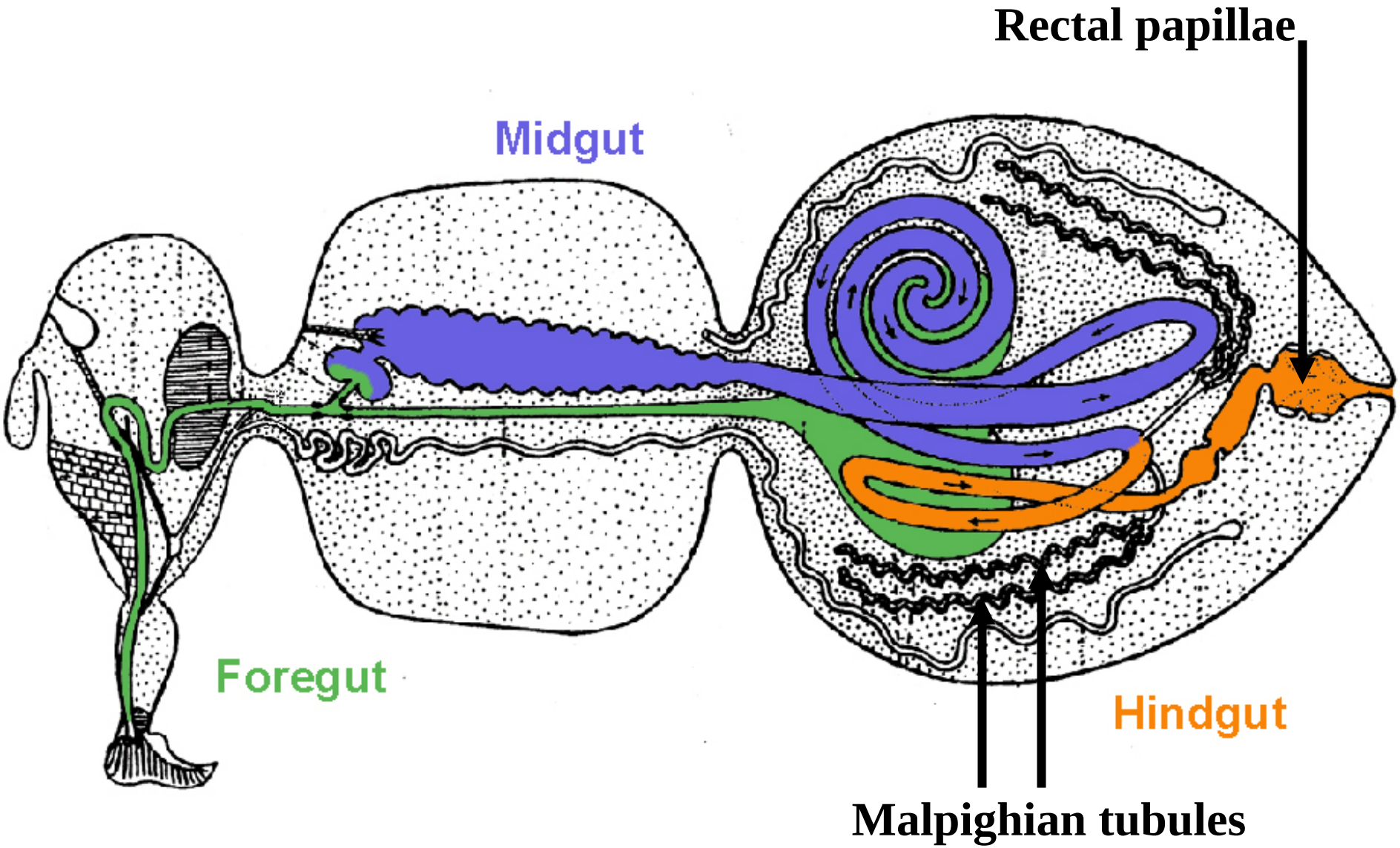
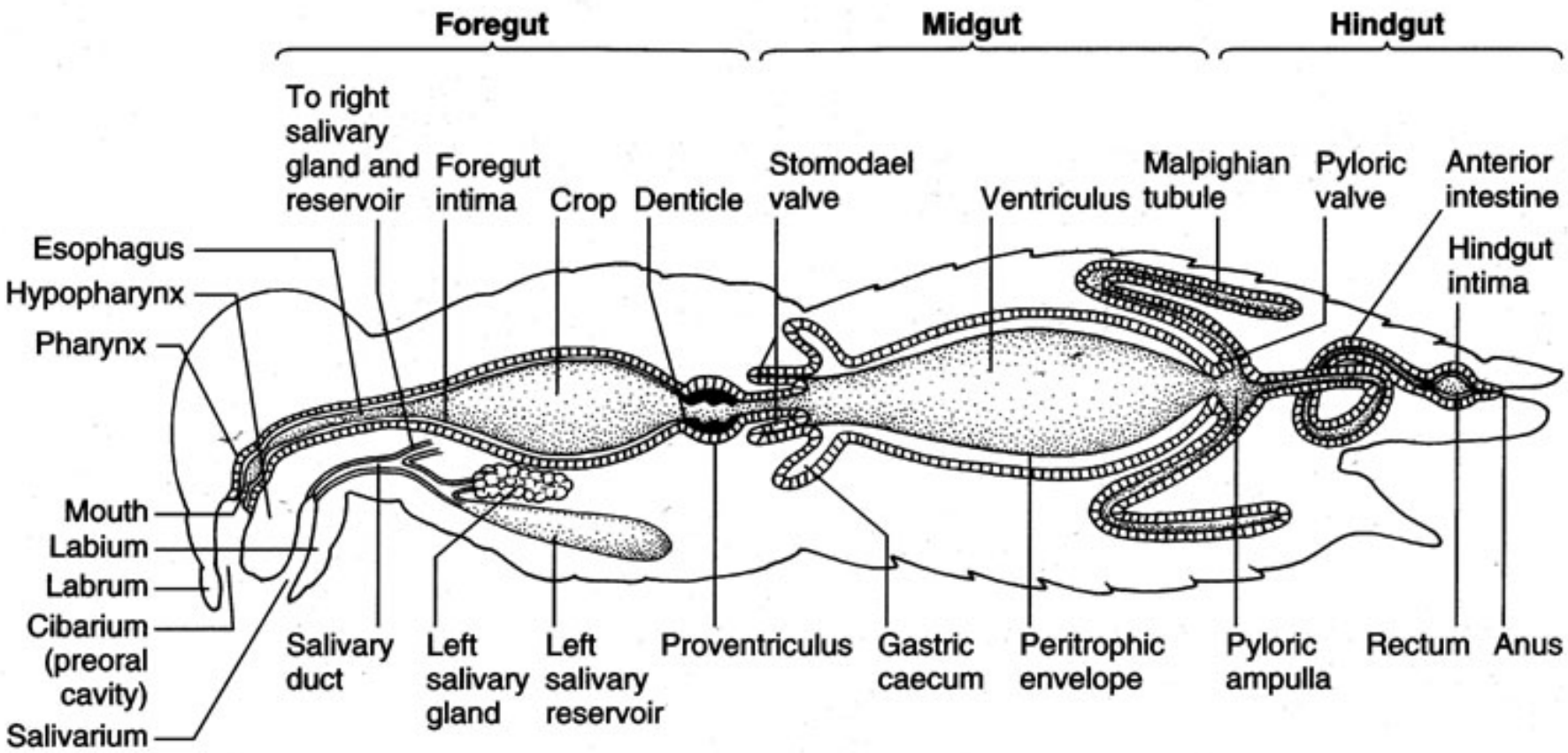
EXCRETORY SYSTEM



Generalized insect alimentary tract, including excretory system



EXCRETORY SYSTEM IN HUMANS AND INSECTS

HUMANS

1. **Liquid system tied in with the circulatory system. Includes kidneys and a urinary bladder**

2. **Main excretory product is urine (all ages)**

INSECTS

1. **System tied in with the digestive tract**

2. **Main excretory product is uric acid (adults)**

FUNCTIONS OF THE EXCRETORY SYSTEM IN INSECTS

Problems insects face in their environments

1. Losing water because of the size/volume ration of being small
2. Controlling the ionic balance of the body fluids
   a. Freshwater insects tend to lose ions to the environment
   b. Insects in salt water tend to gain ions

THESE PROCESSES BASED ON OSMOSIS AND DIFFUSION

Maintain a nearly constant internal osmotic environment of the hemolymph (**HOMEOSTASIS**), tissues, and cell environment by:

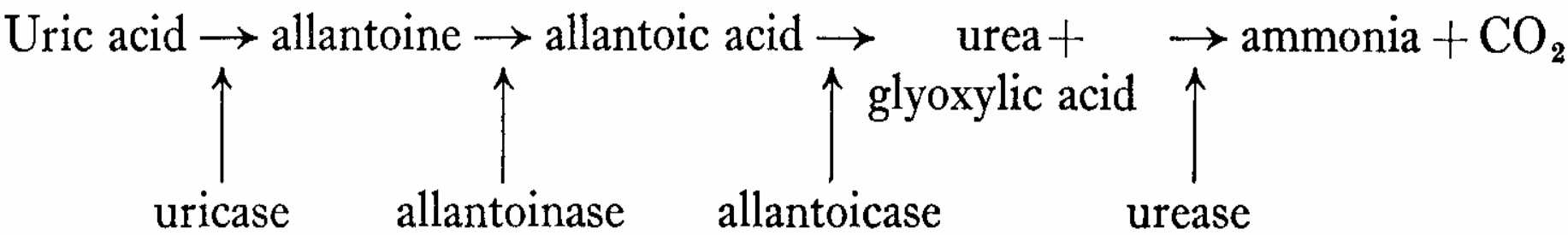
1. Elimination of excretory products
2. Reabsorption of water from the feces
3. Reabsorption and elimination of various ions
4. Absorption of materials produced by the symbionts in the hindgut of those insects housing them

What is one of the major problems facing insects?

What kinds of excretory products would one expect to find in insects and why would one expect these to be the kind of products they would produce?

WATER LOSS-INSECTS, BECAUSE OF THEIR SIZE MUST CONSERVE WATER

Cuticle and excretory system maintain proper water and ion balance



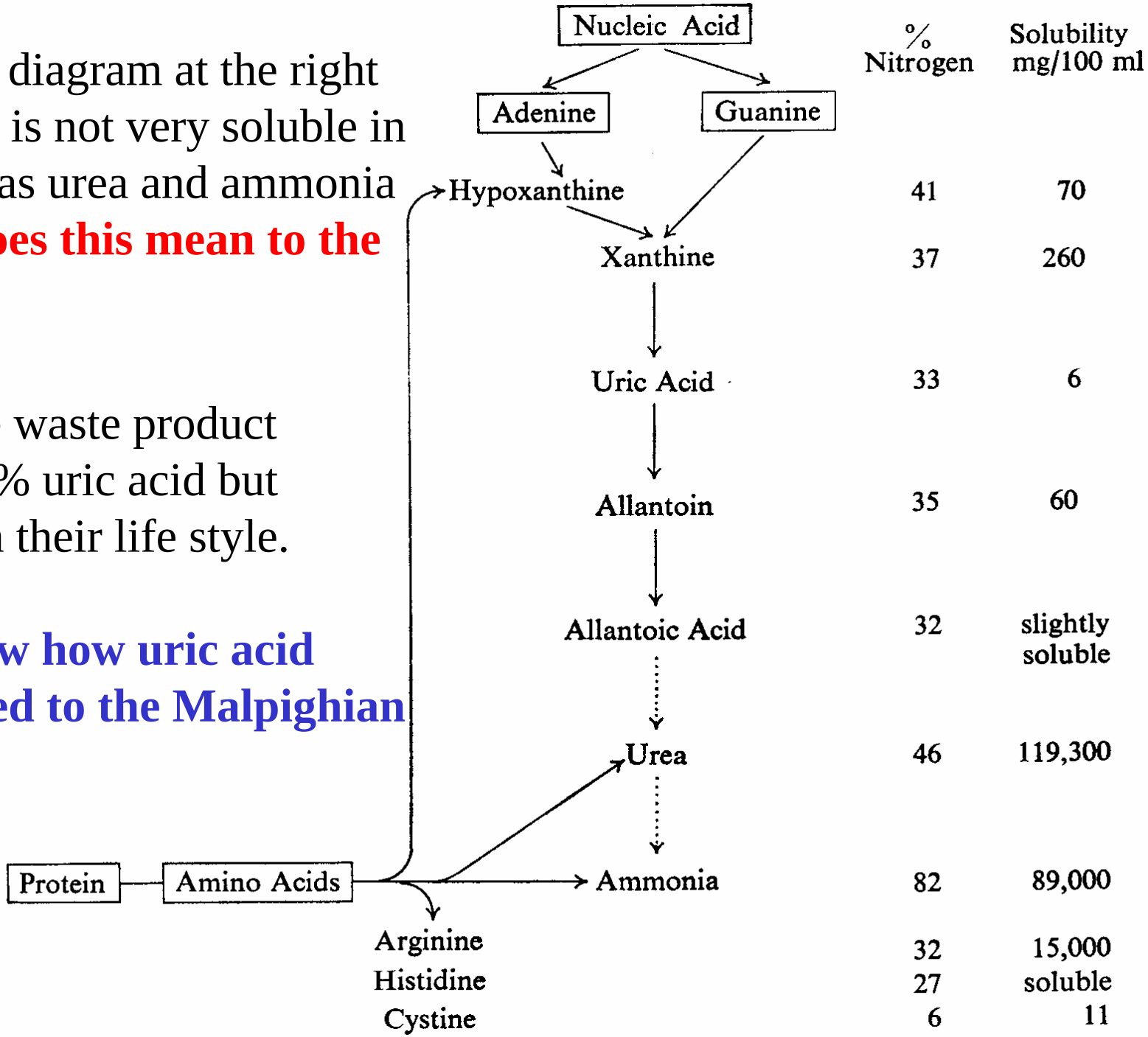
The excretory product in insects is usually colorless, it may be yellow or greenish in color depending on the food. Malpighian tubules may be whitish in color (Uric acid) or contain a yellow pigment, thus they appear yellow.

Amino acids are derived from proteins in foods. They are used by cells for synthesis of new body protein or other nitrogen-containing molecules. The amino acids not used for synthesis are oxidized to generate energy or are converted to fats or carbohydrates that can be stored. In either case, the amino groups (-NH2) must be removed because they are not needed for any of these purposes. Once the amino groups have been removed from the amino acids, they may be excreted from the body in the form of ammonia, urea, or uric acid, depending on the species.

Notice in the diagram at the right that uric acid is not very soluble in water, whereas urea and ammonia are. **What does this mean to the Insect?**

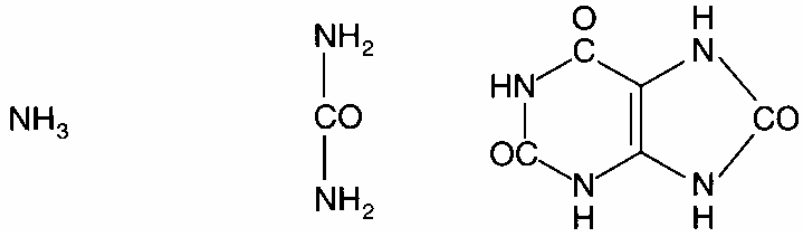
In insects the waste product is usually 80% uric acid but this varies on their life style.

It is not know how uric acid is transported to the Malpighian tubules.



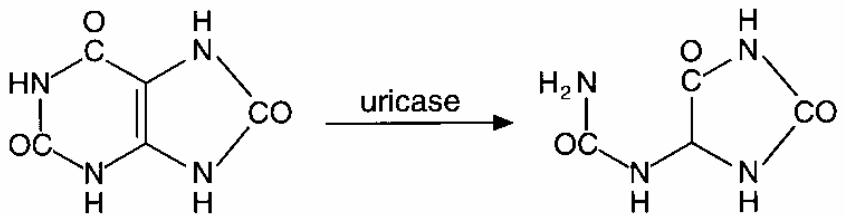
The synthesis of uric acid occurs primarily in the fat body

Since ammonia has 3 hydrogens for every nitrogen, compared to uric acid having 1 to 1, the hydrogen for the ammonia must come from somewhere. It may from water. Thus, it takes more water to get rid of ammonia.

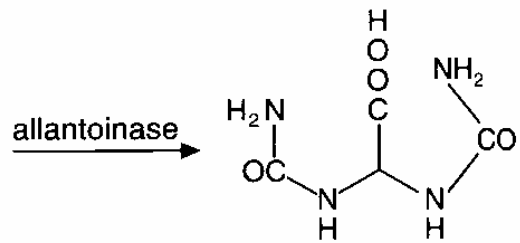


ammonia H:N 3:1 | urea H:N 2:1 | uric acid H:N 1:1

The synthesis of uric acid occurs primarily in the fat body, although synthesis in other tissues may also occur. Its *de novo* synthesis involves the progressive addition of amino groups. It may be further metabolized to allantoin and allantoic acid.



uric acid → allantoin



allantoic acid

This chart shows the type of excreta used by different insects. One should be able to correlate the life style of the insect with that of the main component of the excreta.

Table 18.1. *The distribution of nitrogen in the excreta of insects expressed as a percentage of the total nitrogen in the excreta*[a]

Insect	Order	Uric acid	Allantoin	Allantoic acid	Urea	Ammonia
Terrestrial insects solid food						
Schistocerca	Orthoptera	55	–	–	+	40
Periplaneta	Blattodea	–	–	–	–	up to 90%
Melolontha (A)	Coleoptera	100	–	–	–	–
Attagenus (A)	Coleoptera	25	–	–	35	20
Pieris (L)	Lepidoptera	95	4	1	–	–
Terrestrial insects liquid food						
Rhodnius	Hemiptera	90	–	–	+	–
Dysdercus	Hemiptera	–	61	–	12	–
Pieris (A)	Lepidoptera	20	10	70	–	–
Lucilia (L)	Diptera	–	10	–	–	90
Glossina (L)	Diptera	100	–	–	+	–
Aedes (A)	Diptera	43	–	–	13	18
Freshwater insects						
Aeschna (L)	Odonata	8	–	–	–	74
Sialis (L)	Megaloptera	–	–	–	–	90

TABLE I

Excretory products in the Orthoptera, Odonata and Dermaptera

	Uric acid	Allantoin	Allantoic acid	Urea	Ammonia	Amino acids	Author
Order ORTHOPTERA							
Schistocerca gregaria	**1·00**	0·00	0·00	+	+	—	Chauvin, 1941; Razet, 1961
Locusta migratoria	**1·00**	0·00	0·10	—	—	—	Razet, 1961; Nation and Patton, 1961
Melanoplus bivittatus	**1·00**	—	—	0·07	0·53	0·20	Brown, 1937
Acheta domesticus	**1·00**	0·01	0·01	0·00	—	—	Razet, 1961; Nation and Patton, 1961
Mantis religiosa	**1·00**	0·01	0·00	—	—	—	Razet, 1961
Periplaneta americana	**1·00**	0·00	0·00	—	—	—	Razet, 1961
Blatta orientalis	0·64	0·64	**1·00**	—	—	—	Razet, 1961
Carausis morosus	0·69	**1·00**	0·44	—	—	—	Razet, 1961
Order ODONATA							
Aeshna cyanea (larva)	0·08	—	—	—	**1·00**	—	Staddon, 1959
Order DERMAPTERA							
Forficula auricularia	**1·00**	—	0·00	—	—	—	Razet, 1961

TABLE IV

Excretory products in the Hymenoptera and Diptera

	Uric acid	Allantoin	Allantoic acid	Urea	Ammonia	Amino acids	Author
Order HYMENOPTERA							
Hemichroa alni (larva)	**1·00**	0·00	0·00	—	—	—	Razet, 1961
Pteronidea salicis (larva)	**1·00**	0·03	0·06	—	—	—	Razet, 1961
Pteronidea ribesi (larva)	0·78	**1·00**	0·90	—	—	—	Razet, 1961
Order DIPTERA							
Compsilura concinnata	**1·00**	0·04	0·00	—	—	—	Razet, 1961
Tipula paludosa	**1·00**	0·30	0·62	—	—	—	Razet, 1961
Lucilia sericata	**1·00**	0·30	—	—	0·30	—	Brown, 1936; 1938a and b
Lucilia sericata (larva)	0·05	0·02	—	—	**1·00**	—	Brown, 1936; 1938a and b
Lucilia sericata (pupa)	**1·00**	0·00	—	—	0·15	—	Brown, 1936; 1938a and b
Bibio marci (larva)	0·45	**1·00**	0·36	—	—	—	Razet, 1961
Aedes aegypti	**1·00**	—	—	0·22	0·18	0·11	Irrevere and Terzian, 1959
Anopheles quadrimaculatus	**1·00**	—	—	0·22	0·18	0·11	Irrevere and Terzian, 1959
Culex pipiens	**1·00**	—	—	0·22	0·18	0·11	Irrevere and Terzian, 1959
Glossina morsitans	**1·00**	—	—	+	—	0·22	Bursell, 1964b

TABLE V

Excretory products in the Lepidoptera (Razet, 1961)

