THE SITE OF POTATO LEAFROLL VIRUS MULTIPLICATION IN ITS VECTOR, *MYZUS PERSICAE*

AN ANATOMICAL STUDY

M. B. PONSEN

M. B. PONSEN

THE SITE OF POTATO LEAFROLL VIRUS MULTIPLICATION IN ITS VECTOR, *MYZUS PERSICAE*

AN ANATOMICAL STUDY
(with a summary in Dutch)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF. DR. IR. H. A. LENIGER
HOOGLERAAR IN DE TECHNOLOGIE
IN HET OPENBAAR TE VERDEDIGEN
OP VRIJDAG 13 OKTOBER 1972 TE 16.00 UUR
IN DE AULA
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

STELLINGEN

I

De door METSCHNIKOW (1866) van *Aphis rosae* en *Aphis pelargonii* beschreven retortvormige organen worden door SAXENA en CHADA (1971) ten onrechte beschouwd als onderdelen van het speekselklier complex.

> METSCHNIKOW, E., 1866. Z. wiss. Zool. 16, 49–80.
> SAXENA, P. N. en H. L. CHADA, 1971. Ann. ent. Soc. Am. 64, 904–912.
> Dit proefschrift.

II

Vermeerdering van een plantevirus in de speekselklieren van een cicade is noodzakelijk, wil de betrokken cicade als vector van het virus kunnen fungeren.

III

De door JAN SWAMMERDAM (1669) vermelde gegevens over het vetweefsel van de vlieg Tabanus *(Asili distae)* maken het zeer waarschijnlijk dat deze auteur al structuren heeft gezien die later door WIELOWIEJSKI (1886) als oenocyten zijn beschreven.

> SWAMMERDAM, J., 1669. Bijbel der Natuure. Historie der insecten.
> WIELOWIEJSKI, H. R. von, 1886. Z. wiss. Zool. 43, 512–536.

IV

Nader dient te worden onderzocht in hoeverre bladluis-honingdauw bijdraagt tot de verspreiding van plantevirussen.

> OSSIANNILSSON, F., 1958. K. LantbrHögsk. Annlr 24, 369–374.
> KIKUMOTO, T. en C. MATSUI, 1962. Virology 16, 509–510.

V

De gangbare mening dat de oesophagale klep dient om terugstromen van voedsel uit de maag te verhinderen, is niet juist.

> FORBES, A. R., 1964. Mem. ent. Soc. Can. 36, 1–74.

VI

Het is waarschijnlijk dat bij bladluissoorten die in compacte kolonies plegen te leven, géén schrikstof in de zin van KISLOW en EDWARDS aanwezig is.

> KISLOW, C. J. en L. J. EDWARDS, 1972. Nature, Lond. 235, 108–109.

M. B. PONSEN
Wageningen, 13 oktober 1972

VII

Phloëem transport berust op massastroming.

CRAFTS, A. S. en C. E. CRISP, 1971. Phloem transport in plants, San Francisco, 481 pp.

VIII

De toepassingsmogelijkheden van een insektevirus ter bestrijding van insekten, worden bepaald door de wijze waarop het virus geproduceerd kan worden.

IX

Het bouwen van kantoorcomplexen tegen de stations van de Nederlandse Spoorwegen aan zou een bewuste poging zijn het autoverkeer sterk te reduceren.
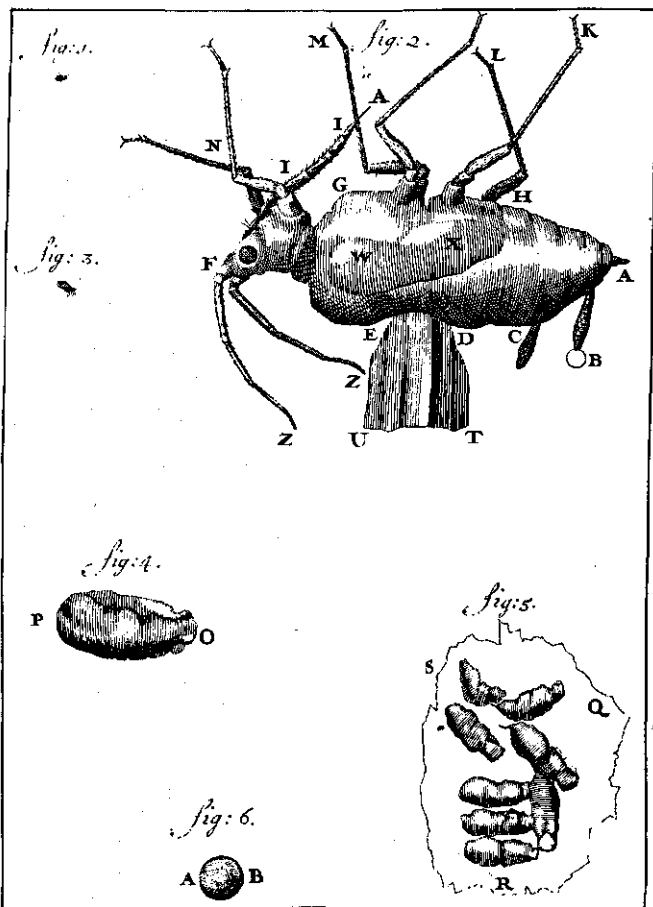
MULDER, K., 1972. Intermediair 29.

Fig. 1. vertoont het zoo genoemde Aelbeffe-Luysje , dat van die foort is , die een ligt groene Couleur hebben , en dat zoo als het in ons bloote oog te vooren komt , en tot zyn volkome grootte is geworden.

Fig. 2. ABCDEFGH. vertoont het gezeyde Dierke , dat mede tot zyn volkome grootte is geworden , en zoo verre al wasgekomen , dat op zyn laarfte vervellen ftond , in welk Dierke men de in een gevouwen wiekjens al quam te zien , die hier in de gezeyde figuur met WX. werden aangewefen.

Met KLMN. werden aangewefen de zes pooten met hare ledekens, welke pooten met zeer veel dunne korte hairrgens zyn bezet , en daar benevens is te fien , hoe yder van defe pootgens met twee klaauwtgens zyn verzien.

Aan F. is afgebeelt een van de twee nette oogjens, welkers ongemene maakfel , zoo het door het vergroot-glas te vooren quam , onmogelijk was om na te volgen.

Met II. werd aangewefen deffelfs fnuyt die het Dierke in de jonge fcheut of in de fteel van het blad fteekt , en daar door zyn voetfel haalt. Ende met FZZ. der zelver twee hoornen.

Aan het agterlyf heeft het twee over eynde ftaande werktuygen , die in 't midden dikft zyn , en zeer aardig met ronde ende in 't verbant leggende fchilfekens omfet zyn , als hier met C. en B. werden aangewefen. Uit welke werktuygen ik verfcheyde malen hebbe zien uit brengen een kleyn druppelrge zeer heldere vogtigheit als aan B. werd aangewefen.

Dit

Dit kleyne druppelrge vogt was voor my een groot vermaak te befchouwen , om dat wanneer dit druppelrge op zekere diftantie voor het vergroot-glas ftond , het zelve voor een tweede vergroot glas verftrekte , om dat de voorwerpen , als Huyfen , en Toorens , door dat druppelrge vogt , het onderfte boven , en dat zoo kleyn en net voorquamen , dat het voor veele onbegrypelijk is.

Ik hebbe ook wel gezien , dat wanneer een kleyn druppelrge vogt , uit een van de gezeyde deelen was geftooten , aanftoncs weder in 't lighaam wierd gebragt.

Met DTVR. werd aangewefen de punét van een fpeld waar op dit Dierke was gekleeft , wanneer het afgeteikent wierd.

Fig. 3. vertoont de grootte van het Dierke , zoo als het van een verhaald Schepfeltge in een Vliegje is verandert.

Terwyl de Teikenaar bezig was met het verhaalde af te teikenen , opende ik weder eenige Dierkens , en ik verfpreyde de ongeboorene Schepfelgens op een dun glas , en voor het vergroot-glas geflelt hebbende een van de ongeboore Dierkens , die ik oordeelde dat van de eerfte uitgeworpen zouden zyn geweeft , liet ik het mede zoo veel als men konde navolgen afteikenen , als hier met Fig 4. OP. werd aangewefen.

Aan dit ongeboore Dierke konde men mede bekennen dat deffelfs lighaam omwonden was met een vlies , dog alle de deelen die daar in te zien ware en konde men niet navolgen , om dat in korte tijd de vogtige ftoffe was weg gewaeffemt , door welke weg waeffeminge de figuur veranderde,

M 3

Ik gaf ook de Teikenaar een ander vergroot-glas in handen,waar voor agt Dierkens by den anderen lagen, aan alle welke men de oogen klaar konde bekennen , die den zelven mede zoo veel heeft nagevolgt als het hem doenlijk was , als hier met Fig. 5. QRS. werd aangewefen.

