PHYSIOLOGY OF SECRETION OF THE SEGMENTED MALPIGHIAN TUBULES OF *CENOCORIXA BIFIDA* (HEMIPTERA-INSECTA)

by

CATHARINE MARY McMAHON SZIBBO

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Department of ZOOLOGY

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date April 25, 1977

ABSTRACT

The secretion of the segmented Malpighian tubules of Cenocorixa bifida (Hungerford) was studied in vitro to determine if the Malpighian tubules of an aquatic predator function in a similiar manner to those of terrestrial insects, and to determine the importance of the different morphological segments in the ion transport of the whole tubule.

Fluid secretion in the Malpighian tubules of C. bifida appears to be governed by the same in vitro factors found important in other insects. As in other insects, the secreted fluid is isosmotic to the bathing medium, while the potassium is hypertonic and sodium hypotonic over a wide range of bathing medium sodium-potassium ratios. At bathing medium potassium concentrations close to that of the insect's haemolymph, potassium and sodium are isotonic in the secreted fluid. The Malpighian tubules of C. bifida produce alkaline secretion when rate of secretion is increased by addition of cyclic AMP.

Differences in ion and fluid transport between the segments of the Malpighian tubules of C. bifida are statistically significant, but only slight. This correlates with the lack of morphological differences in apical and basal infoldings between the segments. The major exceptions to this trend are (1) the high pH of Segment II fluid, (2) the transport of dyes by Segment III and (3) the production of 'secretory granules' by Segment III. Segment III is the most distinct ultrastructurally.

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I. INTRODUCTION

Cenocorixa bifida occurs in a wide range of environmental salinities and is exposed to osmoregulatory problems varying in magnitude with seasonal changes in its milieu (Scudder, 1969a,b). In waters hypo-osmotic to its haemolymph, C. bifida regulates haemolymph volume and osmotic pressure well, but it can tolerate hyperosmotic waters on a short term basis only (Scudder et al., 1972). In this respect it is similiar to other corixids studied (Knowles and Williams, 1973; Frick and Sauer, 1974a,b), except Trichocorixa verticalis interiores Sailer (Tones and Hammer, 1975). The osmoregulatory system of C. bifida must display some lability of function in response to changes in external salinity. Whether and how the rate of isosmotic secretion of the Malpighian tubules of C. bifida is altered is not known; external salinity may alter the secretory activity of tubules by release of neurosecretory products (Jarial and Scudder, 1971; Scudder, 1976).

Cenocorixa bifida must also be able to cope with variable and often extreme ion concentrations in its environment; it lives in water with varying amounts of magnesium, sulfate, sodium, bicarbonate, etc. (Scudder 1969a,b: Topping and Scudder, 1977). Ionic regulation requires adaptations independent of those needed for osmoregulation (Beadle, 1969). For example, the Malpighian tubules of Aedes campestris Dyar and Knab possess special adaptations for the elimination of

excess magnesium and sulfate (Phillips and Maddrell, 1974; Maddrell and Phillips, 1975a). Sodium is an important constituent not only of the environment but also of the food of C. bifida; C. bifida is a predator (Reynolds, 1975; Jansson and Scudder, 1972) and has a high haemolymph sodium-potassium ratio (Scudder et al., 1972) typical of that of a predator (Jeuniaux, 1970). Sodium will thus be more available to the Malpighian tubules of this animal than potassium, and ought to be more important in the composition of Malpighian tubule secretion than it is in the terrestrial phytophagous insects Calliphora erythrocephala (Meig.), Carausius morosus Br., Schistocerca gregaria Forskål, Tipula paludosa Mg., Pieris brassicae L., and Calpodes ethlius Stoll. (Berridge and Oschman, 1969; Ramsay, 1955; Maddrell and Klunsuwan, 1973; Coast, 1969; Nicholson, 1976; Irvine, 1969), but less important that in Rhodnius prolixus Stål and Glossina morsitans Westw. (Maddrell, 1969, 1971a; Gee, 1976).

The Malpighian tubules of C. bifida have four morphologically distinct regions, and ultrastructural studies (Jarial, 1967; Jarial and Scudder, 1970) show that there is one cell type only per segment (Figure 1). Segment I cells are the most unique in terms of their apical and basal infoldings; the basal infoldings are only slightly elaborated and the apical microvilli are few and far between. The apical surface is covered with mucopolysaccharide filaments. The whole segment has a length of 0.9 mm and has a thin wall but wide lumen. Jarial and Scudder (1970) suggest that it is a simple conduit for tubule fluid

Fig. 1

Appearance of the segmented Malpighian tubules of C. bifida. The schematic diagrams of cell structure at the right were taken from Jarial and Scudder (1970). n = nucleus. v = vesicle. m = mitochondria. bm = basement membrane. bb = brush border. mv = microvillus. sg= secretory granule. I = Segment I. II = Segment II III = Segment III. IV = Segment IV.

gut
(1 mm)
I
II
III
IV
bb
bm
m
n
sg
mv
v

and possibly also a site of proximal reabsorption.

Segments II, III and IV are clearly of different lengths: Segment II is 2.2 mm long, Segment III is the longest (3.7 mm), Segment IV is 1.3 mm long. While Segments II and IV have a hyaline appearance, the surface of Segment III looks globular and the cells are coloured green by the presence of many concentrically laminated 'secretory granules'. Segment IV also contains an occasional secretory granule. All three distal segments are very similiar in the structure of apical and basal infoldings. Jarial (1967) suggests that these three segments secrete fluid in a manner similiar to the Malpighian tubules of other insects, while Segment III, in addition, may be concerned with the formation, storage and discharge of secretory granules.

Thus, study of the function of the Malpighian tubules of C. bifida is worthy of attention for two reasons. (1) C. bifida is an aquatic predator. All generalizations of insect Malpighian tubule function come from studies on terrestrial phytophagous and terrestrial blood-feeding insects, except for the information obtained by Phillips and Maddrell (1974) on aquatic filter-feeding mosquito larvae. (2) The Malpighian tubules of C. bifida have four morphologically distinct regions. What is the function of these segments and how does this relate to the secretion of the Malpighian tubule as a whole?

The purposes of this study were (1) to determine the basic excretory properties of the Malpighian tubules of C. bifida and to relate them to its unique environment and haemolymph regime and (2) to determine

whether the distinct morphological segments are similiar in ion transport properties. Section III of this thesis is devoted to the presentation of data and discussion relevant to the first goal; Section IV deals with the ion transport of individual segments.

II. MATERIALS AND METHODS

A. Experimental Animals

Overwintering adults used in this investigation were collected from saline lakes of conductivity range 7,000 to 13,000 mmhos in the Chilcotin and Clinton area of British Columbia. All animals were kept in one gallon thermos jugs half-filled with lake water during transport to the laboratory; here they were kept unfed in dechlorinated tap water at 5°C in constant temperature cabinets. Water was changed bimonthly. Male and female adults, flying or non-flying, were used in experiments.

B. Dissection Procedure

Corixids were dissected in dissecting solution (Table I) under a stereomicroscope using small scissors, finely pointed forceps, and improvised tools made from hand-pulled glass needles. After removal of the abdominal dorsum, the fat body and reproductive organs were dissected away, and gentle pulling of the rectum extended the gut and broke the major tracheae. The extensive tracheae joining tubules to each other and to the gut were carefully broken using glass needles only. Each isolated tubule was then cut free from the gut at the most proximal segment and transferred to the bathing medium. Because the distal ends of the Malpighian tubules of *C. bifida* are fastened to the rectum by short tracheae, it was difficult to free them without damage. Where necessary, the distal ends were repaired with terminal ligations. Other breakages of the tubule could be observed under the microscope;

TABLE I. Composition of bathing media

Compound	Medium A (mM)	Medium B[1] (mM)	K-free Medium (mM)	Na-free Medium (mM)
NaCl	145	70	80	0
KCl	10	10	0	80
NaH_2PO_4	7	7	7	0
KH_2PO_4	0	0	0	7
$MgCl_2 \cdot 6H_2O$	2	2	2	2
$CaCl_2$	2	2	2	2
Glutamine	2.7	2.7	2.7	2.7
Glucose	10	10	10	10
Sucrose	+	0	0	0

[1] Dissecting medium

thus broken tubules could be quickly discarded. In all experiments, any of the four tubules that were removed from the animal intact were used for analysis. Tubules from four or more animals were used in each experiment.

C. 'In vitro' Methods

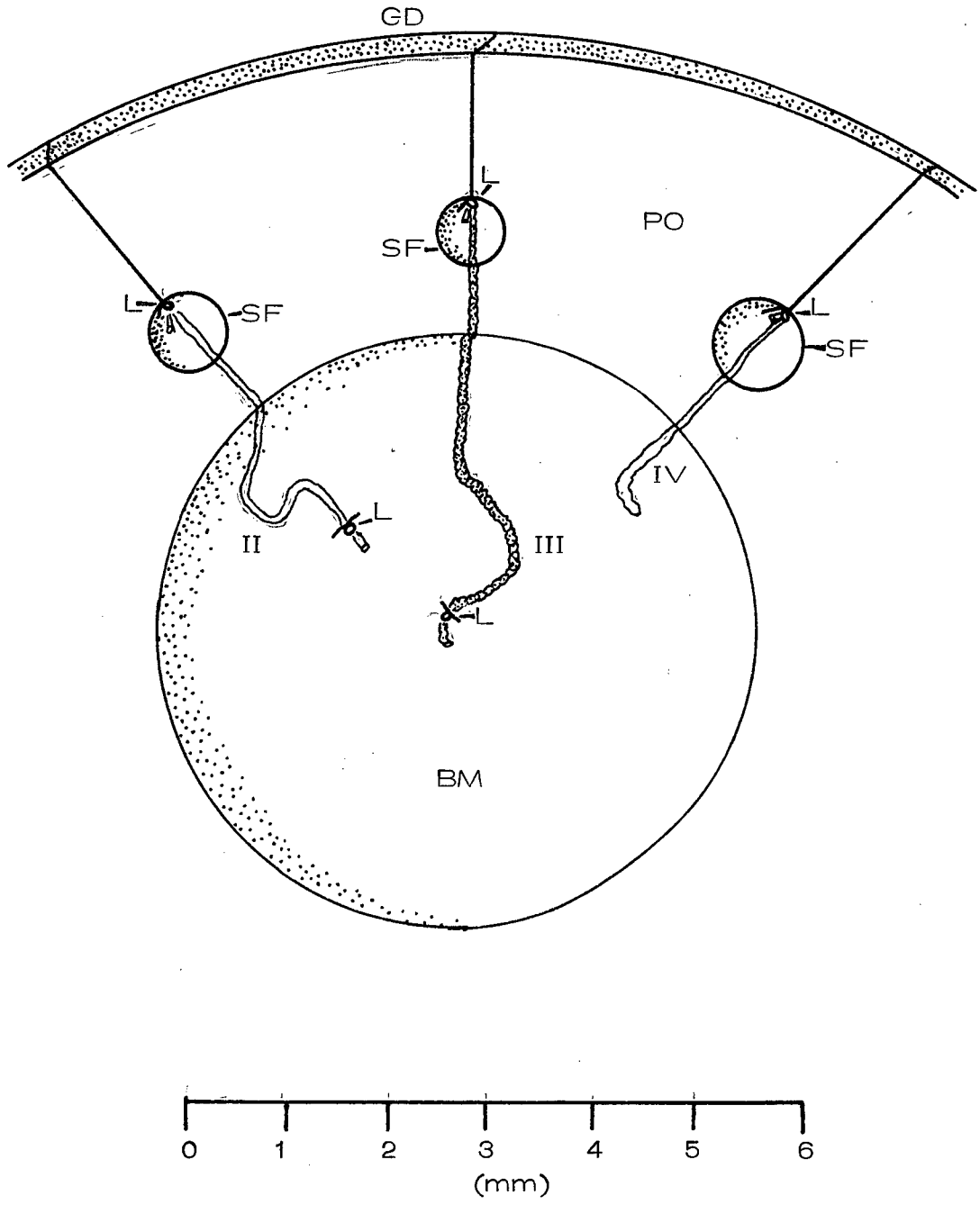
The secretion of all tubules was studed in vitro using the methods of Ramsay (1954). Each tubule was placed in a 50 ul drop of medium held under paraffin oil (Fisher light White) on the slightly wettable surface of a siliconed glass dish. After proximal ligation and before nicking with fine forceps made from tungsten wire sharpened by electrolysis, undamaged tubules were obviously distended with fluid. At no time was any part of the proximal segment (Segment I) bathed in saline; it was too delicate and too small to be successfully studied using the above techniques. The following results that are referred to as 'whole' tubule results were , in fact, from experiments performed upon Segments II , III and IV.

Where the secretion of individual segments was studied, one of two in vitro sampling methods was used. It was preferable to sample from all segments in one tubule at the same time to facilitate comparisons; therefore a technique of immediately cutting the tubule into its component parts was used. Proximal and terminal portions of each segment were ligated, and the proximal end of each segment was pulled into paraffin oil and treated like an entire tubule (Figure 2). In this way all three

Fig. 2

Schematic diagram of preparation of isolated ligated segments of tubules of C. bifida.

II = Segment II. III = Segment III. IV = Segment IV. L = ligation. BM = bathing medium. PO = paraffin oil. SF = secreted fluid. GD = glass dish.


GD
L
SF
PO
L
SF
L
SF
IV
L
II
III
L
BM
0
1
2
3
4
5
6
(mm)

isolated segments of each tubule were maintained in a single droplet, and the bathing medium of all could be changed simultaneously. Timing of segmental and whole-tubule secretion started when the initial droplet of secreted fluid had been removed from the cut proximal end. One disadvantage of this method is that part of the tubule is wasted in the ligature and in the extension into the paraffin oil. The length of each segment immersed was measured in order to correct for variations in amount used in ligation. This method is similiar to that devised by Ramsay (1955) and is hereafter referred to as the 'segmental method'.

The second method of testing for regional differences between segments was more useful for determining the net effect of secretion of proximal segments on fluids produced distally. This is the technique developed by Irvine (1969). Secretion was first collected from the whole tubule, then the tubule was religated close to the junction of Segments II and III and pulled further out of the bathing medium so that only secretion from Segments III and IV was obtained. Finally, the process was repeated and secretion from Segment IV alone collected. This technique is hereafter referred to as 'sequential' sampling of tubule segments. Any change in the rate of secretion with time must be considered as a second variable when using this method.

All experiments were performed at room temperature.

Owing to the relatively small size and correspondingly low rates of secretion of the tubules of C. bifida, it was necessary to collect fluid for periods of several hours.

D. Bathing Solutions

The bathing solutions used in these experiments were based on the haemolymph content of C. bifida as determined by Scudder et al., (1972). A modified Berridge (1966) Ringer was used for a few preliminary experiments, but since tubules could secrete equally well in a simpler medium, all experiments were performed in the salines listed in Table I. The pH of all media was adjusted to 7.1 with 10% NaOH or KOH, raising the concentration of sodium or potassium about 10 mM. Unless otherwise stated, small amounts of phenol red were added to the bathing media to monitor pH. A bathing medium of osmotic pressure equal to that of the haemolymph (350 mOsm) was used in initial experiments, but because tubules secreted much faster at lower osmotic pressures, osmotic pressure was reduced by eliminating sucrose and reducing NaCl. The osmotic pressure of this saline (Medium B) was approximately 175 mOsm. Sucrose was added to create solutions with increased osmotic pressure. Varying sodium and potassium concentrations were obtained by mixing appropriate proportions of sodium-free or potassium-free media.

Bathing media were changed using micropipettes.

E. Sample Analysis

Secreted droplets were removed from the tubule end with a clean glass needle and allowed to fall to the bottom of the sample dish. Diameters of all droplets were measured with an eyepiece micrometer. Volumes of fluid secreted were calculated from diameter measurements;

rate of secretion could be determined where length of secretory period was recorded. Tubule lengths were also estimated with the eyepiece micrometer. Samples for measurement of osmotic pressure were sandwiched between paraffin oil layers in disposable micropipettes made from Fisher alkali-free coagulation capillary tubes. Samples for cation analysis were collected with 1 or 5 ul disposable Drummond Microcaps.

Osmotic pressure measurements were made on the day of sample collection using a Clifton Technical Physics Nanoliter Osmometer with frequent checking by NaCl standards. Each measurement reported here is the mean of 3 to 6 droplets excepting those for fluid produced by Segment III in high potassium saline. In this case sometimes only one or two droplets were obtained.

Sodium and potassium measurements were made with a Techtron Model AA 120 Atomic Absorption spectophotometer used in emission mode; magnesium was measured with this instrument used in absorption mode. For sodium analysis, secreted drops were placed in 1 ml of distilled water, and 1 ul bathing medium samples were placed in 3 ml of distilled water. The samples for potassium measurement were placed in sodium swamp, while those for magnesium were placed in 1.5 % EDTA.