	Uric acid	Allantoin	Allantoic acid
1. ADULTS			
Mammestra brassicae	**1·00**	—	**0·00**
Agritos comes	**1·00**	—	0·01
Aglais urticae	**1·00**	0·00	0·06
Trigonophora meticulosa	**1·00**	0·01	0·05
Pieris brassicae	**1·00**	0·04	0·01
Vanessa atalanta	0·89	0·14	**1·00**
Conistra vaccinii	0·12	—	**1·00**
2. PUPAE			
M. brassicae	**1·00**	—	0·03
A. comes	**1·00**	—	0·01
A. urticae	**1·00**	0·29	0·43
T. meticulosa	**1.00**	—	0·19
P. brassicae	**1.00**	0·03	0·05
V. atalanta	**1.00**	0·14	0·29
C. vaccinii	**1·00**	0·04	0·04
3. LARVAE			
M. brassicae	0·49	0·11	**1·00**
A. comes	0·67	0·02	**1·00**
A. urticae	0·56	0·18	**1·00**
T. meticulosa	**1·00**	0·02	0·12
P. brassicae	0·28	0·16	**1·00**
V. atalanta	**1·00**	0·26	0·20
C. vaccinii	**1·00**	0·06	0·05

This slide shows several things:

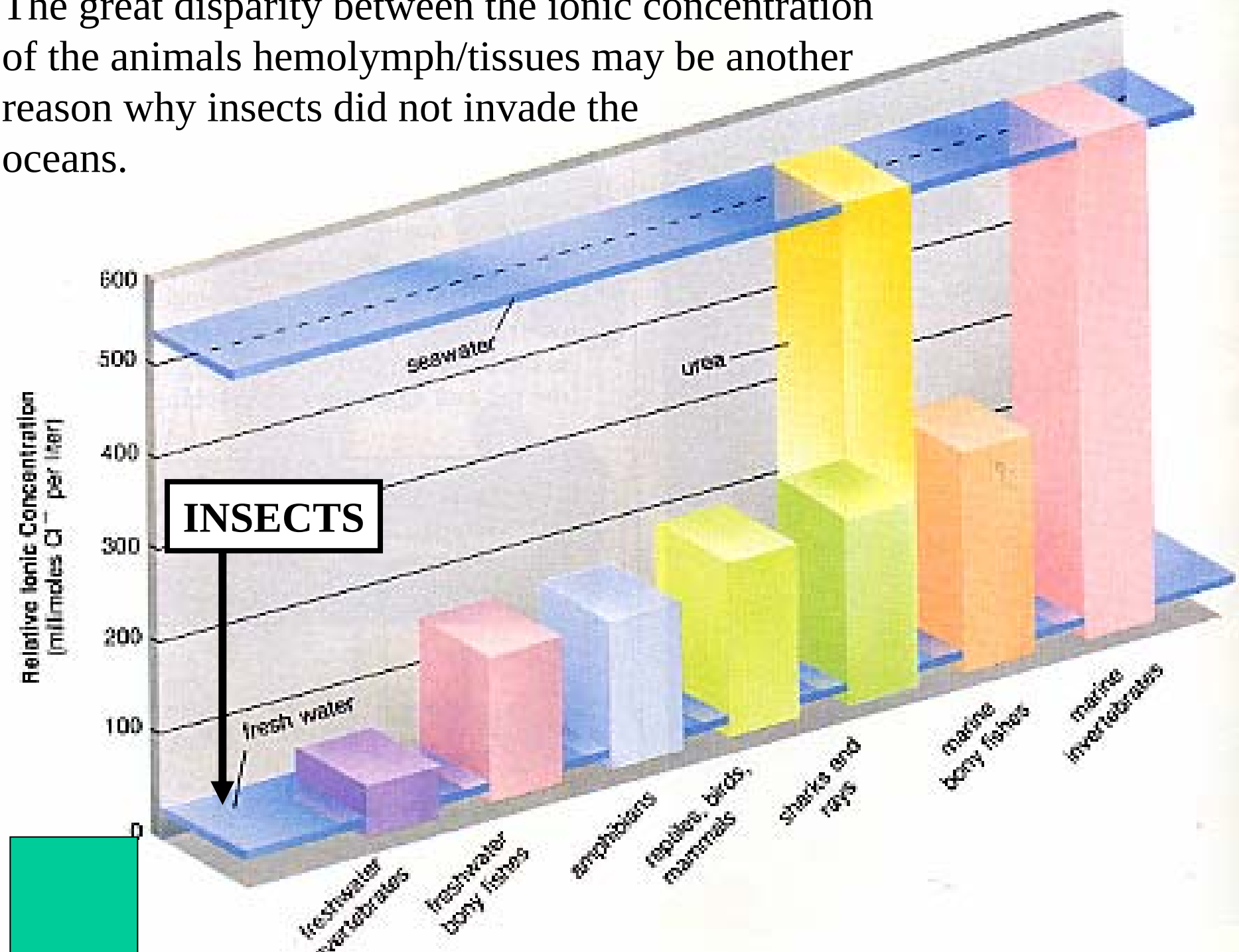
1. The hemolymph is a major storage area for amino acids.
2. Potassium is actively pumped into the Malpighian tubule, as is proline
3. Notice NO TREHALOSE IN THE Malpighian tubule.

TABLE 1 Composition of body fluids (mM) from *Schistocerca gregaria*[a]

Constituent	Hemolymph	Malpighian tubule fluid[b]
Na^+	103	47
K^+	12	165
Mg^{2+}	12	20
Ca^{2+}	9	7
Cl^-	107	88
HCO_3^-	13	NM
Phosphate	6	12
Alanine	1·0	1·0
Aspartate	0·1–0·9	0·5
Asparagine	1·0	0·0
Arginine	1·5	0·0
Glutamate	0·1–1·0	0·8
Glutamine	4	0·5
Glycine	14	4·0
Histidine	1·4	0·0
Isoleucine	0·4	0·0
Leucine	0·4	0·0
Lysine	1·0	0·0
Methionine	0·4	0·0
Phenylalanine	0·7	0·0
Proline	13	38
Serine	2–4	1·0
Threonine	0·5	0·0
Tyrosine	1·0	0·0
Valine	0·6	0·0
Glucose	2·5	4·6
Trehalose	20	NM
Acetate	2–9	4
Citrate	2	NM
Malate	<0·1	NM
pH	7·1	7·0

[a] Data from Chamberlin and Phillips (1982b), Baumeister *et al.* (1981), Hanrahan (1982), and Speight (1967).
[b] Collected by gut ligation *in situ*.
NM: not measured.

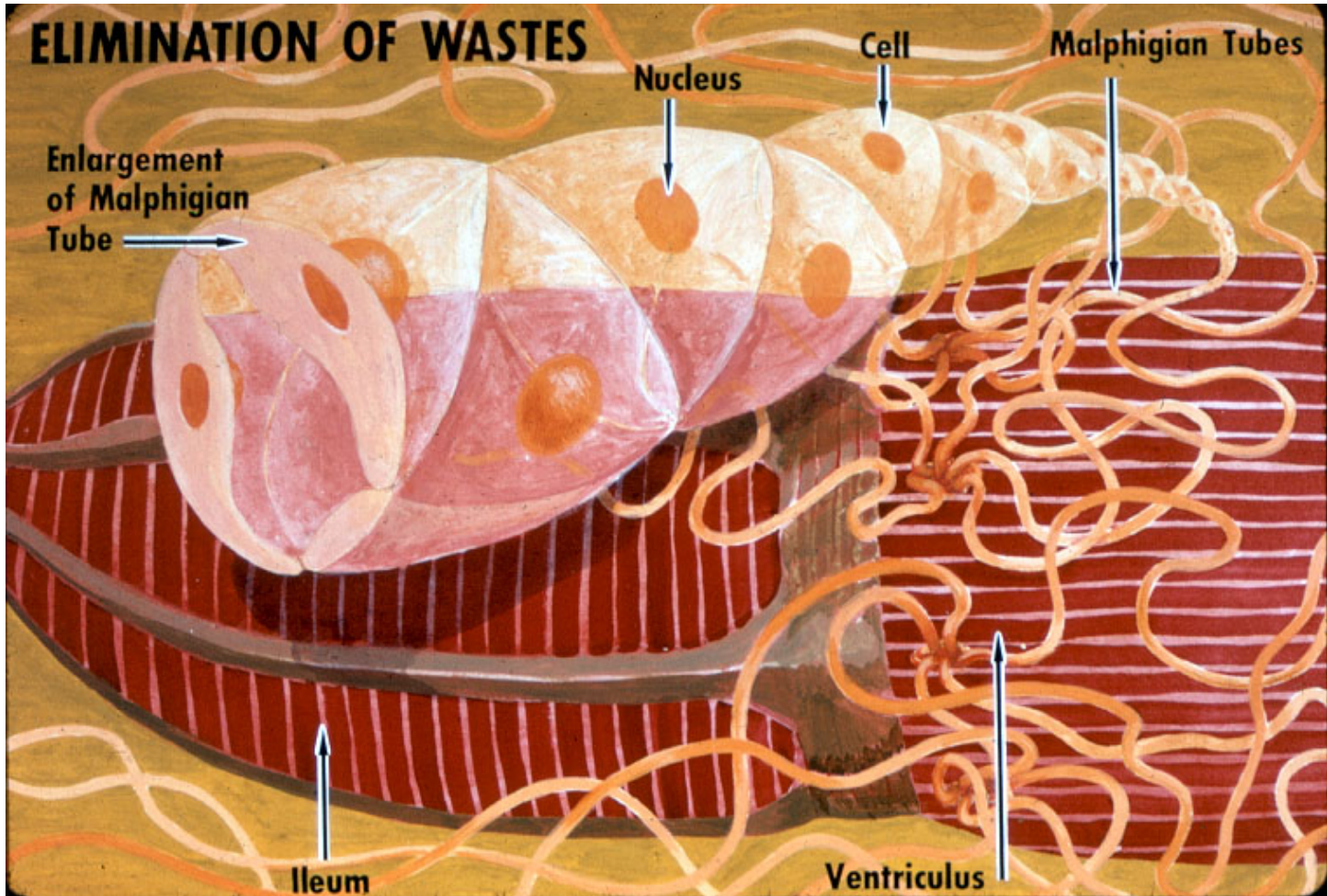
The great disparity between the ionic concentration of the animals hemolymph/tissues may be another reason why insects did not invade the oceans.



Structure of the Malpighian tubules

The Malpighian tubules are surrounded by muscles. They actually are moving in the hemolymph and can carry out peristaltic movements to move material from the terminal end to the opening in the hindgut. The Malpighian tubules produce the primary urine while the hindgut produces the secondary or final urine. They are absent in aphids and Collembola.

In Diplura and Protura they are represented only by papillae. No. varies from 2 in coccids to 250 in desert locust.



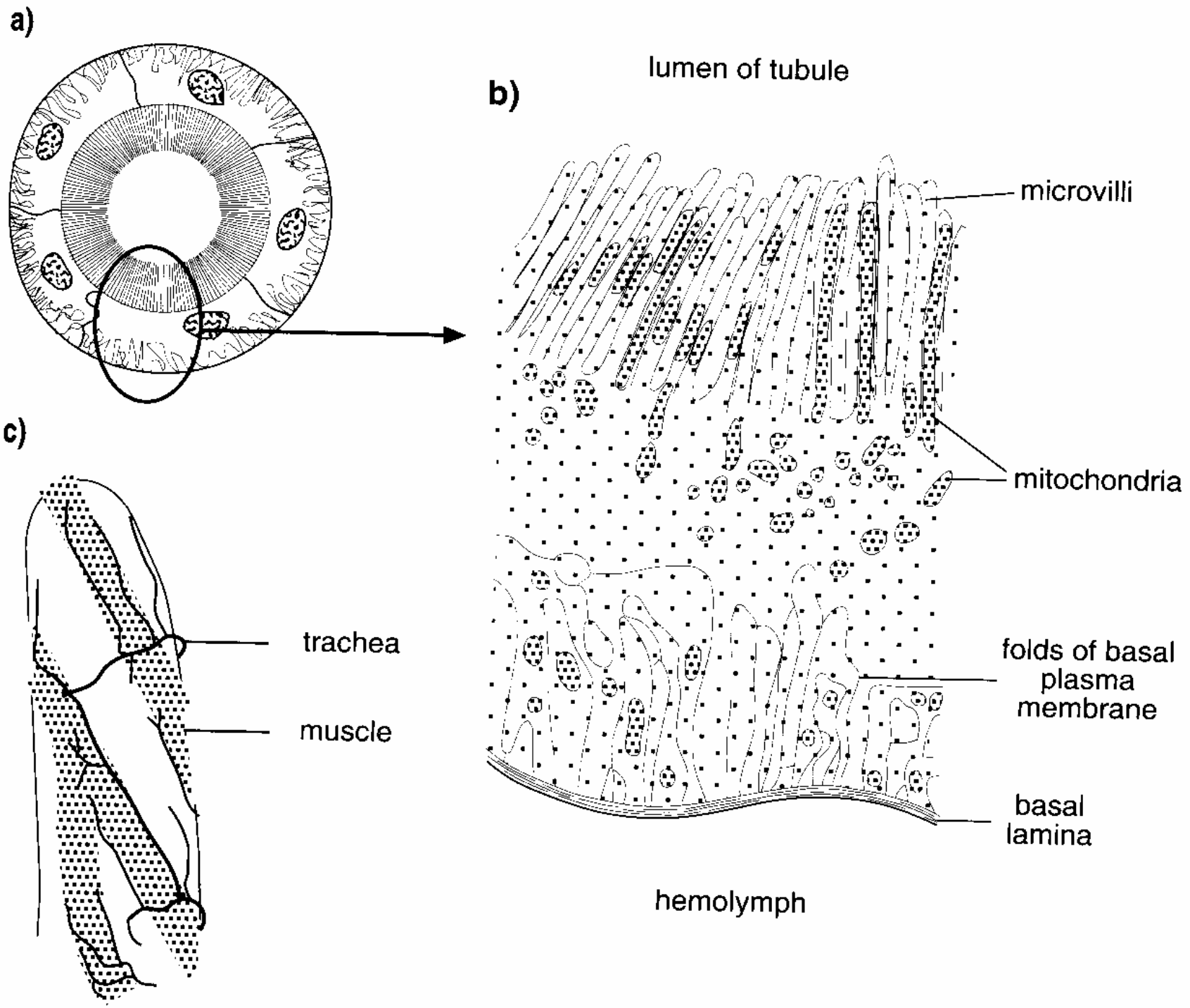
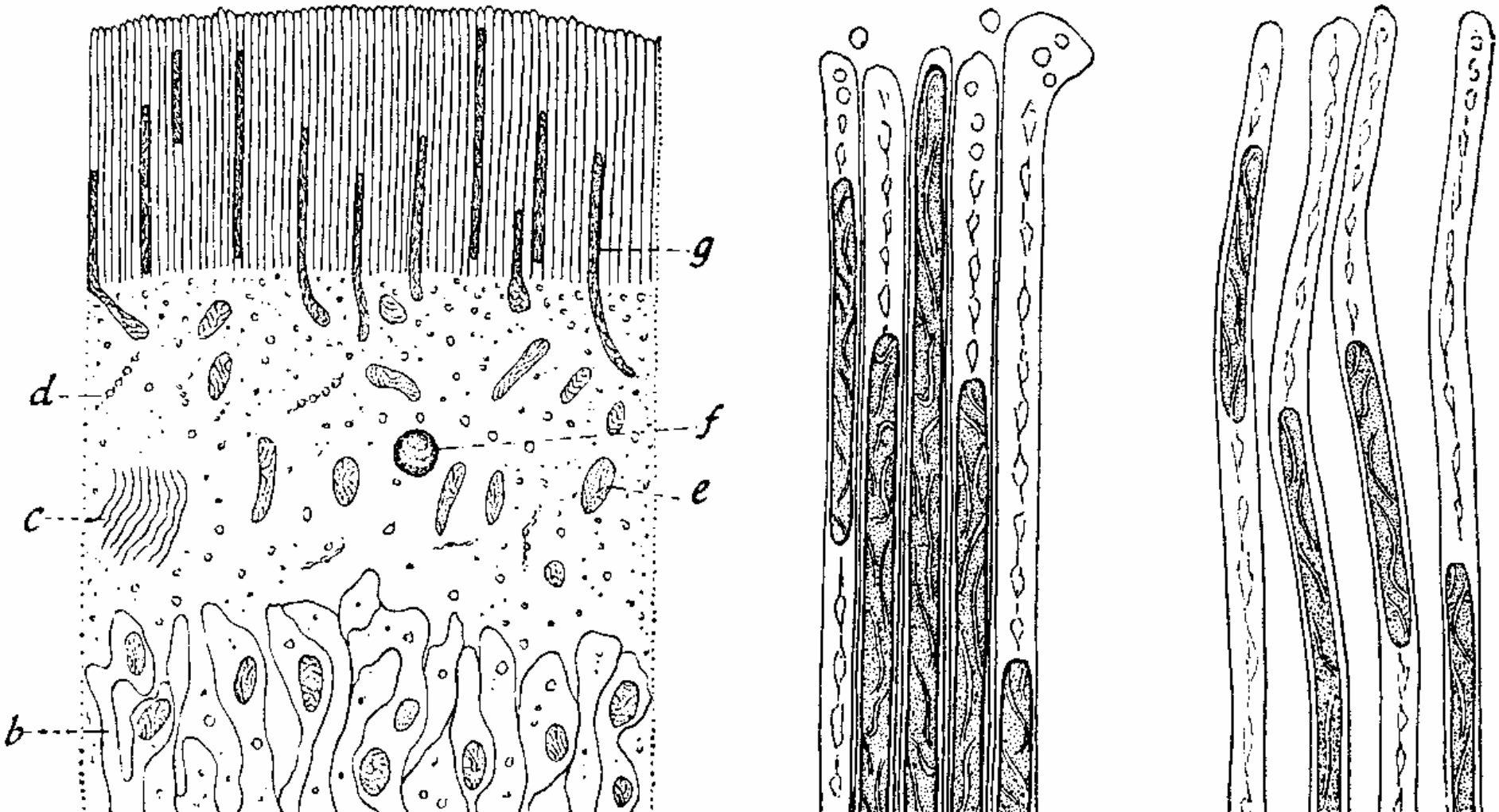
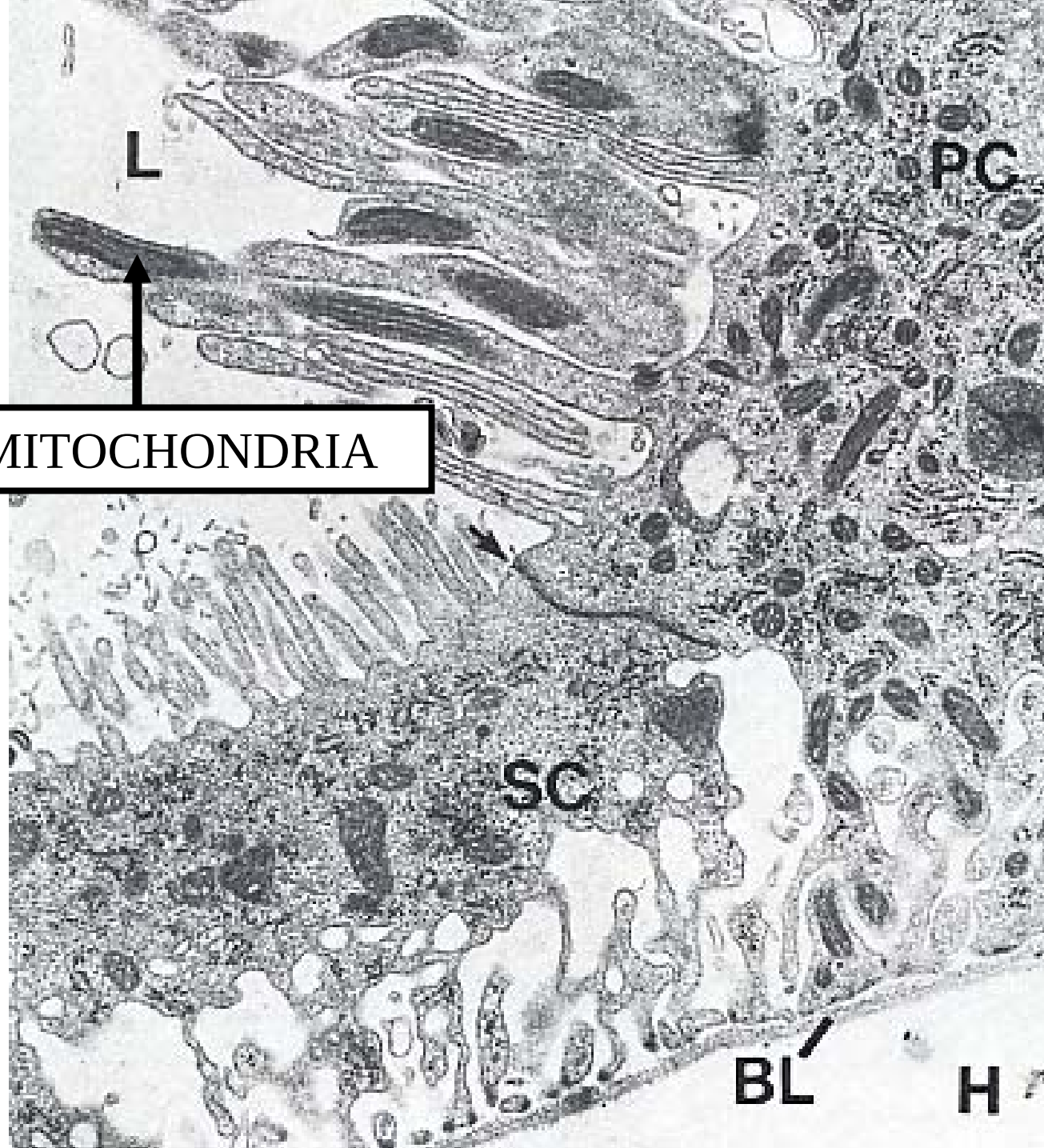

a)
b)
lumen of tubule
microvilli
mitochondria
folds of basal plasma membrane
basal lamina
hemolymph
c)
trachea
muscle