Ik hebbe hier vooren Fig. 2. met F. aangewefen een van de twee nette oogjens (dat mede uit veele gezigten beftaat) dog alzoo my een afgeftroopt huytge te vooren quam , waar in het nette maakfel van het oog nog meerder was uitftekende , hebbe ik goet gedagt het zelvige te laten afteikenen , op dat de volmaaktheyt van zoo een veragt kleyn Schepfel nog meer mogte blijken. Fig. 6. AB. verbeeld het afgeftroopte huytge af menbrane , van het oog , dat door een meerder vergrootende glas is geteikent , als waar voor het Dierke Fig. 2. afgeteikent is.

Sedert defe ontdekkinge onledigde ik nog Dierkens , alleen om te zien of ik aan de Dierkens , die ik uit 's moeders lighaam quam te halen , geen leven konde bekennen , in welk doen ik onder andere een Dierke ontmoette , welkers ingewant in 't lighaam ik niet alleen zag bewegen , maar het maakte met deffelfs pooten hoewel die nog in gefchikte ordre voor het lyf lagen geplaatft , zoo een beweginge , dat de omleggende ftoffe daar door bewogen wierd.

Het was nu den 6. en 7. Juny wanneer ik zag dat verfcheyde Dierkens die op den 31. Mey geworpen waren in vliegende Schepfels veranderden , en nu waren ook al eenige bladeren van de Aelbeffe-takjens aan het verdorren.

Ik

CONTENTS

1. INTRODUCTION

QUANJER (1916) demonstrated that potato leafroll is an infectious disease, and considered it to be caused by a virus. This virus, named potato leafroll virus (PLRV), appeared to be transmittable from plant to plant by the green peach aphid, *Myzus persicae* SULZ. (OORTWIJN BOTJES, 1920). ELZE (1927) found that after a certain acquisition feed a period exists during which *M. persicae* is not able to transmit PLRV; after this period of latency the vector is infectious for a long time of its life, a condition which this author presumed to be due to virus multiplication within the aphid vector. The occurrence of this multiplication was proved by STEGWEE and PONSEN (1958). In this case the ingested virus presumably passes through the gut wall to the haemocoel and is subsequently transported towards the site of multiplication. From here it is carried into the salivary glands of the vector, whence it is injected into the plant.

The present study was undertaken to examine the distribution of PLRV throughout the various organs and tissues of the vector. Although some investigators studied the anatomy of *M. persicae* it appeared necessary to supplement the information found in the literature. Moreover, a special study of the anatomy of the larval stages of this insect was made. The various organs and tissues of viruliferous aphids were dissected and their homogenates tested for infectivity by injecting aphids.

2. SURVEY OF LITERATURE

When after about 1760 the potato plant was introduced as a cultivated crop, it appeared that the yield of the potato in repeated culture gradually decreased as a result of 'degeneration' which was in fact due to the effect of various infectious diseases (ROZENDAAL, 1946). It was in 1906 that APPEL selected from this group of diseases the so-called 'Blattrollkrankheit', the leafroll disease.

To QUANJER (1913) belongs the credit of being the first to discover that the leafroll disease was characterized by necrosis of the phloem. The infectious nature of the leafroll disease was demonstrated by means of stem and tuber grafting (QUANJER, 1916). In analogy with the incitant of the mosaic disease of tobacco QUANJER named this microscopically invisible, infectious matter: 'virus'. In 1920 OORTWIJN BOTJES obtained transmission of PLRV from diseased to healthy potato plants by the green peach aphid, *M. persicae*. This finding was established independently by SCHULTZ and FOLSOM (1921).

The first phase in the transmission process is the acquisition feeding period, i.e. the minimum time during which the nonviruliferous aphids must feed on a PLRV source to acquire so much virus, that transmission can take place. SMITH (1931) obtained transmission of PLRV after a minimum acquisition feeding period of 6 hours on a leafroll diseased potato plant. Thereafter many investigators worked on this subject. Their results are summarized in Table 1. From these results it can be concluded, that the minimum acquisition feeding period of PLRV by its vector is 10–15 minutes. However, LOUGHNANE (1943)

TABLE 1. The length of the minimum acquisition feeding period of *Myzus persicae* in the transmission process of the potato leafroll virus according to different authors.

Leafroll source	Minimum acquisition feeding period	Author
Solanum tuberosum cv. British Queen	6 hours	SMITH (1931)
Datura stramonium	6 hours	WEBB et al. (1952)
D. stramonium var. *tatula*	2 hours	KASSANIS (1952)
S. tuberosum cv. Netted Gem	2 hours	MACCARTHY (1954)
D. stramonium	1 hour	MIYAMOTO and MIYAMOTO (1966)
D. stramonium var. *tatula*	1 hour	MIYAMOTO and MIYAMOTO (1966)
Physalis floridana	1 hour	MIYAMOTO and MIYAMOTO (1966)
D. stramonium	30 min.	KIRKPATRICK and ROSS (1952); WILLIAMS and ROSS (1957a)
P. floridana	30 min.	WILLIAMS and ROSS (1957a)
P. floridana	15 min.	DE MEESTER-MANGER CATS (1956)
P. angulata	10 min.	KLOSTERMEYER (1953)
P. floridana	10 min.	STEGWEE (1960)
S. tuberosum cv. Up-to-Date	5 min.	LOUGHNANE (1943)
S. tuberosum cv. Arran Cairn	5 min.	LOUGHNANE (1943)

2

found that nonviruliferous aphids previously starved for 4 hours could transmit the virus after an acquisition feeding period of 5 minutes on a leafroll diseased potato plant, but KASSANIS (1952) could not reproduce these results. A starvation period before the acquisition feeding period of 2 hours (WEBB et al., 1952), 4 hours (KASSANIS, 1952), or 18 hours (MacKINNON, 1962) had no effect on the efficiency of the transmission. This is in contrast to the non-persistent (stylet-borne; KENNEDY et al., 1962) plant viruses, where a starvation of the aphid before the acquisition has an effect on the transmission of the virus (WATSON and ROBERTS, 1939).

The following phase in the transmission process of PLRV is the infection feeding period of its vector; this period is the minimum time during which the infective aphid must be feeding on the healthy plant to obtain virus transmission. The results of many investigators on this topic are summarized in Table 2. It can be concluded that the minimum infection feeding period of *M. persicae* which had been fed on a PLRV source for several days is 10–15 minutes.

The above mentioned results show that nonviruliferous aphids can acquire PLRV in a minimum acquisition feeding period of 10–15 minutes; using infective aphids the minimum infection feeding period likewise amounted to 10–15 minutes. The explanation given above for these minimum acquisition and infection feeding periods is acceptable on account of the following investigations.

Histological investigations concerning the course of the aphids' stylets in tissues of *Freesia refracta* KLATT., *Digitalis purpurea* L. (HORSFALL, 1923), *Solanum tuberosum* L. (SMITH, 1926; DYKSTRA and WHITAKER, 1938; SORIN, 1960), *Allium cepa* L. (TATE, 1936), *Beta vulgaris* L. (BENNETT and WALLACE, 1938; ESAU et al., 1961), *Nicotiana tabacum* L. cv. *White Burley* (BRADLEY, 1953), tobacco (GUTHRIE et al., 1962), *Prunus persica* BATSCH var. *vulgaris* (SORIN, 1966), *Vicia faba* L. (LOWE, 1967), *Raphanus* sp. (KISLOW et al., 1971), and *Tulipa gesneriana* L. (POLLARD, 1971) showed that *M. persicae* fed mainly in the phloem. DYKSTRA and WHITAKER (1938) found a percentage of 98 % for *M. persicae* which had been feeding in the phloem of potato leaves. SMITH (1926) suggested that the PLRV could only be transmitted when *M. persicae* was feeding in the phloem.

DAY and IRZYKIEWICZ (1953) established that *M. persicae* could not acquire any food from the plant during the first minutes. The aphids were allowed to feed during several acquisition feeding periods on Chinese cabbage leaves, which were put in a solution with labeled phosphorus (^{32}P). In similar experiments WATSON and NIXON (1953) observed an increased concentration of ^{32}P in *M. persicae* after 1 hour feeding on leaves of *Brassica rapa* L. and *B. vulgaris*. According to these authors the aphids could not acquire food before reaching the phloem with their stylets.

ROBERTS (1940) ascertained in her histological work, that the minimum time, necessary for *M. persicae* to reach the phloem with their stylets, was 15 minutes on *N. tabacum* cv. *White Burley* and *B. vulgaris*; 2 out of 18 aphids could reach the phloem within these 15 minutes. Furthermore she found that it

TABLE 2. The length of the minimum infection feeding period of viruliferous *Myzus persicae* to transmit potato leafroll virus according to different authors.

