ testes	20.0
	ovary/ testes	22.7
Replete	synganglion/ hemolymph	8.7
	synganglion/ salivary gl.	18.7
	synganglion/ ovary	16.7
	hemolymph/ salivary gl.	28.0
	hemolymph/ ovary	25.9
	salivary gl./ ovary	40.0

TABLE 5. Isoelectric focusing electrophoresis protein similarity comparisons of selected adult *D. variabilis* tissues, cultured cells, and embryonic cells using the Coefficient of Jaccard.

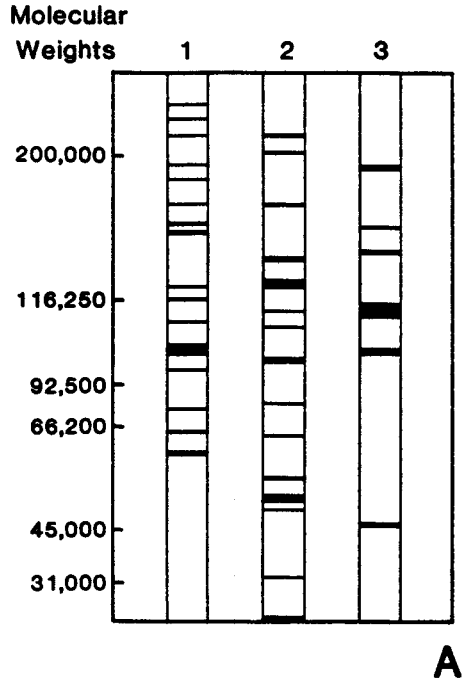
A. Same tissue, different feeding stages

Sample	Comparison	% similar
Cell	cultured/ embryonic	13.3
Synganglion	unfed/ part-fed	18.2
	unfed/ replete	3.3
	part-fed/ replete	13.3
Hemolymph	unfed/ part-fed	20.4
	unfed/ replete	23.8
	part-fed/ replete	8.3
Salivary	unfed/ part-fed	21.2
gland	unfed/ replete	34.2
	part-fed/ replete	22.2
Ovary and	unfed/ part-fed	20.8
Testes	unfed/ replete	30.6
	unfed/ testes	19.4
	part-fed/ replete	12.2
	part-fed/ testes	18.6
	replete/ testes	14.3

B. Different tissues, same feeding stage

Sample	Comparison	% similar
Unfed	synganglion/ hemolymph	20.0
	synganglion/ salivary gl.	19.6
	synganglion/ ovary	19.4
	hemolymph/ salivary gl.	37.7
	hemolymph/ ovary	32.6
	salivary gl./ ovary	29.6
Part-fed	synganglion/ hemolymph	15.8
	synganglion/ salivary gl.	20.5
	synganglion/ ovary	17.4
	synganglion/ testes	18.2
	hemolymph/ salivary gl.	19.5
	hemolymph/ ovary	21.7
	hemolymph/ testes	13.9
	salivary gl./ ovary	47.5
	salivary gl./ testes	15.8
	ovary/ testes	18.6
Replete	synganglion/ hemolymph	11.5
	synganglion/ salivary gl.	24.0
	synganglion/ ovary	12.9
	hemolymph/ salivary gl.	25.9
	hemolymph/ ovary	11.8
	salivary gl./ ovary	29.0