The pH of secreted fluid was estimated to the nearest 0.1 unit by adding small volumes of concentrated solutions at pH = 7 of phenol red or cresol red to the tubule fluid under paraffin oil. Colour comparison of these drops was made to a series of freshly prepared buffers from pH 6.8 to 8.2 containing phenol red or to a series of similiar buffers from

pH 7.0 to 8.8 containing cresol red. Phenol red was not used in the bathing medium during pH determinations because it is concentrated by Segments II and III.

Transport of dyes was recorded by placing segments in dilute solutions of each, and noting whether or not colour appeared in the fluid produced by each of the segments. Phenol red was not included in these media.

F. Statistical Considerations

There was great variability observed between tubules in the secretion rate, and although as a general rule there was no difference in variability in secretory rate among the tubules of one insect compared to between tubules from different insects, the dissection of some animals yielded four tubules that did not secrete at all. Possibly such tubules were damaged during dissection, but it is likely that lack of secretion was because of some inherent property of the tubule, reflecting the hormonal state of the animal before dissection. Because little or no secretion could be obtained from such tubules, they were discarded. No attempt was made to record the frequency of 'non-secretory' tubules under any treatment.

Because the great variability in tubular secretion rate and the presence of nonsecretory tubules made rate comparisons between different groups of tubules difficult, the rate of secretion of each tubule was compared to that of itself under different treatments wherever possible. Likewise, rates of secretion of all segments in each tubule were measured

simultaneously. Determinations of statistical significance of differences between segments in each tubule were made with Friedman's non-parametric two-way analysis of variance followed by multiple comparisons using a modification of the Neuman-Keuls test (Siegel, 1956; Zar, 1974). A 0.05 level of significance was used. All tables and graphs that do not plot individual results show the mean plus or minus one standard error of the mean with the number of measurements (n) in parentheses or beside error bars. Where sample size was constant in a set of determinations, n was shown beside one error bar only. For points calculated from two or more means each with their own variability, the original variability of each mean was added directly for addition of means, but percent variability added for multiplication of means. In such cases sample size was not indicated.

Correlation analysis was done using the Spearman Rank Correlation Coefficient (Zar, 1974).

III. SECRETORY PROPERTIES OF THE WHOLE TUBULE

A. RESULTS

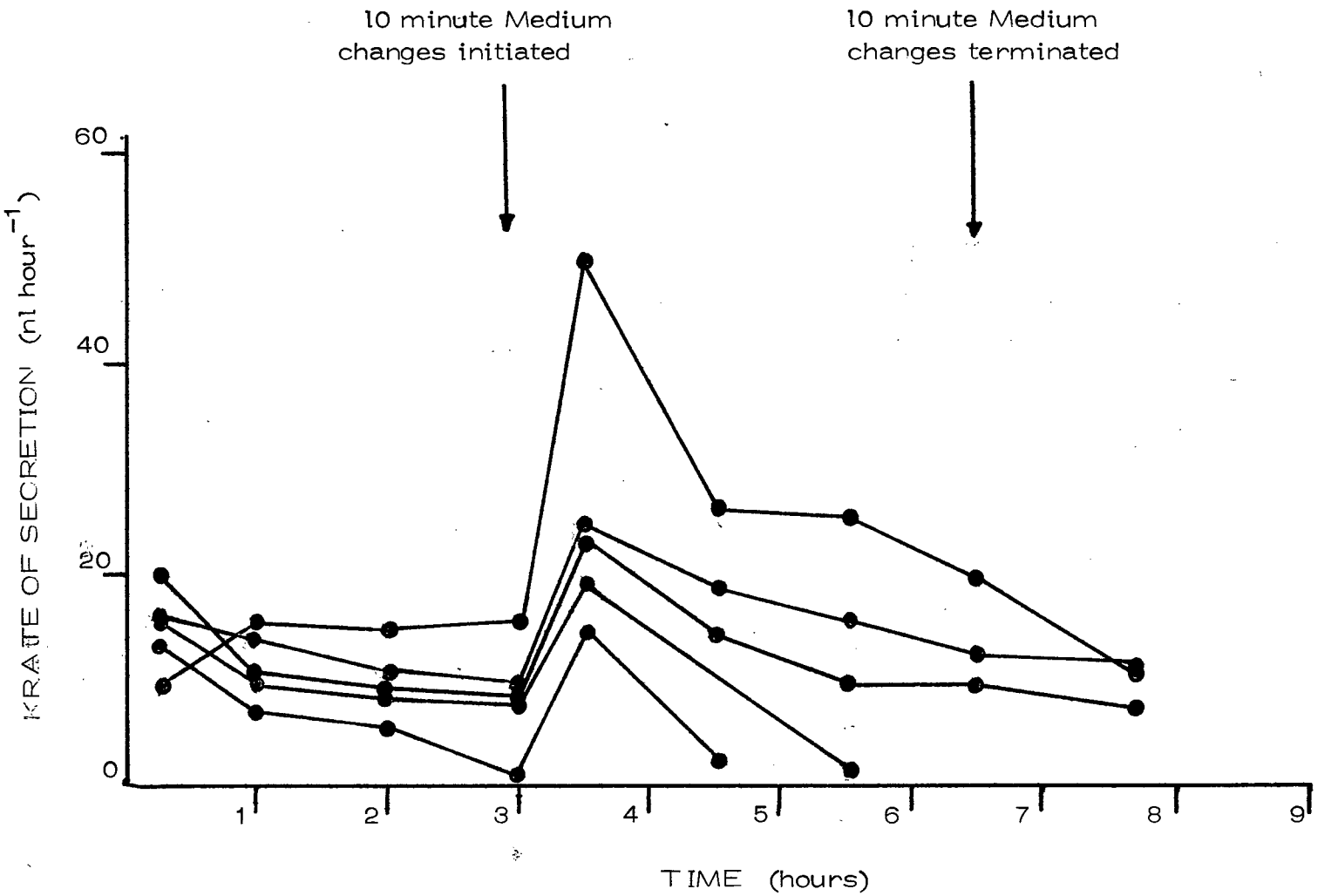
1. Rates of Secretion

The Malpighian tubules of Cenocorixa bifida are comparatively small and secrete at relatively low rates. In order to collect sufficient volumes of fluid for analysis, it was necessary to determine the optimal conditions for secretion. The following preliminary experiments indicate that the bathing fluid conditions found to be stimulatory in several other insects also promote fluid secretion in tubules of C. bifida. Note that 'whole' tubule secretion is actually collected from the distal three segments only.

The Malpighian tubules of C. bifida secreted at low but constant rates (mean rate approximately 15 nl per hour) for periods as long as 24 hours. During this time the bathing medium became markedly acidic and somewhat cloudy. The rate of secretion was enhanced slightly by periodic renewal of the bathing medium (Figure 3). Fluid secretion for five tubules was measured without any change of saline for three hours. Thereafter, renewal of bathing medium at ten minute intervals caused the tubules to secrete faster. This increase was not maintained, and after two hours the rate of secretion declined to the rate before renewal. Termination of the ten minute renewals did not cause any further decrease in secretory rate. Because the tubule is squeezed and stretched during medium changes, bathing media were not changed more

Fig. 3

Effect of medium renewal on rate of tubule secretion. Tubules were maintained in Medium A for three hours without medium change, after which a regime of ten minute medium changes was initiated. Medium was not changed after 6.5 hours


10 minute Medium changes initiated
10 minute Medium changes terminated
RATE OF SECRETION (nl hour^{-1})
60
40
20
0
1
2
3
4
5
6
7
8
9
TIME (hours)

often than every half hour to one hour in subsequent experiments. This regime was sufficient to prevent the medium bathing the tubules from becoming acidic and probably also from becoming depleted of oxygen; tubules maintained in freshly oxygenated medium did not secrete faster. With renewal of the bathing medium, the rate of secretion of tubules isolated in bathing medium A was constant with time, and the mean rate was 25 nl per hour.

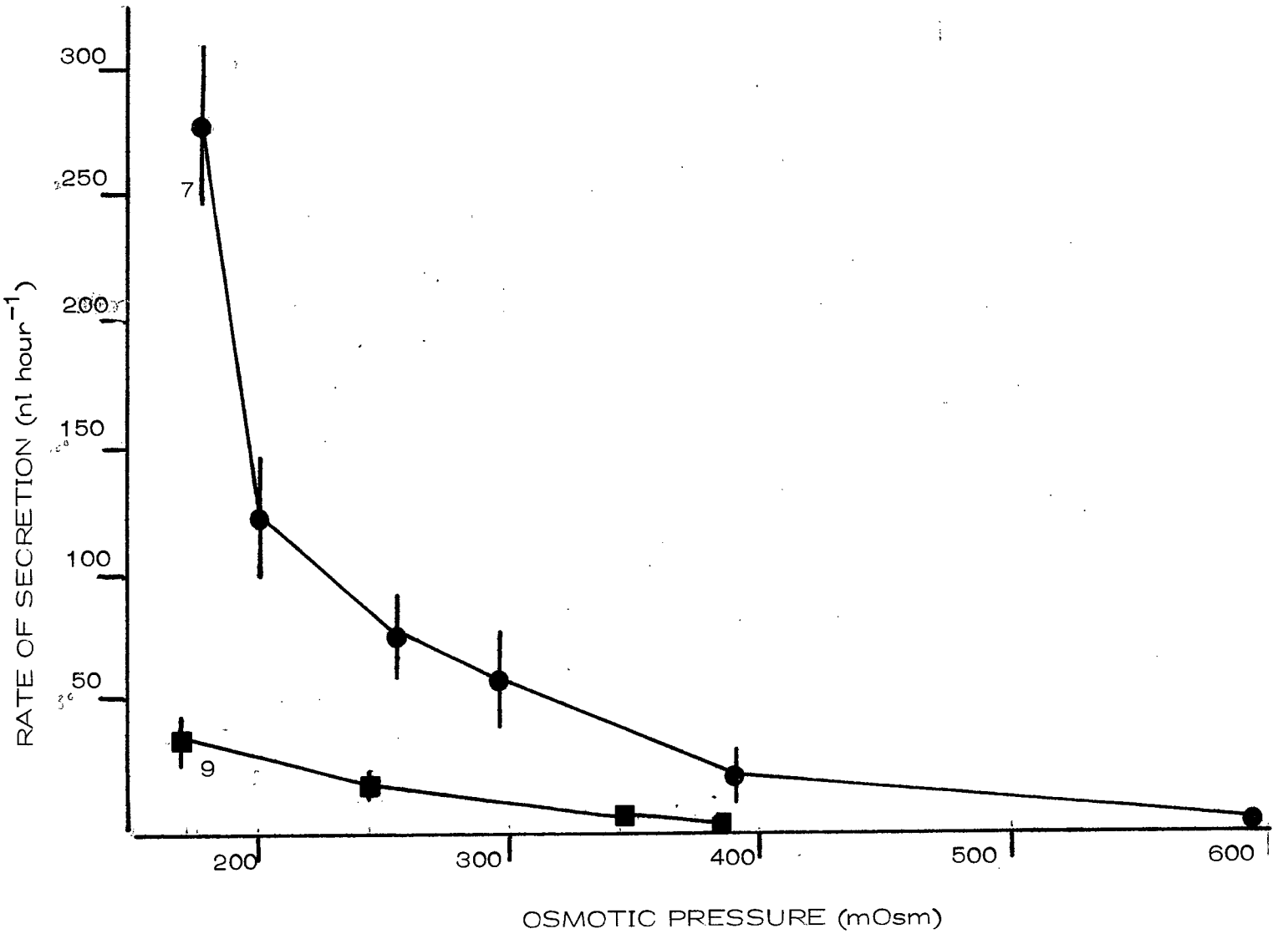
Hyaline granules were commonly found in the secreted fluid, and were often observed moving downstream in the tubule lumen. Because they were seen under a variety of experimental conditions, and in viable tubules, their presence was probably not an artifact of the *in vitro* technique.

2. Effect of Osmotic Pressure

Malpighian tubules of *C. bifida* secreted more rapidly when bathed in a saline of reduced osmotic concentration. The average rate of secretion of a set of nine tubules declined when a saline of reduced osmotic pressure made by dilution of Saline A was replaced by Saline A (340 mOsm) or by salines of even higher osmotic pressure made by sucrose addition (Figure 4). The inhibitory effect of high osmotic pressure was reversible as tubules secreted rapidly when the low osmotic pressure saline was replaced. Note that dilution of medium to reduce osmotic pressure also reduced the concentration of medium components.

Fig. 4

The effect of bathing medium osmotic pressure on rate of secretion. Tubules were placed sequentially in media of increasing osmotic pressure and rate of secretion was determined at each step. The two curves are from tubules under different experimental conditions. The lower curve (■) is from tubules for which osmotic pressure of the medium was varied either by adding distilled water or sucrose to Medium A. No cAMP was present in the bathing medium. The upper curve (●) is from tubules for which osmotic pressure was raised by sucrose addition to Medium B. Cyclic AMP was present in the bathing medium. Bars indicate $\pm$ one standard error. The numbers beside the bars show the sample size (n) for the set of determinations.


300
250
200
150
100
50
RATE OF SECRETION (nl hour^{-1})
7
9
200
300
400
500
600
OSMOTIC PRESSURE (mOsm)

3. Effect of Cyclic Adenosine Monophosphate (cAMP)

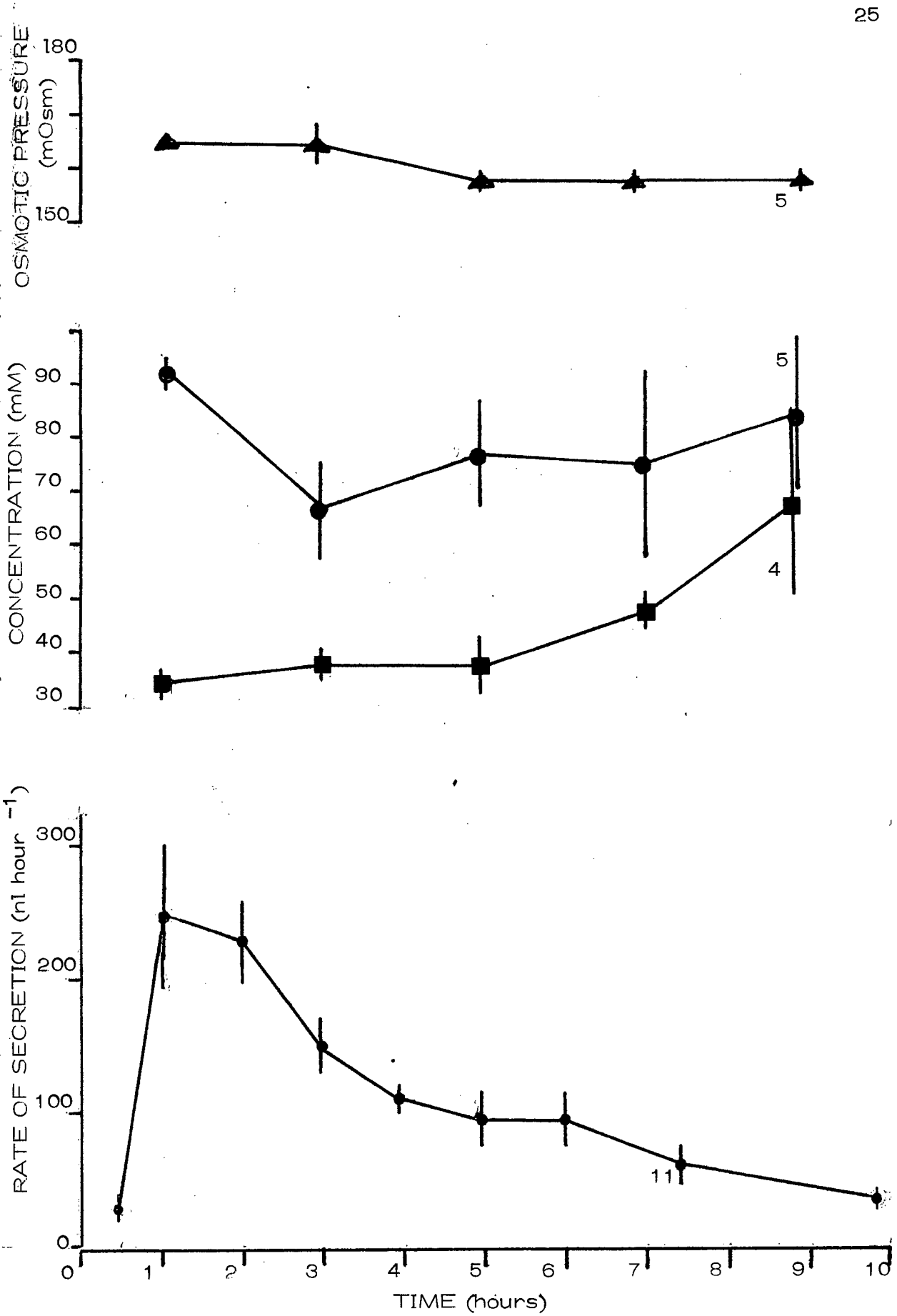
Cyclic AMP had a marked effect on secretion. Preliminary experiments showed that 2.5×10^{-4}M was the minimum concentration necessary to obtain maximum response. 5-Hydroxytryptamine stimulates secretion by tubules of various insects (Maddrell et al., 1969b) but no clear stimulation of secretion was seen with 5 HT at any of several concentrations between 10^{-8} and 10^{-5} M. in tubules of C. bifida.