Leafroll source	Number of days on leafroll source	Minimum infection feeding period	Testplant	Number of aphids/testplant	Author
Datura stramonium	many	4 hours	*Physalis angulata*	1	KIRKPATRICK and ROSS (1952)
Solanum tuberosum cv. British Queen	7 or longer	2 hours	*S. tuberosum* cv. President	many	SMITH (1931)
D. stramonium	many	2 hours	*Physalis floridana*	10	WEBB et al. (1952)
D. stramonium	many	30 min.	*P. floridana*	1	KIRKPATRICK and ROSS (1952)
S. tuberosum cv. Netted Gem	4	30 min.	*P. floridana*	1	MacCARTHY (1954)
D. stramonium	many	15–30 min.	*P. floridana*	5	WILLIAMS and ROSS (1957a)
D. stramonium	many	15–30 min.	*D. stramonium*	5	WILLIAMS and ROSS (1957a)
D. stramonium var. *tatula*	many	15 min.	*D. stramonium* var. *tatula*	20	KASSANIS (1952)
S. tuberosum cv. Russet Burbank	many	10 min.	*P. angulata*	5	KLOSTERMEYER (1953)
D. stramonium	many	10 min.	*P. floridana*	5	MIYAMOTO and MIYAMOTO (1966)

took 5 minutes for the aphid to penetrate the epidermis. MITCHELL (1937), and WATSON and ROBERTS (1939) showed that *M. persicae* could not reach the vascular tissues within 5 minutes. From these observations it could be concluded that the PLRV must be both acquired from and introduced into the phloem; to reach the phloem *M. persicae* needs at least 15 minutes which agrees with the minimum acquisition and infection feeding period of the aphid. According to KASSANIS (1952) these observations suggest that PLRV has to be introduced into the phloem for infection to occur.

ELZE (1927) was the first to observe that PLRV could not be transmitted directly by *M. persicae* after a distinct acquisition feeding period. He used the term latent period, which had already been introduced in medical science regarding the transmission of yellow-fever by the mosquito vector, *Aëdes aegypti* L. (REED, 1902). ELZE's view found the support of many observers, who reported that the aphids having acquired PLRV, undergo a latent period during which they are unable to infect healthy plants. From these results which are summarized in Table 3, the latent period of PLRV in its vector can be defined as the time the aphids passed on the leafroll source plus either the time they passed on the testplant without infecting it (Table 3, nr. 1–8), or the time they passed on successive testplants before infection succeeded (Table 3, nr. 9–10), or the time they passed on an immune hostplant (Chinese cabbage) before transmission was performed on testplants (Table 3, nr. 11–12).

However, KLOSTERMEYER (1953) and DE MEESTER-MANGER CATS (1956) (Table 3, nr. 13–14) have reported transmission of PLRV without a latent period. They could transmit the virus directly following acquisition and infection feeding periods of both 10 and 15 minutes. KIRKPATRICK and ROSS (1952) demonstrated that PLRV could be transmitted by *M. persicae* after a minimum latent period of 1.5–4.5 hours.

DAY (1955) questioned the absence of a latent period when he stated 'hundreds of tests using 5 minutes feeding periods have not resulted in a single inoculation of potato leafroll virus'. The results were also negative with feeding periods of 10, 60, and 120 minutes. In one case (out of 33) he obtained transmission with acquisition and infection feeding periods of 3 and 4 hours respectively. The results of KLOSTERMEYER (1953) were doubted by DAY (1955), as he reported: 'some evidence that KLOSTERMEYER was dealing with a virus differing from typical leafroll is suggested by his statement that visitors from several eastern States and from foreign countries declare such pronounced symptom expression does not occur elsewhere'. CADMAN (1957) too could not confirm such short latent periods; he wrote 'tests duplicating those recently described in N.America and the Netherlands failed to confirm that transmission of PLRV is effected in less than 2 hours', probably aiming at the results of the investigations of KIRKPATRICK and ROSS (1952), KLOSTERMEYER (1953), and DE MEESTER-MANGER CATS (1956). STEGWEE and PONSEN (1958, unpublished results) confirmed the results of DAY (1955) and CADMAN (1957), but showed that after an acquisition feeding period of 15 minutes on a leafroll diseased *Physalis floridana* RYDB. plant the virus could be transmitted rarely to seedlings

TABLE 3. The length of the latent period of potato leafroll virus in *Myzus persicae* according to different authors.

No.	Leafroll source	Acquisition feeding period	Testplant
1	*Solanum tuberosum* cv. Paul Kruger	14 hours	*S. tuberosum* cv. Paul Kruger
	S. tuberosum cv. Paul Kruger	24 hours	*S. tuberosum* cv. Paul Kruger
2	*S. tuberosum* cv. British Queen	6 hours	*S. tuberosum* cv. President
3	*Datura stramonium* var. *tatula*	2 hours	*D. stramonium* var. *tatula*
4	*D. stramonium*	6 hours	*Physalis floridana*
5	*S. tuberosum* cv. Netted Gem	2 hours	*P. floridana*
	S. tuberosum cv. Netted Gem	12 hours	*P. floridana*
6	*D. stramonium*	1 hour	*P. floridana*
	D. stramonium	5 hours	*P. floridana*
	D. stramonium	10 hours	*P. floridana*
7	*P. floridana*	6 hours	*P. floridana*
8	*P. floridana*	6 hours	*P. floridana*
9	*P. floridana*	30 min.	*P. floridana*
10	*P. floridana*	2 hours	*P. floridana*
	P. floridana	6 hours	*P. floridana*
11	*D. stramonium* var. *tatula*	1 hour	*P. floridana*
	D. stramonium var. *tatula*	6 hours	*P. floridana*
12	*S. tuberosum* cv. Bintje	3 hours	*P. floridana*
	S. tuberosum cv. Bintje	6 hours	*P. floridana*
13	*Physalis angulata*	10 min.	*P. angulata*
14	*S. tuberosum* cv. Red Star	15 min.	*P. floridana*
	P. floridana	15 min.	*P. floridana*

of the same species with an infection feeding period of 5 days (5 aphids per testplant), when between the acquisition and infection feedings the aphids were placed during 16 days on Chinese cabbage which is immune to PLRV; 1 out of 11 testplants showed leafroll symptoms.

Summarizing it appears that nonviruliferous *M. persicae* become infective after an acquisition feeding period on a PLRV source, but during a period of 1–5 days the infected aphids can not transmit the virus; only after this period the aphids are able to infect healthy plants. The variations in the latent period of PLRV in its vector seem to depend on several ill-defined factors.

Some publications show that the efficiency of virus transmission depends on the virus source and the testplant. KASSANIS (1952) found that young or recently infected *Datura stramonium* L. var. *tatula* and potato plants were much better virus sources for *M. persicae* than older plants. He supposed that 'the leafroll virus content of the infected plants reaches an early maximum and then falls'. The results of BEEMSTER (1960) pointed in the same direction. According to KIRKPATRICK and ROSS (1952) the older leaves of a full-grown leafroll diseased potato plant contained more virus than young leaves. Furthermore they stated that *P. floridana* was a better PLRV source than *D. stramonium*, the latter in turn being better than *Physalis angulata* L. and potato. MACKINNON (1962) reported that the top leaves of *P. floridana* were better sources of PLRV than

6

Number of aphids/ testplant	Infection feeding period	Latent period		Author
many	10 hours	24	hours	Elze (1927)
many	24 hours	48	hours	Elze (1927)
many	24 hours	30	hours	Smith (1931)
10	48 hours	50	hours	Kassanis (1952)
10	24 hours	30	hours	Webb et al. (1952)
1	48 hours	50	hours	MacCarthy (1954)
1	24 hours	36	hours	MacCarthy (1954)
1	24 hours	25	hours	Williams and Ross (1957a)
1	36 hours	41	hours	Williams and Ross (1957a)
1	12 hours	22	hours	Williams and Ross (1957a)
5	18 hours	24	hours	Stegwee and Ponsen (1958)
3	24 hours	30	hours	Miyamoto and Miyamoto (1966)
1	30 min.	1.5–4.5	hours	Kirkpatrick and Ross (1952)
1	24 hours	50	hours	MacKinnon (1964)
1	24 hours	30	hours	MacKinnon (1964)
1	4 hours	73	hours	Day (1955)
1	4 hours	20	hours	Day (1955)
1	4 hours	123	hours	Ponsen (1958, unpublished)
1	4 hours	54	hours	Ponsen (1958, unpublished)
10	10 min.	10	min.	Klostermeyer (1953)
5	15 min	15	min.	De Meester-Manger Cats (1956)
5	15 min.	15	min.	De Meester-Manger Cats (1956)

either middle or bottom ones. The tip areas of leaves from these three positions were poorer sources of PLRV than middle or basal areas (MacKinnon, 1963a).

The testplants mentioned in Table 3 are all susceptible to PLRV, but they differ in sensitivity. So it appeared that *P. floridana* was more sensitive to the virus than *P. angulata* (MacCarthy, 1954), and also more sensitive than *D. stramonium* and potato (Kirkpatrick, 1948; Williams and Ross, 1957b).

Some publications deal with the efficiency of PLRV transmission by *M. persicae*. Elze (1927) found that in some cases a very small number of aphids achieved the infection, whereas in other cases relatively many aphids could not bring about infection. Smith (1929) obtained 100% infection in his experiments with potato sprouts when he used 18 infective aphids per plant. Working with *P. floridana*, the same results were obtained with 10 infective aphids per plant (Webb et al., 1952). Hovey and Bonde (1948) obtained with *P. angulata* 75% successful infections using 5 infective aphids per plant. Kirkpatrick (1948), who worked with 1 infective aphid per plant, found infections with *P. floridana*, *P. angulata*, and *D. stramonium* to be successful for 70–100%, 20–40%, and 40–70% respectively. Furthermore he reported for *P. floridana* no difference if 1 or 5 infective aphids per plant were used. According to Kirkpatrick and Ross (1952), and Williams and Ross (1957b) the efficiency of PLRV transmission by its vector decreased when the number of infective aphids per plant in-

creased. BINDRA and SYLVESTER (1961) could not confirm these results, and did not show any difference in leafroll transmission between 5 and 10 infective aphids per plant (*P. floridana*) with infection feeding periods of 30 and 60 minutes.