Fig. 3. Comparison of the same tissues of *D. variabilis* in different stages of the lifecycle, by polyacrylamide gel electrophoresis (PAGE). A) Synganglion 1. unfed 2. part-fed 3. replete; B) Hemolymph 1. part-fed 2. replete (hemolymph from unfed ticks unattainable); C) Salivary gland 1. unfed 2. part-fed 3. replete; D) Ovary and Testes 1. unfed 2. part-fed 3. replete 4. part-fed testes.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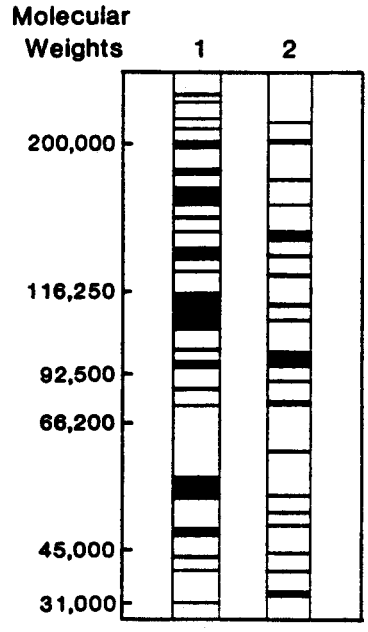
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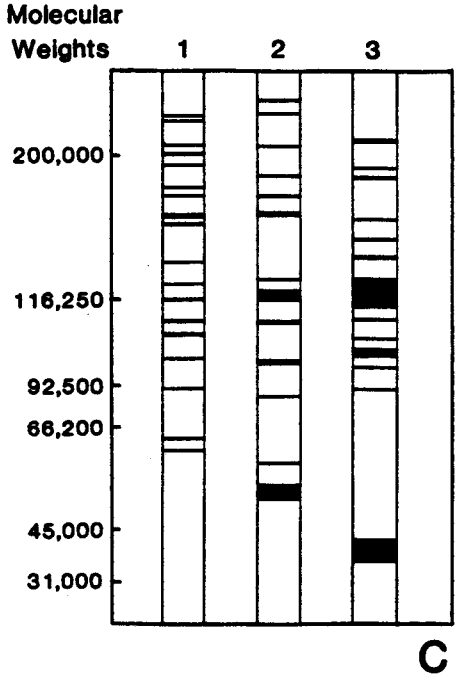
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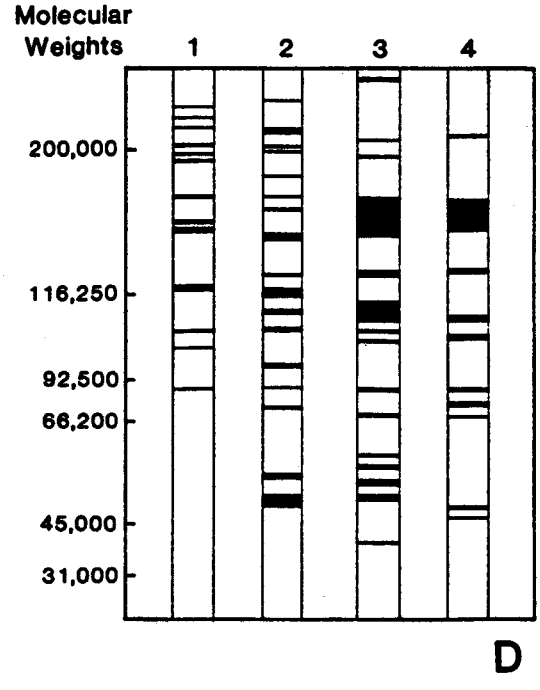
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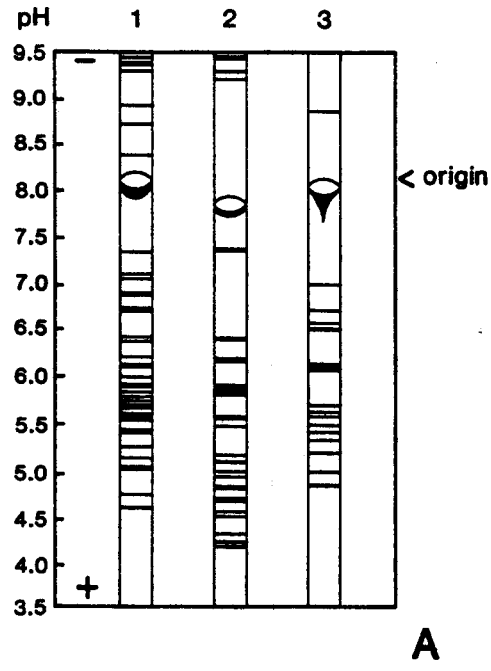
Fig. 4. Comparison of the same tissues of *D. variabilis* in the different stages of the lifecycle by isoelectric focusing separation. A) Synganglion 1. unfed 2. part-fed 3. replete; B) Hemolymph 1. unfed 2. part-fed 3. replete; C) Salivary gland 1. unfed 2. part-fed 3. replete; D) Ovary and Testes 1. unfed 2. part-fed 3. replete 4. part-fed testes.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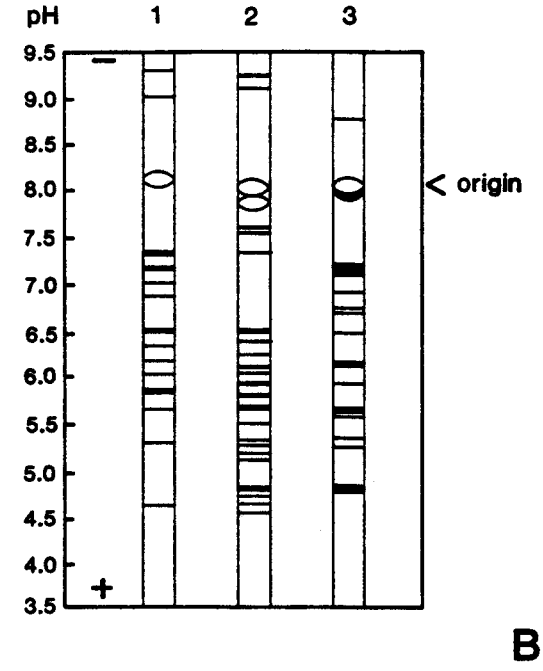
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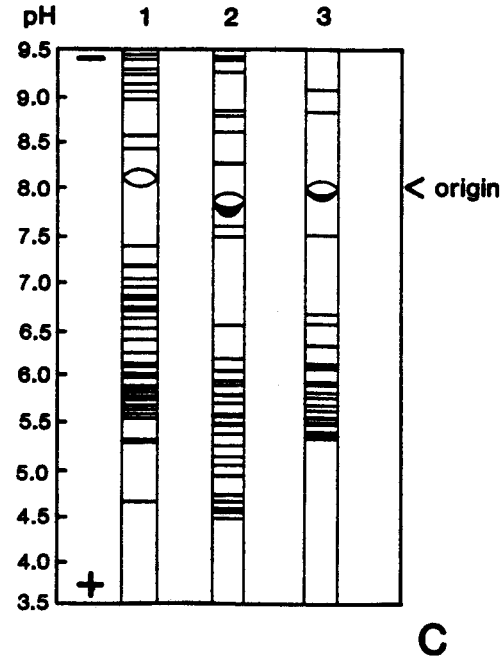
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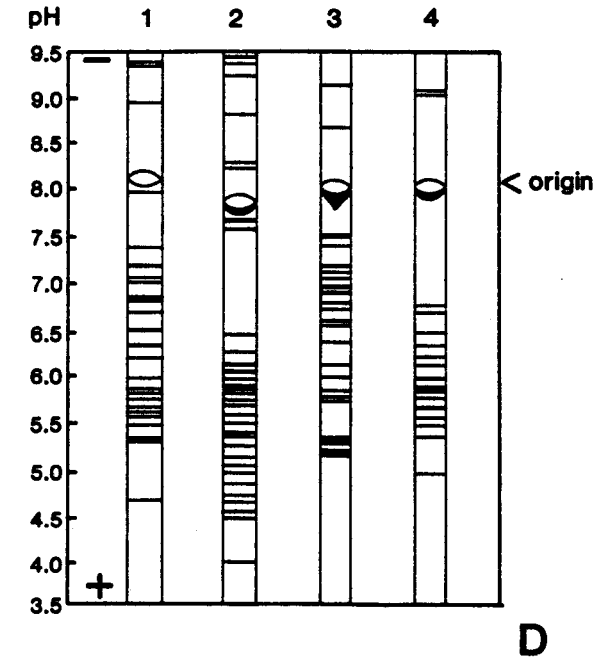
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soluble protein concentration increased 5.7 times as the number of bands in polyacrylamide gel samples decreased and the band number in isoelectric gel samples increased. Both the mean molecular weight and mean pI value of the proteins decreased in this stage of feeding (Tables 2 and 3). In later stages of feeding (part-fed to replete) the concentration of total soluble protein decreased per mg tissue (Table 1), and the number of bands in both polyacrylamide and isoelectric gels decreased while the mean molecular weight and pI values of the proteins increased (Tables 2 and 3). In the polyacrylamide gel samples, there was one dark staining band corresponding to proteins of approximately 95 Kd in unfed ticks which decreased in staining intensity in the part-fed samples and remained about the same in replete samples. At 100 Kd, protein concentrations were low through part-fed samples and increased dramatically in replete samples. Lastly, protein concentrations at 120 Kd increased when comparing unfed to part-fed then disappeared in replete samples (Fig. 3A). In isoelectric gel samples, four band modifications were observed. The major protein concentration difference showed up at approximately pH 7.3 where a substantial increase occurred when part-fed and replete samples were compared. The other three were relatively minor bands that increased only slightly as feeding continued to repletion (Fig. 4A).