Changing osmotic pressure had the same effect on tubules bathed in saline containing cAMP as it had in saline lacking stimulant. The upper curve in Figure 4 shows the rates of secretion of seven tubule placed sequentially in salines of increasing osmotic pressure. This time all media components were kept constant; sodium content was reduced from 145 mM to 90 mM to reduce osmotic pressure (Medium B). All other media were made by the addition of increasing amounts of sucrose. The enhancement of secretory rates at low osmotic pressures was marked. Even with stimulant present, however, the rate of secretion in media of osmotic pressure close to that of the haemolymph (350 mOsm) was low. In all further experiments, tubules were maintained in Medium B or modifications thereof(175 mOsm).

Tubular rates of secretion did not remain elevated after stimulation by cyclic AMP (Figure 5). Application of 2.5×10^{-4}M cAMP caused a one hundred fold increase in secretory rate, but after this stimulation the rate of fluid secretion declined, even though cAMP was maintained in the bathing medium. This change of secretory rate with time makes

Fig. 5

Osmotic pressure, sodium and potassium concentration and rate of secretion as functions of time. Cyclic AMP was added at 0.5 hours at a concentration of 2.5×10^{-4}M. Media were renewed every half hour. Samples for sodium and potassium analysis were collected during the halfhour or hour preceding each measurement. Potassium (●). Sodium (■). Bars indicate ± one standard error. The numbers beside the bars show the sample size for that set of determinations.


OSMOTIC PRESSURE (mOsm)
180
150
5
CONCENTRATION (mM)
90
80
70
60
50
40
30
5
4
RATE OF SECRETION (nl hour $^{-1}$)
300
200
100
0
11
0
1
2
3
4
5
6
7
8
9
10
TIME (hours)

experiments measuring the before and after effects of different treatments on the secretory rate of one group of tubules difficult.

As well as increasing the rate of secretion, cAMP caused a pH change in the fluid secreted. As monitored by phenol red, the pH at rest was 7.1 (orange) but during stimulation it was greater than or equal to 8.0 (purple-pink). The osmotic pressure of secreted fluid was not altered by cAMP.

Although rate of secretion declined with time, there was no significant change in osmotic pressure, potassium or sodium concentration of the tubular secretion with time (Figure 5), although sodium concentration did appear to rise as rate of secretion fell. Potassium and sodium concentrations and osmotic pressure of fluid produced by tubule segments sampled sequentially could therefore be compared without fear that the composition of fluid would change during the course of sampling. All experiments were completed within nine hours.

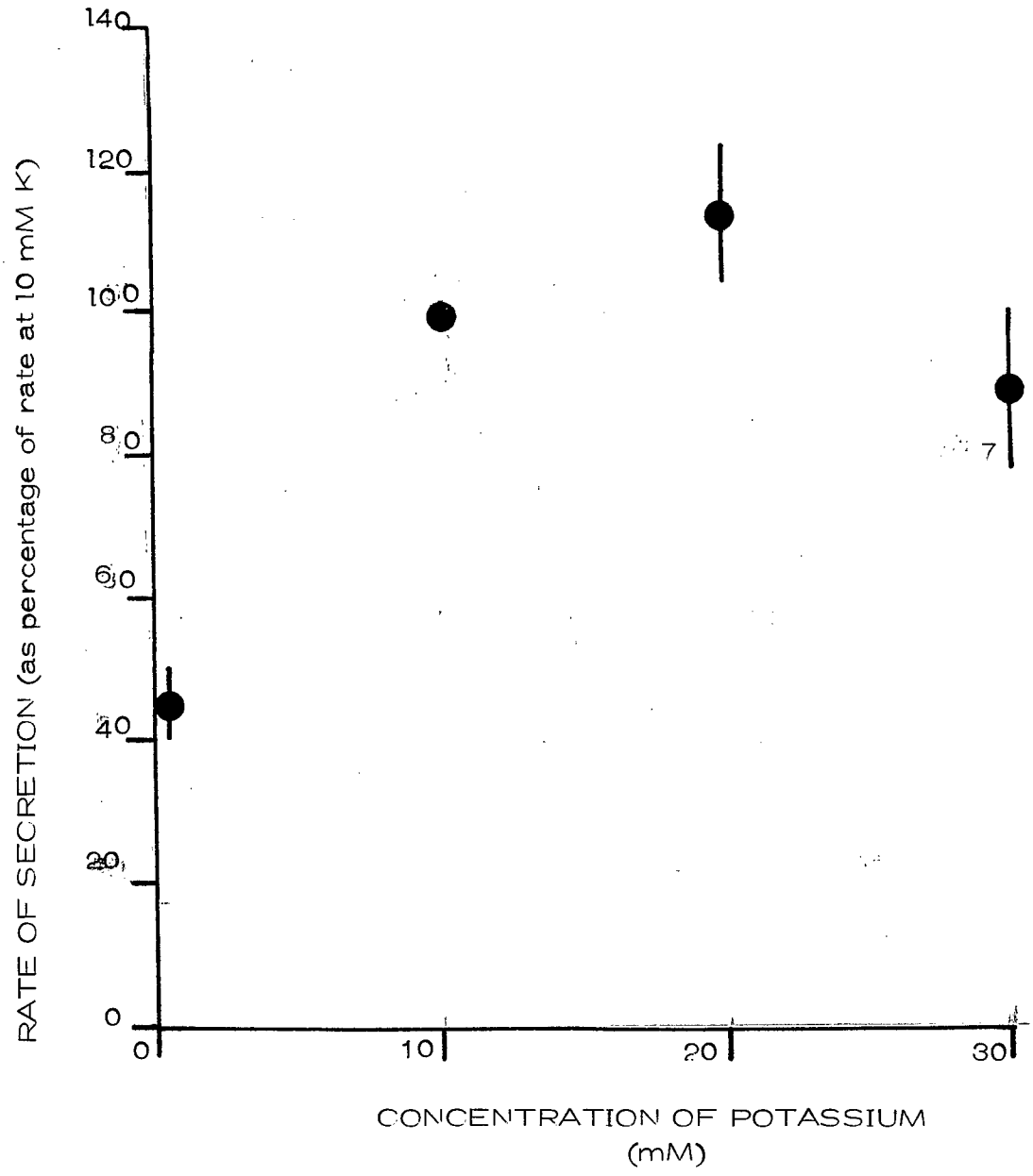
A concentration of 2.5×10^{-4} M cAMP was maintained in media in all further experiments unless otherwise stated.

4. Effect of potassium

The lowest bathing medium potassium concentration tested that supported maximal rates of secretion by Cenocorixa tubules was 10 mM (Figure 6). The rate of secretion was not stimulated further by concentrations of potassium greater than this. To allow the rate of secretion of each tubule to be compared to that of itself in the presence of different potassium concentrations in the bathing medium, and yet

Fig. 6

The influence of bathing medium potassium concentration on rate of secretion. The rate of secretion of each tubule was determined for one half hour in 10 mM K saline, then for one half hour in test saline (0, 20, 30 mM) and then again in 10 mM K saline. The rate of secretion in test saline was recorded as a percentage of the rate in 10 mM K saline averaged between the before and after periods. The bars indicate plus or minus one standard error. Sample size (n) was 7.


140
120
100
80
60
40
20
0
RATE OF SECRETION (as percentage of rate at 10 mM K)
0
10
20
30
7
CONCENTRATION OF POTASSIUM
(mM)

correct for any inherent decline of secretion with time, a method from Kaufman and Phillips (1973) was used. The rate of secretion of each tubule was determined for one half hour in 10 mM K saline, then for one half hour in test saline (0, 20, 30 mM) and then again in 10 mM K saline. The rate of secretion in test saline was recorded as a percentage of the rate in 10 mM K saline averaged between the before and after periods.

The effect of higher concentrations of potassium on secretory rate is not clear. Although Figure 7 shows that addition of 90 mM K saline to tubules previously maintained in 10 mM K did not cause tubules to secrete at mean rates any different from those of control tubules (ionic balance maintained with NaCl) whether or not cAMP was present in the medium, there was a large variation in the response of individual tubules to high potassium. Some individual tubules showed a brief increase in secretory rate in the 15 minute period following addition of 90 mM K, especially in media lacking cAMP (Figure 7a) but it was not maintained. Some tubules were obviously irreversibly inhibited within one hour of high potassium treatment, whereas others continued to secrete at high rates. Rate of secretion of experimental tubules was not significantly different from that of control tubules at any time.

The rate of secretion of tubules isolated in 90 mM K saline declined with time after an initial accelatory response to cAMP but the decline with time was qualitatively similiar to the decline observed in 10 mM K saline (Figure 8). The average rates of secretion of tubules sampled

Fig. 7

Effect of 90 mM K saline on tubular secretion rate. Saline containing 90 mM K was added to tubules previously secreting in 10 mM K and mean rate of experimental tubules (●) compared to that of control tubules (■) maintained in 10 mM K saline throughout. (a) Bathing medium lacks cAMP. (b) Bathing medium contains cAMP. The bars indicate either plus or minus one standard error. Sample size for each set of determinations is shown beside the error bars.

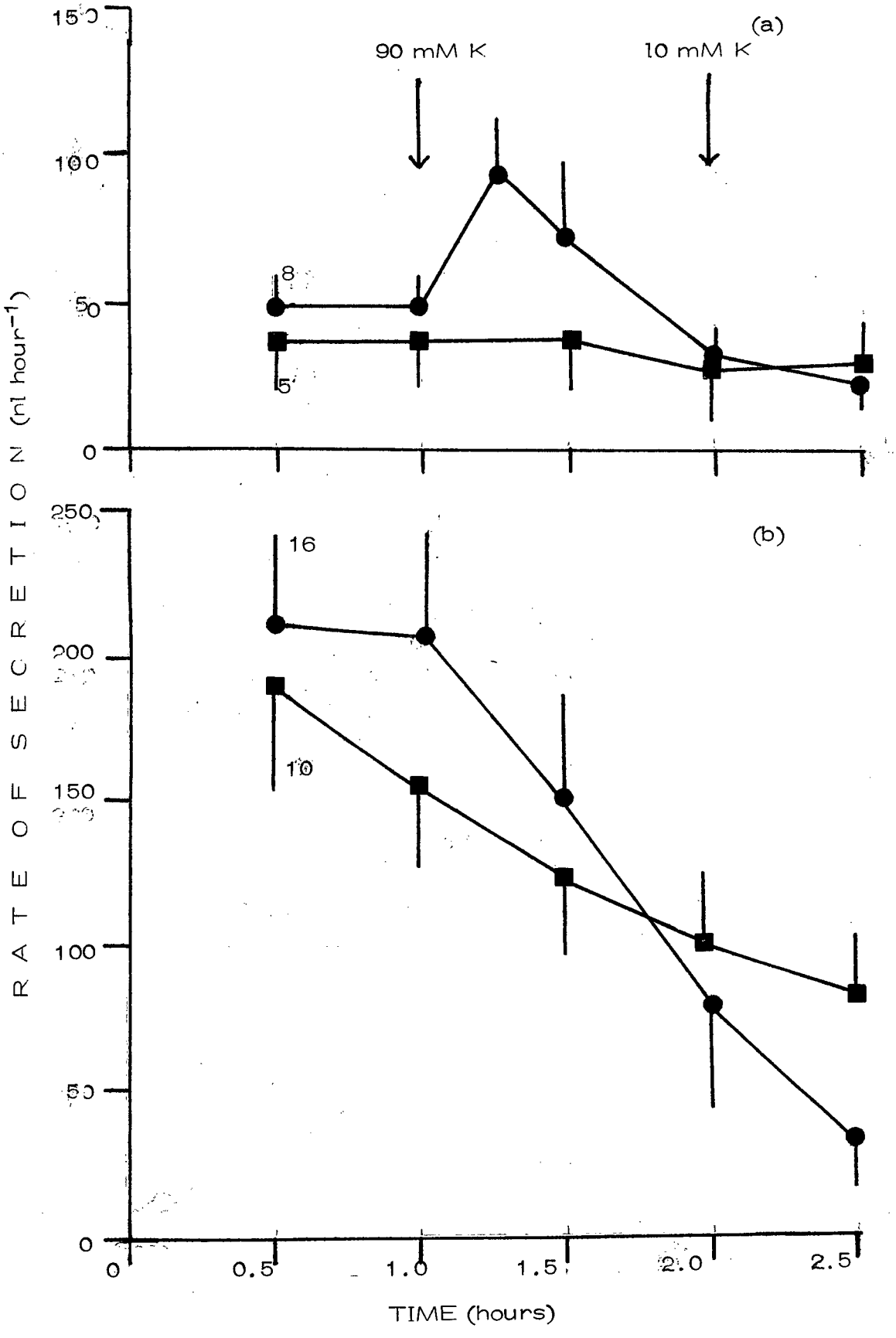
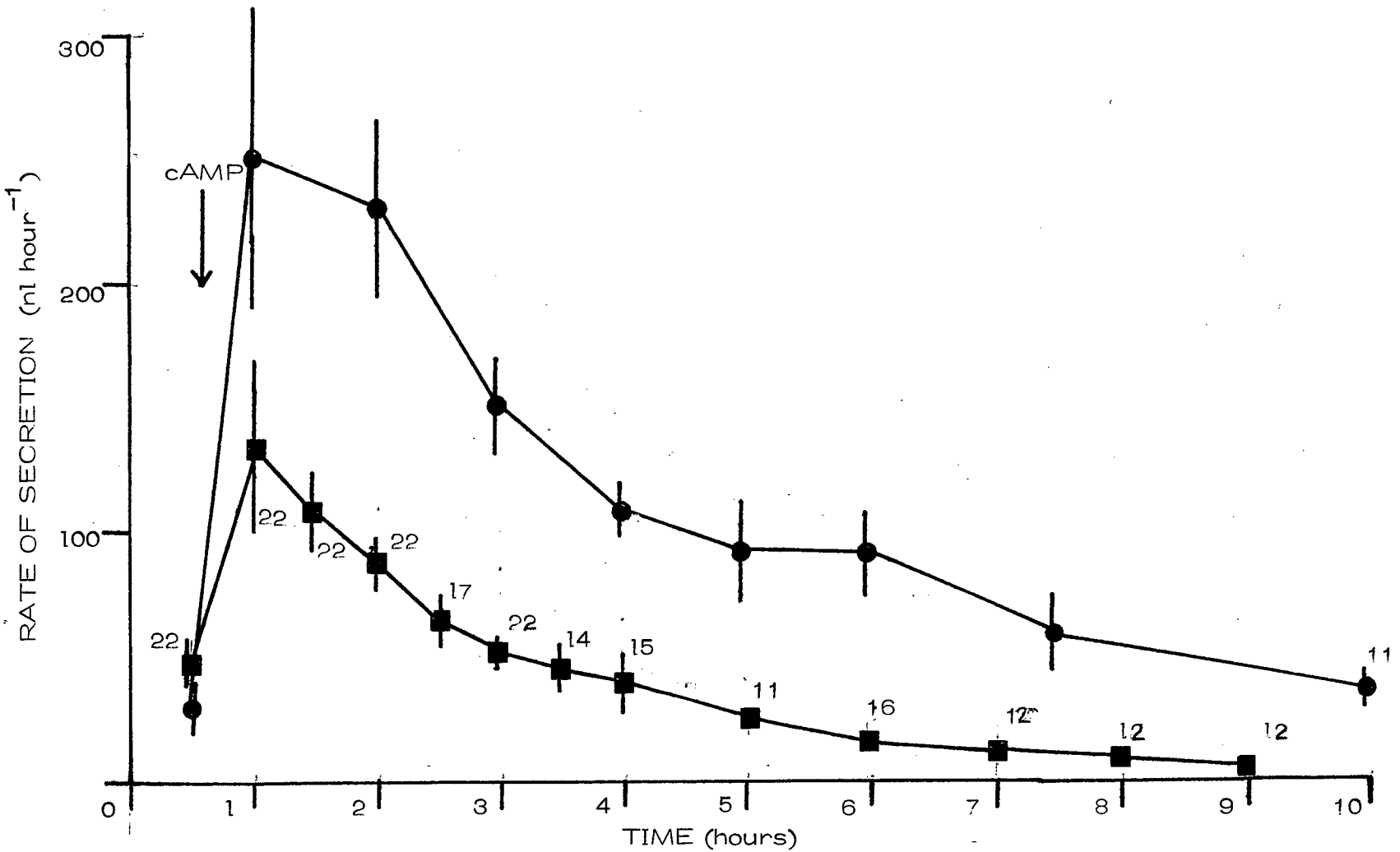

(a)
90 mM K
10 mM K
8
5
(b)
16
10
RATE OF SECRETION (nl hour⁻¹)
TIME (hours)
0
50
100
150
200
250
0.5
1.0
1.5
2.0
2.5

Fig. 8

Rate of secretion as a function of time in 10 mM K saline (●) and 90 mM K saline (■). Cyclic AMP was included in the bathing media after 0.5 hours, and the medium was renewed periodically. The bars indicate ± one standard error unless smaller than the dimensions of the point. Sample size at 10 mM K saline was constant at 11. The figures beside bars for 90 mM K determinations show sample size (n). Fewer tubules were measured for long periods of time.