The differences in infection ability of aphids could probably be explained from the investigations of BJÖRLING and OSSIANNILSSON (1958), who reported that within the species *M. persicae*, strains with a constant difference in their ability to transmit PLRV occurred. Previously DAY (1955) found some indications for the existence of clonal differences. BJÖRLING and OSSIANNILSSON reported for one strain 46.3% successful infections, and for the other one 71.3% with an acquisition and infection feeding period of 3–5 and 3 days respectively (*P. floridana* as virus source and testplant). However, they did not mention the age of the aphids used.

The age of *M. persicae* probably plays an important part in PLRV transmission. In most papers cited up till now nearly nothing was mentioned concerning the age of the aphids used in the experiments. According to KIRKPATRICK and ROSS (1952) adult *M. persicae* transmitted the virus better than larvae, while MACKINNON (1962), MIYAMOTO and MIYAMOTO (1966) and ROBERT et al. (1969), observed the contrary. Later on MACKINNON (1963b) observed that two-day-old larvae born on *P. floridana* transmitted PLRV more readily than did larvae born on *Brassica napus* L. On the other hand, HOVEY and BONDE (1948) and DAY (1955) did not show any difference in the transmission process of PLRV between larvae and adults. STEGWEE and PONSEN (1958) demonstrated large variations in the infection ability of PLRV infected aphids during their lifetime. The percentage infections by using 1 aphid per testplant varied of 10–70%; these variations were somewhat higher at 30°C than at 25°C.

STEGWEE (1960) showed that in aphids which were allowed a 6 hours' acquisition feeding at 20°C on a leafroll diseased *P. floridana* plant, no infectivity developed when *M. persicae* were subsequently kept on Chinese cabbage at 35°C. At 30°C and 25°C a longlasting infectivity developed. Also, when larvae reared on diseased plants at 20°C were transferred to Chinese cabbage at 35°C, they rapidly lost their transmitting ability. After an acquisition feeding period of 24 hours on a diseased plant the aphids remained infective for the next two days on Chinese cabbage at 35°C, but after 4 days the transmitting ability was lost. However, restoration of infectivity was possible by putting the aphids back at 20°C for two days. CADMAN and HARRISON (1956) could not obtain virus transmission at 20°C after the infective aphids had stayed at 32°C during 3–6 days. It appeared that PLRV was inactivated in its vector by exposure to a temperature of 32°C or higher. That was also found for PLRV in potato tubers in which the virus was inactivated after 4 weeks at 38°C (KASSANIS, 1949), or 3 weeks at 36°C (CADMAN and HARRISON, 1956).

ELZE (1927) found that the capacity of an infective aphid to transmit PLRV was not influenced by moulting, and that the infective aphid retained its infectivity probably for the rest of its life. From these results ELZE concluded that PLRV was not transmitted mechanically by *M. persicae*. This conclusion was

8

supported by SMITH (1929, 1931), DAVIES (1932), MACCARTHY (1954), and DAY (1955). SMITH (1926, 1931) and ELZE (1931) supposed that PLRV was ingested with the food and passed from the gut into the haemolymph, and from there into the salivary glands to be injected finally into the plant with the saliva. Another possibility might be the regurgitation of the infective stomach content of *M. persicae* into the plant. SMITH (1931) and WATSON (1938) considered this to be impossible because the oesophageal valve of the aphid would prevent the regurgitation of the stomach content. DAY and IRZYKIEWICZ (1953) confirmed with ^{32}P that *M. persicae* could not regurgitate the ingested food.

The supposition that PLRV ingested by *M. persicae* passed from the gut into the haemolymph was supported by the work of DAY (1955), HEINZE (1955), STEGWEE and PONSEN (1958), VAGO (1961, personal communication), and CLARK and ROSS (1964). They succeeded in transmitting haemolymph from viruliferous aphids with glass capillaries into the body cavity of nonviruliferous ones which could then infect testplants. How the virus is transferred from the haemolymph to the salivary glands to be injected into the plant with the saliva of the aphid has not yet been explained.

According to ELZE (1927) an aphid once infected with PLRV probably would remain infective for the rest of its life. Infective *M. persicae* could transmit PLRV after a stay of 7 or 10 days on an immune hostplant (*Spinacia oleracea* L.) to healthy potato plants. SMITH (1929) reported transmission after a stay of 4–5 days without food, or of 7 days on an immune cabbage plant (*Brassica oleracea* L. var. *capitata*). DAY (1955) obtained PLRV transmission after a period of 8 days on an immune cabbage plant (*Brassica chinensis* L.), and MACCARTHY (1954) after a period of 24 days on an immune cabbage plant (*Brassica juncea* COSS.) to *P. floridana* testplants. When viruliferous aphids including apterous adults and first to fourth instar larvae were reared on detached leaves of Chinese cabbage in petri dishes under the conditions of 20–25°, 5°, and 30°C, the aphids were still infective 21, 32, and 11 days after leaving the virus source plants (*P. floridana, D. stramonium,* or *D. stramonium* var. *tatula*) (MIYAMOTO and MIYAMOTO, 1966).

Summarizing it appears that the characteristics of the transmission of PLRV by its vector, *M. persicae*, are: (1) no influence on virus transmission after a previous starvation period; (2) a positive correlation between the duration of the feeding period and the ability to transmit the virus; (3) a latent period; (4) no loss of infectivity after a moult by its vector; and (5) sustained infectivity of the vector during a considerable long time. Since PLRV is present in the haemolymph of *M. persicae*, this virus, according to BLACK (1959), belongs to the group of the circulative viruses. This in contrast to the group of the stylet-borne viruses, where the virus disappears after a moult of the aphid (KENNEDY et al., 1962).

As early as 1931 ELZE expressed the view that PLRV would multiply in *M. persicae*, since a once infected aphid retains its infectivity for a long time. DAY (1955) was the first who obtained an indication for the existence of multiplication. He gave large numbers of *M. persicae* acquisition feeding periods of 1,

3, and 6 hours on leafroll diseased *D. stramonium* var. *tatula*; these aphids were then transferred to Chinese cabbage plants which are immune to leafroll. At 24 hours intervals groups of aphids were singly placed on *P. floridana* testplants and allowed an infection feeding period of 4 hours. The results of these experiments showed that the number of leafroll diseased testplants increased with the time the aphids had stayed on the Chinese cabbage. From these observations DAY concluded that 'the virus concentration in the haemolymph continued to increase after the ingested virus was distributed through the tissues of the vector'.

STEGWEE and PONSEN (1958) demonstrated that, when nonviruliferous *M. persicae* were injected with saline extracts obtained from viruliferous aphids, nearly 50% of the injected aphids became infective and transmitted the virus to *P. floridana* seedlings after a latent period of approximately 20 hours. When, instead of undiluted extracts, saline dilutions were used as inoculum, the latent period of PLRV in its vector could be prolonged to as much as 7–10 days. MUELLER and ROSS (1961) could obtain transmission when the aphids had fed constantly for at least 3 days after injection with haemolymph extracts from viruliferous aphids. These findings also point to virus multiplication in its vector.

On the other hand HARRISON (1958) reported the failure to detect any multiplication of PLRV in *M. persicae*. He showed that if aphids were allowed a 24 or 48 hours' acquisition feeding on a leafroll diseased potato, the presence of virus in extracts from these aphids could be demonstrated by injecting the extract into nonviruliferous aphids. This could be done only immediately after the acquisition feeding period, but after a period of 1–4 days on *B. rapa* he failed to show infectivity. According to HARRISON this should be taken as evidence in favour of a decrease in virus content of the aphids and against the possibility of virus multiplication. Experiments after the publication of these results showed that after a longer period on *B. rapa* the presence of virus could be detected with certainty (personal communication: HARRISON to STEGWEE, 1959). This indicates that the virus concentration in the aphid during the time on *B. rapa* must have increased. A support for this assumption is given by HARRISON (1957) who found that aphids after injection sometimes transmitted leafroll to testplants within the first day after injection; usually however, transmission did not occur until the second or third day: aphids transmitted the virus up to 20 days after they were injected.

In 1958 STEGWEE and PONSEN confirmed the multiplication of PLRV in *M. persicae*. They succeeded in serial transmission experiments, in which injections of haemolymph were practised. Nonviruliferous aphids, maintained on Chinese cabbage, were injected with haemolymph of viruliferous aphids and kept for the following 7 days on Chinese cabbage. Afterwards small amounts of haemolymph were taken from these injected aphids and in turn injected into another group of nonviruliferous aphids. This procedure was repeated 15 times at weekly intervals. In every passage the presence of virus could be demonstrated by removing a few injected aphids from Chinese cabbage, and testing

10   *Meded. Landbouwhogeschool Wageningen 72-16 (1972)*

them on *P. floridana* seedlings. After 15 serial passages it was found that the theoretical dilution of the original virus inoculum amounted to approximately 10^{-21}. In view of the fact that the haemolymph of viruliferous *M. persicae* diluted beyond 10^{-4} no longer rendered nonviruliferous aphids infective, they concluded that the virus had multiplied in the injected aphids during the 15 serial passages. It is interesting to note that the dilution end-point of juices of leafroll diseased *P. floridana* also amounted to 10^{-4} (MURAYAMA and KOJIMA, 1965). A detailed explanation about PLRV multiplication in its vector was later on given by STEGWEE (1961).