Hemolymph

The protein content of the hemolymph was higher than any other tissue studied and as feeding continued the protein concentration increased (Table 1). The number of bands decreased in both polyacrylamide and isoelectric gels when comparing unfed through replete hemolymph samples (unfed samples for polyacrylamide gels were unattainable). In early feeding, the proteins analyzed on isoelectric gels decreased in mean pI values, then increased slightly in the replete samples (Table 3). In the polyacrylamide gels, the mean molecular weight of the proteins decreased when comparing part-fed samples to replete (Table 2). Some of the darker staining bands decreased in size when comparing part-fed to replete, but there were two bands (150 Kd. and 93 Kd.) that exhibited moderate increases in size in the replete samples (Fig. 3B). For the hemolymph samples on isoelectric gels there was one major band (pH 6.15) that increased in size as feeding continued (Fig. 4B). In the hemolymph, the degree of similarity between the polyacrylamide gel samples was one of the highest values measured (Table 4A) and for the isoelectric gel samples, comparison of part-fed to replete showed one of the lowest values for similarity (Table 5A).

Salivary Gland

The total soluble protein content in the salivary gland increased 4.5 X in early feeding stages, unfed to part-fed, (Table 1), and there was a decrease in band number for both polyacrylamide and isoelectric gel samples for this period (Tables 2 and 3). There was also a decrease in mean molecular weight and pI value for proteins in the bands. In late feeding stages (part-fed to replete) the total soluble protein concentration decreased (Table 1). The number of bands continued to decrease for the samples analyzed on both polyacrylamide and isoelectric gels, and the mean molecular weight also continued to drop while the mean isoelectric point of the proteins increased slightly (Tables 2 and 3). When comparing the three feeding stages of the salivary gland there was a high degree of similarity for polyacrylamide gel samples and slightly elevated percentages in the isoelectric gel samples (Tables 4A and 5A). In polyacrylamide gel samples there were two major proteins in the salivary gland that increased in concentration as feeding progressed (116 Kd and 93 Kd). The lowest protein in each sample period decreased in molecular weight, from 64 Kd in unfed to 52 Kd in part-fed to 32 Kd in replete, while it increased in concentration (Fig. 3C). In the isoelectric gel samples, there were two

proteins (pH 6.1 and 5.9) that increased in concentration in the replete stage (Fig. 4C).

Ovary

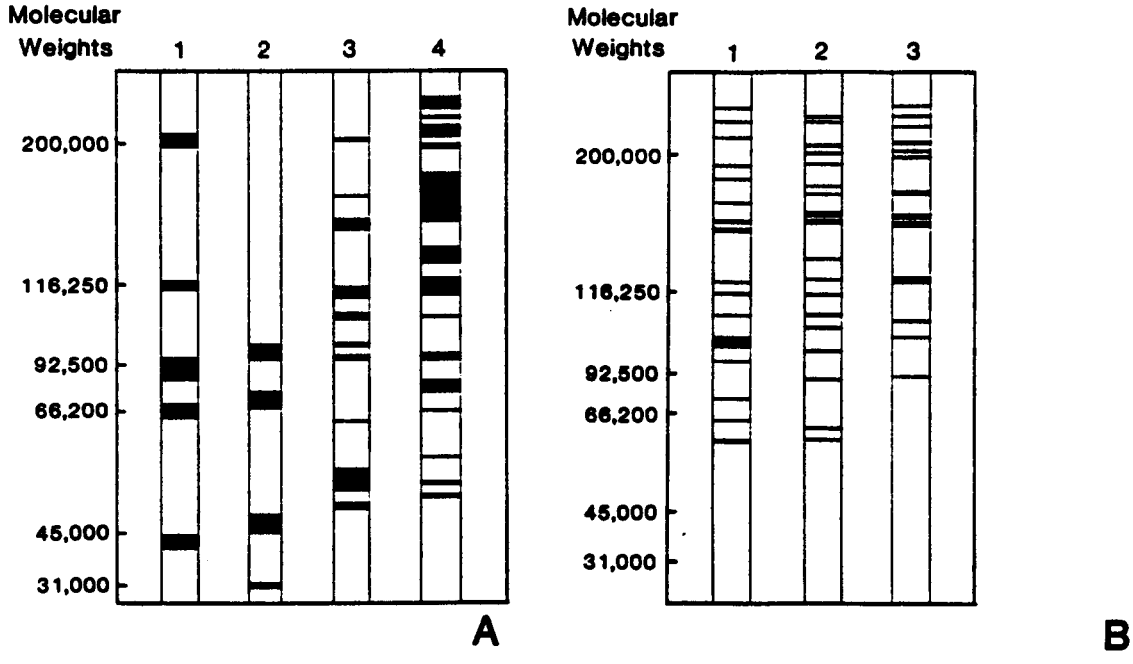
The total soluble protein content in the ovary increased throughout feeding. As tissue weight increased from 0.13 mg to 4.64 mg, the protein content/organ increased from 0.017 ug/organ in unfed tissues to 2.22 ug/organ in the replete tissue (Table 1). During the early feeding stages, in both polyacrylamide and isoelectric gel samples, the number of bands increased while the mean molecular weight and pI value of the proteins in the bands decreased. Comparing part-fed to replete ovary samples, in both the polyacrylamide and isoelectric gels the number of bands decreased. The mean molecular weight also decreased while the mean pI value increased (Tables 2 and 3). One comparison that had a high percent of similarity was unfed to part-fed ovary samples in polyacrylamide gels (Table 4A). Also in polyacrylamide gels, there were three major protein changes in the ovary samples. The first protein was approximately 150 Kd and started out at a low concentration, which increased slightly in part-fed and finally was very concentrated in the replete samples. The second protein was approximately 116 Kd and the concentration remained constant throughout feeding. The