300
200
100
0
RATE OF SECRETION (nl hour^{-1})
cAMP
22
22
22
22
17
22
14
15
11
16
12
12
12
11
1
2
3
4
5
6
7
8
9
10
TIME (hours)

in both these cases became significantly different after one hour, although such a comparison may not be valid because different groups of tubules were used. One cannot rule out the possibility that 90 mM K had an inhibitory effect that was not generally manifested until after one hour of exposure.

Some visual observations confirm that raised potassium had a definite effect on the Malpighian tubules. They became extremely swollen; the cells enlarged and broke very easily. Phenol red transport appeared to be inhibited, more hyaline bodies were seen in the secreted fluid, and the pH was reduced. When rupture of the tubule wall occurred, hyaline bodies similiar to those seen in small numbers in secreted fluid flowed out into the bathing medium in great quantities.

5. Composition of the Secreted Fluid

Malpighian tubules of C. bifida produced fluid that was isosmotic to the bathing medium over the range 170 to 420 mOsm. All measurements are shown as single points in Figure 9. The concentration of major constituents of fluid secreted by tubules bathed in 10 mM potassium, 90 mM sodium medium (Medium B) is given in Table II. The potassium and sodium concentrations of the Malpighian tubule secretion are almost equal when the tubules are bathed in a saline containing a more haemolymph-like sodium-potassium ratio (170 mM Na, 20 mM K). They were 110 mM K and 105 mM Na.

A range of bathing medium sodium and potassium concentrations was obtained by mixing aliquots of potassium-free and sodium-free salines.

Fig. 9

Effect of osmotic pressure of bathing fluid on osmotic pressure of tubular secretion. Tubules were maintained in either 10 mM K saline (○) or 90 mM K saline (■). The solid line indicates the isosmotic line.

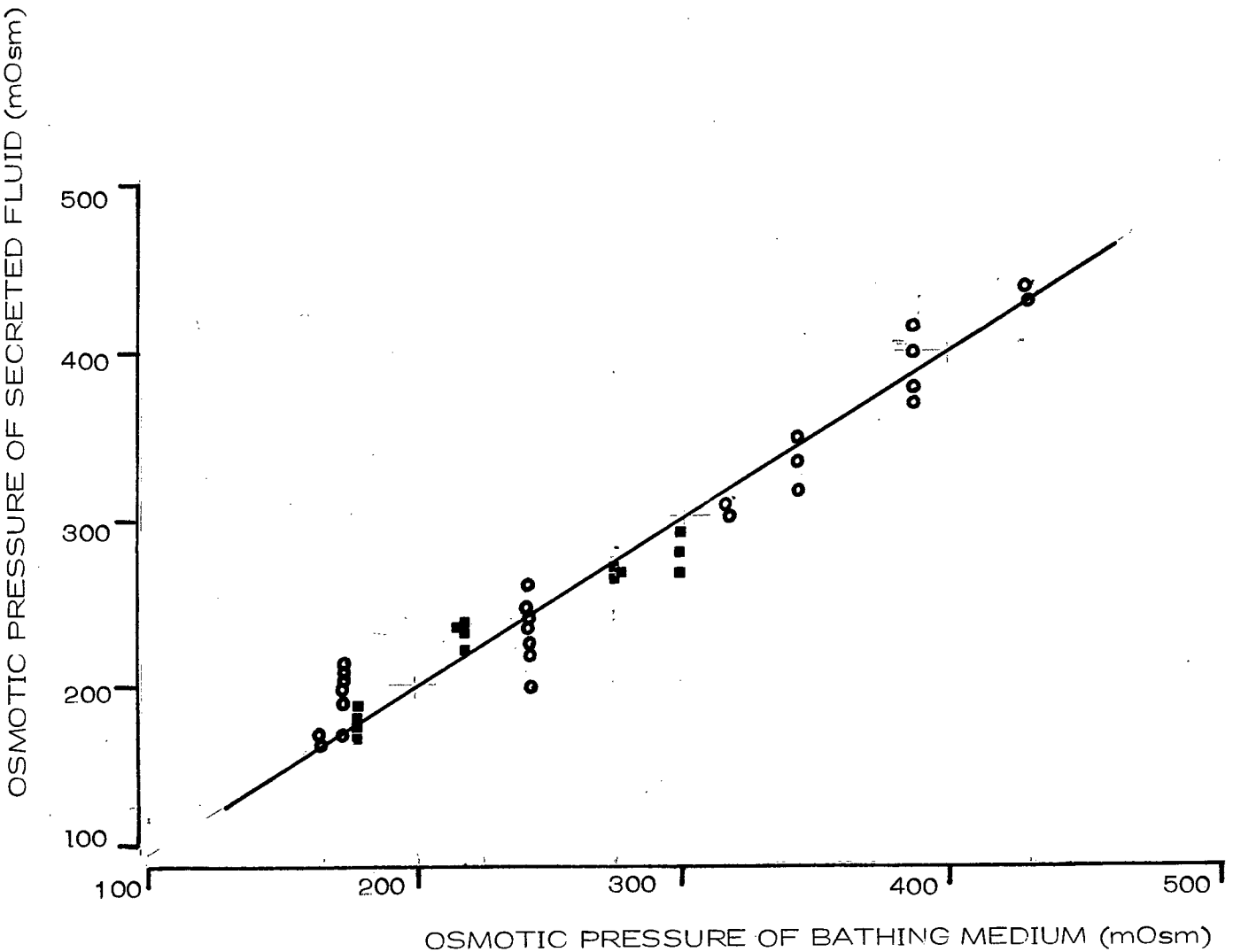

OSMOTIC PRESSURE OF SECRETED FLUID (mOsm)
500
400
300
200
100
100
200
300
400
500
OSMOTIC PRESSURE OF BATHING MEDIUM (mOsm)

TABLE II. The composition of the secreted fluid[1]

	mean	±	S.E.	(n)
Na^+ (mM)	60	±	5	(11)
K^+ (mM)	61	±	2	(26)
Cl^- (mM)	88	±	2	(8)
Mg^{++} (mM)	0.55	±	0.04	(8)
Osmotic pressure (mOsm)	177	±	2	(4)
pH	approximately 8.0			

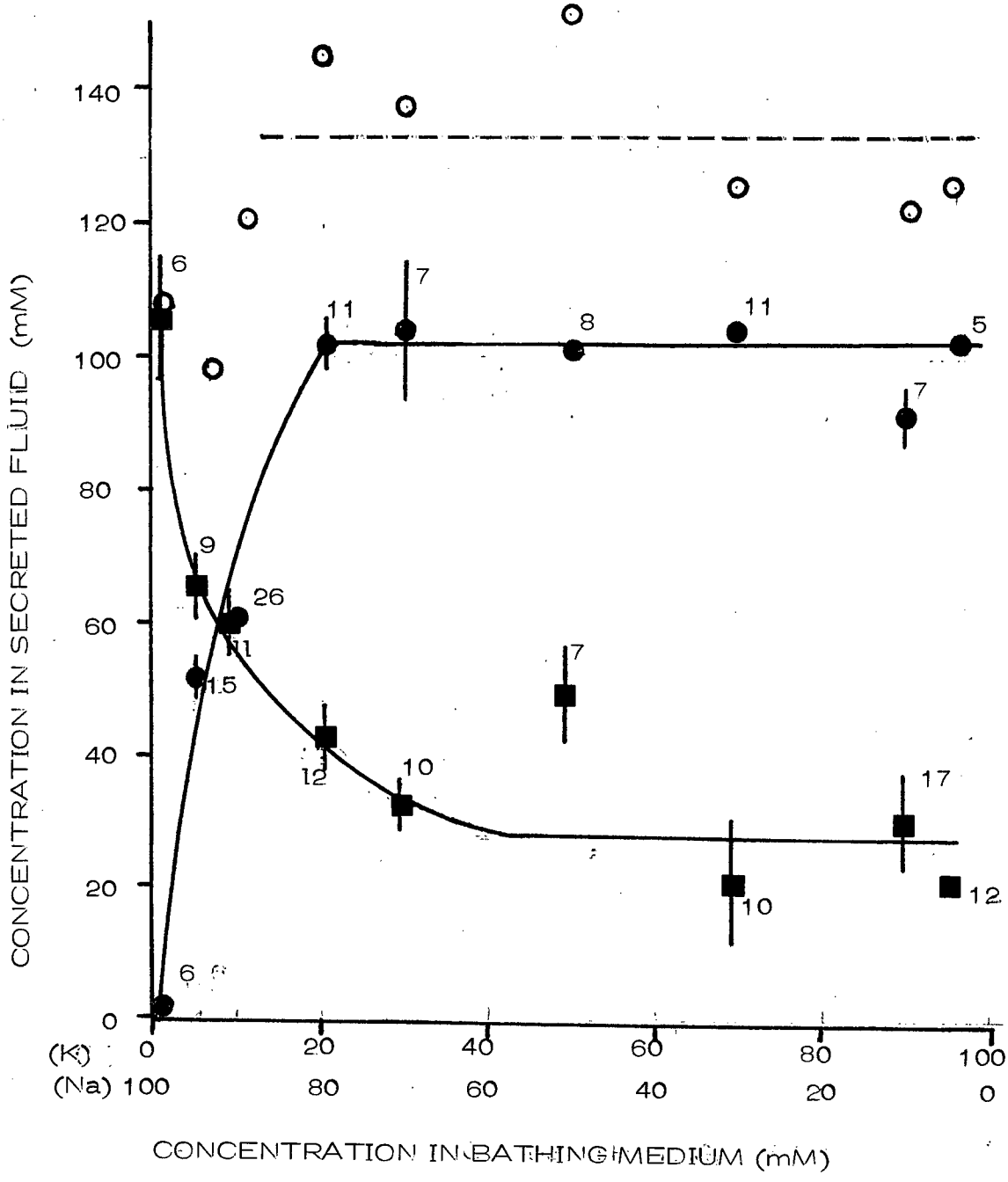
[1] Tubules were maintained in Saline B in the presence of 2.5×10^{-4}M cAMP.

The sum of sodium plus potassium in these media was maintained at 101 $\pm$ 8 mM (n=8). Different groups of tubules were isolated into each medium and volumes of fluid of at least 100 nl were collected for sodium analysis from each tubule. Pooling of samples from two to three tubules was necessary to obtain adequate volumes for analysis for some tubules. Figure 10 shows that in 0 and 5 mM K saline, the concentration of sodium in secreted fluid was greater than that of potassium. In 10 mM potassium saline, the sodium and potassium concentrations were equal, while at higher potassium concentrations, the tubules concentrated potassium preferentially. The sum of the mean sodium and potassium concentrations in fluid produced by tubules in each saline was relatively constant (Figure 10).

Inspection of mean sodium concentrations in secreted fluid produced by tubules bathed in 20 mM and higher potassium salines shows that these values do not decline as rapidly towards zero as they do in other insects, but appear to stabilize at about 30 mM. Sodium concentration in secreted fluid was elevated in fluid secreted by tubules bathed by salines in the range 5 mM to 30 mM Na. Because there was some rise in sodium concentration in secreted fluid with time as rate of secretion dropped (Figure 5), and because only low rates of secretion were obtained for tubules sampled in salines with greater than 20 mM K, it was suggested that the higher than expected sodium concentrations could be related to the low rates of secretion by tubules in these experiments. Because rate of secretion as well as

Fig. 10

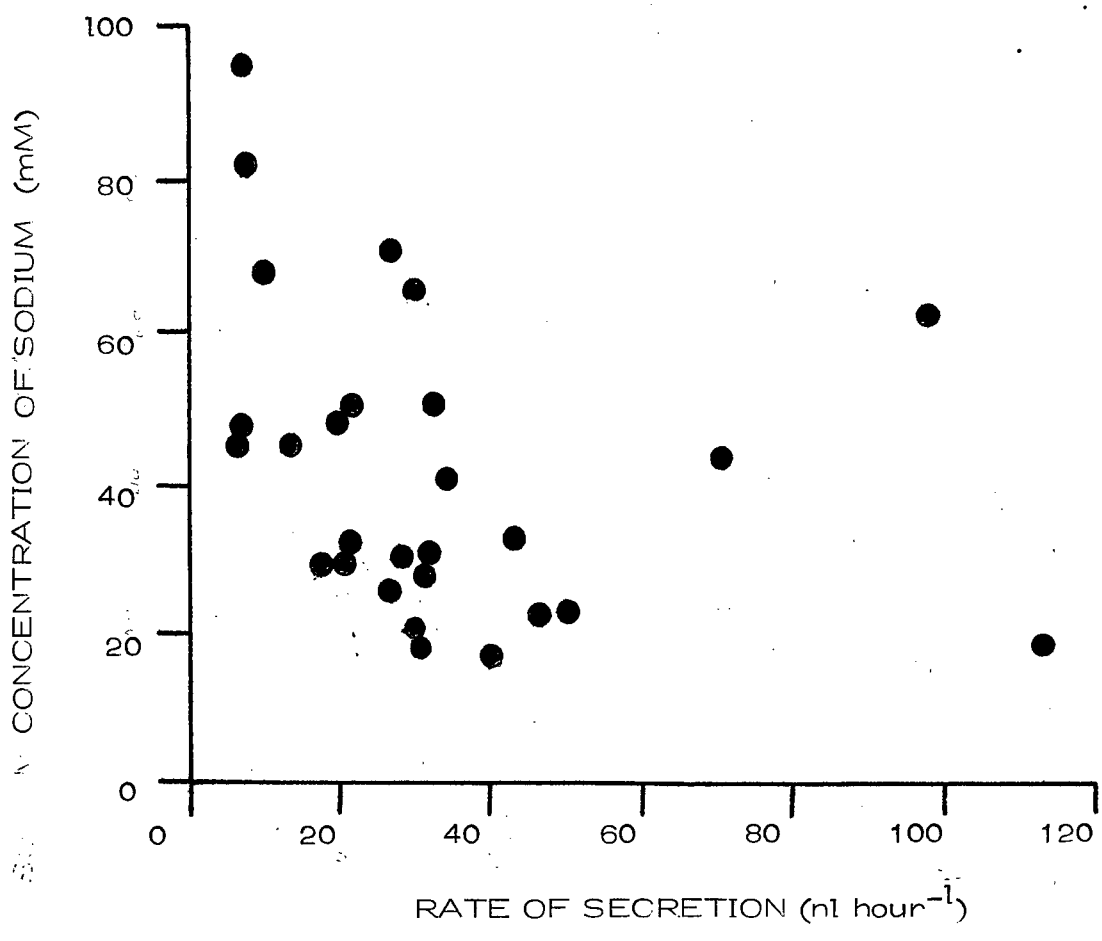
Composition of secreted fluid when bathing medium contains different sodium-potassium ratios. Fluid from different tubules was measured in each medium and potassium (●) or sodium (■) concentration determined in each. The sum of mean potassium and sodium values (O) in each medium remains constant as indicated by the dashed line. The curves were fitted by eye. Bars indicate plus or minus one standard error unless it is smaller than the dimensions of the point.


CONCENTRATION IN SECRETED FLUID (mM)
140
120
100
80
60
40
20
0
6
11
7
8
11
5
7
9
26
11
15
7
12
10
17
10
12
6
(K) 0 20 40 60 80 100
(Na) 100 80 60 40 20 0
CONCENTRATION IN BATHING MEDIUM (mM)

sodium concentration was determined for tubules secreting in 30, 50, and 70 mM K, it was possible to plot a scatter diagram of sodium concentration as a function of secretory rate (Figure 11). Correlation analysis of these data shows that low rate of secretion was correlated with higher sodium concentration in the secreted fluid ($p < 0.05$). This was particularly evident at low secretion rates. Variability in the rate of secretion could thus contribute to the variability observed in sodium concentrations of secreted fluid. In contrast, potassium concentration of fluid secreted by tubules bathed in 30, 50, and 70 mM K bathing solutions was found to be independent of rate of secretion.

Fig. 11

Scatter diagram of sodium concentration in tubular secretion as a function of rate of secretion in 30, 50 and 70 mM potassium salines.


CONCENTRATION OF SODIUM (mM)
100
80
60
40
20
0
0
20
40
60
80
100
120
RATE OF SECRETION (nl hour^{-1})

B. DISCUSSION

Preliminary studies of the Malpighian tubule function of C. bifida confirm that fluid secretion in this insect is governed by the same in vitro factors found to be important in other insects like Calliphora erythrocephala, Schistocerca gregaria, Carausius morosus and Pieris brassicae (Berridge, 1968; Maddrell and Klunsuwan, 1973; Pilcher, 1970; Nicholson, 1976). In these insects the rate of urine formation is inversely proportional to the osmotic pressure of the bathing medium, is increased by cAMP, and is proportional to potassium concentration over at least part of the range studied. As expected, the Malpighian tubule secretion of C. bifida is isosmotic to the bathing medium over a wide range of osmotic pressures, and as predicted, sodium is an important component of the secreted fluid at haemolymph-like potassium concentrations. The Malpighian tubules of C. bifida do not appear to tolerate high potassium concentrations in the bathing medium. The pH of the secretion was high.