Summarizing it appears that PLRV circulates and multiplies in its vector, *M. persicae*; this enables us to explain the characteristic way of transmission. The question where in the aphid virus multiplication takes place is not yet explained. It does not seem unacceptable to suppose that wherever the virus is multiplying in the tissues of the aphid cytopathogenic changes are produced.

Many observers have attempted to find changes in the tissues from viruliferous *M. persicae* by histological sections. BLATTNY (1931) reported a difference in the structure of the cytoplasm in the salivary glands of PLRV infected *M. persicae*. The nonviruliferous aphids all had a clear zone around,the nuclei, which was lacking in some viruliferous aphids. On the other hand VAGO (1958) could only detect a difference in the structure of the fat cells of viruliferous aphids and nonviruliferous ones. The fat cells from aphids infected with PLRV exhibited hypertrophy of the cytoplasm, which coincided with deformations of the nucleus; this phenomenon was not observed in nonviruliferous aphids. In ultra-thin sections from viruliferous aphids 'plages' occurred in the cytoplasm of the fat cells, which were not found in nonviruliferous aphids. SCHMIDT (1959) reported the presence of stellate nuclei in the fatbody of viruliferous aphids which were lacking in nonviruliferous ones. According to SCHMIDT the most important difference between viruliferous and nonviruliferous aphids was found in the stomach cells. The nuclei in the stomach cells of viruliferous *M. persicae* were bigger; in these enlarged nuclei the chromatin was lacking, and besides the nucleolus several small partly dark-coloured, partly refractive bodies provided with vacuoles were seen. However, FORBES (1964a) demonstrated the same bodies in the nuclei of the epithelial cells in the stomach of nonviruliferous aphids. Previously PONSEN (STEGWEE, 1960) working with adult *M. persicae*, was unable to detect any significant histological differences between viruliferous and nonviruliferous aphids. The phenomena observed by the above-mentioned authors are not necessarily cytopathogenic alterations as a result of virus multiplication; they may be also due to the age of the aphid, because the histological investigations were performed on aphids of unknown age.

Using electron microscopy HEINZE (1955) found in the haemolymph of viruliferous *M. persicae* long threads, which varied between 1.66 and 5 μ, with a diameter of about 17.5 nm. Ultra-thin sections from salivary glands of viruliferous *M. persicae* revealed the presence of rod-shaped inclusions (30 × 200

nm), which were not to be seen in nonviruliferous aphids (MOERICKE, 1961). Later on MOERICKE (1963) described the occurrence of the same inclusions in nonviruliferous *M. persicae*. Besides in the salivary glands he could detect these rod-shaped inclusions in the cells of the gut and in those of the follicle epithelium. However, FORBES (1964b) reported that in the stomach of *M. persicae* infected with PLRV, masses of rod-shaped viruslike particles occurred within the cytoplasm and outside the cell at the haemocoel surface; similar particles were absent in stomachs of nonviruliferous aphids. None of the observers mentioned the possible presence of spherical particles in the investigated tissues (e.g. haemolymph, salivary glands, gut, and follicle epithelium) from viruliferous *M. persicae*, which would not occur in those from nonviruliferous ones.

Many attempts have been made to isolate PLRV from infected plants and viruliferous *M. persicae*. By means of the electron microscope BAWDEN and NIXON (1951) could not detect any difference between purified juice from healthy and that from leafroll diseased potato plants and *D. stramonium*. SPRAU (1952) demonstrated long threads of 150–4250 nm in the juice from leafroll diseased potato plants which could not be observed in juice from healthy ones. The same results were obtained by HEINZE (1955) for leafroll diseased *P. floridana*, *D. stramonium*, and *S. tuberosum*. However, DAY and ZAITLIN (1958) could not confirm the results of SPRAU and HEINZE. Purified extracts from leafroll diseased *P. floridana* plants showed the presence of many spherical particles with a diameter of about 10–20 nm which were not to distinguish from those in healthy controls. According to DAY and ZAITLIN the extracts from leafroll diseased plants appeared to be infective after injection in nonviruliferous aphids which were allowed to feed on *P. floridana* testplants. HILLE RIS LAMBERS (1959) could observe spherical particles in the sieve-tube sap from both leafroll diseased and healthy *P. floridana*; similar results were obtained with dippreparations from both these plants. Only the sieve-tube sap obtained from cut stylets of *M. persicae* feeding on leafroll diseased *P. floridana* appeared to be infective when it was injected into nonviruliferous aphids (PONSEN and STEGWEE, 1960). Infectivity of *M. persicae* macerates was demonstrated by several investigators (DAY, 1955; HARRISON, 1958; STEGWEE and PONSEN, 1958; STEGWEE and PETERS, 1961; MURAYAMA and KOJIMA, 1965). It was PETERS (1967a, b) who isolated the PLRV from viruliferous *M. persicae* macerates and described it as being a spherical particle with a diameter of about 23 nm and showing a hexagonal outline. Similar results were obtained from leafroll diseased *P. floridana* plants (KOJIMA et al., 1968, 1969). They also found these particles in ultra-thin sections of *P. floridana* which usually appeared in degenerated phloem cells of petioles and veins of diseased leaves.

3. GENERAL MATERIALS AND METHODS

In this chapter the general materials and methods which were used throughout this study will be described. Additional procedures and techniques will be mentioned in the relevant chapters.

Virus

The experiments were carried out with the isolate of PLRV used by STEG-WEE and PONSEN (1958). This virus was maintained in *Physalis floridana* RYDB. by repeated aphid transfers. To produce uniform leafroll diseased plants, virus-free larval aphids reared on Chinese cabbage (*Brassica pekinensis* RUPR. cv. *Granaat*) were allowed to feed for 24 hours on a leafroll source (*P. floridana* plants about 5 weeks old), after which they were placed singly for another 24 hours on one-leaf-stage seedlings of *P. floridana*.

The symptoms caused by this isolate on *P. floridana* were already described by PETERS (1967b) as moderate. According to him this isolate contaminated with 'virus-like particles', which did not appear to be pathogenic for several plants, e.g. *P. floridana* and Chinese cabbage.

Aphid

All experiments were done with the aphid vector *Myzus persicae* SULZ. A stock culture of virus-free aphids, maintained on Chinese cabbage which is immune for PLRV (MACCARTHY, 1954; DAY, 1955; MIYAMOTO and MIYA-MOTO, 1971), was kept in the Laboratory of Virology. In winter the aphids were exposed to additional light during 18 hours a day.

The plants with the virus-free aphids were placed in an aphid-proof cage. At regular times the plants which were crowded with aphids, were replaced by aphid-free ones to prevent stunting and the occurrence of winged aphids by overcrowding.

Throughout this study apterous viviparous aphids were used. New-born larvae were picked up from excised leaves of *P. floridana* or Chinese cabbage plants with a fine paint brush under a dissecting microscope to prevent damage.

Plants

P. floridana was used as a test plant for PLRV and as a virus source. Seeds were germinated in seed-boxes with steamed soil under double-glass on bottom-heating of 25–28 °C, and planted singly into 10-cm pots with steamed soil as soon as the cotyledons developed. Chinese cabbage was germinated in pots; after the development of the cotyledons the plants were transplanted singly into 10-cm pots. Before use the flats and pots were steamed during 30 minutes at 100 °C, and the soil during 1 hour at the same temperature.

The plants were maintained in an insect-proof greenhouse provided with thermostatically controlled heating and cooling at a temperature varying between 18° and 22°C and with a relative humidity of 60–80 %. During winter the plants received additional light to provide a day-length of 16 hours. The plants were covered with perspex cages (8 × 13.5 cm) during the acquisition

and infection feeding period; daily each test plant was examined during the infection feeding to determine whether or not the aphid was alive. When larvae were dead or lost within the fixed infection feeding period, these plants were not counted. At the end of the infection feeding periods the aphids were removed from the test plants and killed. The inoculated test plants were kept for 5 weeks in an insect-proof greenhouse and examined for symptoms. Healthy seedlings were interspaced among the inoculated test plants as controls. At regular intervals the plants were fumigated with different insecticides.