third major protein was approximately 100 Kd. It was not present in the unfed ovary samples, weak in the part-fed ovary samples, and very concentrated in replete ovary samples (Fig. 3D). In the isoelectric gel samples there were two major proteins at pH 5.0 and 5.5 in the replete ovaries which matched two weak-staining proteins in part-fed samples. There was only one of these proteins in the unfed ovaries (Fig. 4D).

Cross Tissue Comparisons

Several similarities occured when examining different tissues in the same feeding stage (Fig. 5 and 6). Comparison of the different tissue types in unfed ticks resulted in a high degree of similarity between the hemolymph and salivary gland (37.7%) and also between the hemolymph and ovary (32.6%) in isoelectric focusing gels (Table 5B). In polyacrylamide gels, the part-fed samples had several tissues with similar data points; the synganglion had a relatively high degree of similarity with both the hemolymph and the ovaries, 40.9% and 34.8% respectively (Table 4B). The isoelectric focusing gel data for these same tissues did not result in elevated percentages of similarity. However, in isoelectric focusing gels the part-fed ovary and salivary gland showed an increased degree of similarity (47.5%). In replete samples the ovary and salivary gland again showed

Fig. 5. Comparison of different tissues of D. variabilis in the same stage of the adult lifecycle by polyacrylamide gel electrophoresis. A) Molecular weight markers (MWM) and cellular comparisons 1. high MWM 2. low MWM 3. cultured cells 4. embryonic cells; B) Unfed tissues 1. synganglion 2. salivary gland 3. ovary (hemolymph from unfed ticks unattainable); C) Part-fed (7 days) tissues 1. synganglion 2. hemolymph 3. salivary gland 4. ovary; D) Replete tissues 1. synganglion 2. hemolymph 3. salivary gland 4. ovary 5. testes.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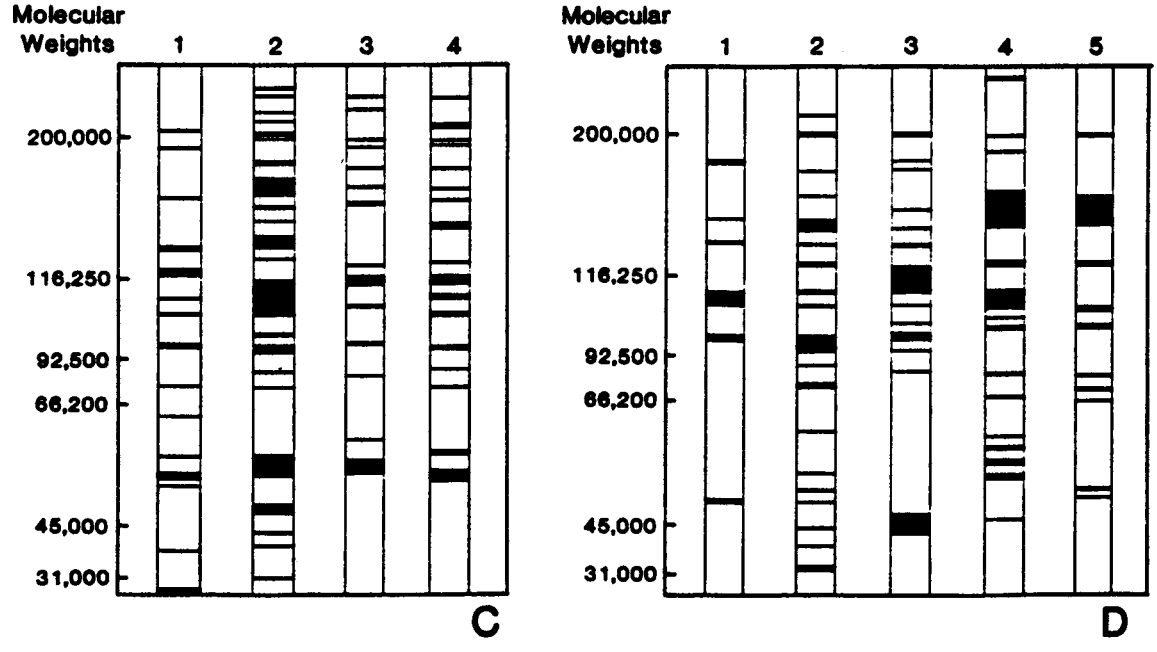
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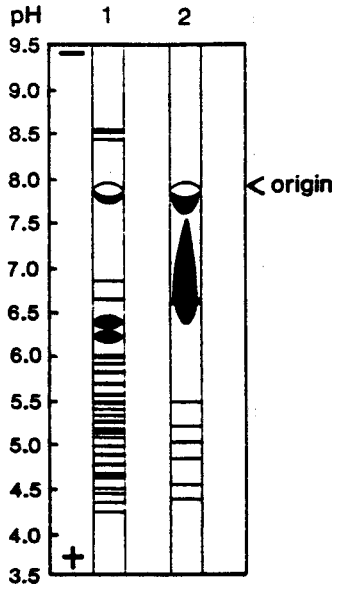
Fig. 6. Comparison of different tissues of *D. variabilis* in the same stage of the adult lifecycle by isoelectric focusing separation. A) Cellular comparisons 1. cultured 2. embryonic cells; B) Unfed tissues 1. synganglion 2. hemolymph 3. salivary gland 4. ovary; C) Part-fed (7 days) tissues 1. synganglion 2. hemolymph 3. salivary gland 4. ovary; D) Replete tissues 1. synganglion 2. hemolymph 3. salivary gland 4. ovary 5. testes.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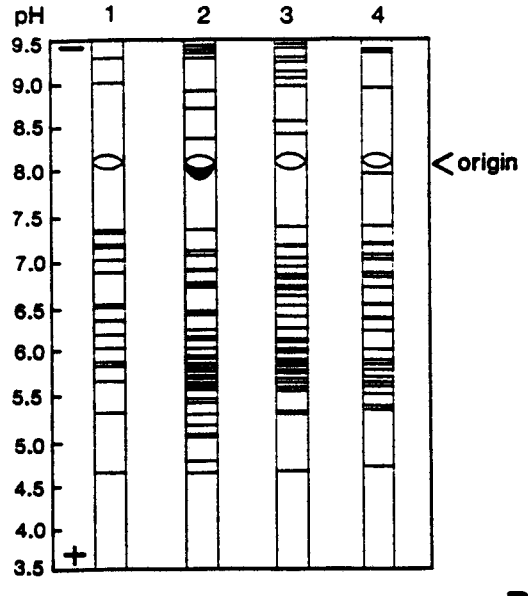
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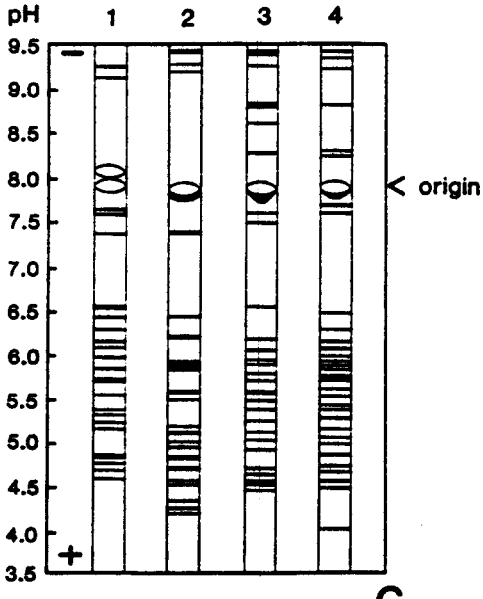
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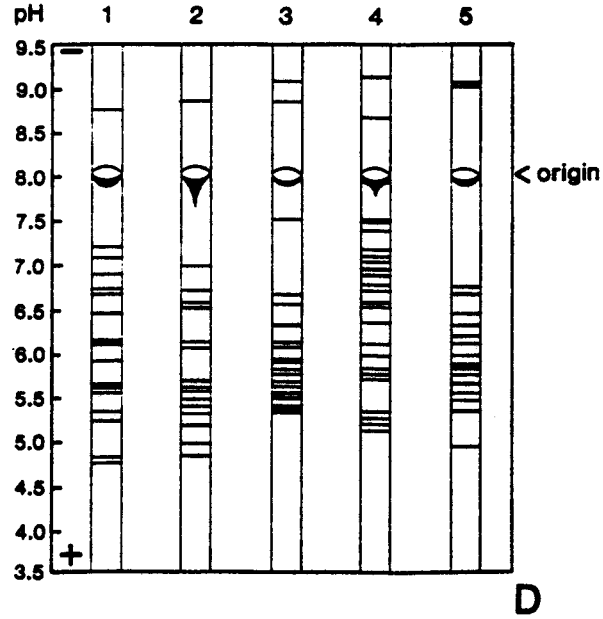
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D