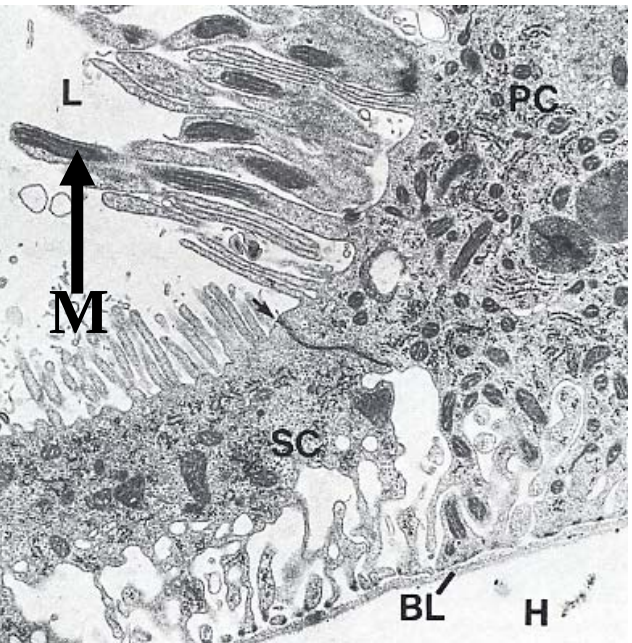
Diagram of structures in *Rhodnius* Malpighian tubules. Note brush border made of microvilli. a=basement matrix, b=invagination of plasma
Membrane with mitochondria (e); c=endoplasmic reticulum; f=mineralized granule; g=microvilli with mitochonria entering the microvilli. Enlarged view of microvilli showing droplets released into
hemolymph

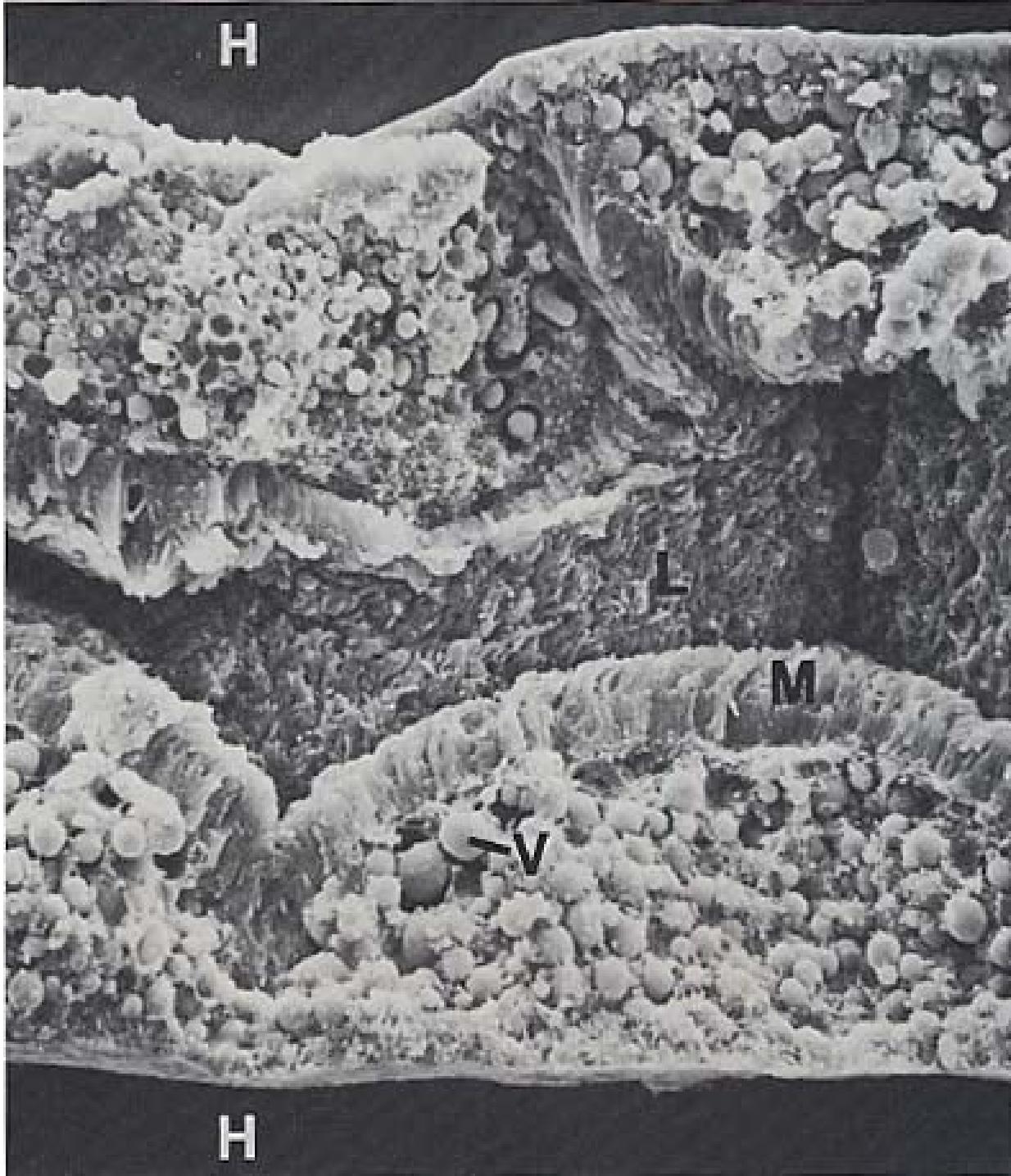


TEM of larval Malpighian tubules of *A. taeniorhynchus*. L=lumen; SC= stellate cell; BL=basal lamella or matrix. PC=primary cells.H=hemolymph SC have wider extra-cellular spaces or infoldings than does the primary cell.

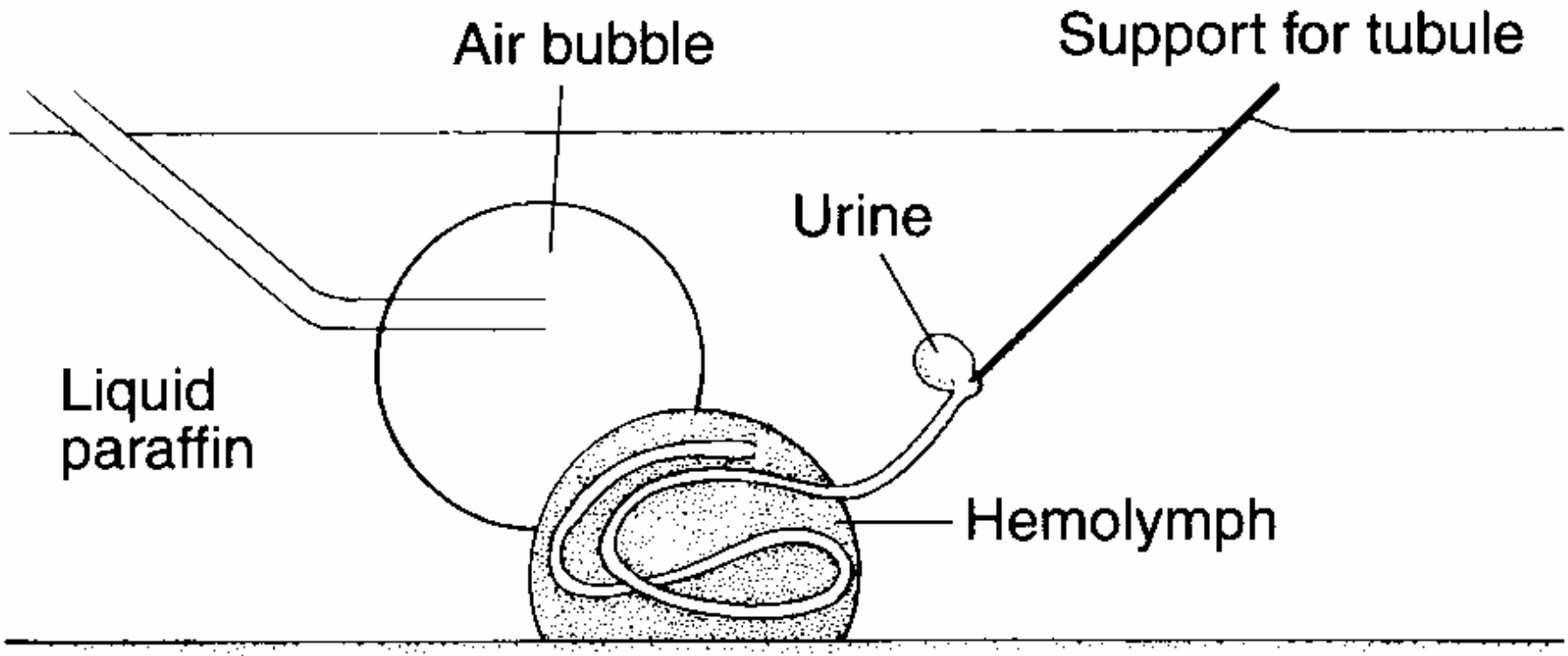


SEM of Malpighian tubule of *Aedes taeniorhynchus*. H= hemolymph; V=spherical vacuoles in the cells of the tubule containing concentric crystals of Ca. L=lumen of the tubule. M=mitochondria in microvilli of the cells.



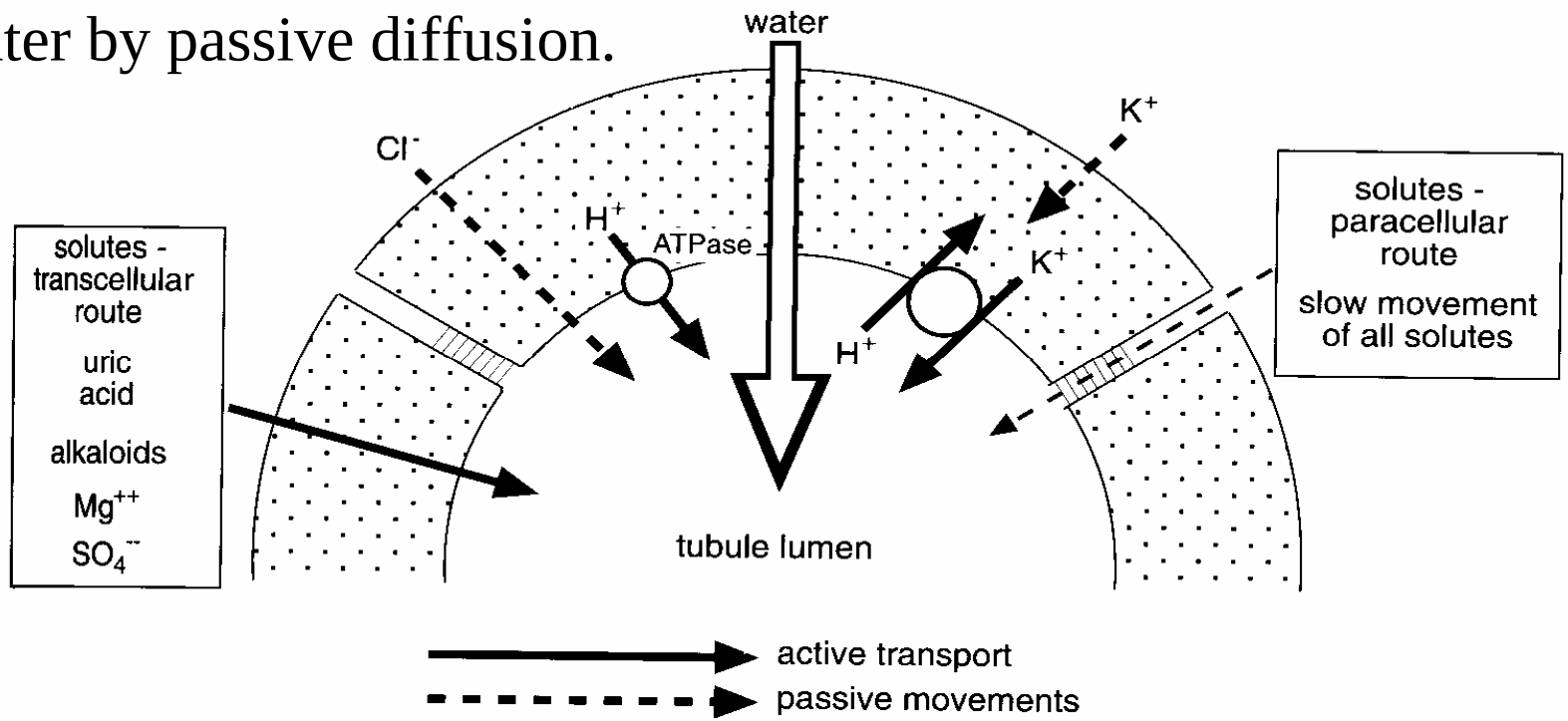


Bioassay technique developed by Ramsay for determining what factors influence excretory rates and secretions by the Malpighian tubules. Air bubble provides the tubule cells with oxygen to respire. Urine is insoluble in the liquid paraffin so it remains as a droplet at the proximal end of the tubule that would lead into the hindgut for excretion.



Schematic of Malpighian tubule. To excrete a liquid or primary urine, water must enter the tubule. This is facilitated by the movement of cations (positively charged ions) across the membrane (hemolymph side). This usually involves the potassium ion but, in blood feeders where there is a lot of Na, it may also involve Na. Hydrogen is pumped into the lumen by an ATPase driven pump (proton pump activated by mitochondria in microvilli) and this hydrogen then leaves and is replaced by the potassium. Increase in ions around microvilli. Water follows by osmosis and a transcellular route. Increase of ions in the lumen allows solutes to enter by passive diffusion.

Can explain how the tubules work with channels, pumps, and carriers



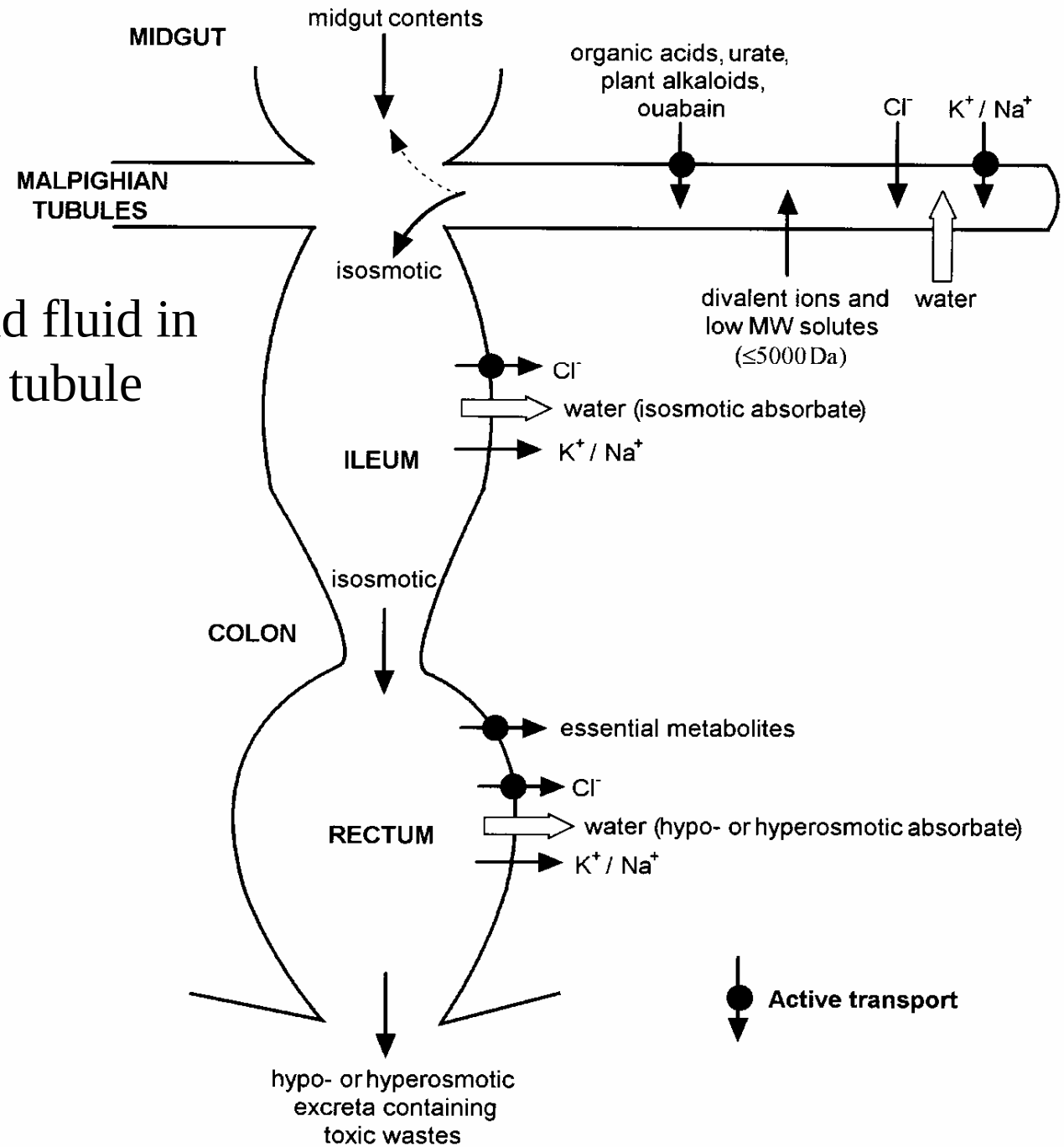
The point to note from the table to the right is that basically, the osmolarity of the hemolymph and that of the primary urine in the Malpighian tubule is nearly equal or isomotic. The exception, however, is for the ions like Na and K that are actively transported across the tubule against concentration gradients.

Potassium and proline are higher in the urine because they are actively excreted.

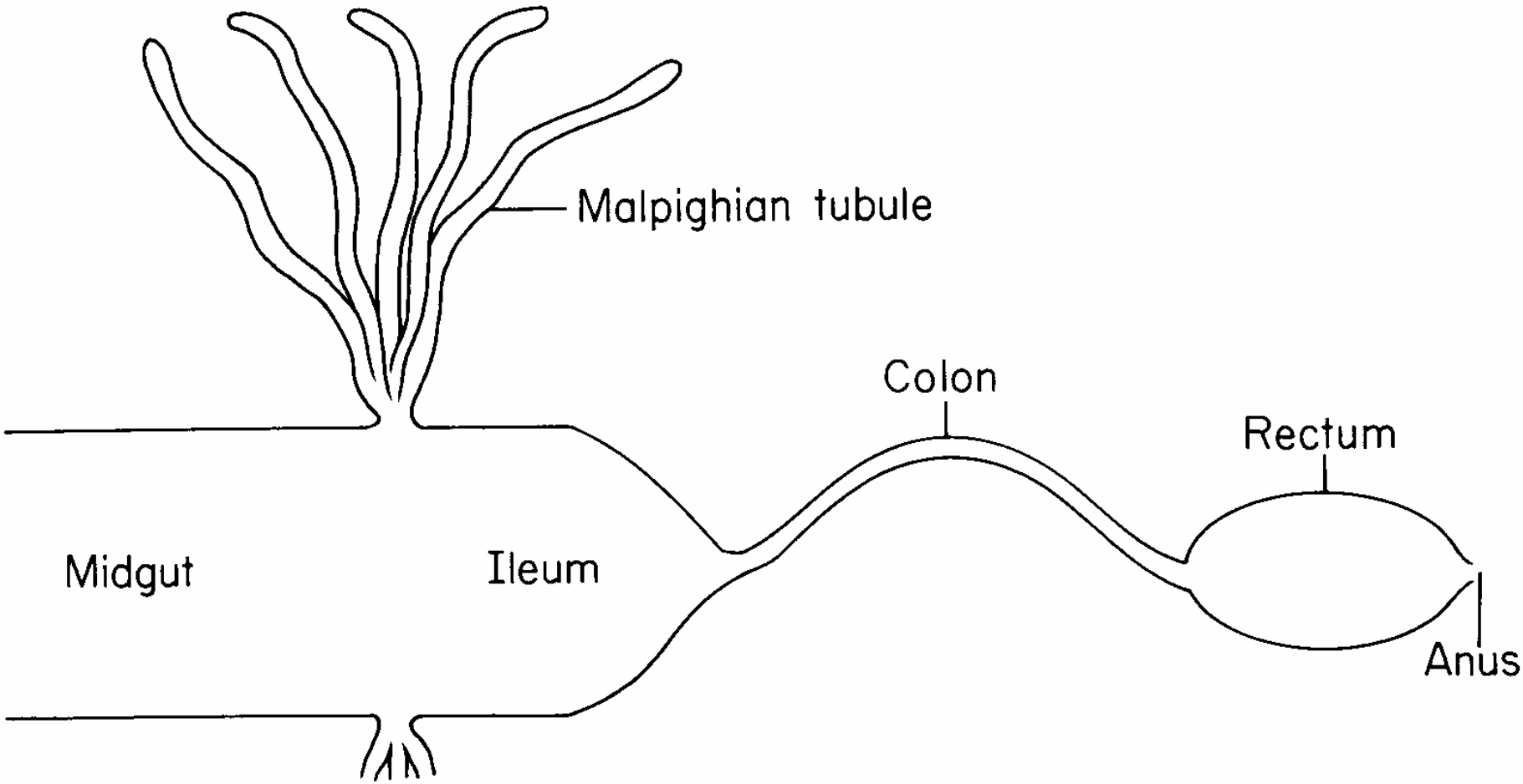
Table 18.2. *Composition (mM) of hemolymph and primary urine of* Schistocerca[a]

Constituent	Hemolymph	Primary urine
Na^+	103	47
K^+	12	165
Mg^{++}	12	20
Ca^{++}	9	7
Cl^-	107	88
Phosphate	6	12
Glucose	2.5	4.6
Alanine	1.0	1.0
Aspartate	0.1–0.9	0.5
Asparagine	1.0	0
Arginine	1.5	0
Glutamate	0.1–1.0	0.8
Glutamine	4	0.5
Glycine	14	4.0
Histidine	1.4	0
Isoleucine	0.4	0
Leucine	0.4	0
Lysine	1.0	0
Methionine	0.4	0
Phenylalanine	0.7	0
Proline	13	38
Serine	2–4	1.0
Threonine	0.5	0
Tyrosine	1.0	0
Valine	0.6	0

Hemolymph and fluid in the Malpighian tubule are isosmotic.