No information was obtained about in vivo regulation of secretion, but there are indications that it is hormonally mediated.

1. Rates 'in Vitro'

Without measurements of in vivo rates of secretion by the Malpighian tubules of C. bifida, it is difficult to determine whether the rates of secretion and variations thereof observed in the in vitro preparation are physiologically meaningful. Lack of an essential

metabolite or stimulant, below normal osmotic pressures in Medium B, reduction of the sodium-potassium ratio in Medium B, and differential tolerance of tubules to such abnormal conditions could be responsible for some of the variations in secretion rate and the decline with time.

Furthermore, because for practical purposes corixids were maintained in very hypoosmotic dechlorinated tap water, it is impossible to say whether the secretory properties of the insects used in these studies are the same as insects in the field. However, C. bifida sometimes occurs naturally in waters of low salinity (Scudder 1969a,b) and can be reared in dechlorinated tap water (Scudder, pers. comm.). The mortality rate was not obviously higher than for animals maintained in lake water. In fact, highest secretory rates were often observed in the Malpighian tubules of animals that had recently had their water changed. Since animals were not used until several weeks after field collection, all animals should have been well acclimated (Scudder et al., 1972). Although it would be preferable to conduct studies of the effect of external salinity on in vivo rate of Malpighian tubule secretion with either freshly-collected corixids or corixids maintained in laboratory conditions carefully simulating field conditions, it was assumed that for the purposes of this study, the Malpighian tubules of C. bifida maintained in dechlorinated water were in a physiologically acceptable state.

The rate of secretion of the Malpighian tubules of C. bifida secreting in a bathing medium of haemolymph-like osmotic pressure without

stimulant are low compared to rates observed in other insects even when the smaller size of the tubules is taken into consideration (Table III). Only a small percentage of tubules dissected secrete in this medium. It is possible that the low secretory rates and lack of secretion by some tubules is owing to the inviability of the Malpighian tubules in this preparation. The fact that secreting tubules had a healthy appearance and could maintain a constant rate of secretion for long periods of time probably indicates, however, that medium A lacked a necessary stimulant.

2. Effect of Osmotic Pressure

The Malpighian tubules of C. bifida secrete faster when the osmotic pressure of the bathing medium is reduced, as do those of other insects (Maddrell, 1969a; Gee, 1976; Maddrell and Klunsuwan, 1973; Berridge, 1968). In vivo variations in haemolymph osmotic pressure could alter tubule secretory rates, although it is improbable that corixid haemolymph is ever diluted to as low as 175 mOsm. Reducing osmotic pressure by reducing total NaCl content in Saline B changed the Na/K ratio. The actual haemolymph activity of sodium in C. bifida may not be as high as the measured concentration of 145 mM owing to binding (Treherne, 1975), thus the sodium content of Saline B, 90 mM, may not be unrealistically low.

3. Effect of cAMP

The hormonal mechanisms supporting Malpighian tubule secretion

TABLE III. Rate of secretion of tubules of C. bifida in comparison to rates in other insects[1]

Animal	Tubule surface area[2] (mm^2)	Mean rate ($nl\ min^{-1}$)	Rate per unit area ($nl\ min^{-1}\ mm^{-2}$)
Calliphora erythrocephala	4	13	3.3
Schistocerca gregaria	4	2	0.5
Manduca sexta	18	18	1.0
Rhodnius prolixus	7	60	5.1
Triatoma phyllosoma	17	60	3.5
Cenocorixa bifida			
Medium A (no cAMP)	1.5	0.45	0.3
Medium B (cAMP, initial rate)	1.5	3.5	2.3

[1] Adapted from original table by Maddrell and Gardiner (1974). Estimated values for C. bifida have been added to the original table.

[2] Differing tubule sizes were taken into account by calculating mean rate per unit surface area.

in aquatic insects have not yet been studied. C. bifida, which lives in waters hypoosmotic to its haemolymph (Scudder et al., 1972) faces osmoregulatory problems different from those of terrestrial insects. Although it is impossible to speculate about the magnitude of secretion rates in vivo, it can be hypothesized that the Malpighian tubules of C. bifida produce urine continuously to eliminate nitrogenous excretory products (Staddon, 1963) and to prevent haemolymph dilution owing to water intake through gut and cuticle. Isotonic Malpighian tubule secretion probably occurs at a constant rate in the intact animal at a rate proportional to this inflow; haemolymph volume has been shown to be closely regulated (Scudder et al., 1972). Variations in external salinity may influence the rate of isosmotic secretion indirectly through release of a neurohormone (Jarial and Scudder, 1971). In terrestrial insects, by contrast, rates of secretion of Malpighian tubules are highly sporadic, occurring at high rates only after drinking or a blood meal (Maddrell, 1969; Wall, 1970).

The accelatory response to cAMP of Malpighian tubules of C. bifida indicates that hormonal stimulation of secretion may be important in the intact animal as postulated by Jarial and Scudder (1971). Cyclic AMP has been implicated as a second messenger in several other insects eg. Glossina morsitans (Gee, 1976), Pieris brassicae (Nicholson, 1976), Rhodnius prolixus and Carausius morosus (Maddrell, 1971a) and Schistocerca gregaria (Maddrell and Klunsuwan, 1973). It has been shown to enhance secretion by Calliphora erythrocephala salivary

glands by raising intracellular calcium which in turn reduces the permeability of the basal membrane to anions (Berridge and Prince, 1971, 1972; Berridge et al., 1975, 1976). Since the apical cation pump of Malpighian tubules is unable to function without a permeant anion in the bathing medium (Berridge, 1968; Maddrell, 1969), it is thought that the rate of secretion of non-stimulated tubules is limited in part by the rate of diffusion of anions into the cell (Berridge et al., 1976).

Rate of secretion of Malpighian tubules of C. bifida declined after initial cAMP stimulation. A decline in secretion rate of Malpighian tubules with time despite renewal of media was observed by Taylor (1973) in Carausius morosus. Taylor (1973) found that this decline occurred whether the tubules were bathed in serum, bathing medium, or a mixture of the two. He attributed the decline to a change in the secretory properties of the tubules with time, postulating that the initial rates were artifically high due to handling, and that rate gradually fell to approximately the in vivo rate. Because in vivo rates of secretion by tubules of C. bifida are not known it is impossible to determine whether initial rates after cAMP stimulation are artifically high or not. Cyclic AMP may fail to maintain secretion at in vivo rates if a) the saline lacks an essential metabolite, b) cAMP is degraded by phosphodiesterase in the tubule cells, or c) cyclic AMP does not produce all the changes necessary to maintain secretion. Cyclic AMP degradation could be prevented by incorporating theophylline into the saline. The mechanism of hormonal stimulation of tubules in C. bifida has not been studied, but

it is possible that exposure to a natural hormone is necessary before an inhibition of secretion is removed.

The large variations in secretory rates of Malpighian tubules observed in these experiments could be owing to varying conditions in the animal before dissection. Factors known to cause variation in rates of Malpighian tubule secretion are age of donor (Nicholson, 1976), stage in oviposition cycle (Berridge, 1968), diet, and whether the animal is fed or fasting (Pilcher, 1970; Wigglesworth, 1972), drinking or dehydrated (Wall, 1970; Taylor, 1973). Season (Farquharson, 1974a) and stage in 'excretory phase' (Berridge, 1966) also influence secretory rates. Because limited numbers of field-collected animals were available, no attempt was made to control sex of animal, morph or lake of origin: as many tubules as possible from each animal were used in the present experiments. All these factors could contribute to experimental variability in the hormonal condition of the corixids before dissection. The corixids used in these experiments were all overwintering adults of the same generation (Jansson and Scudder, 1974). Overwintering adults do not feed in nature (Scudder, pers. comm.) but metabolize their fat body as an energy source. Perhaps first or second generation animals with greater metabolic rates would have Malpighian tubules capable of secreting at higher rates.

4. The Effect of Potassium

The lack of proportionality between rate of secretion and potassium

concentrations above 10 or 20 mM K was unexpected considering the widespread importance of this proportionality in other insects. The relation can probably be rationalized when the fact that C. bifida is a predator with a high sodium, low potassium haemolymph regime typical of a carnivorous insect (Jeuniaux, 1970) is considered. Scudder et al.,(1972) determined that haemolymph potassium concentration is maintained at approximately 5 to 15 mM, while the sodium concentration is about 145 mM. Thus the lowest potassium concentration that produces maximal stimulation of fluid secretion is close to the maximal haemolymph concentration. Such a relation holds true for Malpighian tubules of Pieris brassicae (Nicholson, 1976) where the maximum rate is achieved at the haemolymph concentration of 40 mM potassium, and for tubules of Carausius morosus bathed in saline where the maximal rate is achieved at 18 mM (Pilcher, 1970). In Carausius morosus serum, however, the rate of secretion is proportional to the potassium concentration over a large range of potassium concentrations (0 to 100 mM). In the Malpighian tubules of Calliphora erythrocephala, the rate of secretion is proportional to potassium over the range 0 to 150 mM while in Rhodnius prolixus, the rate is completely independant of the potassium concentration (Berridge, 1968; Maddrell 1969a). It may be that the rate of secretion is more obviously affected by potassium in phytophagous insects where potassium is an important constituent of the haemolymph (Jeuniaux, 1970).

Changing the concentration of potassium in the bathing medium from

10 mM to 90 mM potassium had little sustained stimulatory effect on the Malpighian tubules of C. bifida whether cyclic AMP was present in the bathing medium or not (Figure 7). Almost all tubules maintained in saline lacking cAMP increased in rate in the half hour following 90 mM K addition although the rate was never significantly different from control rates. Berridge et al. (1975, 1976) found a marked difference in the effect of potassium between salines with and without stimulant. When 5-hydroxytryptamine was absent, the rate of secretion was enhanced by potassium concentrations up to 90 mM, whereas in the presence of stimulant, rate of secretion was proportional to potassium concentration over the range 0 to 5 mM only. It would be interesting to learn more about the proportionality between rate of secretion and potassium concentrations in Malpighian tubules of C. bifida in media without cAMP. Perhaps tubules timulated by cAMP are already secreting at maximal rates which can only be slightly increased when potassium concentration in the bathing medium is raised (Prince, pers. comm.).

Figure 8 suggests that 90 mM potassium may be inhibitory to tubules of C. bifida during long term exposure. Tubules isolated in 90 mM K followed the same pattern of decline in secretory rates after cAMP as those in 10 mM K, but the average rates were much less than those in 1o mM K after 1.5 hours. Although Figure 7 does not show that potassium has any average inhibitory effect within one hour, many individual tubules showed irreversible inhibition. Ninety mM potassium

is probably toxic on a long term basis.

Inhibition of Malpighian tubule secretion by elevation of potassium concentration in the bathing medium has not previously been demonstrated in any insect; 90 mM is a concentration routinely used in Malpighian tubule experiments (Maddrell, 1969a; Gee, 1976; Maddrell and Klunsuwan, 1973; Berridge, 1968). The tubules of Calliphora erythrocephala are capable of producing a secretion hypertonic in potassium even when the potassium concentration in the bathing medium is as high as 600 mM (Berridge, 1968). In the highly permeable Malpighian tubules of the pill millipede, Glomeris marginata (Villers), however, high potassium may be inhibitory (Farquharson, 1974b). Concentrations of potassium higher than 90 mM have a toxic effect on the salivary glands of Calliphora erythrocephala, although exposure to 120 and 150 mM causes a short-lived pulse in secretory rate (Berridge et al., 1975).

Without additional data it is impossible to explain the mechanism of toxicity of potassium to the Malpighian tubules of C. bifida. It is believed that potassium increases can alter basal membrane permeability to anions and lead to intracellular increases in potassium as well as calcium (Berridge et al., 1975; 1976). All tubules immersed in 90 mM K initially showed great distension of the tubule and tubule cell coupled with increased cell fragility. The varied responses in secretory rate to elevated potassium may be due to a varied ability to prevent or tolerate increases in cell volume.

It is doubtful whether the high potassium effect has any physiological significance. Concentrations above 15 mM probably never occur in the haemolymph of C. bifida; the mean potassium concentration in the haemolymph remained below this value even when potassium in the external medium was raised to abnormally high values (Scudder et al., 1972). Furthermore, the effect of high K is not distinguishable from that of low sodium in any of the above experiments (the ionic balance was maintained with NaCl).

6. Composition of the Secreted Fluid---pH

Alkaline Malpighian tubule secretion has been found in other insects eg. Carausius morosus (Ramsay, 1956) and Pieris brassicae (Nicholson, 1976). Malpighian tubules of Rhodnius prolixus produce alkaline fluid immediately after feeding, but later the pH drops gradually (Wigglesworth, 1931). The final acidic secretion of Rhodnius prolixus is thought to be owing to resorption of potassium and bicarbonate in the proximal region of the tubule. Wessing and Eichelberg (1975) suggest that carbonic anhydrase activity may be responsible for the production of alkalinity in secretion by the initial segment of the Malpighian tubules of Drosophila melanogaster Meig. via a dehydration of the contents of cytopemptic vesicles. Carbonic anhydrase was localized cytochemically in the Malpighian tubules of D. melanogaster (Wessing and Eichelberg, 1975). The carbonic anhydrase inhibitor acetazolamide, however, does not inhibit fluid secretion in the Malpighian tubules of Rhodnius prolixus or Calliphora erythrocephala (Maddrell, 1969 ; Berridge, 1968) and carbonic

anhydrase is not thought to be important in invertebrate renal function (Maren, 1967).

Several possible explanations could account for the high pH in the Malpighian tubule fluid of C. bifida, but the dependence of alkaline production on water transport must be taken into account. The alkalinity may be explained by the passive movement of HCO_3^- or $HPO_4^=$ into the secreted fluid as the permeant anion in addition to chloride. When the rate of secretion is low, the lumen contents equilibrate with the bathing medium, and the pH of both fluids is equal. When the rate of secretion is high, fast rates of fluid flow towards the lumen prevent HCO_3^- backflux into the bathing medium by solute entrainment. It is also possible that the high pH of the secreted fluid is due to (1) a non-electrically neutral apical K/H exchange or (2) an active lumen-directed bicarbonate pump.

Whatever the mechanism is that is responsible for increasing the pH of tubular secretion compared to the bathing medium, the phenomenon may be of great importance if it occurs in vivo. Since C. bifida occurs in saline waters of high bicarbonate concentrations and hence high alkalinity (Topping, 1969) the elimination of excess bicarbonate in the Malpighian tubules could help to regulate haemolymph pH.

6. Composition of Secretion ---Na, K, Mg

Sodium and potassium concentrations in the Malpighian tubule fluid of C. bifida were similiar to expected concentrations as predicted by studies on other insects (Maddrell, 1971a; Pilcher, 1970; Berridge

1968; Maddrell and Klunsuwan, 1973; Nicholson, 1976; Phillips and Maddrell (1974) (Figure 10). Over a range of sodium and potassium bathing medium concentrations, potassium concentrations in the secreted fluid were elevated while those of sodium were depressed compared to the bathing medium. This pattern is also displayed by Malpighian tubules of Calliphora erythrocephala (Berridge, 1968; Maddrell, 1971a), Schistocerca gregaria (Maddrell and Klunsuwan, 1973) and Pieris brassicae (Nicholson, 1976). There are two observations of significance in Figure 10. At high potassium concentrations, the sodium content of secretion did not approach zero as rapidly as expected by comparison to data from other insects. (2) Over the normal haemolymph range of potassium concentrations (5 to 15 mM K) the sodium concentration in the secretion was approximately equal to that of the potassium.

The correlation of low rates of secretion in the fluid sampled at low sodium-potassium bathing medium ratios with high sodium concentrations in the secreted fluid (Figure 11) may indicate that the stabilization of sodium at 30 mM would not have been observed if tubules with higher rates of secretion had been sampled. Pilcher (1970) also found that sodium content of urine tended to increase when rates of secretion fell. These observations indicate that sodium movements across the epithelium, whether active or passive are constant despite water flow, whereas those of potassium are linked to water flow. In the range 0 to 30 mM Na, the concentration of sodium in the tubule fluid was

greater than that of the bathing medium. If the lumen is positive with respect to the bathing medium as in most other insects (Maddrell, 1971a,b; 1972) then a part of the sodium movement must be because of active pumping.

In media of haemolymph-like potassium and sodium ratios the sodium and potassium concentrations of the secreted fluid are equal. In the saline lakes that are inhabited by C. bifida, sodium is much more plentiful than potassium (Topping and Scudder, 1977). Although the Malpighian tubules of C. bifida do not concentrate sodium except at low bathing medium potassium concentrations, they could be important in eliminating a portion of the excess sodium. Insects with a high availability of sodium in the haemolymph probably have a part of the fluid movement linked to the pumping of sodium by the apical cation pump (Maddrell, 1976 as cited by Nicholson, 1976).