4. THE ANATOMY OF MYZUS PERSICAE

INTRODUCTION

The green peach aphid, *Myzus persicae*, is a vector of PLRV and a host for its multiplication. To investigate the site of virus multiplication in this vector it is necessary to study its anatomy. MORREN (1836), who was the first to publish on the anatomy of *Aphis persicae* (= *Myzus persicae* SULZ.), paid special attention to the alimentary, respiratory, and reproductive system, intra-ovarian development of the embryo, and cornicles. More than a century later, a brief description of the anatomy of this aphid was given by SCHMIDT (1959). Since then, detailed histological studies with the electron microscope have been performed on the stylets (VAN HOOF, 1957; FORBES, 1966, 1969), alimentary system (FORBES, 1964a), salivary glands (WOHLFARTH-BOTTERMANN and MOERICKE, 1959, 1960; MOERICKE and WOHLFARTH-BOTTERMANN, 1960a, b, c), pericardial cells (BOWERS, 1964), and mycetome (HINDE, 1971a, b).

In the present study the anatomy is investigated of larval stages. Much information was obtained from the work of WITLACZIL (1882), MORDWILKO (1895), DAVIDSON (1913, 1914), WEBER (1928), and ROBERTI (1946) on other aphids, and of PESSON (1944) on coccoids. Aphid names have been used in the text in the spelling of the respective authors who applied them. The modern names are summarized in Table 9 (page 94-98).

MATERIALS AND METHODS

New-born larvae of *M. persicae* were selected from a virus-free colony on Chinese cabbage and transferred to one-leaf-stage Chinese cabbage seedlings. The culture of Chinese cabbage plants has been described in chapter 3. Each seedling received 5–10 larvae. This procedure was repeated in several parallels in order to get enough aphids of the same age. The individually caged plants were kept in a cabinet at 20°C (PONSEN, 1969) and the aphids spent their whole life on the same cabbage plants. After 1 day, 3, 5, 7, and 9 days the larvae were removed from the plants to prepare them for serial paraffin sections and cornicle secretion smears. These intervals were chosen because of the irregular moults of the larvae.

Light microscopy
The larvae were fixed in DUBOSQ BRASIL's fluid. Prior to embedding in paraffin the larvae were dehydrated with ethanol, cleared in methyl benzoate followed by methyl benzoate celluidin (2%) for 3 days or longer. Paraffin sections of 5 μ thickness were made. They were finally stained with EHRLICH's haematoxylin for 5 minutes, and then rinsed in tap-water, SCOTT's solution for 2 minutes,
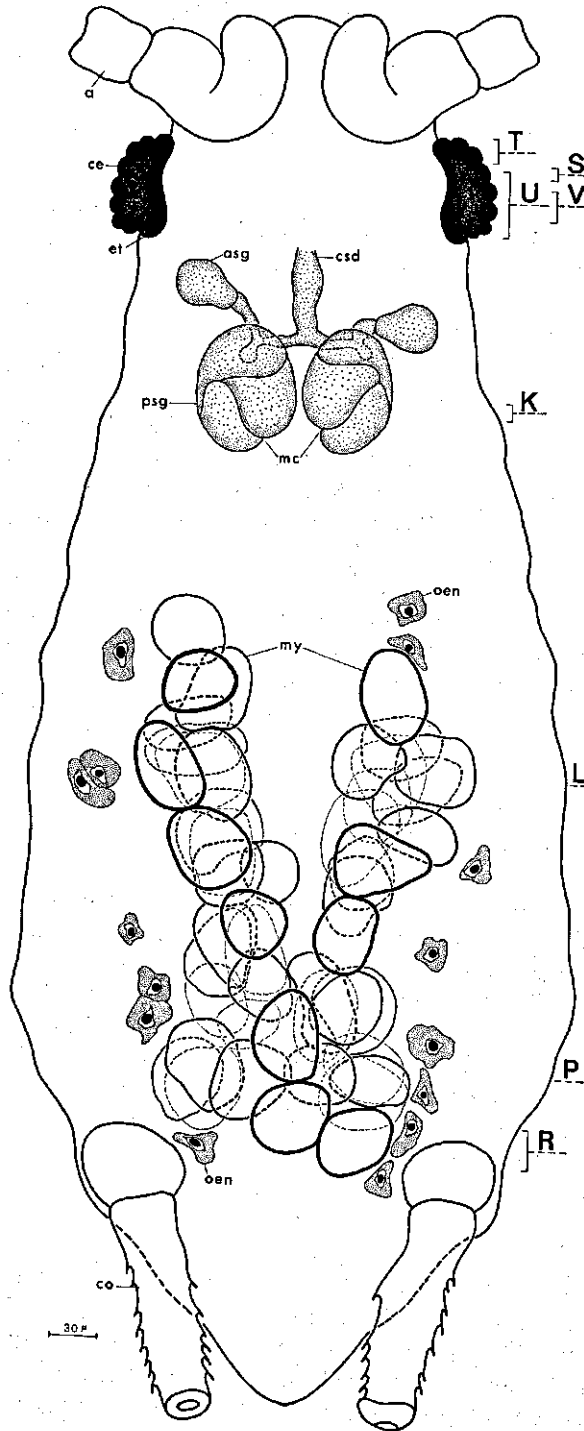
FIG. 1. Dorsal aspect of a graphic reconstruction of a one day old *Myzus persicae* larva showing position of cornicles (co), salivary glands and their myoepithelioid cells (mc), mycetome (my), and oenocytes (oen). The letters K, L, P, R, S, T, U, and V correspond with transverse sections given in Figs. 20, 25–28, and 30–32. For explanation of abbreviations see pages 144-147.
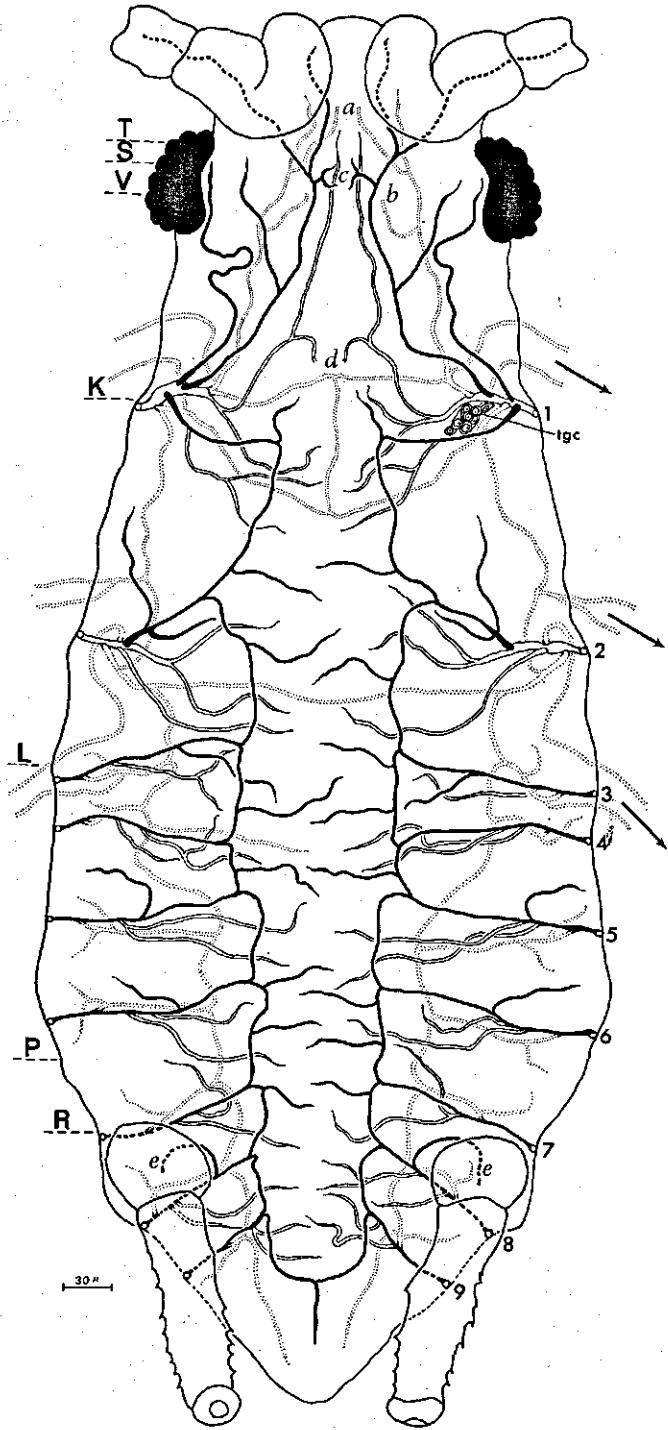
FIG. 2. Graphic reconstruction of the tracheal system of a one day old *Myzus persicae* larva. The tracheae (a) run to the clypeus, (b) to the clypeo-labrum, (c) to the corpora cardiaca, (d) to the proboscis, and (e) to the cornicles. The latter structures are outgrowths of the fifth abdominal segment. 1–2, meso and metathoracic spiracles; 3–9, abdominal spiracles. The arrows represent the three pairs of legs. Note the situation of the thoracic gland cells (tgc). The tracheal system of *M. persicae* agrees largely with that of *Acyrthosiphon malvae* (WITLACZIL, 1882) and *Pentatrichopus tetrarhodus* (GROVE, 1909).

and distilled water. To remove the excess of haematoxylin the sections were differentiated in 0.25% hydrochloric acid for 30 seconds and washed in running tap-water of 25°C during 30 minutes. Counterstaining was done in a 5% aqueous solution of eosin during 5 minutes; the sections were directly transferred to 70%, 90%, 96% ethanol, methyl benzoate, xylene, and mounted in xylene-dammar.