an increase in percent similarity in both polyacrylamide and isoelectric focusing gels, 40.0% and 29.0% respectively (Tables 4B and 5B).

Discussion

Cellular Comparisons

In view of the data presented for the embryonic and cultured cells it would seem that there are few similarities. The similarities that do exist appear in the polyacrylamide gels which would indicate that at least some of the proteins in the two samples have similar molecular weights. The large differences in the parameters of the isoelectric focusing gels (i.e. number of protein bands and mean pI value) but similarity of the molecular weights would indicate differences in amino acid composition between these two tissues. These data could be explained by changes in amino acid composition that would produce changes in the total charge of the polypeptide but not in the molecular weights. Proteins used in the growth medium of the cultured cells may account for some of the dissimilarities, but the possiblity that there is a divergence in cell type between the developing embryonic cells and the undifferentiated cells in culture can not be ruled out.

Adult Tissues Comparisons

Synganglion

The protein patterns for the synganglia fit the general trend of production and storage in early feeding stages and either enzymatic degradation or release of proteins into the hemolymph in later feeding stages. These proteins and other substances are then secreted into the hemolymph and transported to areas where they could be used in several ways including control of egg production, digestion, molting, and several other physiological functions in the tick. Dhanda, (1967) also presented evidence in H. dromedarii that, as feeding continues, neurosecretory cells become active, the cytoplasm fills with secretory substances and, after oviposition, some types of these neurosecretory cells appear to be empty.

Hemolymph

Preliminary studies using polyacrylamide gel electrophoresis on part-fed female D. variabilis hemolymph shows 21 protein bands (Ben-Yakir and Barker, 1985). These data are very similar to the data presented in this paper including the molecular weights and concentrations of numerous proteins. Both polyacrylamide and isoelectric focusing gel data indicate that, as feeding progresses, total soluble protein concentration

increases as smaller peptide subunits are being synthesized. This production may represent the transformation of host proteins into subunits that could be used to develop tick proteins such as vitellogenin and vitellin needed for oocyte (yolk) production. This is one of many hypotheses since the hemolymph is the transport system for many of the substances produced in the different organ systems of the tick.