The Malpighian tubules of C. bifida do not concentrate magnesium (Table II) as do those of Aedes campestris (Phillips and Maddrell, 1974). C. bifida could regulate haemolymph magnesium by other means such as, for example, storage excretion in the magnesium-positive secretory granules (Jarial and Scudder, 1970).

C. CONCLUSIONS

The results of preliminary experiments on the Malpighian tubules of *C. bifida* indicate that fluid secretion operates according to proposed models of insect excretion. Although there is some question as to the similiarity of the *in vitro* preparation used in these experiments to haemolymph conditions, the tubules retained a healthy appearance and were able to secrete for long periods of time, and thus studies of fluid composition were probably valid. The high variability in secretory rates and the decline of secretory rates with time could be explained by the failure to exactly duplicate hormonal conditions maintaining secretion *in vivo*. Hormonal regulation of excretion has not yet been studied in an aquatic insect; it would be interesting to determine the hormonal control mechanism of the Malpighian tubules of *C. bifida*.

The toxicity of high potassium concentrations casts doubt on the lability of tubules of *C. bifida* to function over a wide range of sodium-potassium ratios in contrast to those of other insects. The composition of the isotonic secreted fluid suggests that although the tubules of *C. bifida* cannot aid in osmotic regulation, they may be important in ion regulation of haemolymph by eliminating excess bicarbonate and sodium.

The experiments also highlight the importance of sodium in Malpighian tubule secretion. Further studies on Malpighian tubule function of insects with alternate haemolymph regimes and environments

may give support to the thought that a common mechanism underlies fluid secretion in all insect Malpighian tubules.

One of the most interesting facts about the Malpighian tubules of C. bifida is their morphological division into four distinct parts. Data gathered on the function of the individual segments clarifies, but also complicates, the patterns revealed by studies on whole Malpighian tubules.

IV. SECRETORY PROPERTIES OF THE SEGMENTS

A. RESULTS

1. Rate of Secretion of Segments

Segments II, III and IV of the Malpighian tubules of C. bifida all became distended with fluid when ligated at both ends, and secreted when bathed in Medium B. Segment I did not swell when treated in the same manner. Because of the length of tubule required for ligation and drawing into paraffin oil, the whole of each segment was not immersed. In addition, the average total length of each segment varied as much as 0.3 mm. Without correction for length immersed average rates of secretion of Segments II, III and IV did not differ significantly, but when the rate of secretion per unit tubule length was calculated, it became clear that it was significantly greater in the short distal segment than in either of Segments II or III (Table IV). To determine the relative contributions of Segments II, III and IV to the overall secretion, each value of rate per unit length was multiplied by the average length of each segment. Table IV shows that in the intact tubule all segments did not make an equal fluid contribution to the final Malpighian tubule secretion. Segment II contributed the least fluid while Segment IV contributed the most.

Average rates of secretion were also measured for the whole tubule, Segments III and IV and finally Segment IV in sequence (sequential method). In 10 mM potassium saline, there was a constant reduction

TABLE IV. Rate of secretion of segments isolated in 10 mM K bathing medium

Segment	Rate of secretion (nl $hour^{-1}$) mean ± S.E. (n)	Rate per unit length (nl $hour^{-1} mm^{-1}$) mean ± S.E. (n)	Rate in intact tubule[1] (nl $hour^{-1}$) mean ± S.E.
II	38 ± 5 (25)	22 ± 3 (24)	49 ± 6
III	34 ± 5 (25)	18 ± 3 (24)	67 ± 12
IV	38 ± 6 (25)	69 ± 9 (24)	90 ± 11

[1] Rate of secretion corrected for length immersed. The rate of secretion per unit length was multriplied by average segment length.

in secretory rate at each step; no more and no less than can be accounted for by decline of rate with time and the reduction of the secreting tubule length. At no time was secretory rate of Segment IV alone greater than from III and IV together or the secretory rate of III and IV greater than that from the whole tubule.

Although no measurements of *in vivo* rates of secretion of the tubules of *C. bifida* have been made in this study, it is assumed that the secretory properties of all segments in each tubule retain the same relations *in vitro* as *in vivo*.

Secretory granules or hyaline globules were most numerous in the fluid produced by Segment III.

2. Osmotic Pressure of Segment Fluid

All isolated ligated segments in Saline B produce fluid that is isosmotic to the bathing medium within the error of the technique (5%) (Table V).

3. Potassium Concentration of Segment Fluid

Isolated Segment III usually produced fluid significantly higher in potassium concentration than either of Segments II or IV alone (Table V). Sampling of fluid sequentially through the three segments confirmed that secretion of III plus IV had a higher potassium concentration than from IV alone. The addition of fluid from Segment II, however, failed to reduce significantly the potassium concentration of whole tubule fluid as compared to that from III and IV only (Table V),

TABLE V. Composition of segmental secretion

Segment	Osmotic Pressure (mOsm) mean ± S.E. (n)	K Concentration (mM) mean ± S.E. (n)	Na Concentration (mM) mean ± S.E. (n)	Cl Concentration (mM) mean ± S.E. (n)	pH mean ± S.E.
II	161 ± 2 (12)	67 ± 4 (24)	47 ± 3 (18)	76 ± 2 (8)	8.6 ± 0.1 (8)
III	166 ± 1 (12)	83 ± 15 (23)	56 ± 7 (17)	83 ± 3 (8)	6.9 ± 0.1 (8)
IV	169 ± 2 (12)	51 ± 4 (9)	61 ± 6 (17)	100 ± 3 (8)	7.1 ± 0.1 (8)
II + III + IV	167 ± 1 (8)	79 ± 3 (15)	32 ± 3 (13)	---	---
III + IV	168 ± 2 (8)	73 ± 5 (14)	40 ± 6 (13)	---	---
IV	171 ± 1 (8)	51 ± 5 (13)	60 ± 6 (11)	---	---
Bathing Medium	165	10	90	100	7.1

A rough calculation of the rate of potassium secretion per unit length of each segment shows that the rate of potassium production in Segment IV was higher than in the other two segments (Table VI). This corresponds with the high rate of fluid secretion in this distal segment.

4. Sodium Concentrations of Segment Fluid

The sodium concentrations in fluid secreted by the three segments were also unequal (Table V). Fluid from Segment IV had a significantly higher sodium concentration than fluid from Segment II. When the sodium concentration in the tubule fluid was analyzed from sequential samples, it was apparent that as the fluid passed downstream, the concentration of sodium was reduced. The rate of sodium secretion per unit tubule length was higher in the distal segment (IV) than in the other two segments (Table VI).

5. Chloride Concentration of Segmental Fluid

The concentrations of chloride in the fluid produced by isolated tubule segments are shown in Table V. Like the sodium concentration, the chloride concentration was highest in Segment IV fluid and lowest in Segment II fluid. The rate of chloride secretion, also like the rate of sodium and potassium secretion, was highest in Segment IV (Table VI).

TABLE VI. Rate of ionic secretion [1]

Segment	Rate of K secretion per unit length (picomoles $hour^{-1}mm^{-1}$) mean ± S.E.	Rate of Na secretion per unit length (picomoles $hour^{-1}mm^{-1}$) mean ± S.E.	Rate of Cl secretion per unit length (picomoles $hour^{-1}mm^{-1}$) mean ± S.E.
II	1.5 ± 0.2	1.0 ± 0.2	1.7 ± 0.2
III	1.5 ± 0.4	1.0 ± 0.4	1.5 ± 0.4
IV	3.5 ± 0.8	4.2 ± 0.9	6.9 ± 1.1

[1] Values calculated from data in Tables IV and V.

6. The pH of Segmental Fluid

When the pH of the fluid produced by Segments II, III and IV was measured by comparison to buffers containing indicator, it was clear that fluid produced by Segment II was very alkaline, while that of Segment III and IV had a pH close to that of the bathing solution. The pH of Segment III was on average slightly more acidic than that of Segment IV (Table V).

The pH of secretion by isolated segments was analyzed before and after cAMP stimulation. Phenol red changed from orange to pink in Segment II only.

7. Segmental Secretion in Elevated Potassium Media

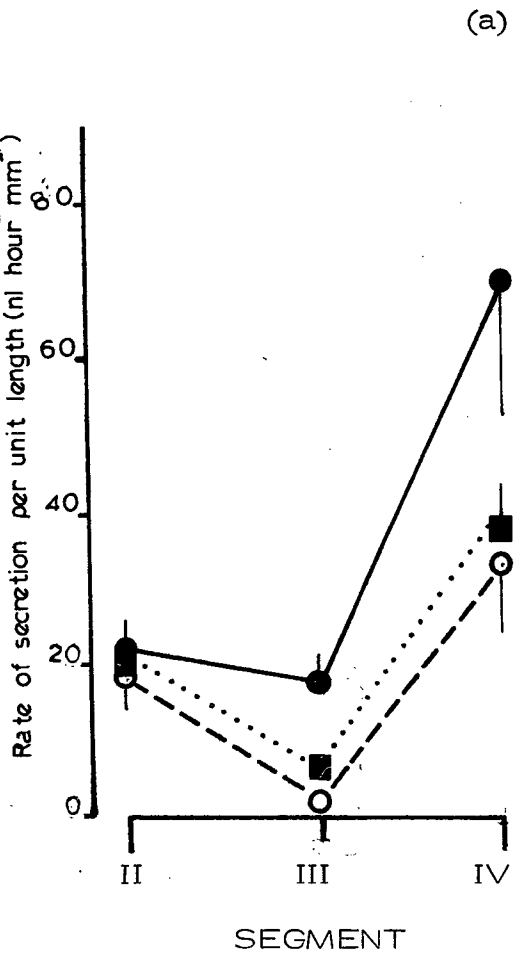
To determine if there was any differential change in segment function as the sodium-potassium ratio of the bathing medium was reduced, some of the preceding measurements were repeated in media with elevated potassium concentrations. The following results show that a change of the sodium-potassium did indeed change the secretory relations of Segments II, III and IV.

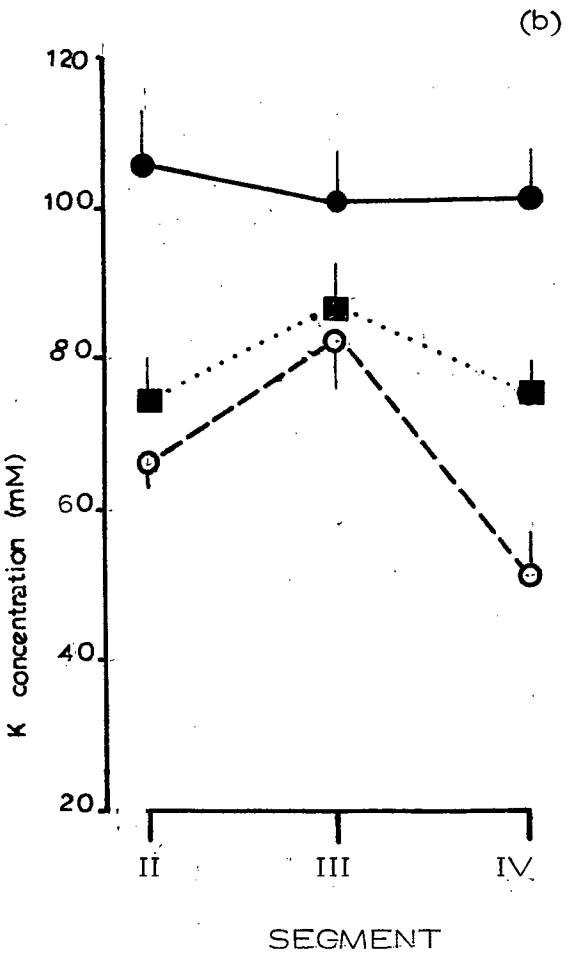
(a) Rates of Secretion.

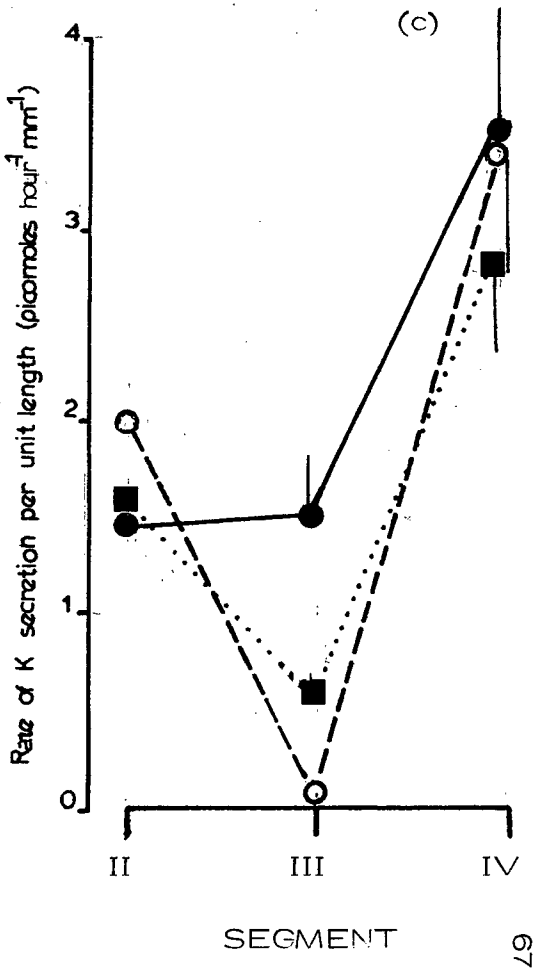
The rate of secretion per unit length of Segment III became increasingly reduced compared to the rate of secretion per unit length of Segments II and IV in 20 mM K and 90 mM K salines (Figure 12a). Although it is not rigorous to compare rate measurements of different tubules, there appeared to be a constant reduction of the rate of secretion of Segment III as the potassium concentration of the bathing

Fig. 12

The effects of increased potassium concentrations on rate of fluid secretion and fluid composition of tubule segments. (a) Rate of fluid secretion per unit segment length in 10 mM K (●), 20 mM K (■) and 90 mM K (○) salines. Rate of secretion per unit length of Segment III is depressed by high potassium. Sample sizes were: 10 mM K (all segments) = 24; 20 mM K, n = 22; 90 mM K, n=20. (b) potassium concentration in segmental fluid in 10, 20, 90 mM K salines. The potassium concentrations in the segment are not significantly different in high potassium saline. Sample size at 10 mM K was 24 for all segments; at 20 mM K, n = 22 (II), 18 (III), 18 (IV); at 90 mM K, n = 22 (II), 6(III) and 6 (IV). (c) Rate of potassium secretion per unit length in all salines. Rate of potassium secretion of Segment III is reduced in high potassium salines. Means and errors are calculated from data in Figs. 12a and b. For all graphs, bars indicate either plus or minus one standard error unless it is less than the dimensions of the point.


(a)
Rate of secretion per unit length (nl hour^{-1} mm^{-1})
80
60
40
20
0
II
III
IV
SEGMENT


(b)
K concentration (mM)
120
100
80
60
40
20
II
III
IV
SEGMENT


(c)
Rate of K secretion per unit length (picomoles hour^{-1} mm^{-1})
4
3
2
1
0
II
III
IV
SEGMENT

medium increased. It is not certain whether decreasing secretion rates in Segment III were caused by damage to cells at high potassium; observation has shown that this segment can break easily unless handled with extreme care in 90 mM K saline.

To determine if the addition of high potassium saline caused any obvious differential inhibition or stimulation of the three segments, 90 mM K saline was added to the segments from three tubules previously isolated in 10 mM K saline. These preliminary experiments did not demonstrate any obvious differences between segments nor any preferential inhibition of the secretory rate of Segment III within one hour of treatment, but the sample size was too small for results to be conclusive. Hypotonic saline (175 mOsm) was used in these experiments.

(b) Osmotic pressure.

When segments of tubules of C. bifida were placed in 90 mM K medium, the fluid produced by Segment III usually had a 10% higher osmotic pressure than fluid produced by either Segments II or IV (Table VII). Because of the systematic errors involved in the measurement of osmotic pressure by freezing point depression (Taylor, 1971) such a small increase cannot be regarded as important.

(c) Potassium concentrations

The potassium concentration of Segment III fluid in 20 K and 90 K media was no longer significantly greater than that of Segments II and IV (Figure 12b). The rate of secretion of potassium per unit segment length was depressed in Segment III when tubules were

TABLE VII. Osmotic pressure of segment fluid from tubules in 90 mM K bathing medium.[1]

Segment	Osmotic Pressure (mOsm) mean ± S.E.(n)
II	181 ± 2 (20)
III	198 ± 4 (20)
IV	183 ± 3 (20)
II+III+IV	174 ± 4 (12)
III + IV	184 ± 3 (12)
IV	180 ± 3 (12)

[1] Tubules were sampled by both the segmental and sequential methods.

bathed in 90 mM K saline (Figure 12c)

8. The Transport of Dyes by the Individual Segments

The transport of a few dyes was followed to determine if tubule segments differed in their treatment of organics as well as of ions. It was observed very early in the course of the experiments that phenol red was concentrated preferentially by Segments II and III. No phenol red was ever observed in the secretion of Segment IV. Accordingly, a series of anionic sulphanone dyes were placed in 10 mM K bathing medium lacking phenol red, and tubule segments tested for their ability to transport them. A few anionic non-sulphanone dyes and two cationic dyes were also tested. Different tubules were used in each experiment, and secreted droplets observed over periods of five hours. A negative response was recorded if colour in the secretion could not be detected. The results of the experiment are shown in Table VIII. Neither of the basic dyes were transported.