The cornicle ejection was produced by pinching somewhat the head of the aphid with watchmaker's forceps. This results in the elevation of the cornicles, followed immediately by the production of a droplet which was smeared on a slide by moving the aphid slowly in one direction. The cornicle secretion smears were dried at room temperature and stained with LEISHMAN's solution for 15 minutes (CARLETON and DRURY, 1957). Then the smears were rinsed twice in methyl alcohol for 3 seconds, blotted with filterpaper, cleared in xylene, and mounted in xylene-dammar.

The sections were examined under a WILD microscope equipped with a universal phase contrast condenser and fluotar phase objectives. The drawings were made with a WILD drawing tube at a magnification of 600 or 1500 times. The morphology of the various organs was determined from dissections, and by graphic reconstruction from a complete series of camera lucida pictures of serial sections of whole larvae.

Electron microscopy

The procedure to remove the salivary glands from larvae has been described in chapter 5. Dissections were carried out in 0.1 M SÖRENSEN phosphate buffer (pH 6.8). The glands were prefixed in 0.1 M phosphate buffered (pH 6.8) 6% glutaraldehyde for 1.5 hours, washed in phosphate buffer, postfixed in 0.1 M phosphate buffered 2% osmium tetroxide for 1.5 hours, and finally washed in phosphate buffer (MOHR and COCKING, 1968), in which they can remain overnight at 4° C. After fixation the glands were dehydrated in 70%, 80%, 90%, and 96% ethanol and stained by a mixture of 3 ml 0.5% uranyl nitrate in ethanol-acetone (1:1) and 7 ml saturated lead acetate in ethanol-acetone (1:1) for 1.5 hours (KUSHIDA and FUJITA, 1966). After staining the glands were washed during 5–10 minutes in ethanol-acetone and absolute ethanol. Prior to embedding in styrene methacrylate (2:3) the glands were put in a mixture of styrene methacrylate in absolute ethanol (1:1) for 1 hour, styrene methacrylate for 1 hour, and styrene methacrylate with initiator for 1–3 days at 4°C. Final embedding was done in gelatin capsules using a fresh lot of embedding medium. The blocks polymerized after approximately 3 days at 48°C. The embedding medium consisted of 2 volumes of styrene and 3 volumes of n-butyl methacrylate (KUSHIDA, 1961). The initiator was 3% w/v benzoyl peroxide.

Sections were cut on a LKB ultrotome 3 using glass knives and were expanded with chloroform vapor. The sections were picked up on formvar covered grids and examined in a SIEMENS Elmiskop 1 at 80 KV.

Alimentary canal

The most anterior part of the alimentary tract is the food canal of the maxillary stylets. From the stylets it passes into the pharyngeal duct which in turn leads into the pharynx. This structure passes upwards through the head, and leads into the foregut (oesophagus) to enter into the stomach, a sac-like dilation of the midgut. The stomach narrows towards its posterior end, and leads into the coiled intestine, from which the hindgut passes to a rectal canal terminating into the anal opening (Figs. 3, 5, and 11).

The entire gut is enclosed by a tunica propria which dorsally extends from the divaricator muscles in the posterior part of the pharyngeal pump and ventrally from the pharyngeal muscles originating from the tentorial bar (Fig. 5). It terminates in the muscles which control the anal opening (Fig. 12). Conspicuous flattened cells occur in this sheath; each of them contains a distinct ellipsoid-shaped nucleus with regular scattered basophilic chromatin and a spherical eosinophilic nucleolus. Around the foregut the sheath is very thick, but around the remaining part of the gut it is very small so that the nuclei protrude in the body cavity. The muscularis of the tunica propria is mainly build up of circular muscle fibres; the hindgut and the rectum muscularis contains also longitudinal muscle fibres. The latter parts of the gut show vigorous peristaltic movements by the action of these both muscle fibres. On the other hand, the peristalsis in the foregut and midgut is more slowly.

During larval life the entire gut retains the same position in the aphid's body cavity. The total length of the alimentary tract is 3 times as long as the aphid's body, and the gut increases in size proportionally to the aphid (Table 4).

Stylets. The stylet bundle lies in a dorsal groove running along all three segments of the proboscis (= labium) (Fig. 3). The fine structure of the stylets of *M. persicae* has been studied by VAN HOOF (1957) and FORBES (1966, 1969). There are two pairs of chitinous stylets, one maxillary, one mandibular. The maxillary pair is always firmly interlocked and appears as a single structure enclosing two minute canals formed by opposing grooves. The dorsal canal or food canal communicates with the pharyngeal duct, while the ventral one joins the salivary pump. The mandibular stylets are closely applied to the maxillary pair and each of them has a minute canal containing a nerve originating from the tritocerebral longitudinal tract (Figs. 4 and 32 V).

Each stylet originates from a retort-shaped organ (METSCHNIKOW, 1866) situated in the posterior part of the head. In the first instar of the larvae these organs are shaped like a flower-bud (Figs. 4, 30, and 31), but during growth of the larva they increase rapidly in size showing elongated, slightly bent structures. The retort-shaped organs consist of two layers of epithelial cells of which the outer layer is a continuation of the epidermis (Fig. 4). The inner layer passes into the matrix cells. These cells are responsible for the secretion of a new stylet at each larval moult. The matrix cells occupy the interior part of the retort-shaped organ and have a spindle-shaped form with a spherical or irregular

nucleus at their base. All these matrix cells show on their distal end a fine, long fibril of a chitinous substance. These fibrils unite to form the final stylet. Similar matrix cells are also found above the salivary and pharyngeal pump (Fig. 8 no. 11 and 13) secreting the cuticular parts of the latter organs.

There are two muscles attached to the basal end of each stylet: (a) a retractor muscle at the internal side originating from the tentorial bar, and (b) a protractor muscle inserting at the external side of the stylet and originating from the ventral wall of the maxillary sclerite (Fig. 31). More proximally on the external side the stylet possesses an insertion of a muscle originating from the lateral wall of the maxillary sclerite. On the internal side there are muscle fibres attached to the pharynx floor (Fig. 8 no. 12–13).

As shown in Fig. 7 no. 2, each stylet is conducted by a groove of the epipharynx on the ventral face of the clypeo-labrum and labrum. They are so arranged that the stylets fit snugly into the grooves, being held firmly in position, at the same time having perfect freedom of movement for protraction or retraction. After leaving the head (Fig. 7 no. 1) the stylets cross over into the longitudinal labial groove and extend along it to the extremity of the rostrum (Fig. 3 no. 1–4). The stylet bundle shows in its way a torsion of 180° which was also observed by SORIN (1966) and FORBES (1969), by RILLING (1960) for *Dactylosphaera vitifolii* (Table 9), and by WEBER (1929) for several psyllids. In *M. persicae* the labium telescopes to expose the stylets during feeding (BRADLEY, 1952; SORIN, 1966).

Pharynx. Morphologically the pharynx can be divided in three parts: the pharyngeal duct, valve, and pump (Figs. 5, 7, and 8), The pharyngeal duct is a prolongation of the food canal and is formed by the epipharynx and the hypopharynx lip (Fig. 7 no. 2–8). This portion does not exert any sucking action, and is simply a duct, which conveys the plant-juices into the pharyngeal pump. The epipharynx is marked by a thick sclerotized plate which also forms the floor of the clypeo-labrum. In longitudinal sections the epipharynx reveals a median row of eight sensillum pores (Fig. 6), which communicate with the pharyngeal duct (Fig. 7 no. 5–8). Behind the second pore the duct dilates abruptly and attains a complicated cross-section up to the valve. At the upper wall of this wide duct, on each side of the epipharynx, an invagination is observed (Fig. 7 no. 7–8), which runs as an arch from the third pore to the valve (Fig. 3); its lumen looks somewhat stellated. Close before the valve the floor of the wide duct passes from the hypopharynx lip into the hypopharynx. In the median line of the floor a narrow gutter runs from the food canal to terminate abruptly in a cup-shaped one which connects with that of the valve. The cup-shaped part of the floor before the valve is provided with two sensillum pores (Fig. 5; Fig. 8 no. 9–10).