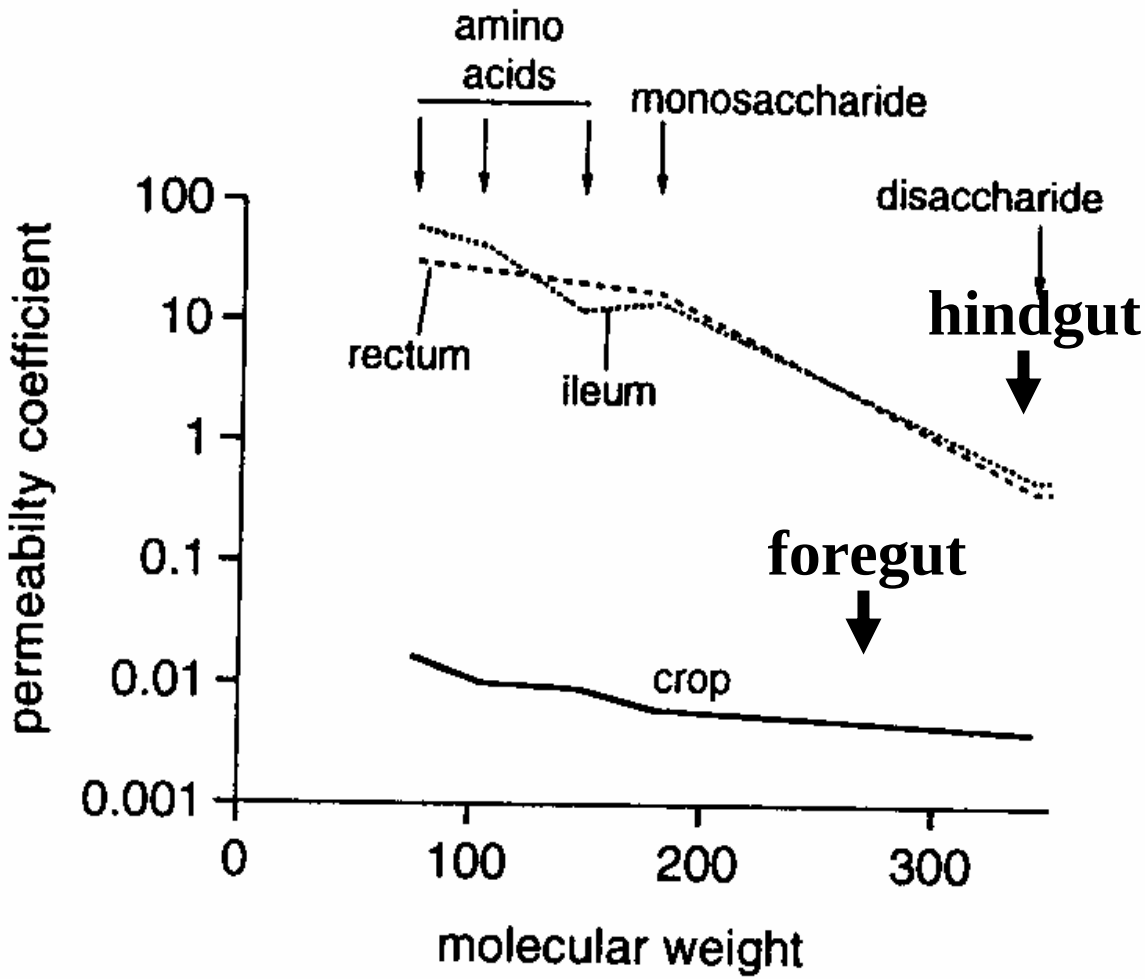


If the hindgut or rectum area of the insect is involved in water uptake, ion movement, and amino acid uptake, what might be the characteristics that have to be met for this to take place?



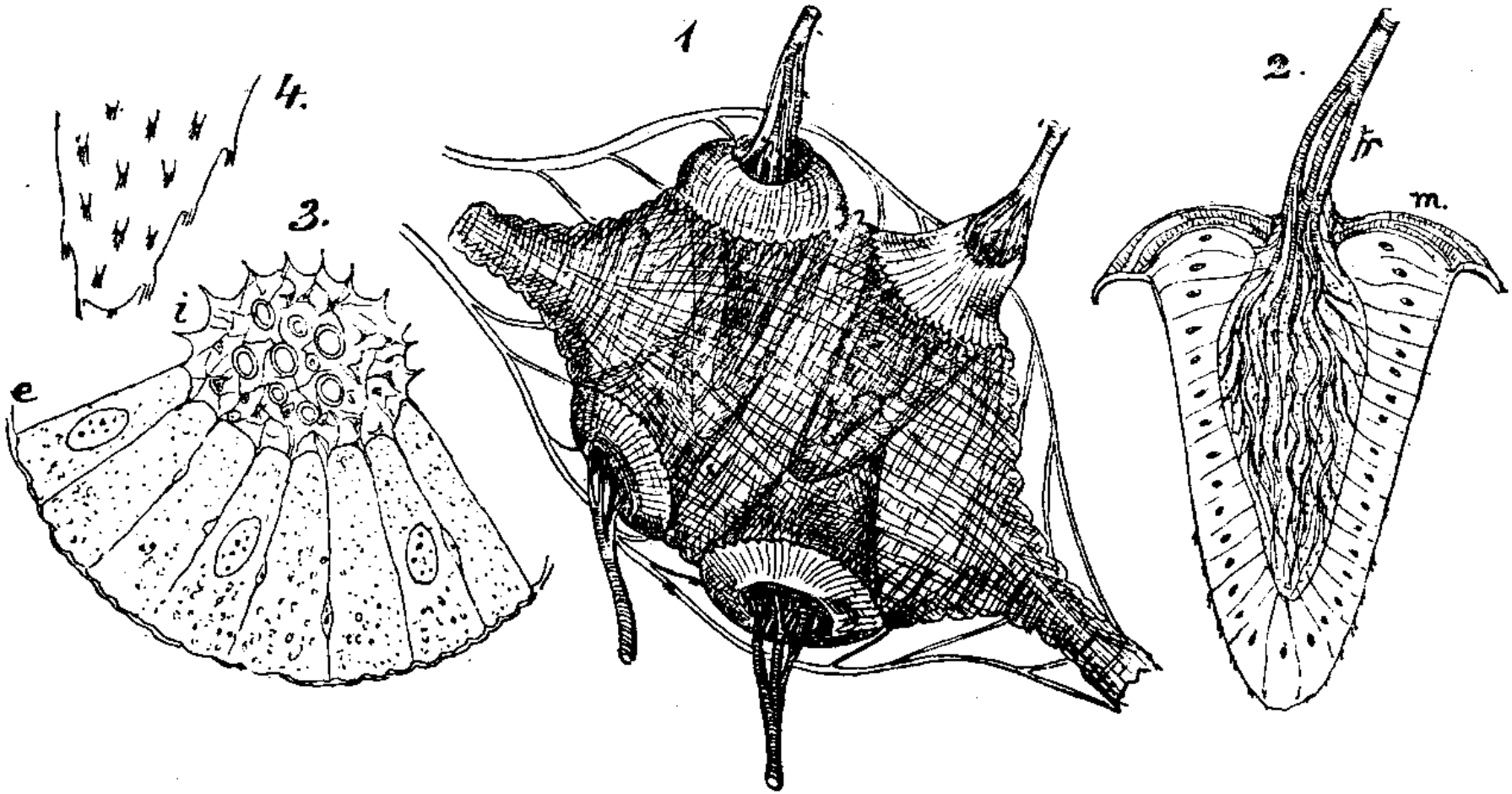
Structure of anal papillae, anal organs and rectal papillae

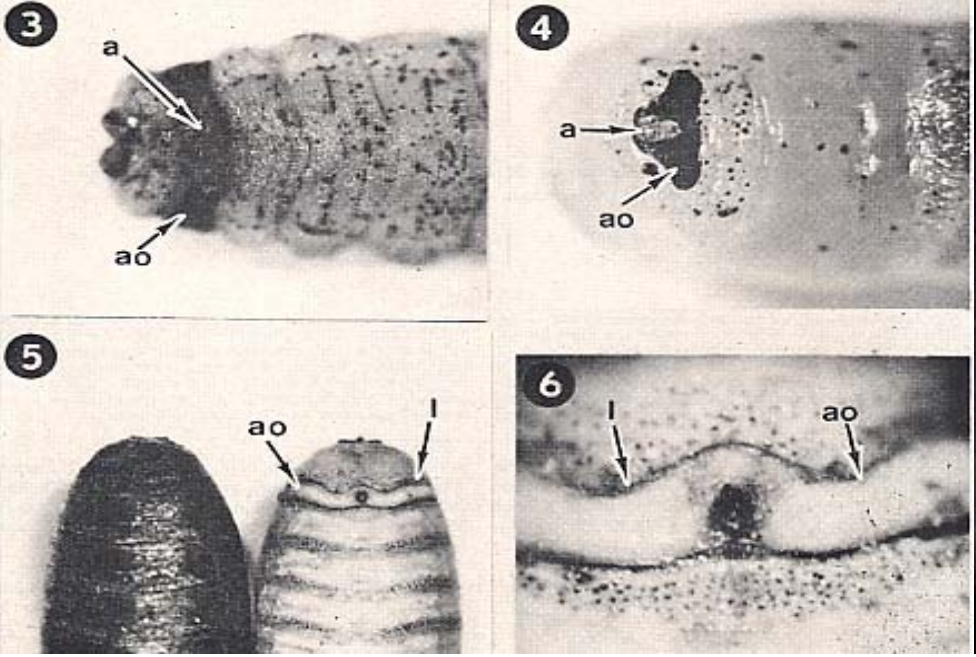
The permeability of the cuticle of the hindgut is highly permeable compared to that of the foregut (i.e. crop here)(see below) and it is usually much thinner.



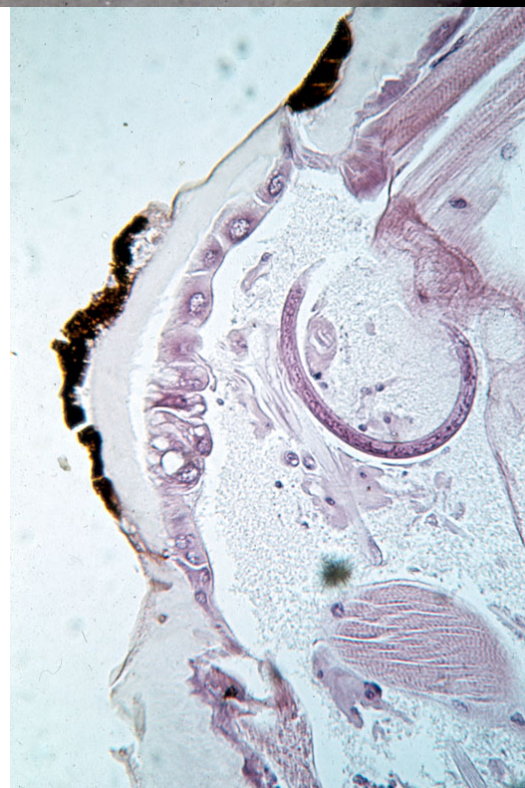
Rectal papillae of flies and rectum

Various types of papillae in the rectum of insects are involved in reabsorption of water and the movement of ions for osmoregulation





Notice the cuticle of the anal gland in the photo on the right is about half as thick as that of the adjacent cuticle. Also, note that it is delineated from the surrounding cuticle, thus preventing materials from moving laterally instead of just in and out/or vice versa of the gland. In the photo above, notice how the anal organ in fig. 6 is delineated from the rest of the cuticle. Remember, its cuticle is produced by epidermal cells that produce it while the adjacent cells produce the normal cuticle.



Terrestrial insects lose water. How do they recoup it?

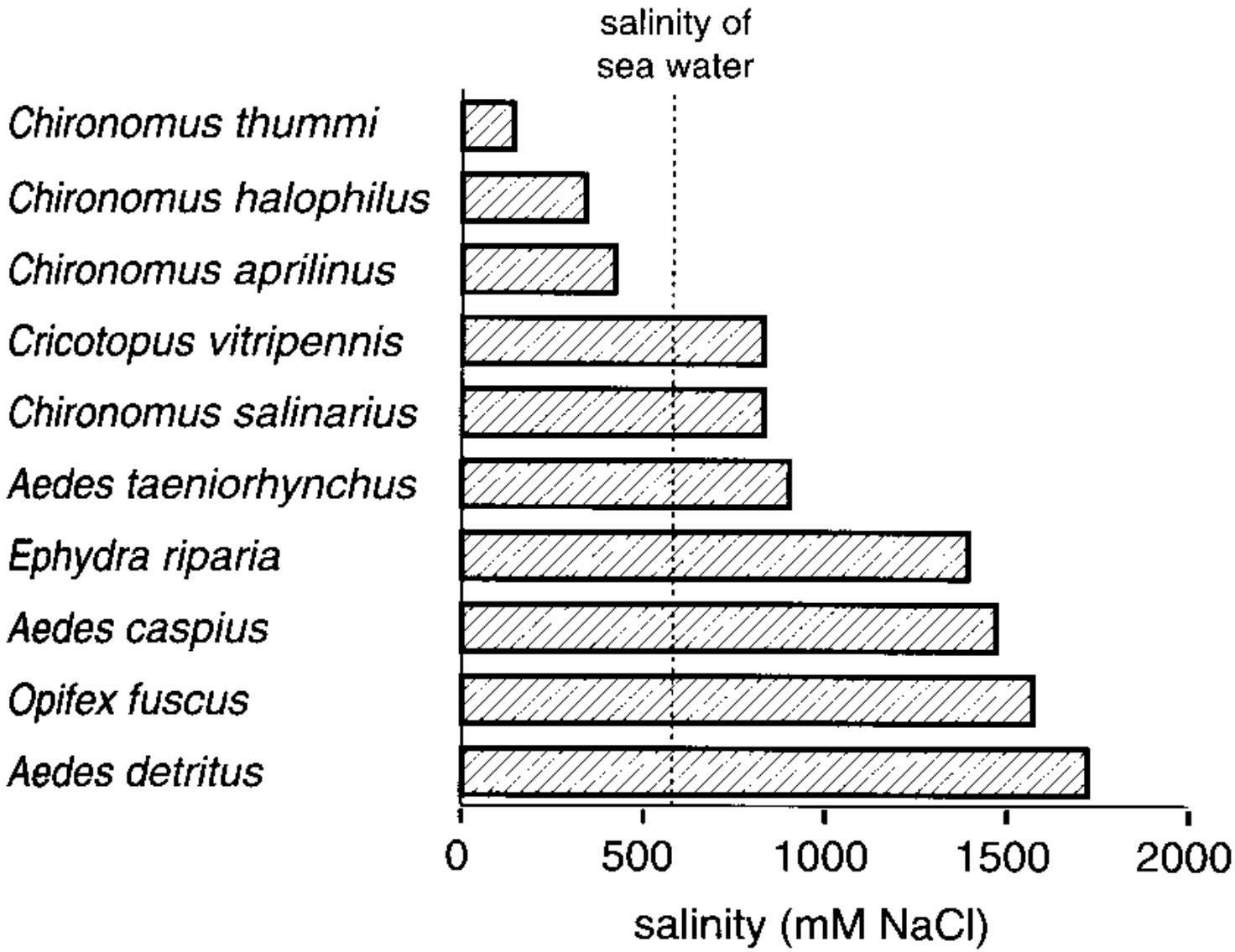
How do they lose water?

1. Through cuticle
2. Water loss from the respiratory surfaces
3. Water loss in excretion

How do they gain water?

1. Drinking
2. Uptake through cuticle
3. Metabolic water (grain beetles)

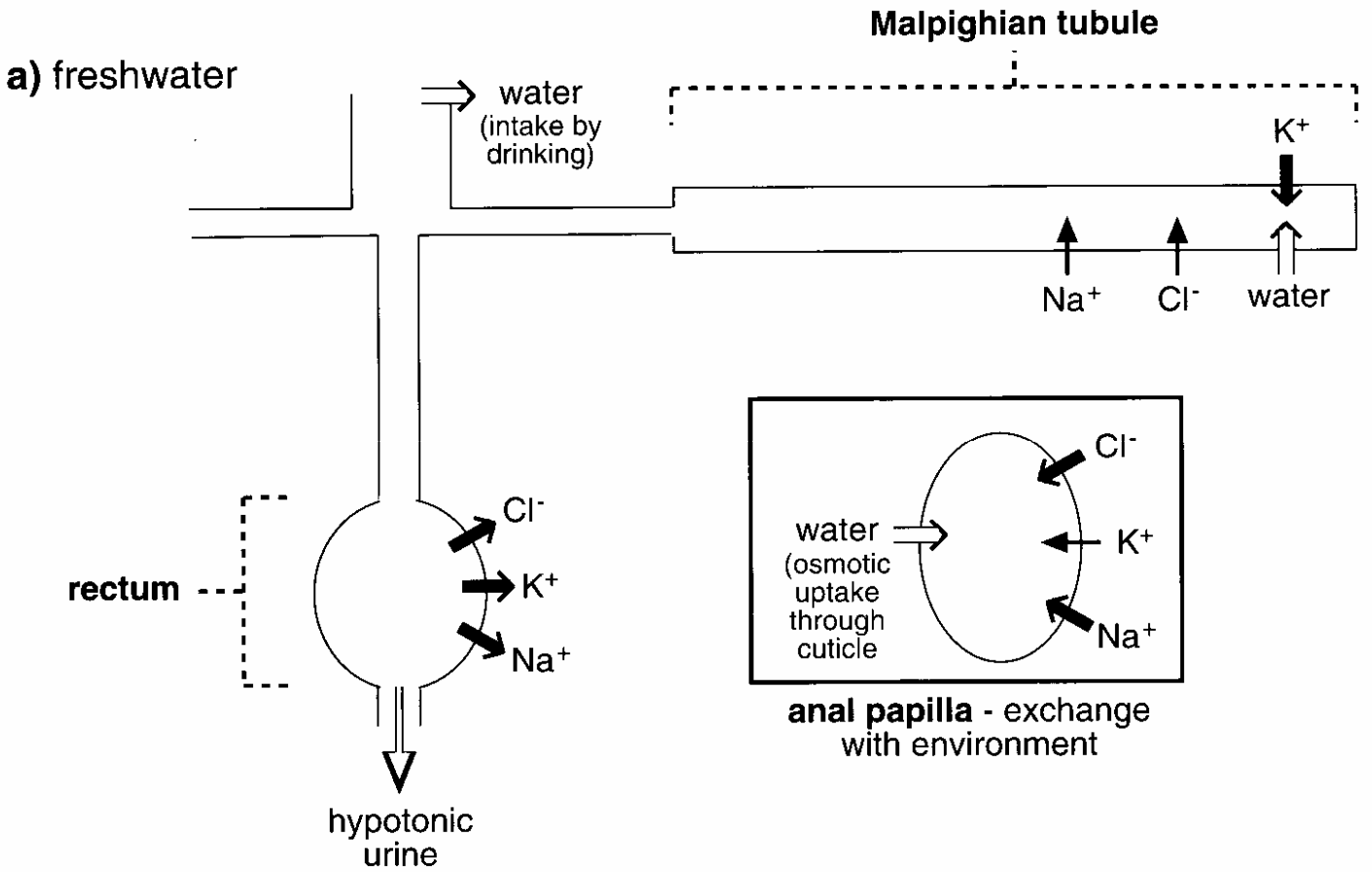
Some dipterous larvae span a broad range of salinity tolerances

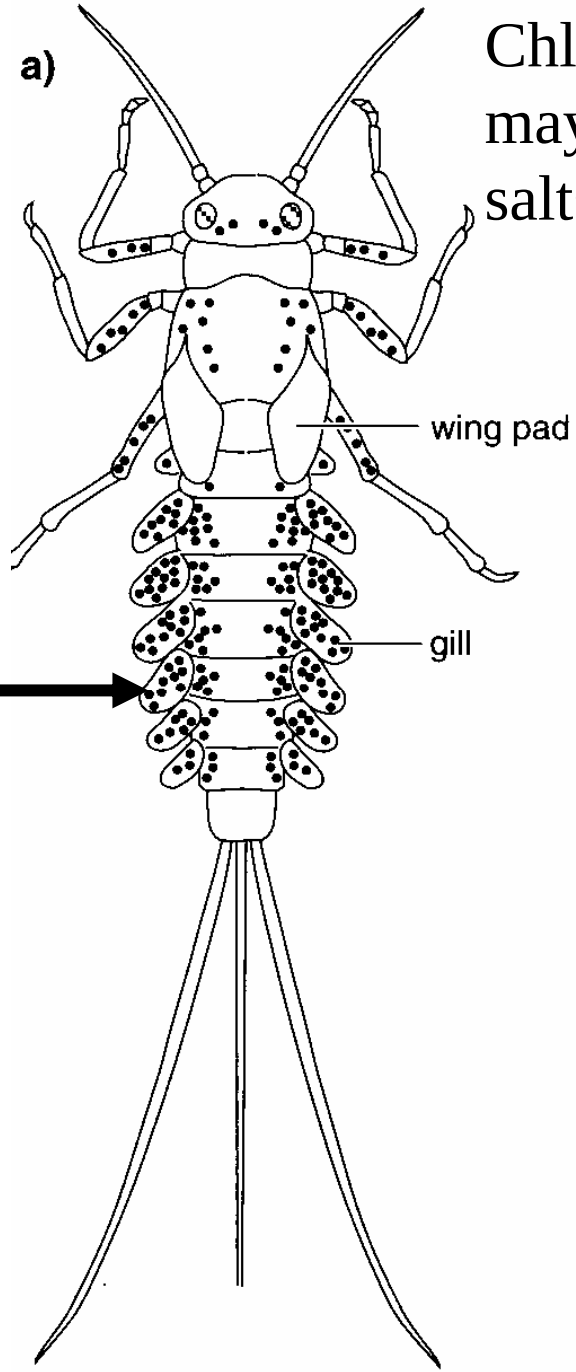


Freshwater insects tend to lose salts to the environment because of their highly permeable cuticle. K, Na, and chloride are reabsorbed in the rectum but water is not.