All acidic dyes were transported only by Segment III, excepting phenol red which was also concentrated by Segment II. Cresol red and chlorophenol red, which differ from phenol red only in the presence of two methyl groups or chloride groups respectively, were not transported by Segment II.

TABLE VIII. Localization of dye transport.

Dye	SEGMENT		
	II	III	IV
ACIDIC			
Phenol Red	+	+	-
Cresol Red	-	+	-
Chlorophenol Red	-	+	-
Bromocresol Green	-	+	-
Indigo Carmine	-	+	-
Amaranth	-	+	-
Chromotrope 2R	-	+	-
BASIC			
Neutral Red	-	-	-
Methyl Green	-	-	-

+ = transport

- = no transport

B. DISCUSSION

1. Importance of Morphological Differentiation

(a) Types of morphological differentiation

The Malpighian tubules of many insects besides C. bifida have morphologically distinct regions. There is a great deal of diversity in terms of how much and what types of Malpighian tubule differentiation occurs. The Malpighian tubules of some Orthoptera and Diptera do not have morphologically distinct regions, eg. Melanoplus differentialis (Thomas), Dissosteira carolina (L.), Aedes aegypti (L.), Aedes campestris, Glossina morsitans (Beams et al., 1955; Tsubo and Brandt, 1962; Ramsay, 1951; Phillips and Maddrell, 1974; Gee, 1976). In these insects all cells of the Malpighian tubule are presumed to be concerned with the production of isosmotic Malpighian tubule fluid (Jarial and Scudder, 1970).

The Malpighian tubules of Hemiptera are characteristically divided into segments of distinct cell types. In Rhodnius prolixus the Malpighian tubules have two morphologically distinct segments (Wigglesworth and Salpeter, 1962) and like the tubules of C. bifida, those of Macrosteles fascifrons Stål have four segments (Smith and Littau, 1960). The tubules of some Cercopidae are regionally specialized for the release of products needed in tube-building (Marshall, 1968; 1973) while certain segments of the Malpighian tubules of some Cicadidae are associated with the filter chamber (Marshall and Cheung, 1974; Cheung and Marshall, 1973).

The lack of distinct segments, but the presence of more than one cell type occurs in the Malpighian tubules of Carausius morosus, (Taylor, 1971 a,b) and Calliphora erythrocephala (Berridge and Oschman, 1969).

The most common Malpighian tubule plan is that where in addition to division into segments, there are two or more cell types in some segments, eg. 'accessory cells', 'stellate cells' or 'mucocytes'. This is the situation in Grillus domesticus L. (Berkaloff, 1960), Gryllotalpa gryllotalpa Latr. (Lhonoré, 1971), Periplaneta americana L. (Wall et al., 1975), Drosophila melanogaster and Ephydra riparia (Fallén) (Wessing and Eichelberg, 1975) and Musca domestica L. (Sohal, 1974). Specialized leptophragma cells are present in the cryptonephridial segments of some Coleoptera (Grimstone et al., 1968).

(b) Functional interpretation of morphological differentiation.

On the basis of ultrastructure and/or histology it has been possible to suggest the potential functions of some Malpighian tubule segments and distinct cell types. For instance, the stellate cells of Calliphora erythrocephala are suggested to play a special role in ion transport (Berridge and Oschman, 1969); Orthopteroid mucocytes and the fibril-producing segments of Cercopid Malpighian tubules probably release mucopolysaccharides (Marshall, 1973; Berkaloff, 1960) and the distal segment of the inferior tubules of Carausius morosus is probably concerned with the storage of Ca for egg cases (Ramsay, 1955). Unfortunately, this sort of speculation is limited by difficulty in

interpreting ultrastructural features. The correlation of morphological segments with localizations of physiological function has been studied in depth in two insects only.

The Malpighian tubules of the blood-sucking insect Rhodnius prolixus have been examined both by the electron microscope and with physiological methods. The distal segment has a structure similiar to that of the basic Malpighian tubule cell plan (Maddrell, 1971a). Its apical border has many closed-packed microvilli containing mitochondria, the basal membrane is very complex and there are concentrically-laminated spherical granules in the cytoplasm (Wigglesworth and Salpeter, 1962). This upper segment produces fluid that is isosmotic to the bathing medium and elevated in potassium concentration, but reduced in sodium concentration, much like that of other insects studied (Maddrell 1969, 1971a). In contrast, the apical microvilli of the proximal segment are wider apart and the basal membrane is less complex (Wigglesworth and Salpeter, 1962). This segment has a completely different role in ion transport, lowering the osmotic pressure of the whole tubule fluid by reabsorption of potassium without water. It has reduced permeability to small organics and its function is hormonally controlled (Maddrell and Phillips, 1975b).

The ultrastructure of the Malpighian tubules of Calpodes ethlius Stoll has not been studied, but the tubule is clearly divisible into a cryptonephridial segment, a rectal lead, and illaeic plexus and a white region and yellow region (Irvine, 1969). Under 'normal'

bathing medium conditions of high potassium and low sodium concentrations, the proximal parts of the tubule are reabsorptive, but when the sodium concentration of the bathing medium is increased, the lower areas produce the bulk of the tubule fluid and sodium is concentrated proximally (Irvine, 1969).

Morphological division into segments is not always correlated with obvious differentiation of physiological function. Nicholson (1976) failed to find any differences in the osmotic pressure, sodium or potassium concentrations of the fluid produced by the three morphological regions of the Malpighian tubules of Pieris brassicae.

The three distal segments of the tubule in C. bifida are similiar in the structure of their apical and basal infoldings. Not surprisingly, the physiological variations among the segments, as discussed in the following sections, are only slight.

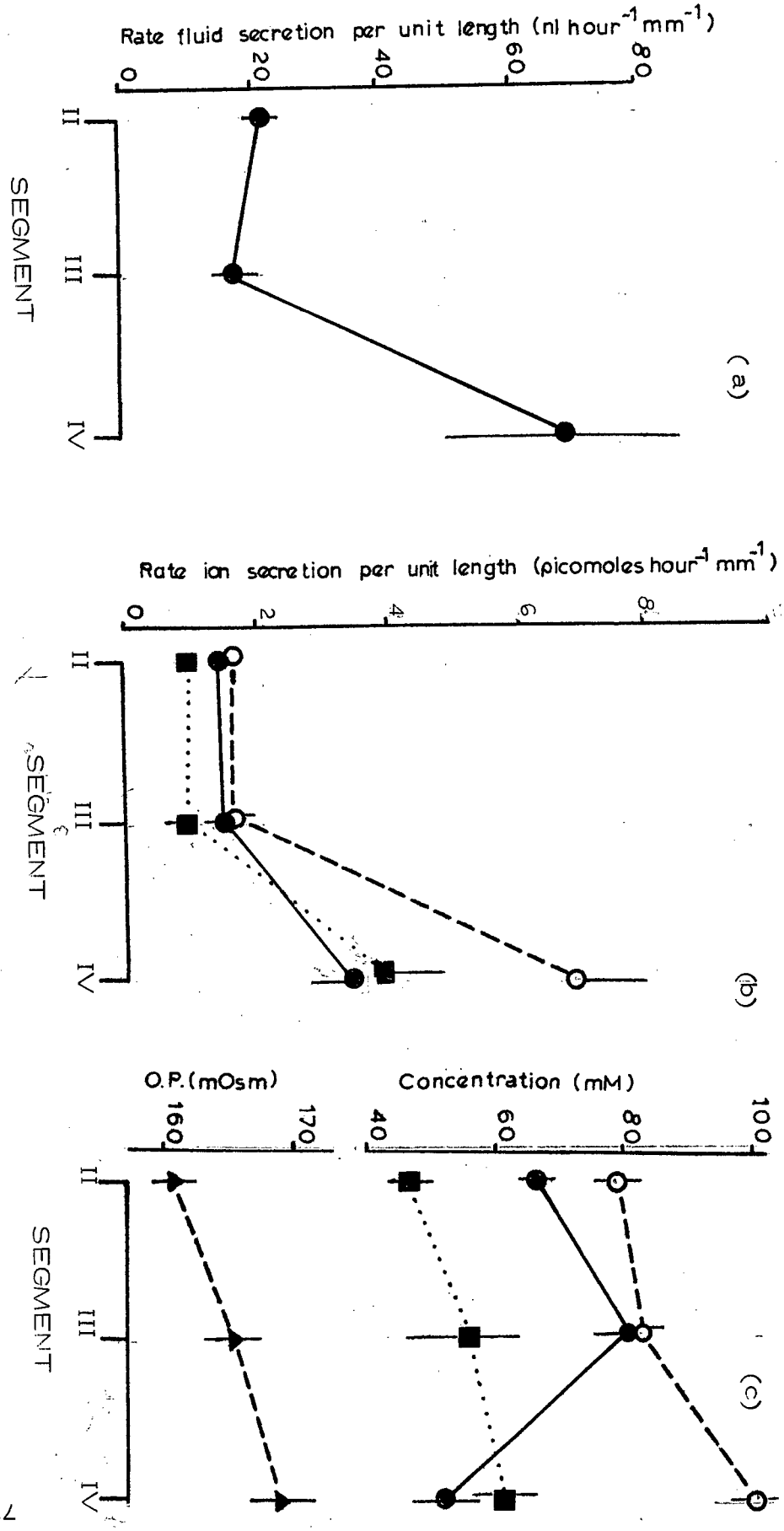
2. Basic Ion Transport Properties of the Segments of C. bifida Tubules

(a) Differences in rate of secretion.

Both the rate of fluid secretion per unit length and the rate of calculated fluid contribution were found to be higher in Segment IV than in either Segment II or III (Table IV, Figure 13a). As predicted by models of Malpighian tubule secretion (Maddrell, 1971a,b,1972,1976 as cited by Nicholson, 1976), the high rates of fluid secretion in Segment IV were correlated with high rates of cation movement (presumably active) and anion movement (presumably passive) (Figure

Fig. 13

Fluid composition and rate of secretion of segments. All segments were maintained in 10 mM K saline. (a) Rate of secretion of segments. (b) Rate of secretion of major ions. Rate of secretion of all ions measured is highest in Segment IV. (c) Composition of secreted fluid. Sodium, chloride and osmotic pressure are highest distally and lowest proximally. Figure (b) and (c): Potassium (●). Chloride (O). Sodium (■). Osmotic pressure (O.P.) (▲). Bars indicate either plus or minus one standard error unless it is smaller than the dimensions of the point. Sample sizes are not shown on the graph, but are given in Tables IV, V, and VI.


Rate fluid secretion per unit length (nl hour^{-1} mm^{-1})
0
20
40
60
80
SEGMENT
II
III
IV
(a)
Rate ion secretion per unit length (picomoles hour^{-1} mm^{-1})
0
2
4
6
8
SEGMENT
II
III
IV
(b)
O.P. (mOsm)
160
170
Concentration (mM)
40
60
80
100
SEGMENT
II
III
IV
(c)

13b). Although the rate of secretion per unit length was lower in the more proximal Segments II and III, these areas were capable of secreting fluid; at no time was there evidence of proximal fluid reabsorption as found in Calpodes ethlius (Irvine, 1969).

There are two possible explanations for the great differences in secretory rate between Segment IV and Segments III and II. Either there are more cation pumps per unit length in Segment IV, enabling higher rates of cation transport, or the rate of lumen-directed cation movement is the same in all segments but the passive permeability of the epithelium to sodium or potassium is higher in Segments II and III. If higher backfluxes of cation occur, the net cation transport would be less, hence less fluid could be elaborated. Information concerning bidirectional sodium and potassium fluxes in all segments is necessary before the most likely of these hypotheses can be determined. Passive fluxes could either follow a transcellular or paracellular route.

The presence of an apical mucopolysaccharide coat, uptake of neutral red and the presence of many vesicles suggest reabsorption by pinocytosis occurs in Segment I (Jarial, 1967). Its small size and reduced apical and basal amplifications suggest it cannot be involved in fluid transport. No data have been gathered on the properties of Segment I in this study, excepting that it does not swell when ligated at both ends. It is thus not possible to comment on the possibilities that it is either a site of proximal reabsorption or a conducting capillary only (Bahadur, 1961, 1968).

(b) Gradient in composition of tubule fluid.

The ionic composition of fluid produced by the Malpighian tubules of C. bifida changed distally to proximally in a step-wise fashion. As indicated by Figure 13c, sodium, chloride and perhaps osmotic pressure were highest in Segment IV and lowest in Segment II. Excepting Segment III, the potassium concentration appeared to follow an opposite trend, i.e. it was higher in Segment II than in Segment IV. When the tubule fluid was sampled sequentially, the proximal to distal decline appeared more obvious (Table V, Figure 14). Thus the sum of potassium plus sodium concentrations were equal in Segments II and IV, but the sodium-potassium ratio changed from 1.2 in Segment IV to 0.7 in Segment II (Table IX). Sodium was an important component of tubular fluid when tubules were bathed in 10 mM K saline. The Malpighian tubules of C. bifida may be able to substitute some sodium for potassium as the actively transported cation promoting fluid secretion (Maddrell, 1976 as cited by Nicholson, 1976). This possibility is especially evident in Segment IV where the rate of sodium secretion per unit length was slightly higher than the rate of potassium secretion (Figure 13b).

A gradient in the secretory properties of Malpighian tubules was observed in the tubules of Carausius morosus by Ramsay (1955). In contrast to C. bifida, however, the sodium-potassium ratio of the tubule fluid of this animal was highest in the proximal area.

Fig. 14

Sodium and potassium concentrations in fluid from tubule segments sampled sequentially. The sodium concentration drops because of fluid addition proximally, while the potassium concentration rises. Sodium (■). Potassium (●). Bars indicate plus or minus one standard error. The sample size for all segments is indicated beside the error bars for Segment II.

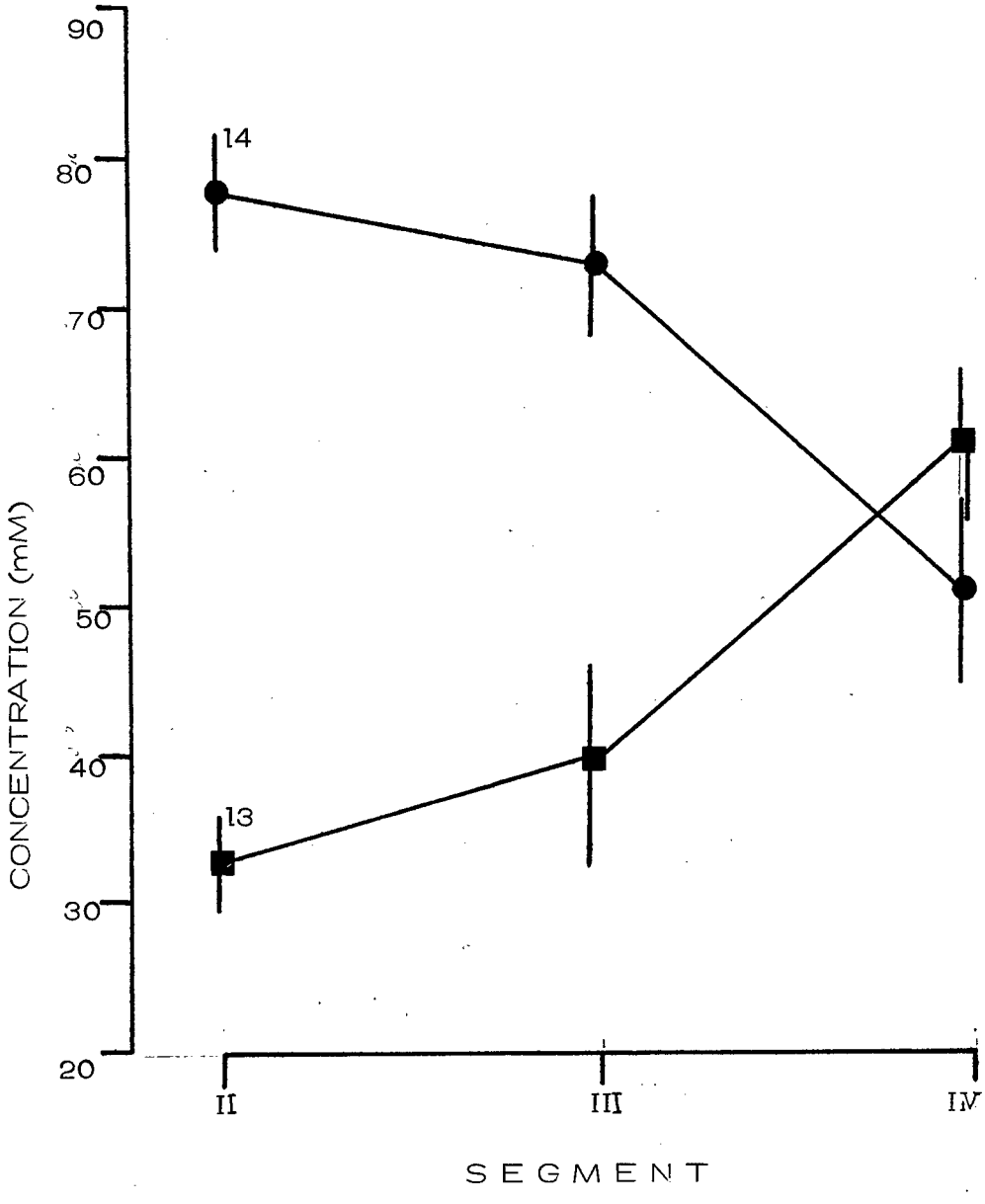

90
80
70
60
50
40
30
20
CONCENTRATION (mM)
14
13
II
III
IV
SEGMENT

TABLE IX. Sodium-potassium ratios, sum of cation concentrations, and chloride concentrations in segmental fluid.