Salivary Gland

Initially, when the tick first attaches to the host the salivary glands increase dramatically in size and protein content. As feeding continues to the second phase the protein content remains high (Table 1) while the number of bands and mean molecular weight decreases (Table 2 and 4) indicating breakdown and/or use of proteins possibly for structural purposes. These results concur with McSwain et al. (1982) who reported production of three groups of salivary gland cells as feeding continues from recently attached to second stage feeding A. americanum. When salivary gland tissue samples from the second feeding stage (growth phase) are compared to third feeding stage samples (rapid engorgement phase) there are decreases in total soluble protein content, number of bands, and mean molecular weight. There is also a high degree of similarity in both polyacrylamide

and isoelectric focusing samples when comparing the part-fed to replete salivary glands, meaning that, although there are changes in the protein amount of certain bands, the molecular weights of most of the proteins in the sample remain the same. An exception to this is the appearance of a large band with a molecular weight of 35 Kd and, since total soluble protein concentration in this tissue decreases, this may indicate the beginning of protein degradation in the replete tick. Kaufman (1986) reported that in the later stages of feeding (3-4 days post-engorgement), the salivary glands deteriorate and they lose 95% of their fluid secretory competence through autolysis or degradation of the tissue. This loss of tissue could explain the decrease in protein concentration as feeding concludes.

Ovary

The ovary has the largest growth rate of any organ tested, and synthesizes the most protein. In the early feeding stage, there are increases in the number of bands, mean molecular weight of the proteins, and mean pI value of the proteins, all of which indicate a build up and storage of proteins (probably vitellogenin and vitellin). Along with the increase in the number of protein bands, concentrations of several proteins increase as the feeding continues which is another

indication of protein production. According to Chinzei et al. (1983) the molecular weights of the polypeptide chains in the vitellogenin of Ornithodorus parkeri O are 215 Kd, 210 Kd, 160 Kd, 140 Kd, 125 Kd, and 100 Kd, while in vitellin the 215 Kd and 210 Kd protein bands are replaced with 64 Kd and 50 Kd protein bands. In this study D. variabilis seems to follow a similar pattern with few exceptions. In the early feeding stages, the ovary samples match four of the six bands mentioned above (215 Kd, 210 Kd, 160 Kd and 100 Kd) while later in feeding 160 Kd, 125 Kd, 100 Kd, 64 Kd, and 50 Kd bands are all present in significant quantities. The sum of the molecular weights of these polypeptides are substantially larger than the molecular weight of either vitellogenin or vitellin. This probably means that the polypeptides are not all subunits of these molecules. Chinzei et al. (1983) reported that some of these polypeptides are produced during the processing of vitellogenin to vitellin.

Cross Tissue Comparisons

When the similarities in banding patterns of different tissues in the same feeding stage are compared, the hemolymph, salivary gland, and ovary are most alike. This might be expected since both the salivary gland and possibly the ovary have some tissues with glandular

functions and the hemolymph is the transport system for the materials they secrete. The results of this study indicate that in early feeding stages there is a higher degree of similarity between these 3 tissues than in later stages. This may be due to the lack of host material in the unfed tick. As feeding continues to part-fed (ca. 7 days) the degree of similarity between ovary and salivary gland continues to increase while the similarity they both share with the hemolymph remains constant. At this point in the feeding cycle these tissues begin to increase in size possibly using stored proteins for structural changes and not secreting as much material into the hemolymph. Finally, in the replete female, the degree of similarity in the salivary gland and ovary remains elevated in both polyacrylamide and isoelectric focusing gels (40.0% and 29.0%, respectively) while the similarity with the hemolymph remains fairly constant.

The ovary and salivary gland have a high degree of similarity when using the Coefficient of Jaccard (Sneath, 1957), which compares tissues according to band number. These two tissues have similar mean molecular weights, but when total protein amounts are examined the ovary has 4X the concentration of the salivary gland. Analysis of these data indicate that while the ovary in the replete

female is converting vitellogenin into vitellin and incorporating this yolk protein into the oocyte, the salivary gland is in the early stages of degradation.

The synganglion, an organ that also has several cell types with secretory functions, shows a high degree of similarity with the hemolymph (40.0%) and slightly less with the ovary (34.8%) in part-fed female tick samples, in polyacrylamide gels. These data support those mentioned earlier in the synganglion section regarding the synganglion releasing products into the hemolymph, some of which may ultimately end up in the ovary.

When examining the band similarity comparisons between tissues the minimum percent similarity found is 3.3% (Tables 4 and 5). This is probably the lowest number possible since there are essential biosynthetic pathways common in all living cells. Some of these activities involve proteins and enzymes that are necessary for the survival of the cell.

The purpose of this study is to present a general overview of the changes which occur to the protein content of several tissue types in different feeding stages of the adult tick and comparison of proteins in embryonic and cultured cells. The next logical step would be identifying and isolating the individual

proteins towards an eventual goal of possible biological control of these medically and veterinarily important zoonoses.

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