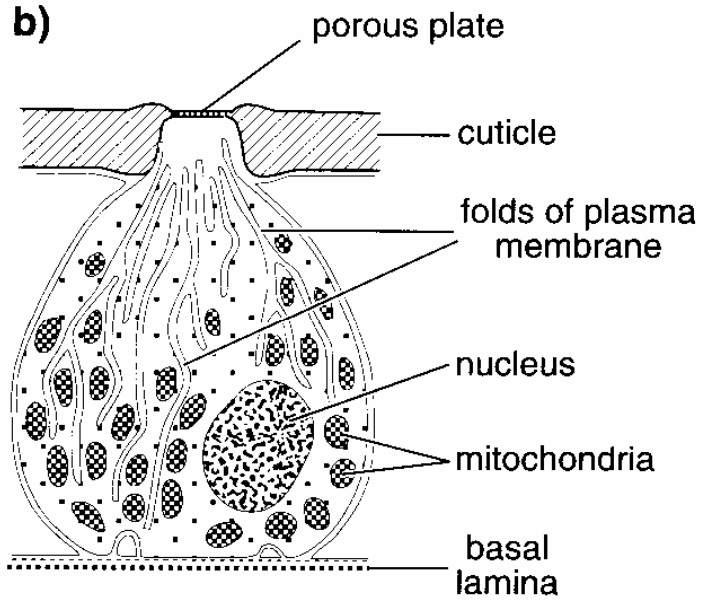
Ways to recoup salts in freshwater larvae

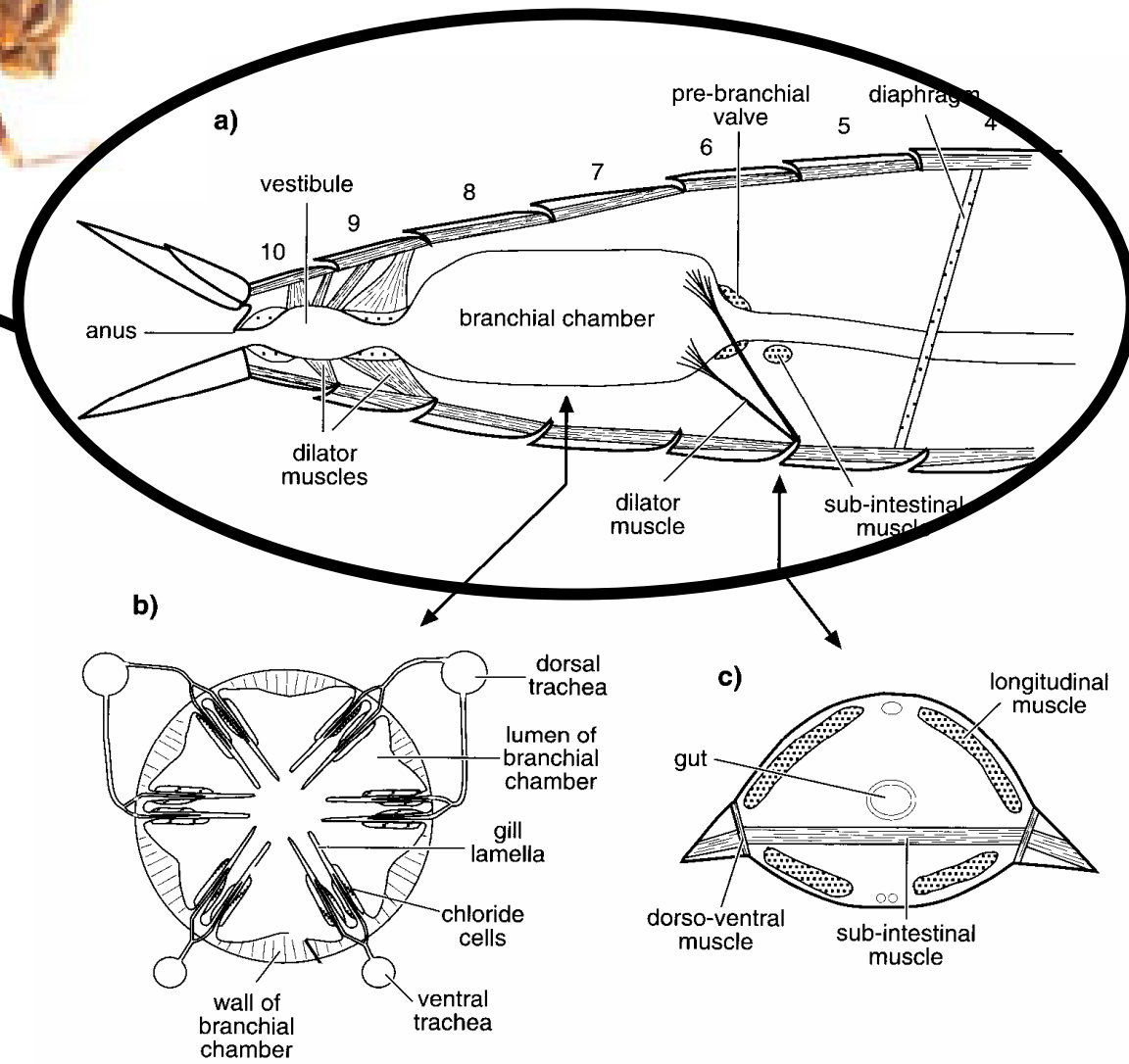
1. Special chloride cells in some aquatic larvae
2. Rectal gills in dragonfly naiads



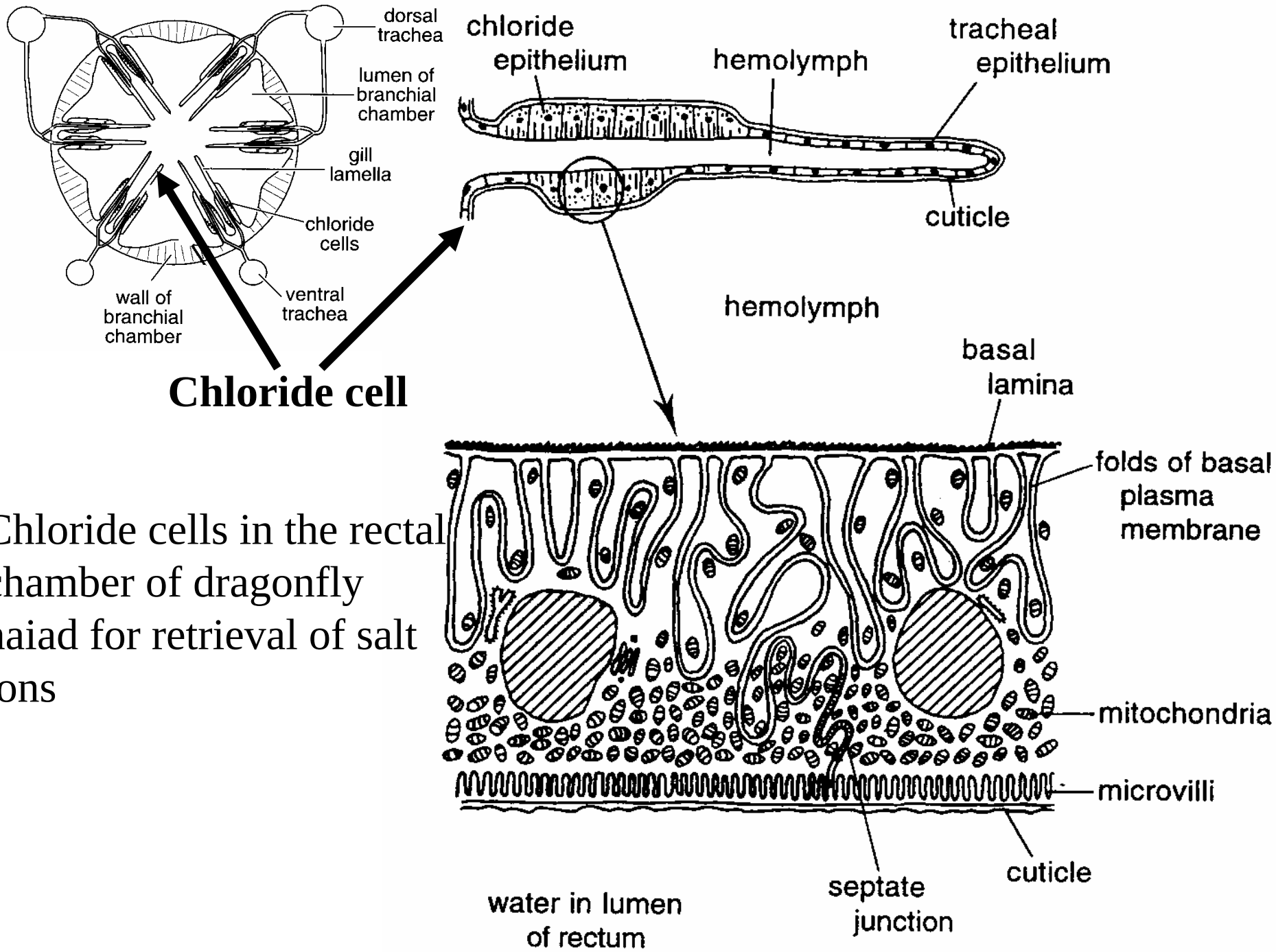


Chloride cells in the gills of the mayfly naiad for retrieval of salts





Chloride cells in the rectal chamber of dragonfly naiad for retrieval of salt ions

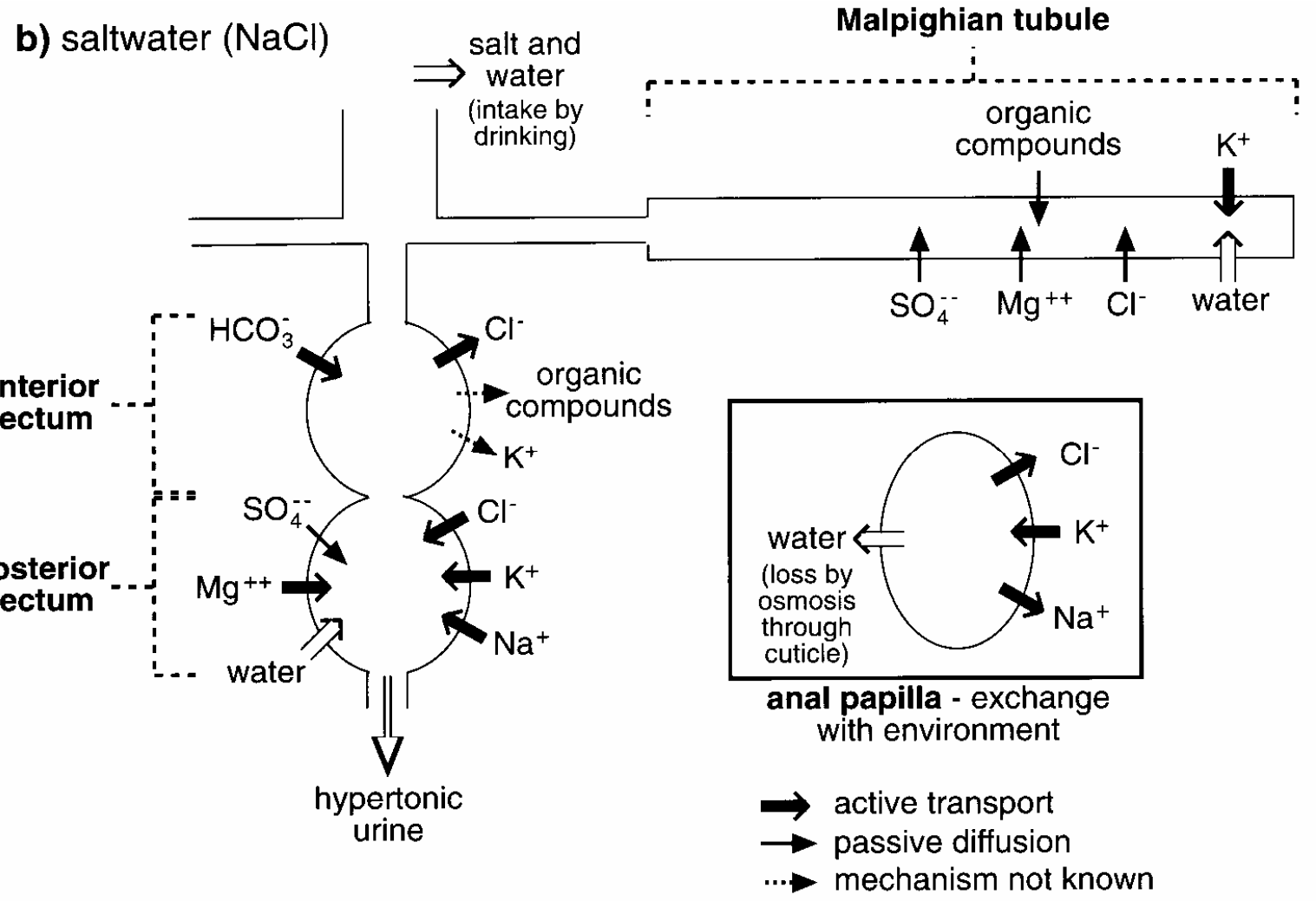


Chloride cell

Chloride cells in the rectal chamber of dragonfly naiad for retrieval of salt ions

Saltwater insects gain salts and water with their food, thus **losing water** osmotically.

Some insects like *Aedes campestris* or *Aedes sollitans* (common along salt marshes of Massachusetts). Also, *Ephydra cinerea* lives in Utah's Salt Lake, which is 20% NaCl.



Diuresis-rapid flow of urine elimination from the body

Many insect species decrease urine output and increase blood volume prior to the molt. WHY?
Following the molt, they increase urine output and decrease blood volume after cuticular expansion.

RATES OF EACH ARE

PRIMARY URINE PRODUCTION
in Malpighian tubules

REGULATION BY DIFFERENT MECHANISMS

RESORPTION OF SALTS
in the hindgut

Insect diuretic and antidiuretic hormones

Coast, G.M., et. al. 2002. Adv. Insect Physiol. 29: 279-409

Diuretic hormones generally act on the Malpighian tubules to stimulate urine production

Antidiuretic hormones generally increase fluid reabsorption by act on the hindgut

Malpighian tubules are not innervated, thus they must be regulated by hormones released in the blood

Muscles of the tubules can be modulated by diuretic hormones and Myotrophic peptides. Increase writhing movement in hemolymph

This made Ramsay's assay a useful bioassay

Using his assay, it was shown that an extract from the fused mesothoracic glanglion mass in *Rhodnius* increased urine production up to 1,000-fold. *A. aegypti* diuretic hormone-loss of 40% of water in the blood meal with 2 hrs of feeding.

1989-1st isolation and identification of a diuretic hormone (peptides) in an insect, *Manduca sexta*.

Since then there have been major technological advances to further the identification, isolation, and purification of peptides (2 or more amino acids linked together).

1. HPLC=high performance liquid chromatography
2. Automated peptide sequencing
3. MS=mass spectrometry
4. Development of routine molecular protocols for
   a. mRNA isolation
   b. amplification and sequencing of genes
   c. gene expression
5. Sequencing the entire genome of *Drosophila melanogaster* (2000) and *Anopheles gambiae*
6. Genomic databases for searching for genes encoding neuropeptides and their receptors

With the exception of serotonin, all of the factors that have been identified as having diuretic or antidiuretic activity are all **NEUROPEPTIDES**

Some insect neuropeptides are similar to those of vertebrates, thus indicating a long evolutionary history as the two groups diverged about 6000 million years ago.

TABLE 1 List of identified diuretic and antidiuretic peptides, detailing the species from which they were first identified, abbreviated name, whether they have been fully sequenced (Seq), primary site of action, second messenger and activity

Peptide	Species	Name	Seq.	Site	Messenger	Activity
CRF-related	*M. sexta*	Manse-DH	Yes	MT CNC	cAMP/Ca^{2+}?	Diuretic/antidiuretic
Kinin	*L. maderae*	Leuma-K	Yes	MT	Ca^{2+}	Diuretic
Cardio-acceleratory	*M. sexta*	Manse-CAP_{2b}	Yes	MT	cGMP	Diuretic/antidiuretic
Calcitonin-like	*D. punctata*	Dippu-DH_{31}	Yes	MT	cAMP	Diuretic
AVP-like	*L. migratoria*	AVP-IDH	Yes	MT	cAMP	Diuretic
Antidiuretic factor	*T. molitor*	Tenmo-ADF	Yes	MT	cGMP	Antidiuretic
Natriuretic peptide	*A. aegypti*	MNP	No	MT	cAMP	Diuretic
Antidiuretic factor	*F. polyctena*	FopADF	No	MT	?	Antidiuretic
Antidiuretic factor	*M. sexta*	Manse-ADF-B	No	CNC	cAMP	Antidiuretic
Ion transport peptide	*S. gregaria*	Schgr-ITP	Yes	Ileum	cAMP/+?	Antidiuretic
Cl^- transport stimulating hormone	*S. gregaria*	CTSH	No	Rectum	cAMP	Antidiuretic
Neuroparsins	*L. migratoria*	Nps	Yes	Rectum	Ca^{2+}	Antidiuretic

MT, Malpighian tubules; CNC, cryptonephric complex.

Insect brain or nervous tissue (ganglia)

a. Neuropeptides with diuretic activity

Corpus cardiacum

a. Storage and release of the peptides into the

HEMOLYMPH

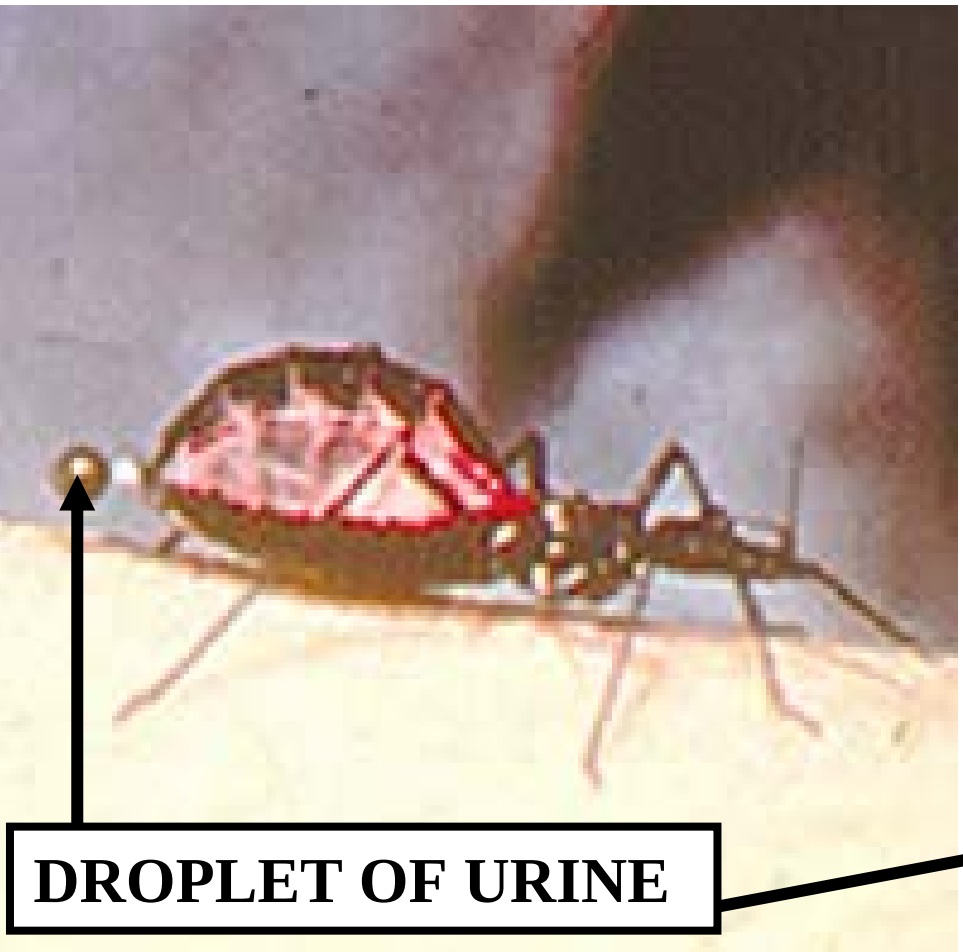
Malpighian tubules

a. Diuretic peptide hormone released into the blood and Icreases in titre in the hemolymph. Goes to the Malpighian tubule and activates diuresis