	Segment II mean ± S.E.	Segment III mean ± S.E.	Segment IV mean ± S.E.
Na/K ratio [1]	0.70 ± 0.08	0.67 ± 0.13	1.20 ± 0.43
Na + K (mM) [1]	114 ± 6	139 ± 14	112 ± 10
Cl (mM)	76 ± 2	83 ± 3	100 ± 3

1: Calculated from data in Table V.

Transwall potential difference was not constant along the length of the tubule. The gradient in physiological properties of the Malpighian tubules of Carausius morosus correlated with a gradation in ultrastructure; a change in the apical to basal surface area ratio and length of microvilli was observed along the length of the tubule (Taylor, 1971a). The superior tubules of Carausius morosus do not have morphologically distinct segments.

No comparative measurements of surface area and microvillus depth are available for C. bifida. Figures 1 and 2 of Plate IV of Jarial (1967) show some differences in cell width and microvillus length between Segments II and IV.

(c) High potassium concentration in Segment III.

The potassium concentration in the fluid secreted by Segment III was higher than in that of either Segments II or IV (Table V). This high potassium value of Segment III occurred in 17 out of 20 tubules analyzed, but when the potassium concentration of the fluid was analyzed sequentially, the addition of fluid from Segment II did not reduce significantly the potassium concentration of the whole tubule fluid as compared to that from Segments III and IV only(Figure 14). Although this could be owing to the fact that much of Segment II was wasted in ligation and the fluid contribution by Segment II is low, it is equally possible that more severe damage to the delicate Segment III cells occurred when tubules were isolated with the segmental method. Maybe damaged cells released their contents, raising the concentration of potassium above

normal. A high potassium concentration could also occur if some of the lumen-directed potassium movement in Segment is not tightly coupled to water flow. For instance, potassium salts may be moved into the lumen in the secretory granules which are released by Segment III. Further studies on the ionic content of the cellular and lumenal secretory granules of Segment III and on the possibilities of cell damage due to handling are required to explain the high potassium concentration in the fluid secreted by Segment III.

(d) Anion content of fluid secreted by segments.

Chloride is clearly not the only anion present in the Malpighian tubule fluid of C. bifida. In Segment IV the total sodium plus potassium concentration was virtually equal to the chloride concentration, but in Segments II and III, the chloride concentration was less that the sum of the concentrations of measured cations (Table IX). Some of the potassium in Segment III fluid may have been present as an artifact of cell damage or as amorphous carbonate or phosphate salts in the secretory granules, and this would account for the ion balance in Segment III fluid. In Segment II, where secretory granules were not produced, and which was less prone to damage, the sum of sodium plus potassium concentrations was higher than the chloride concentration and the fluid was markedly alkaline (Table V). The presence of another anion like bicarbonate in solution, could explain both the anion-cation imbalance and the high pH of the fluid.

(e) The high pH in Segment II fluid

The simplest hypothesis explaining the high pH of Segment II fluid and the dependence of the high pH on high rates of fluid flow is that Segment II has a low permeability to chloride; metabolically-produced carbon dioxide from the cell may pass into the lumen as bicarbonate instead of chloride to maintain electroneutrality of the secreted fluid. As discussed earlier, there is also a possiblity that the high pH of the Malpighian tubule fluid in C. bifida is due to the action of a K/H exchange pump or an active HCO_3^- or $HPO_4^=$ pump; these pumps would have to be localized in Segment II.

Segmental variations in pH of Malpighian tubule fluid has been found in both Drosophila melanogaster and Rhodnius prolixus (Wigglesworth, 1931; Wessing and Eichelberg, 1975). In these insects the fluid is alkaline distally and acidic proximally which is the reverse of the situation in C. bifida. Since these are both terrestrial insects, it is thought that proximal acidification is related to the elimination of uric acid as a waste product (Wigglesworth, 1931). Is proximal alkalinization of advantage to an aquatic insect like C. bifida?

(f) The effect of a change in the sodium-potassium ratio.

Unlike the cryptonephridial tubules of Calpodes ethlius where changes in the sodium-potassium ratio of the bathing medium change the proximal segments of the tubules from reabsorptive to secretory function (Irvine, 1969), none of the tubule segments of C. bifida showed major changes in tubule function when the potassium concentration

of the bathing medium was elevated. Elevated bathing medium potassium concentrations did appear to inhibit the secretion of delicate Segment III preferentially, and prevented potassium concentration in Segment III fluid from being elevated compared to Segment II and IV fluid, but the phenomenon was probably just an artifact of differential toxicity of high potassium to the tubule cells and is thus not physiologically meaningful.

3. Transport in Segment III

Segment III is unique in two other properties besides sensitivity to high potassium. This is the only area of the tubules of C. bifida to produce luminal 'secretory granules' and it is the main area in which transport of dyes occurs. Segment III differs in ultrastructure from Segments II and IV by the presence of cytoplasmic concentrically-laminated secretory granules (Jarial and Scudder, 1970). These cytoplasmic granules are probably related to lumenal granules and may be important in the storage and elimination of amorphous salts and large organics.

(a) Secretory granule production.

Hyaline globules or 'secretory granules' were also present in the fluid secreted by the entire tubule. Often they were seen moving downstream along the tubule lumen and into the droplet of secreted fluid. Formed body production is considered a normal part of Malpighian tubule secretion in Carausius morosus and Rhodnius prolixus (Riegel, 1966, 1970a,b,1971; Maddrell 1971a), but it is not

certain (a) whether initial formation occurs within the tubule wall , (b) what the composition of lumenal secretory granules is nor (c) of what importance this mechanism is in secretion.

Some workers feel that initial crystallization occurs within the endoplasmic reticulum of the the tubule cells. Crystals are then released by exocytosis or plasma membrane breakage into the lumen (Lhonoré, 1971; Wall et al., 1975) where additional substances may be added (Ballan-Dufrançais, 1970; Lhonoré, 1971; Berkaloff, 1958). Other workers believe that lumenal granules are formed entirely by crystallization of substances passed into the lumen, and are not related to the granules in the tubule wall which are stationary (Wigglesworth, 1972; Bayon and Martoja, 1974; Wigglesworth and Salpeter, 1972; Sohal et al., 1976). Both mechanisms may in fact occur; in Drosophila melanogaster, the terminal Malpighian tubule segment is specialized for formed body production within the tubule wall, while in the main segment, granules are produced in the lumen only (Wessing and Eichelberg, 1975).

The mineral content of neither the cytoplasmic nor lumenal secretory granules of C. bifida tubules have been determined although Jarial and Scudder (1970) found that the latter are magnesium-positive. The low concentrations of magnesium found in the secreted fluid indicates that if cytoplasmic granules are released into the luminal fluid the movement of magnesium is not quantitatively important. In the Malpighian tubules of other insects, both lumenal and cytoplasmic

granules have been shown to contain a variable composition, including elements like Mg, K, Ca, P, Na, S, Cl, N, Ba, Cu, Sr, Fe, and other components like carbonates and phosphates deposited on an organic stroma of mucopolysaccharides; sometimes urates are present, other times they are not (Ballan-Dufrançais, 1970,1972; Ballan-Dufrançais et al., 1971 Waterhouse 1950,1951; Bayon and Martoja, 1971,1974; Ballan-Dufrançais and Fichelson,1974; Sohal et al., 1976; Berkaloff, 1960; Wessing and Eichelberg, 1975; Stadhouders and Jacobs 1961; Martoja and Seureau, 1972; Lhonoré, 1971). The luminal and cytoplasmic granules in the tubules of C. bifida probably contain several of these components. Formed bodies in aquatic insects usually lack puric wastes and potassium deposits (Bayon and Martoja, 1974).

The importance of formed bodies in Malpighian tubule secretion is not generally agreed upon. Cytoplasmic formed bodies undergo a maturation process (Jarial and Scudder, 1970; Wigglesworth and Salpeter, 1962; Sohal et al., 1976) and could provide a means of storage of substances useful in the haemolymph at a later time (Bayon and Martoja, 1974) or simply could be a site of storage excretion for excess minerals (Gouranton, 1968). It has also been suggested that the granules act as pH buffers for the cell or secreted fluid (Threadgold, 1976). The passage of formed bodies to the lumen and their subsequent dialysis has been proposed as a mechanism causing isosmotic fluid secretion (Riegel, 1970a,b,1971; Riegel and Cook, 1975), but as formed bodies are not found in all insects

(Berridge and Oschman, 1969) and as in any case the rate of secretion is usually inversely proportional to the rate of formed body production this is not considered an acceptable hypothesis (Maddrell, 1971a). Formed body production and release into the lumen may simply provide an alternate route for material transport into the lumen (Maddrell 1971a, Wessing and Eichelberg, 1975).

(b) Dye transport by Segment III.

Transport and concentration of all anionic dyes except phenol red occurred in Segment III (Table VIII). Both chlorophenol red and cresol red differ from phenol red by the presence of two functional groups only. Segment II may have a transport system for structurally smaller molecules with sulfanone-like structure. Dye transport is thought to be an active process (Maddrell et al., 1974).

Localization of dye transport in Malpighian tubule segments has been demonstrated previously (eg. Srivastava 1961; Nijhout, 1976) and may be indicative of localizations in mechanisms important in excretion of large molecules formed by conjugation of toxins (Maddrell et al., 1974). Jarial (1967) suggested that transport and accumulation of pigments occurred in Segment III, and that the secretory granules of this segment were involved in the process. He originally thought that the green colour in the secretory granules was owing to storage of breakdown products of chlorophyll. It has recently been shown that C. bifida is predatory, rather than phytophagous (Reynolds, 1975). However, Corixids feed on Chironomids selectively (Reynolds, 1975).

Chironomid haemolymph contains haemoglobin, and the green pigment in the secretory granules in the Malpighian tubules of C. bifida may be owing to the presence of a breakdown product of haemoglobin in the secretory granules. Haemoglobin is taken up unaltered by the gut and is temporarily stored as a greenish coloured breakdown product in the Malpighian tubules of several insects prior to the release of biliverdin into the tubule lumen (Wigglesworth, 1943). Secretory granules in other insects have been shown to contain vitamins and pigments (Hartman-Goldstein et al., 1976; Mello and Bozzo, 1969) and eye pigment precursor is stored in the Malpighian tubules of Drosophila melanogaster (Wessing and Eichelberg, 1972). Perhaps the Segment III secretory granules are important in the storage or elimination of large organics like pigments.

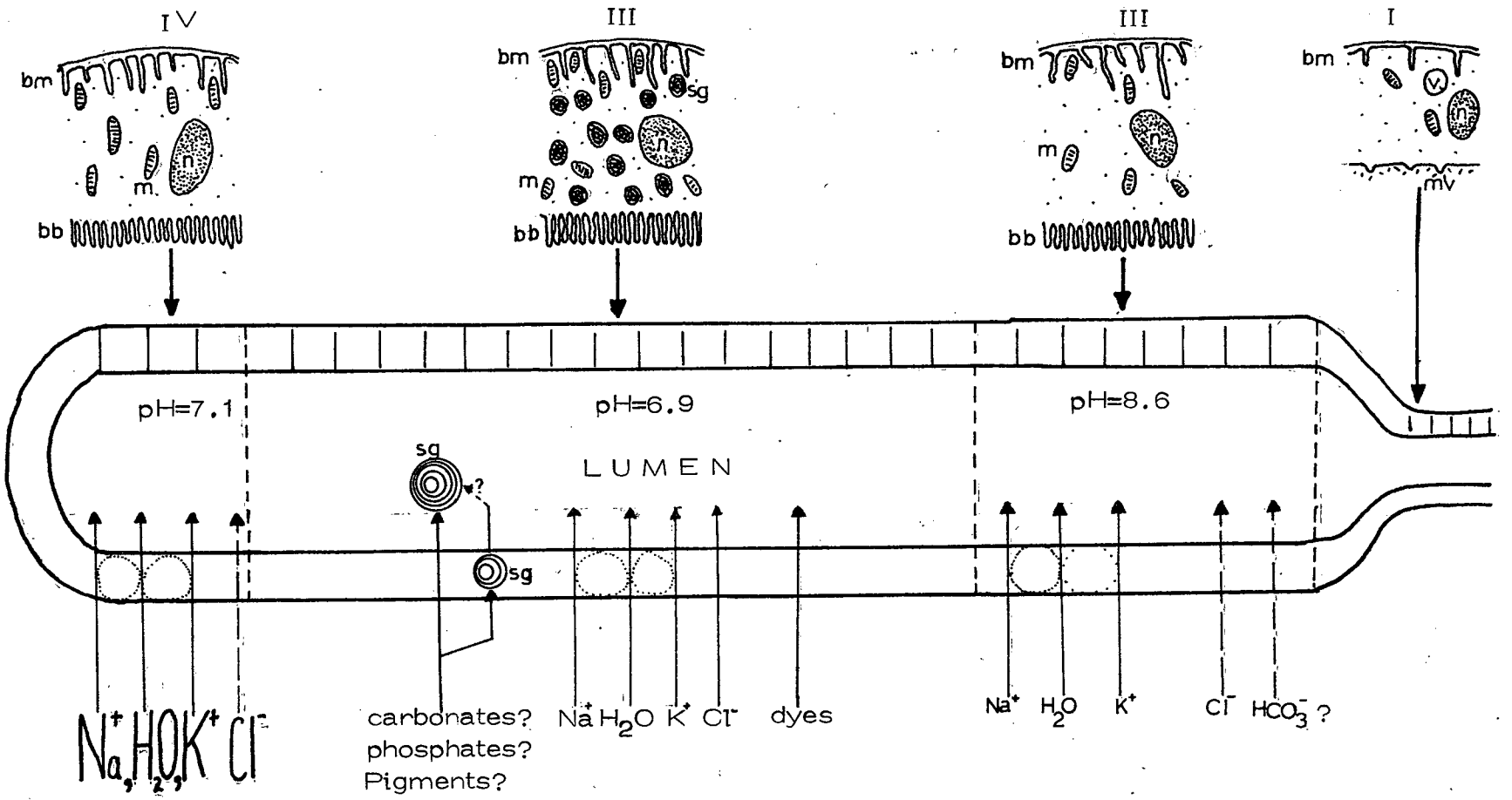
C. CONCLUSIONS

The morphological and physiological segmental differences in the Malpighian tubules of C. bifida discussed in this study are summarized in Figure 15. The cell structure diagrams are taken from Jarial and Scudder, (1970). The only striking morphological difference in the three segments is the presence of cellular secretory granules in Segment III which may be precursors of the lumenal secretory granules. Dyes and possibly other large organics like pigments are secreted in Segment III. The rate of fluid secretion and the rates of potassium, chloride and sodium secretion are greatest in the distal segment. Electroneutrality is maintained in Segment II by a passive influx of bicarbonate which also raises the pH of fluid in this area.

Except for the proximal alkalinization at Segment II, the physiological variations between the individual segments of C. bifida tubules probably do not have much importance in the ion and fluid transport of the whole tubule. Segment III, however, is implicated as a site of localization of some organic transport properties.

Fig. 15

Summary of morphological and physiological properties of the Malpighian tubule segments of C. bifida. Magnitude of rate of secretion of ions and water is indicated by the relative size of letters. Hypothesized active movements are represented by solid arrows; passive by dashed lines. Circles joining arrows indicate the possible coupling of water flows to cation movements. bm = basement membrane. m = mitochondrion. n = nucleus. bb = brush border. mv = microvillus. v = vesicle. sg = secretory granule. The ultrastructure diagrams are taken from Jarial and Scudder, (1970).


SEGMENT
IV
III
III
I
bm
m
n
bb
sg
mv
pH=7.1
pH=6.9
pH=8.6
LUMEN
?
carbonates?
phosphates?
Pigments?
dyes

IV. BIBLIOGRAPHY

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