Cuticular plasticization in blood feeder and rapid excretion of water

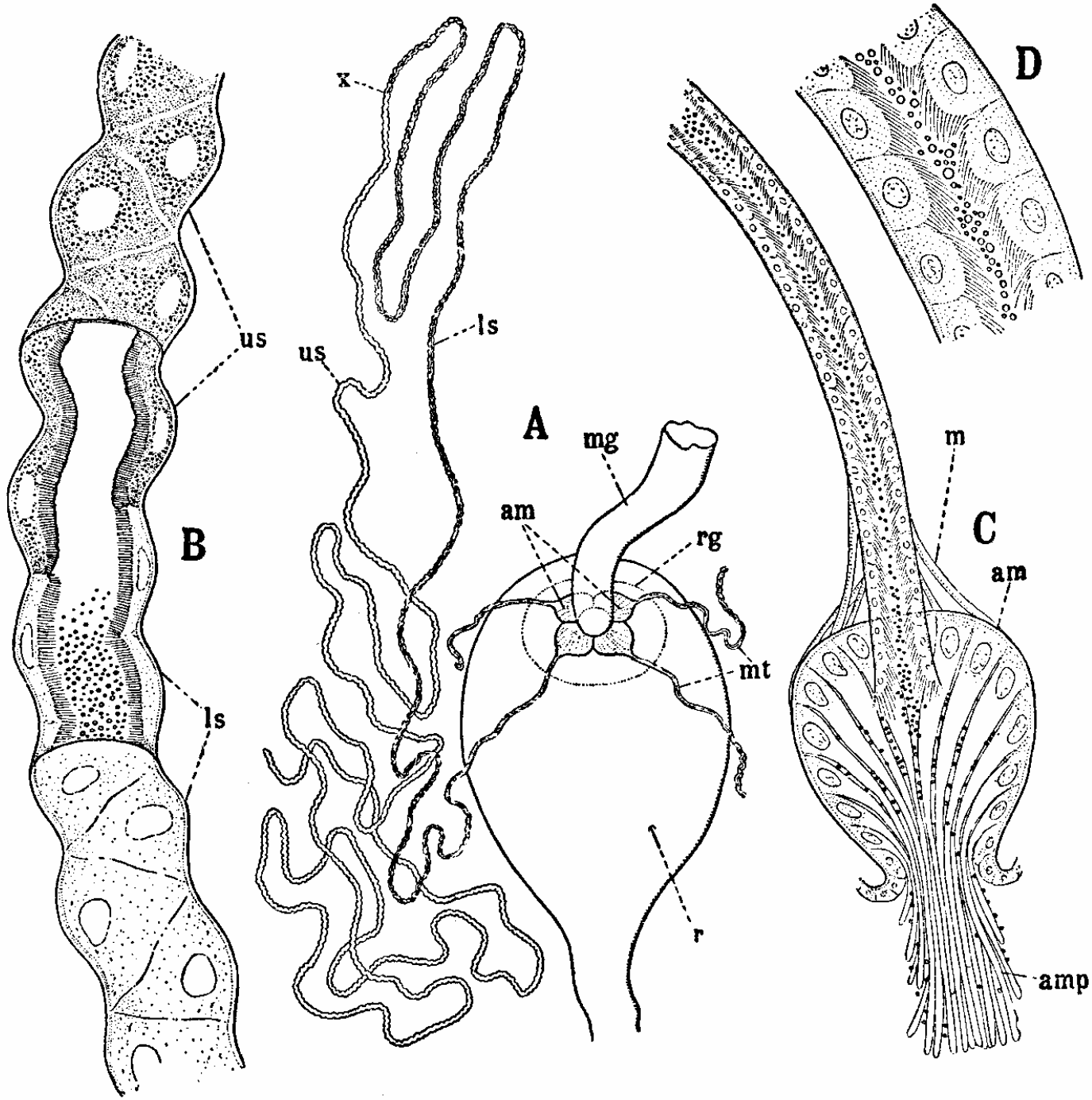
***Rhodnius prolixus*-kissing bug and vector of trypanosome that is causative agent of Chaga's**

Occurs as a result of the action of hormones or neurohormones.



DROPLET OF URINE

The rapid acquisition of a blood meal by hematophagous insects could produce a major osmotic problem if all of the water in the bloodmeal were to get into the hemolymph and stay there. Also, such a load greatly hinders the movement, especially flying, of these insects. They have solved this problem by using diuretic hormones that are released by stimulation of stretch in the abdomen in *Rhodnius*. These hormones cause rapid movement of water from th bloodmeal into the hemolymph where it rapidly moves into the Malpighian tubules for elimination as a droplet of urine (see photos).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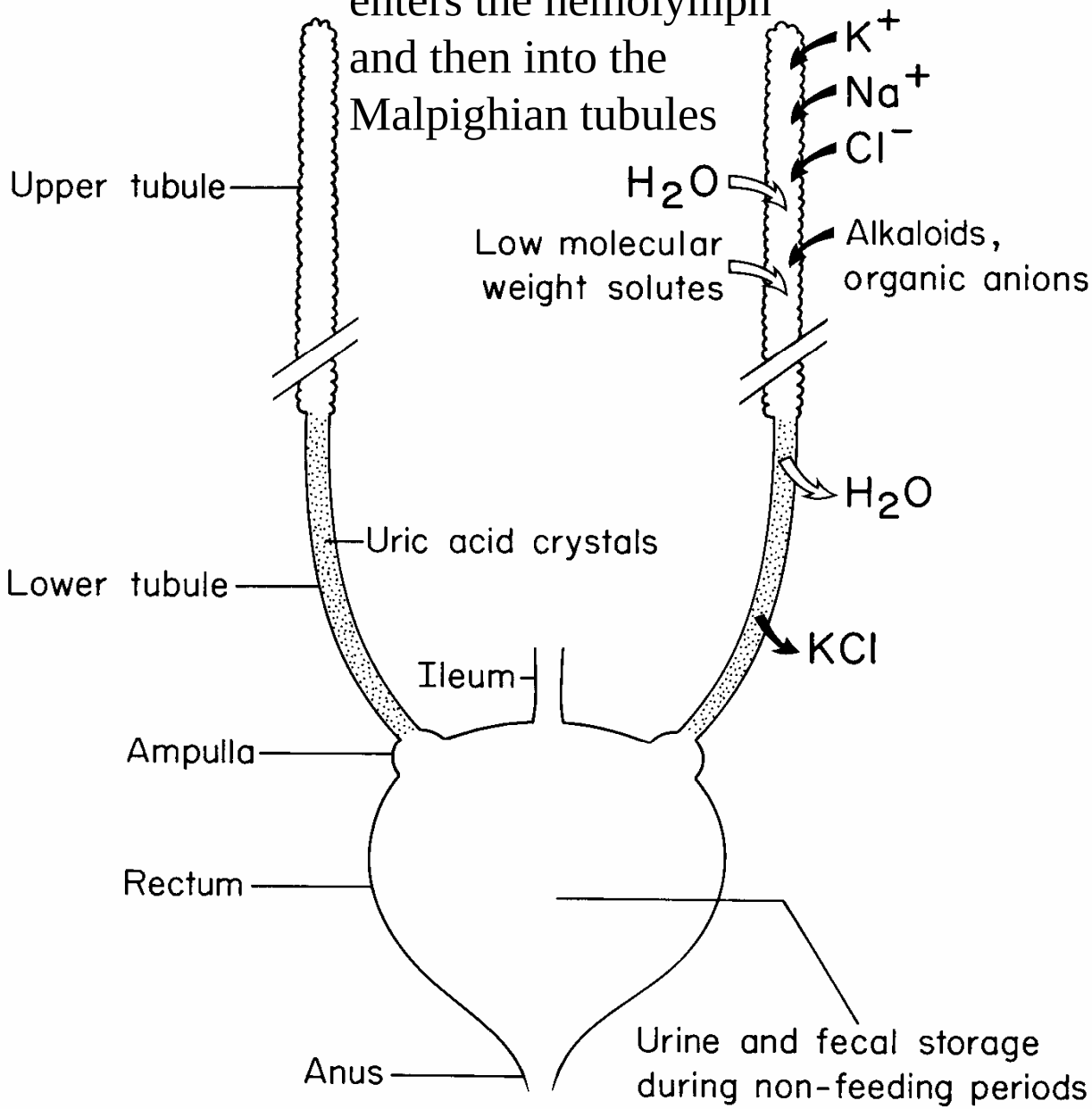
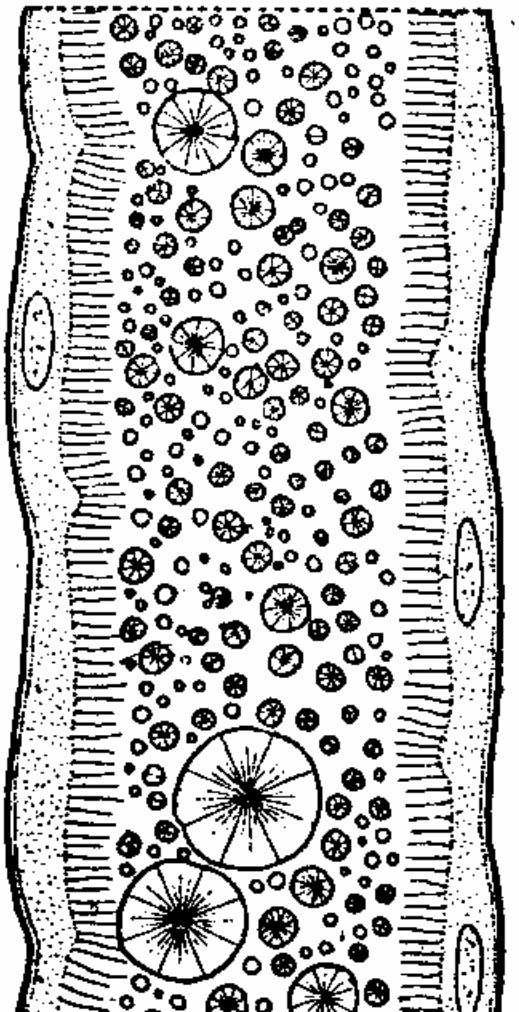
Excess water from the blood meal enters the hemolymph and then into the Malpighian tubules

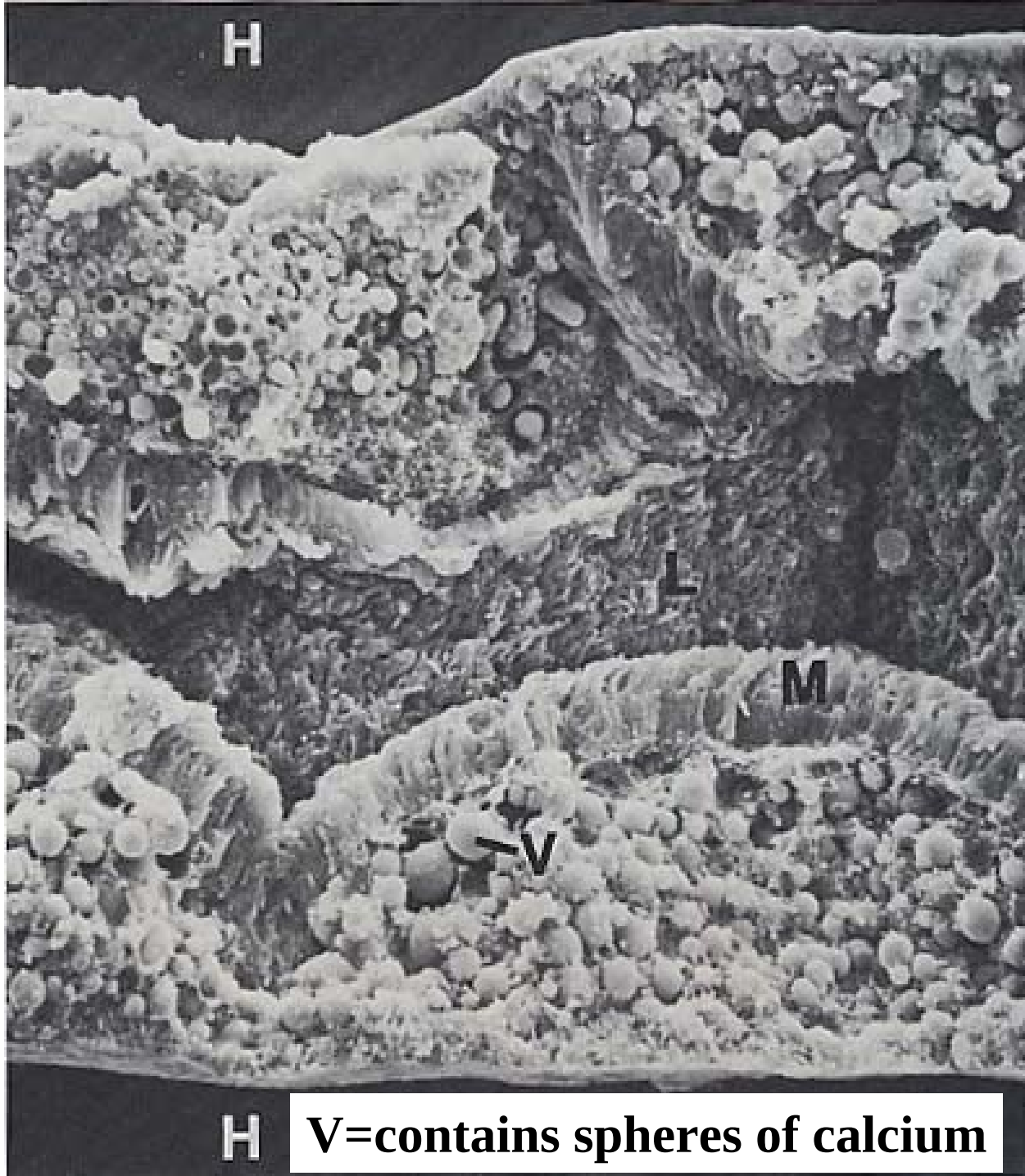


Rhodnius prolixus as a model

1. Extremely rapid loss of water from the bloodmeal in blood feeders
2. The rate of water movement across the midgut must somehow match that entering the Malpighian tubules otherwise their will be a drastic change in the osmotic balance of the insects hemolymph.
3. Maddrell removed some of the Malpighian tubules from *Rhodnius* and did the measurements. Somehow, water leaving the bloodmeal across the midgut slowed down to match what was coming in.
4. Evidence suggests that hormonal control over the midgut is the same a that over the Malpighian tubule takeup.
5. Human blood contains a lot of calcium, which *Rhodnius* stores in crystaline form in the Malpighian tubules.
6. It is the stretch of the abdomen (monitored by stretch receptors in the abdomen) by the bloodmeal that triggers the release of serotonin from the abdominal nerves in the hemocoel.
7. At the same time, a diuretic hormone is released. Serotonin and the diuretic hormone act synergistically to regulate primary urine production by the Malpighian tubules.

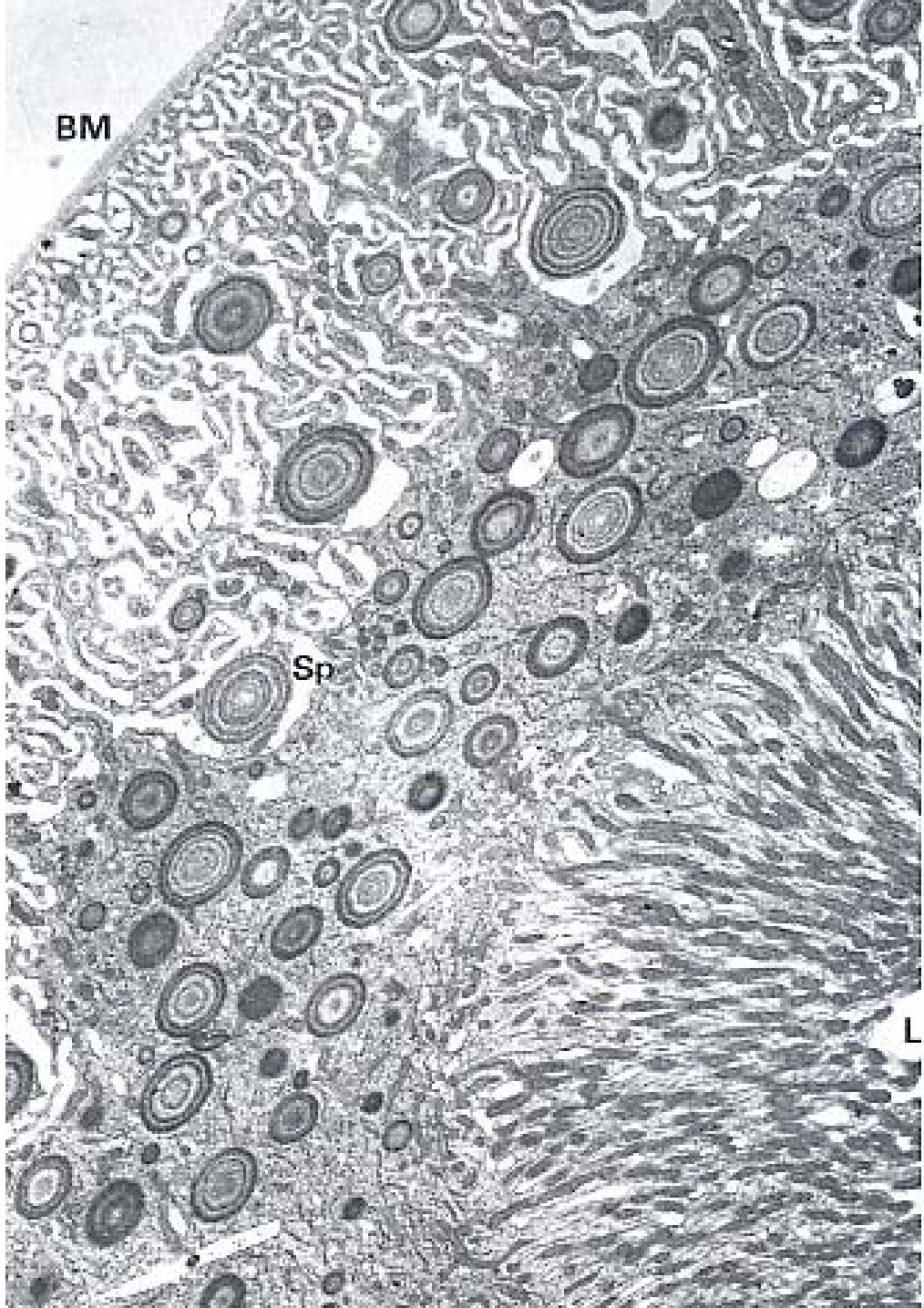
Drawing of histology of Malpighian tubule of *Rhodnius* and an SEM of the same. Note spheres of calcium.





V=contains spheres of calcium

TEM of principal cell of the Malpighian tubule of *Calpodes ethlius* larva. Note the presence of spherocrystals that are produced from materials taken up from the hemolymph and packaged into these spherules that can contain uric acid, Ca, Mg, and/or Phosphates



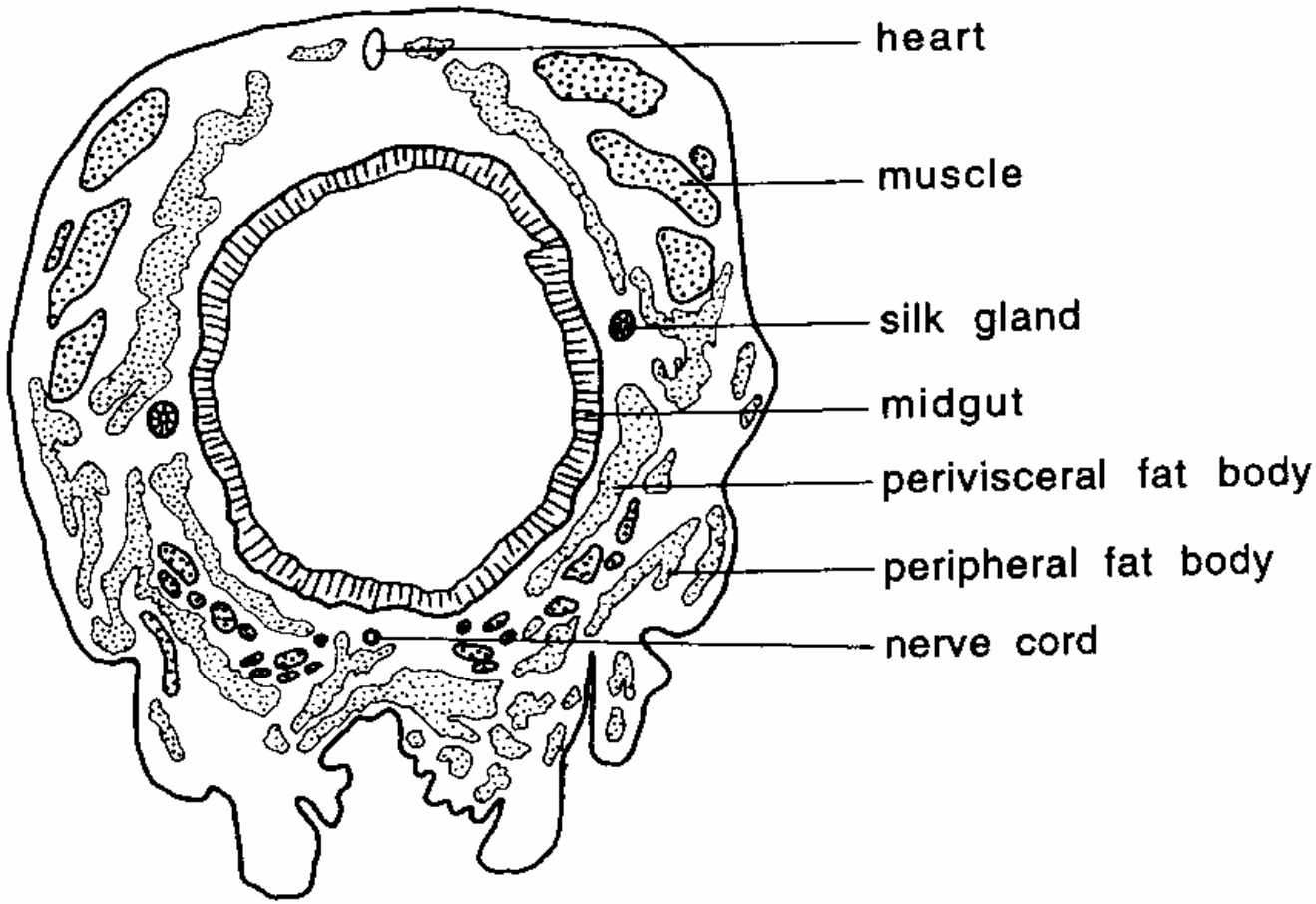
FAT BODY revisited-

Fat body cells are involved in:

1. **Intermediary metabolism** (glycogen to glucose; glycerol production; synthesis of trehalose from glucose)
2. Contain MFOs or cytochrome P450 enzymes (similar to vertebrate liver)
3. Fat body as a protein factory. It takes precursors from the hemolymph and produces the female specific protein or vitellogenin (Vg) and puts it into the hemolymph
4. Takes wastes out of hemolymph and produces uric acid
5. Production of antibacterial proteins known as Cecropins
6. Serves as a storage organ for lipids, etc.
7. Hormonal modulation of fat body (JH makes the fat body competent to make Vg)
8. Can house mycetocytes
9. Can house uric acid in special cells called urocytes, which are found amongst the fat body cells

Fat body can be categorized on where it is found

1. **Subcuticular or peripheral fat body**
2. **Perivisceral fat body**



1. Fat body cells

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1. Intermediary metabolism (glycogen to glucose; glycerol production)
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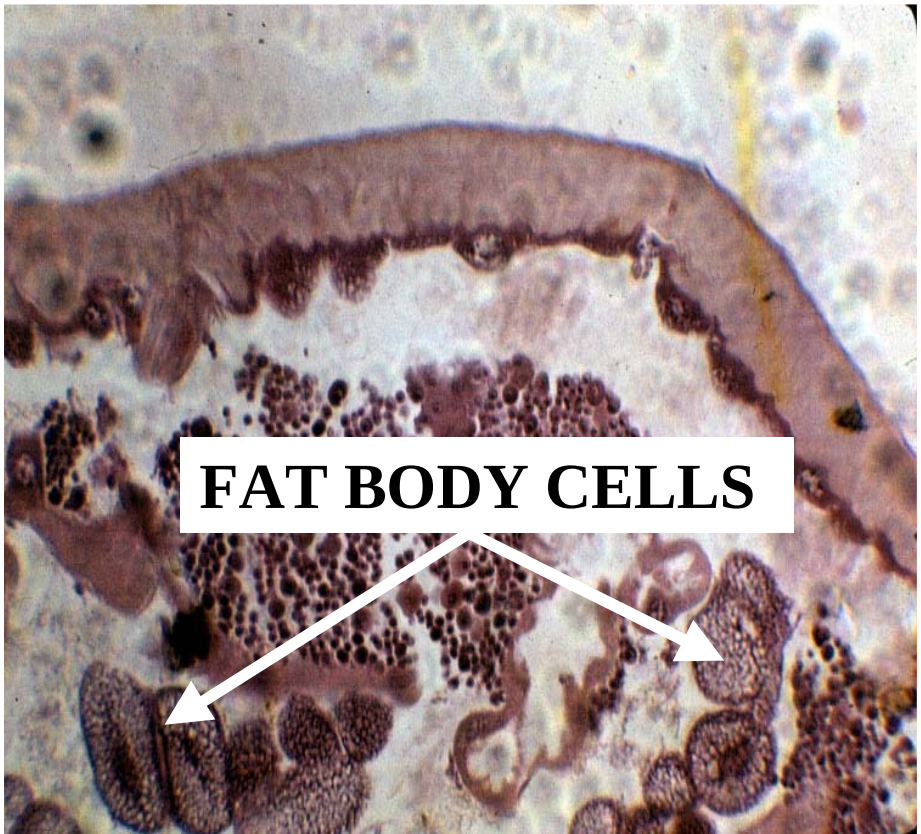
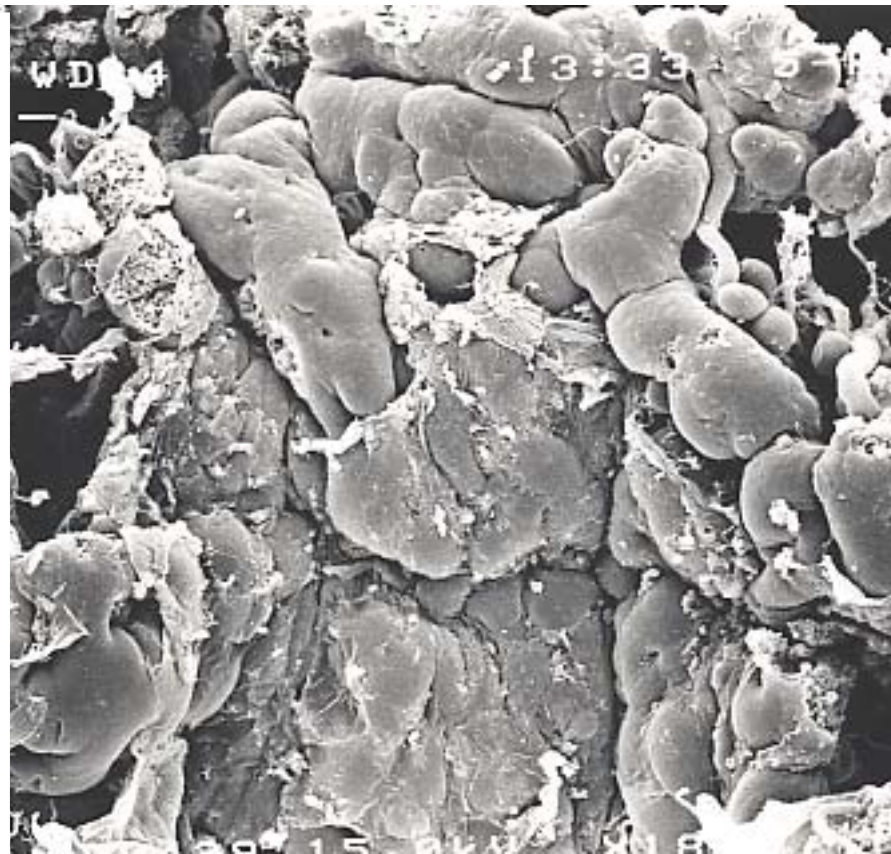
3. Take precursors from the hemolymph and produce the female specific protein c vitellogenin and put it into the hemolymph
4. Takes wastes out of hemolymph and produces uric acid
5. Production of antibacterial proteins known as Cecropins and defensins

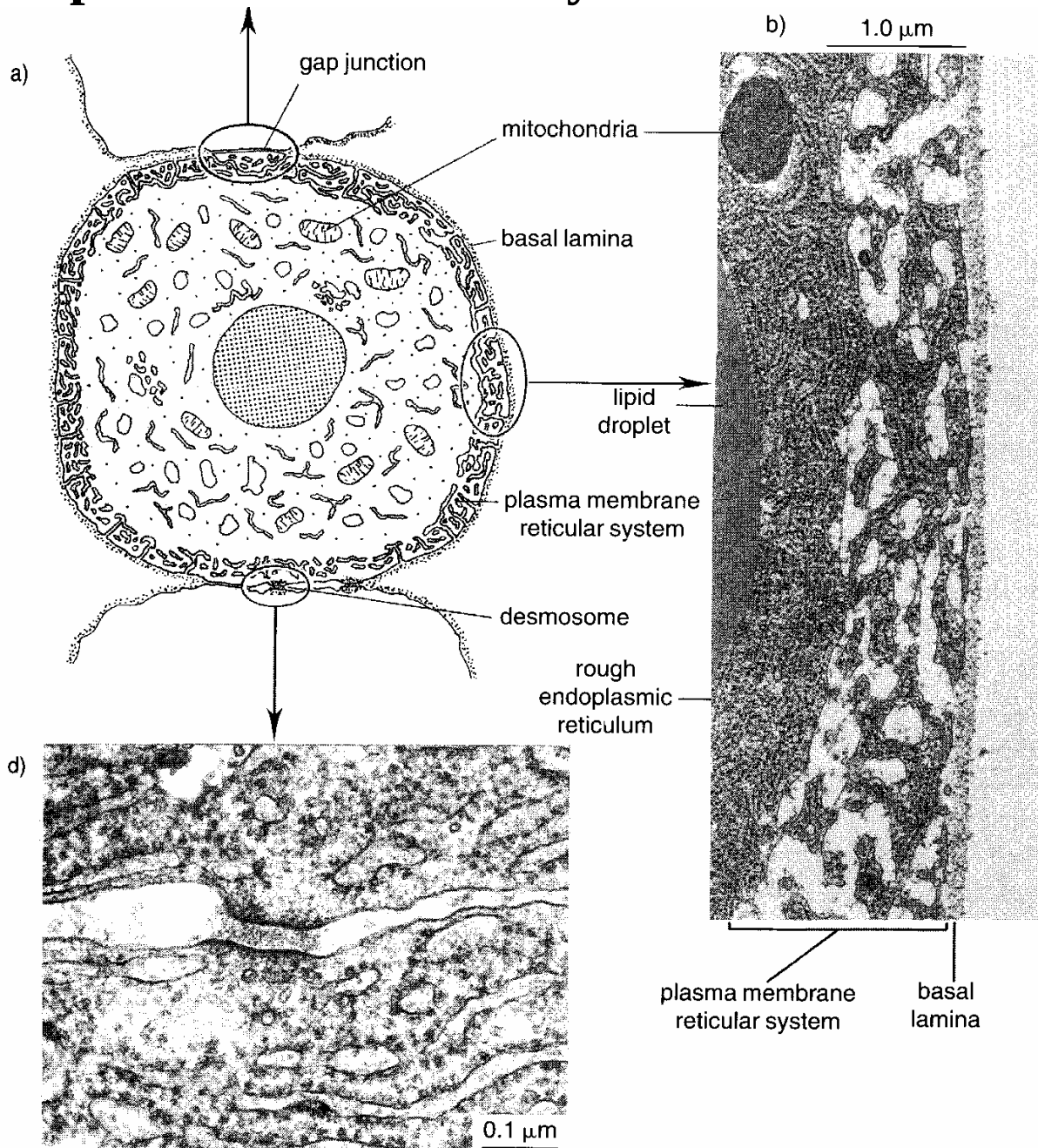




Fat body, at one time was considered to be composed of only one cell type. Now we know that this is not true. The principal cell type of the fat body is the trophocyte. The fat body may also contain bacteriocytes (=mycetocytes), urate cells and hemoglobin

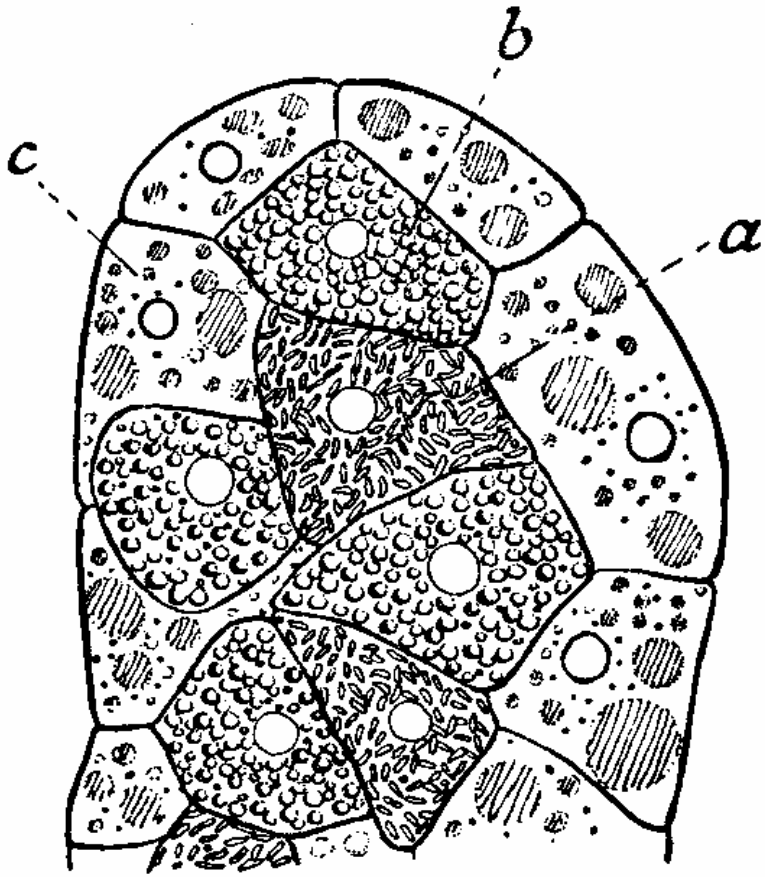
Trophocytes-the principle cell of the fat body

Trophocytes are held together by desmosomes and are surrounded by a basal lamina, thus the appearance that they are one mass of tissue.



Bacteriocytes (=Mycetocytes in fat body)

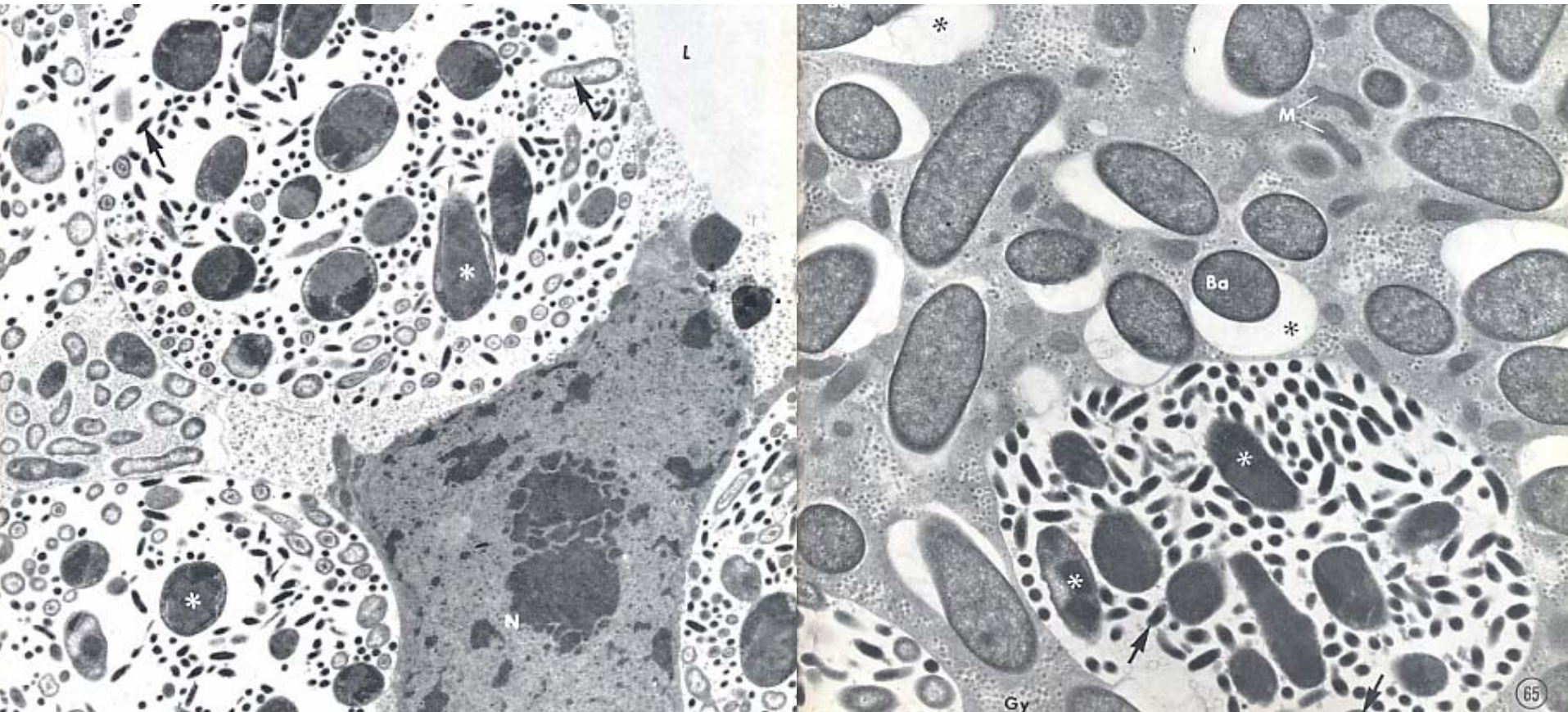
Many insects contain specific micro-organisms, which are present in every individual, and are transmitted from one generation to the next by elaborate mechansims. Associated a with gut, gonads or fat body. Key role in the nitrogen economy of the host. Provide host with amino acids and vitamins.



Mycetocytes: Located in various tissues

1. **Midgut**
   - **a. *Glossina* in wall of**
2. **Gonads**
   - **a. *Cimex*, Formica**
3. **Malpighian tubules**
4. **Fat body**
   - **a. Aphids, Coccids, Aleuroidids, Cicadids (Most Homoptera)**

Bacteriotyces, or cells containing micro-organisms, are found in various parts of the body in a number of insects. Bacteria and/or yeast seem to be the major endosymbionts. These organisms, shown below in a TEMr reveal bacterioids in bacteriotyces of the fat body in the American cockroach. Presumably they are able to use uric acid produced by the fat body.



Most endosymbionts are passed from one generation to the other via transovarial transmission.

1. **Symbionts are released from the bacteriocytes during egg development of the host.**
2. **They migrate from fat body to developing ovaries**
3. **Gain access to the developing oocytes**
4. **Are taken into the oocytes by photocytosis, the process that the female specific yolk protein, Vitellogenin, is taken up by the developing oocyte.**

Among bacterial endosymbionts of insects, the best studied are the pea aphid *Acyrthosiphon pisum* and its endosymbiont *Buchnera sp.* APS, and the tsetse fly *Glossina morsitans morsitans* and its endosymbiont *Wigglesworthia glossinidia brevipalpis*. As with endosymbiosis in other insects, the symbiosis is obligate in that neither the bacteria nor the insect is viable without the other. Scientists have been unable to cultivate the bacteria in lab conditions outside of the insect. With special nutritionally-enhanced diets, the insects can survive, but are unhealthy, and at best survive only a few generations.
The endosymbionts live in specialized insect cells called *bacteriocytes* (also called *mycetocytes*), and are maternally-transmitted, i.e. the mother transmits her endosymbionts to her offspring. In some cases, the bacteria are transmitted in the egg, as in *Buchnera*; in others like *Wigglesworthia*, they are transmitted via milk to the developing insect embryo.
The bacteria are thought to help the host by either synthesizing nutrients that the host cannot make itself, or by metabolizing insect waste products into safer forms. For example, the primary role of *Buchnera* is thought to be to synthesize essential amino acids that the aphid cannot acquire from its natural diet of plant sap. The evidence is (1) when aphids' endosymbionts are killed using antibiotics, they appear healthier when their plant sap diet is supplemented with the appropriate amino acids, and (2) after the *Buchnera* genome was sequenced, analysis uncovered a large number of genes that likely code for amino acid biosynthesis genes; most bacteria that live inside other organisms do not have such genes, so their existence in *Buchnera* is noteworthy. Similarly, the primary role of *Wigglesworthia* is probably to synthesize vitamins that the tsetse fly does not get from the blood that it eats.
The benefit for the bacteria is that it is protected from the environment outside the insect cell, and presumably receives nutrients from the insect. Genome sequencing reveals that obligate bacterial endosymbionts of insects have among the smallest of known bacterial genomes and have lost many genes that are commonly found in other bacteria. Presumably these genes are not needed in the environment of the host insect cell. (A complementary theory as to why the bacteria may have lost genes, Muller's ratchet, is that since the endosymbionts are maternally transmitted and have no opportunity to exchange genes with other bacteria, it is more difficult to keep good genes in all individuals in a population of these endosymbionts.) Research in which a parallel phylogeny of bacteria and insects was inferred supports the belief that the obligate endosymbionts are transferred only vertically (i.e. from the mother), and not horizontally (i.e. by escaping the host and entering a new host).
Attacking obligate bacterial endosymbionts may present a way to control their insect hosts, many of which are pests or carriers of human disease. For example aphids are crop pests and the tsetse fly carries the organism (trypanosome protozoa) that causes African sleeping sickness. Other motivations for their study is to understand symbiosis, and to understand how bacteria with severely depleted genomes are able to survive, thus improving our knowledge of genetics and molecular biology.

Developmental Origin and Evolution of Bacteriocytes in the Aphid–*Buchnera* Symbiosis

Christian Braendle[1 2 ¤] , Toru Miura[3] , Ryan Bickel[1] , Alexander W. Shingleton[1] , Srinivas Kambhampati[4] , David L. Stern[1*]

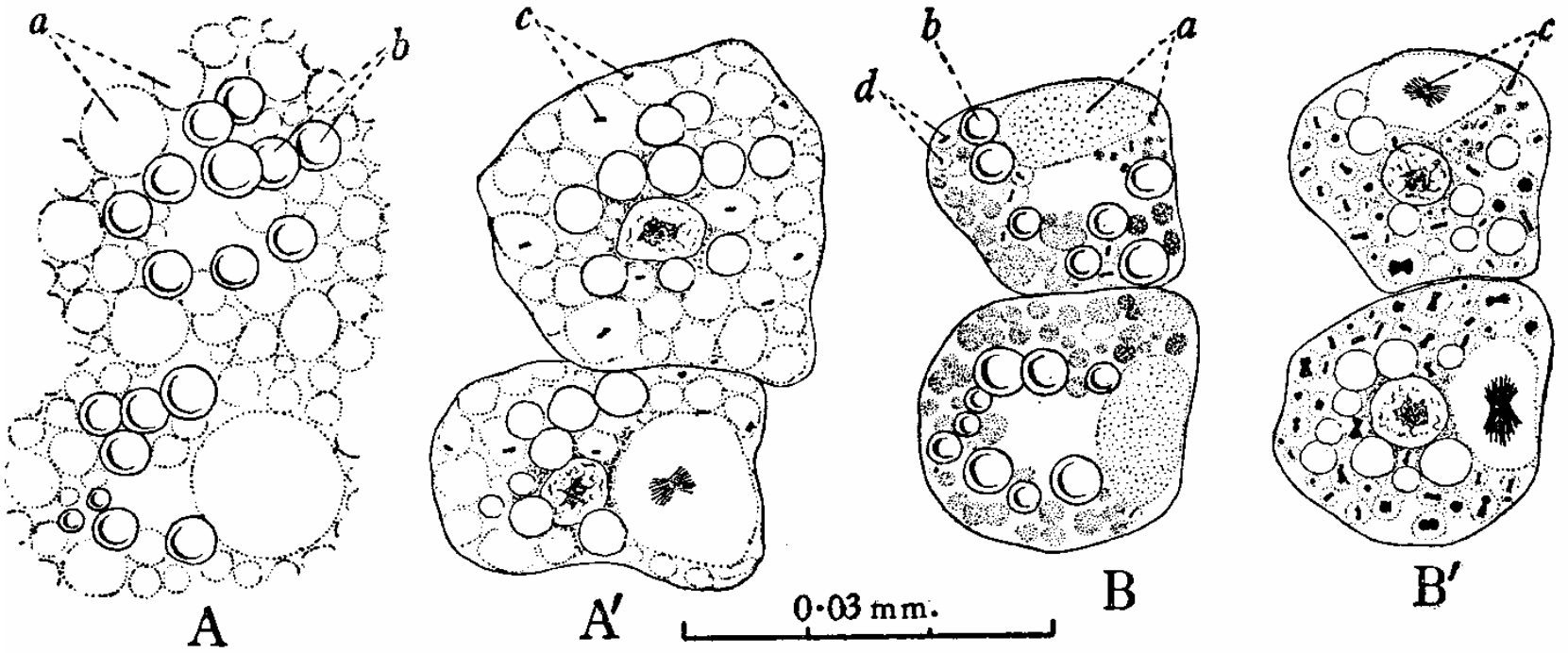
1 Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, United States of America, **2** Laboratory for Development and Evolution, University Museum of Zoology, Cambridge, United Kingdom, **3** Department of Biology, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan, **4** Department of Entomology, Kansas State University, Manhattan, Kansas, United States of America

Symbiotic relationships between bacteria and insect hosts are common. Although the bacterial endosymbionts have been subjected to intense investigation, little is known of the host cells in which they reside, the bacteriocytes. We have studied the development and evolution of aphid bacteriocytes, the host cells that contain the endosymbiotic bacteria *Buchnera aphidicola*. We show that bacteriocytes of *Acyrthosiphon pisum* express several gene products (or their paralogues): Distal-less, Ultrabithorax/Abdominal-A, and Engrailed. Using these markers, we find that a subpopulation of the bacteriocytes is specified prior to the transmission of maternal bacteria to the embryo. In addition, we discovered that a second population of cells is recruited to the bacteriocyte fate later in development. We experimentally demonstrate that bacteriocyte induction and proliferation occur independently of *B. aphidicola*. Major features of bacteriocyte development, including the two-step recruitment of bacteriocytes, have been conserved in aphids for 80–150 million years. Furthermore, we have investigated two cases of evolutionary loss of bacterial symbionts: in one case, where novel extracellular, eukaryotic symbionts replaced the bacteria, the bacteriocyte is maintained; in another case, where symbionts are absent, the bacteriocytes are initiated but not maintained. The bacteriocyte represents an evolutionarily novel cell fate, which is developmentally determined independently of the bacteria. Three of five transcription factors we examined show novel expression patterns in bacteriocytes, suggesting that bacteriocytes may have evolved to express many additional transcription factors. The evolutionary transition to a symbiosis in which bacteria and an aphid cell form a functional unit, similar to the origin of plastids, has apparently involved extensive molecular adaptations on the part of the host cell.

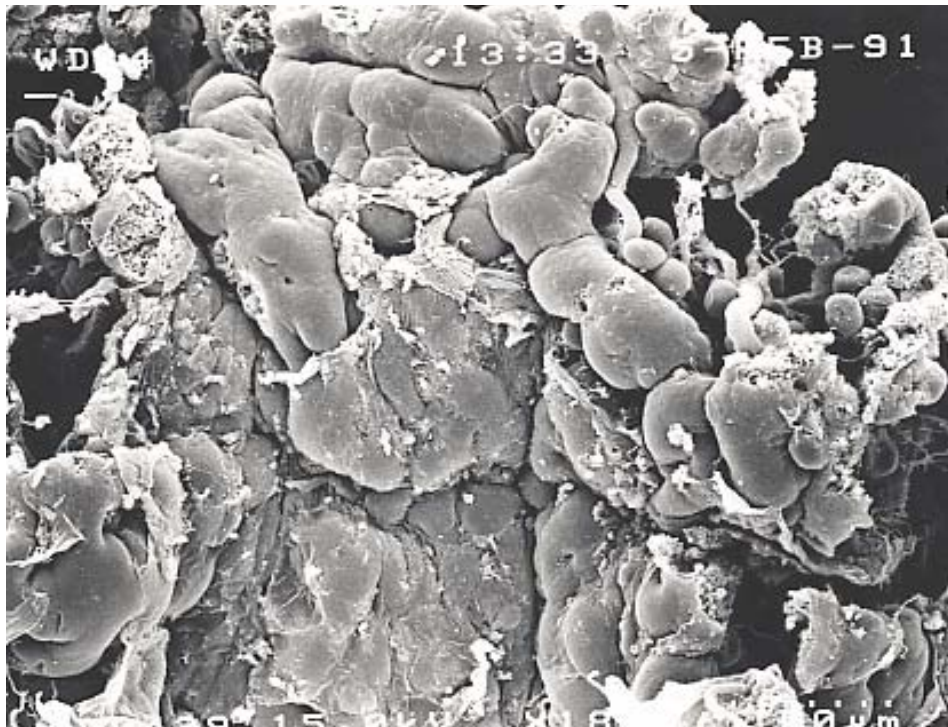
Urate cells-

Are found in Collembola (lack Malpighian tubules), Thysanura, Blattodea and larval Apocrita (bees and wasps). Contain large spherules of uric acid. Probably for storage, which is based on some aspect of the insects life cycle or development.

Cockroaches do not excrete uric acid. Instea, they store it in the urate cells.



Waste products are taken up by the fat body from the hemolymph where they are converted to uric acid crystals (c) or uric acid in vacuoles (d).



STORAGE EXCRETION-waste products are stored in the body rather than eliminating them. Uric acid is not toxic and requires little water. It is stored in special cells (called urocytes) amongst the fat body in cockroaches and some other insects in crystalline form.

Cockroaches and uric acid (Coby Schal work)

Males of several species have special storage glands called uricose glands that store uric acid and are part of their accessory reproductive glands. When males transfer the spermatophore to the female he deposits uric acid solution on it. This passes onto the female. When the sperm are released and the spermatophore is voided by the female, she eats it, thus getting the nitrogen rich uric acid from the male. Also, other species feed on a urate 'slurry' found in the male's genital chamber.

.

Storage of waste in the embryo-uric acid increases

Storage in the pupa also occurs

Permanent deposits in epidermal cells-Cotton stainer bug (uric acid) and pterins in white cabbage butterfly.

Insect diversity provides for wonderful examples of how a particular tissue or organ, that is used for a specific function becomes modified to serve another function. Following are examples of how the Malpighian tubules have become modified to serve various functions in different insects.

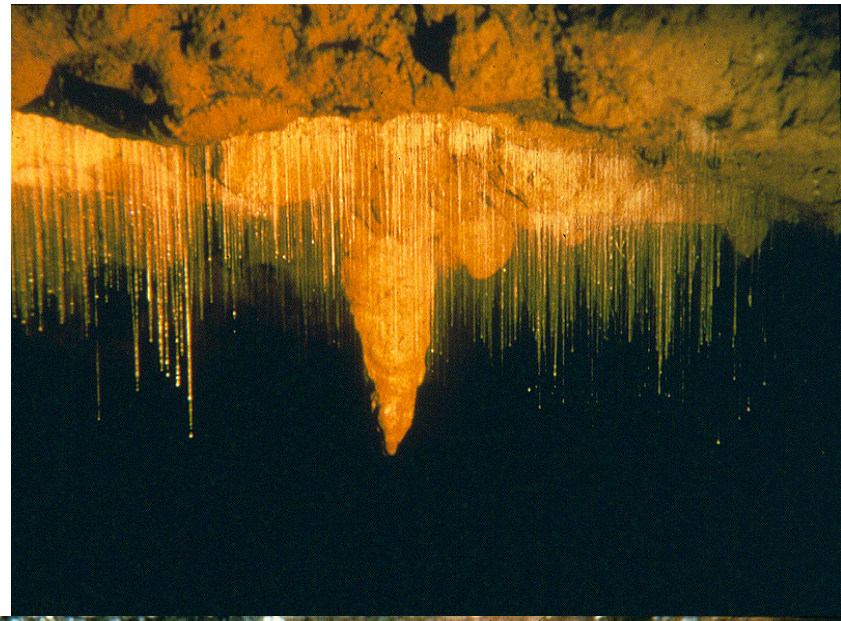
Hemoglobin-

Hemoglobin synthesis occurs in the fat body of larval midges (Chironimidae).

Go to the website below to see some excellent photos of the larvae
http://www.wdaweb.com/bloodworms.htm

Larva of the fly *Arachnocampa luminosa*, Malpighian tubules are modified as light producing organs

In Waitomo Caves of New Zealand about 600,000 tourist/yr for a total of 4 million US dollars.



Sticky fishing lines produced by fly larva

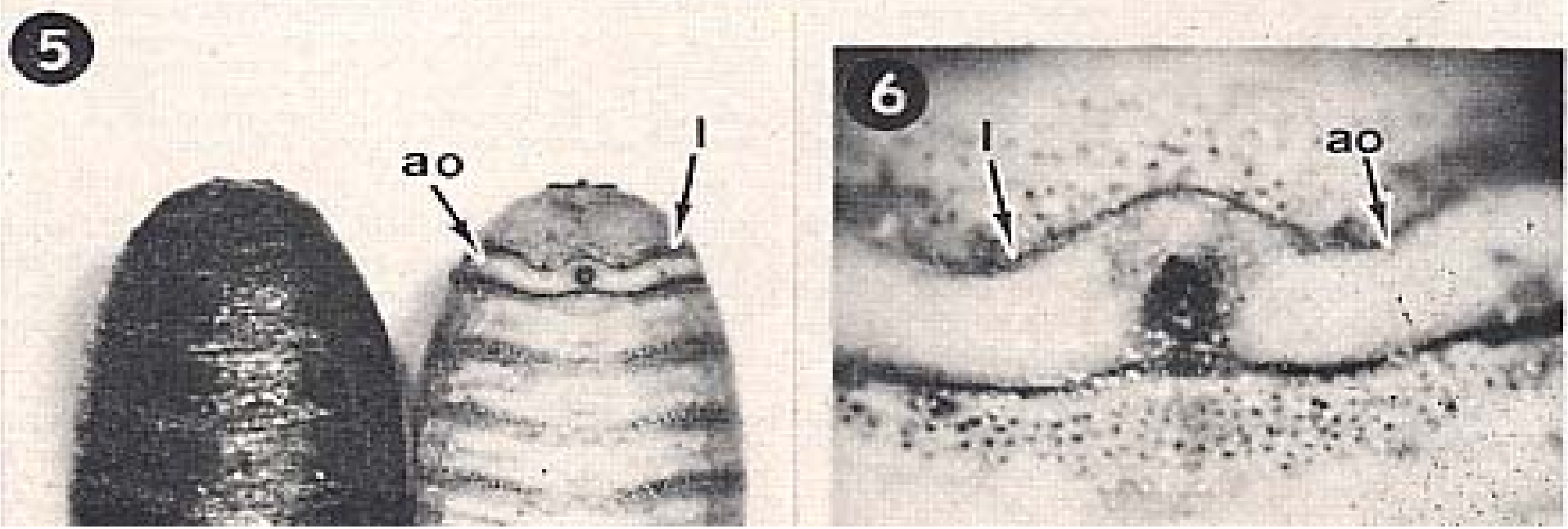
Enlarged distal ends of Malpighian tubules form the luminous organ that that produces the light. Tissue Cell. 1979;11(4):673-703.**Regional specialization in the Malpighian tubules of the New Zealand glow-worm Arachnocampa luminosa (Diptera: mycetophilidae). The structure and function of type I and II cells.**

Various structures that normally serve a particular function, such as excretion with the Malpighian tubules, evolve in various insects to serve a totally unrelated function (I.e., here a light organ). This pattern repeats itself throughout the insect world.

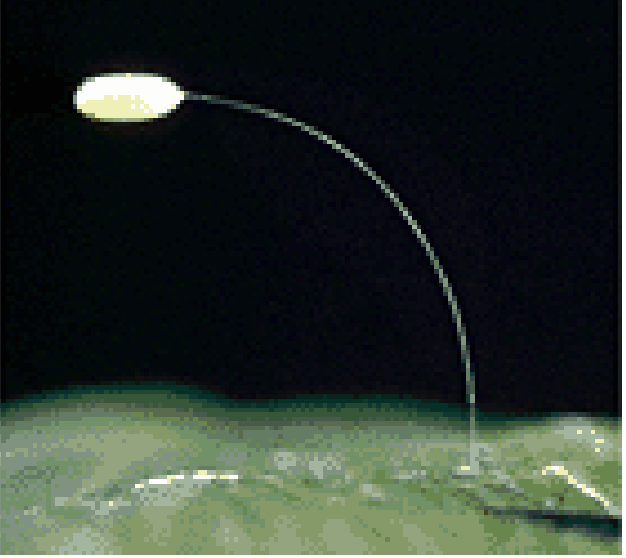
Fat body cells in *Keroplatus trstaceus*, the glowworm known as the northern fungus gnat, produces its light from modified cells in the fat body.

In spittle bugs, the Malpighian tubules are involved in producing the spittle, which then comes out of the anus.

Structure and Function of the Malpighian Tubules, and Related Behaviors in Juvenile Cicadas: Evidence of Homology with Spittlebugs (Hemiptera: Cicadoidea & Cercopoidea)
Author: Rakitov R.A.[1]
Source: Zoologischer Anzeiger, May 2002, vol. 241, no. 2, pp. 117-130(14)



In the above fig. 5 one can see the puparium of the house fly on the left, which is melanized and darkened. The one of the right is of face fly, which is calcified. The calcium for puparium formation in this species comes from the transfer of calcium from the Malpighian tubules to the cuticle at the time of pupariation. Several other dipteran species have white, calcified pupal cases.



In *Chrysopa* larvae, material from the Malpighian tubules is involve with forming the silk of the pupa (see lower left). Also antlion larvae do the same thing in the Malpighian tubule modification.

What is a MECONIUM?

It is the waste products produced by a human, unborn baby or by an insect during the pupal stage. It contains wastes usually produced by the developing organism that is unable to pass it out because it is still inside the mother or the pupal case.

Every expectant parent hopes for an uncomplicated birth and a healthy baby. Some babies, however, do face delivery room complications. One fairly common condition that may affect your newborn's health is meconium aspiration. Meconium aspiration, also referred to as meconium aspiration syndrome (MAS), is a common cause of illness in newborns, but there is good news. Most cases are not severe, and one new treatment is saving the lives of some of the most seriously ill infants. See website below for excellent info about meconium in humans http://ak.essortment.com/whatismeconium_rhmq.htm

INSECT MECONIUM

Ommochromes (yellow, brown and red pigments) color the meconium of Lepidoptera, especially the painted lady, which is red.
Uric acid is also a large part

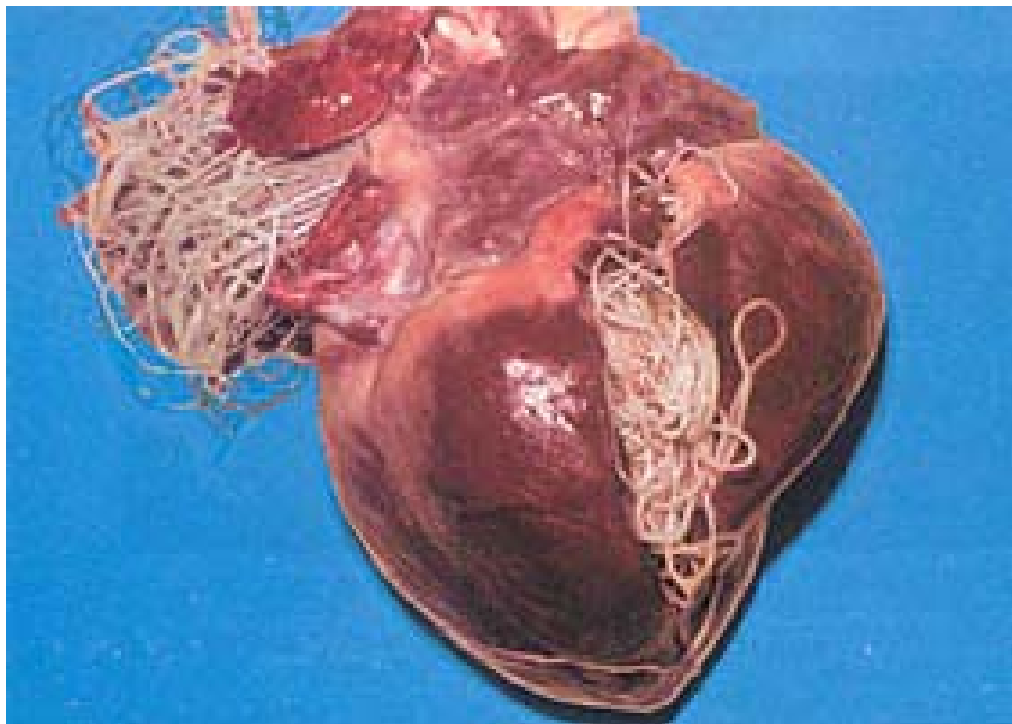
Is that blood coming from the newly emerged Painted Butterfly?

- **Is that blood dripping from our emerging butterflies?**

No, that bright pink fluid, called “meconium,” contains the last traces of the caterpillar’s liquefied body and some other wastes that have collected during metamorphosis. The butterfly expels meconium through its anal opening.

Parasitic hiding in the Malpighian tubules, thus escaping the host response:

1. **Microfilaria of pathogenic nematodes do this in Dog heartworm and Elephantiasis. When they enter the hemolymph of the mosquito, they find their way to the Malpighian tubules and enter the epithelia cells where they avoid the host response.**



J Parasitol. 1984 Feb;70(1):82-8.

Early cellular responses in the Malpighian tubules of the mosquito Aedes taeniorhynchus to infection with *Dirofilaria immitis* (Nematoda).

Bradley TJ, Sauerman DM Jr, Nayar JK.

Early ultrastructural changes in the Malpighian tubules of the mosquito, Aedes taeniorhynchus, were examined following infection with the nematode, Dirofilaria immitis. After ingestion by the mosquito, the microfilariae enter the cells of the Malpighian tubules, becoming intracellular. During early development, the filarial prelarvae reside in the cell cytoplasm surrounded by a clear zone without a delimiting membrane. Cells infected with prelarvae differed from uninfected cells and from cells in uninfected mosquitoes in that the volume of the apical microvilli was reduced and mitochondria were retracted from these microvilli. Morphometric analysis was used to quantify the ultrastructural consequences of infection. In infected cells, microvillar volume, the percent of microvillar volume occupied by mitochondria, and volume of mitochondria within the microvilli were significantly reduced.

Neuropeptide Analogs

- control and research applications

- peptides are not useful for control because they are:
  - susceptible to environmental degradation
  - susceptible to enzymatic degradation
  - unable to pass through insect cuticle

- nonpeptidal and pseudopeptidal agonists can overcome these obstacles

EXCRETORY SYSTEM AS A TARGET FOR PEST CONTROL

1. For terrestrial insects
    a. use diuretic agonists and/or antidiuretic antagonists

2. Problem-separate control of Malpighian tubule from that of the hindgut. If try to regulate one then the other may compensate for what has been done.

Molecular engineers

a. Design molecules that can be applied topically. Development of peptide analogues that can be applied topically and still work. Has been done with the PBAN (pheromonotropic activity) in moths and it works.

PBAN

Pheromone biosynthesis activating neuropeptide

Synthesized Pseudopeptide:

- lipid moieties
- fortified peptide bonds

Results:

- cuticle penetration (esp. foregut)
- hemolymph persistence
- pheromone production

Heliothis virescens

SUMMARY

INSECT EXCRETORY SYSTEM INCLUDES:

1. **Malpighian tubules**
2. **Hindgut**

They regulate water and ion composition of the cells, tissues, and hemolymph and hemolymph volume

The rates of water and ion movement are controlled by diuretic and antidiuretic hormones

Some insects do not excrete uric acid but rather store it and use it with the aid of endosymbionts, which are housed in special cells know as bacteriocytes (=mycetocytes).