The pharyngeal duct is separated from the pharyngeal pump by a valve, of which both the dorsal wall and ventral wall are marked by two cuticular dome-shaped prominences, the pharynx 'protuberanzen' named by KRASSILSTSCHIK (1893) and 'Mundknöpfe' by WEBER (1928). The dorsal wall of the valve is controlled by two pairs of divaricator muscles, each pair being attached to a tendon

20
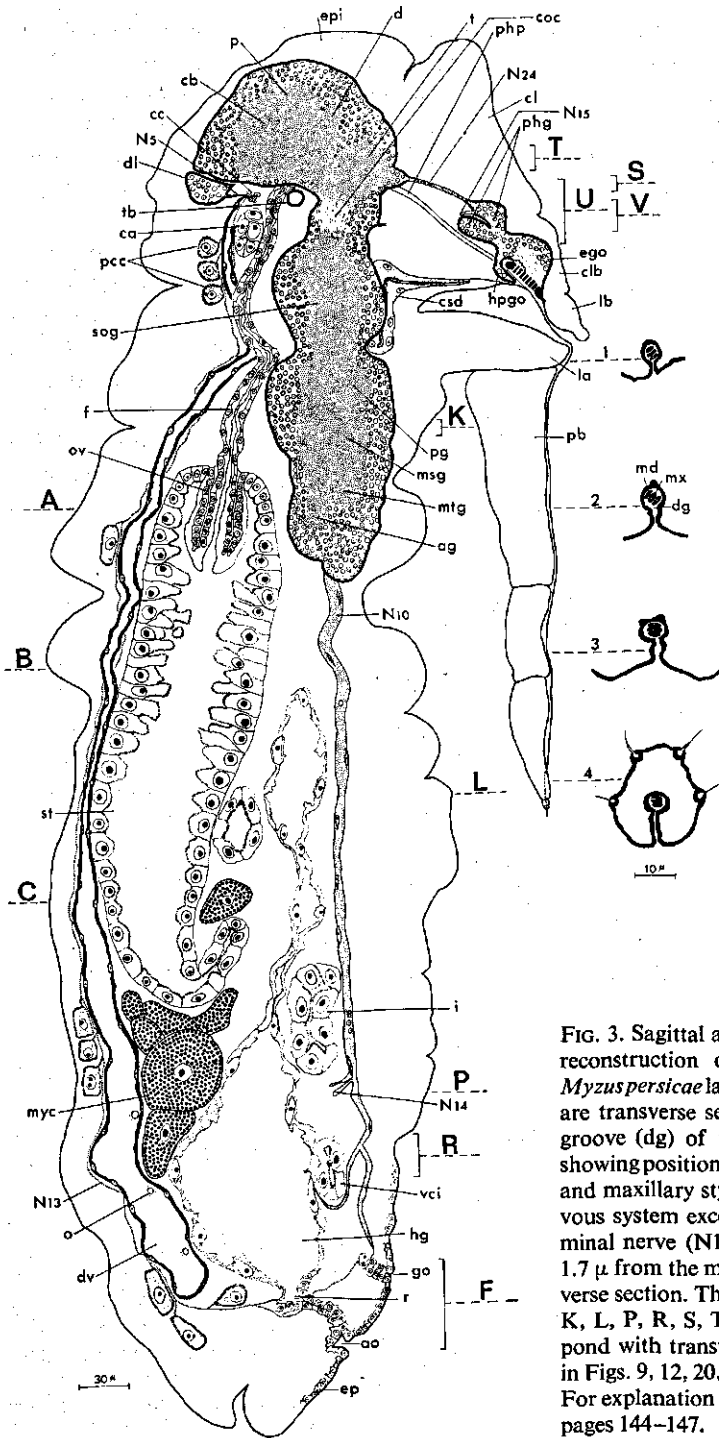
FIG. 3. Sagittal aspect of a graphic reconstruction of a one day old *Myzus persicae* larva. The figures 1–4 are transverse sections of the dorsal groove (dg) of the proboscis (pb) showing position of mandibular (md) and maxillary stylets (mx). The nervous system except the main abdominal nerve (N10) is reconstructed 1.7 μ from the midline of each transverse section. The letters A, B, C, F, K, L, P, R, S, T, U, and V correspond with transverse sections given in Figs. 9, 12, 20, 25–28, and 30–32. For explanation of abbreviations see pages 144–147.

(Figs. 5 and 8 no. 11–12). The muscles diverge from each side of this tendon and become attached bilaterally of the clypeo-labrum. On the lateral side of the valve a muscle (lateral pharyngeal valve muscle) is inserted originating from the lateral wall of the clypeus. In embryos the valve is open (Fig. 8 no. 10–12), but in larvae it is always found in a closed position (Fig. 8 no. 10′–11′). In the latter situation the opposite pharynx protuberances and the valve itself fit closely together.

The pharyngeal pump or sucking pump continues from the valve and traverses the middle region of the head through the oesophageal connectives to join the foregut in front of the tentorial bar (Fig. 5). It acts as a pumping organ to bring the liquid food through the food canal and to force it into the foregut. The movement of the flexible dorsal wall of the sucking pump is controlled by divaricator muscles. In the anterior part they are attached to 13 long tendons arising from the midline of the dorsal pump-wall (Fig. 8 no. 13). The muscles pass from each side of the tendon; the first 5 pairs of muscles become attached to the wall of the clypeo-labrum, while the next 8 pairs to the wall of
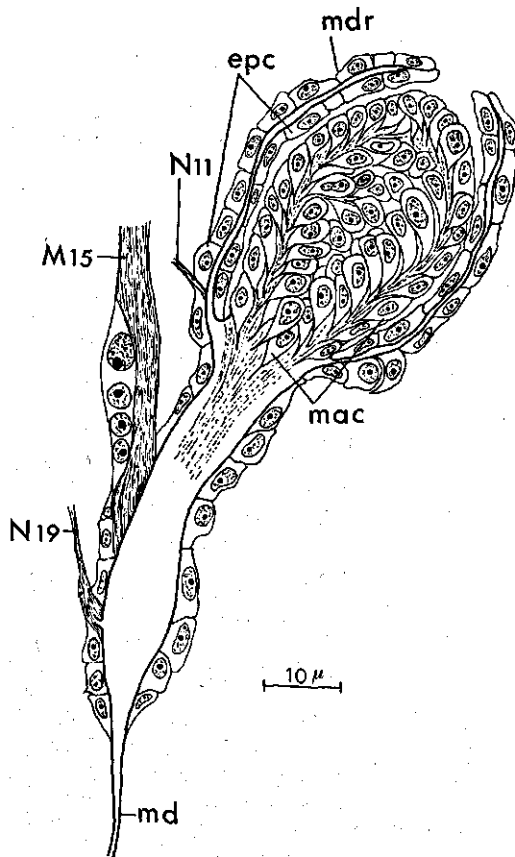

FIG. 4. Sagittal section of a mandibular stylet (md) and its retort-shaped organ (mdr) of a one day old *Myzus persicae* larva showing position of the epidermal cells (epc) and the matrix cells (mac.) For explanation of abbreviations see pages 144–147.

22

the clypeus (Figs. 5 and 6). The middle part of the pump is provided with short tendons placed in 14 pairs, each of them inserted with a muscle originating from the clypeal wall (Fig. 8 no. 14). In the posterior part of the pump 2 pairs of muscles are directly inserted on the dorsal wall originating from the wall of the epicranium (Fig. 8 no. 15). They run between the tritocerebral lobes and are innervated by branches from the recurrent nerve (Fig. 30). All dorsal pump muscles have their origin on each side of the mediodorsal line of the head. Close to the tentorial bar the floor of the pharyngeal pump is directly attached by muscles, one pair originating from the tentorial bar (ventral pharyngeal pump muscles), and one pair from the piston of the salivary pump (retractor muscle of salivary pump piston) (Figs. 5 and 19).

Foregut. The foregut (oesophagus) is a uniform thin tube which runs posteriad between the two principal salivary glands and dorsally of the suboesophageal ganglion to terminate into the oesophageal valve half-way the thoracic ganglion (Figs. 3 and 9). The foregut of the larva consists of a single layer of squamous epithelial cells resting upon a basement membrane (Fig. 32 Z). The cells contain homogeneous, basophilic cytoplasm in which occur few small vacuoles. The inner walls of the cells protrude into the lumen and are lined with an intima forming a stellate narrow lumen. At the connection with the midgut, where the foregut continues into the oesophageal valve, the lumen gradually dilates (Fig. 9). The cells of the larval foregut were observed to increase in size during larval life (Fig. 32 Z'). This fact agrees with the increase of the length and width of the foregut, and the absence of cell divisions; the latter fact corresponds with only a small variation in cell numbers during larval life (Table 4). By ageing the cells flatten and become situated around a wide lumen; the optical properties remain the same although the number of vacuoles increases somewhat. Their elliptical nuclei and spherical nucleoli which also increase in size (Table 4), retain their original structure, characterized by a regularly scattered basophilic chromatin and an eosinophilic nucleolus.

Oesophageal valve. The oesophageal valve marks the junction of the foregut and the stomach (Fig. 9). The role of the valve is to prevent regurgitation of ingested food from the stomach to the foregut (DAVIDSON, 1914; SMITH, 1931; WATSON, 1938; DAY and IRZYKIEWICZ, 1953), although it does not have any muscle fibres (WEBER, 1928; FORBES, 1964a). This was clearly demonstrated when in dissections the alimentary canal was cut half-way the foregut in dissecting fluid. Even then, no contents of the mid and hindgut were lost. Only after intersecting of the intestine, the contents of the alimentary canal flew out. Further it was observed that the valve has an apple-shaped structure closely adjacent to the anterior part of the stomach cells (WATSON, personal communication).

The valve consists of two layers of cells of which the inner layer is the continuation of the foregut into the stomach (Fig. 9A). This layer consists of squamous epithelial cells being somewhat flattened and of which the optical composition is similar to those of the foregut. The outer layer consists of cuboidal epithelial cells; they contain homogeneous basophilic cytoplasm in which occur

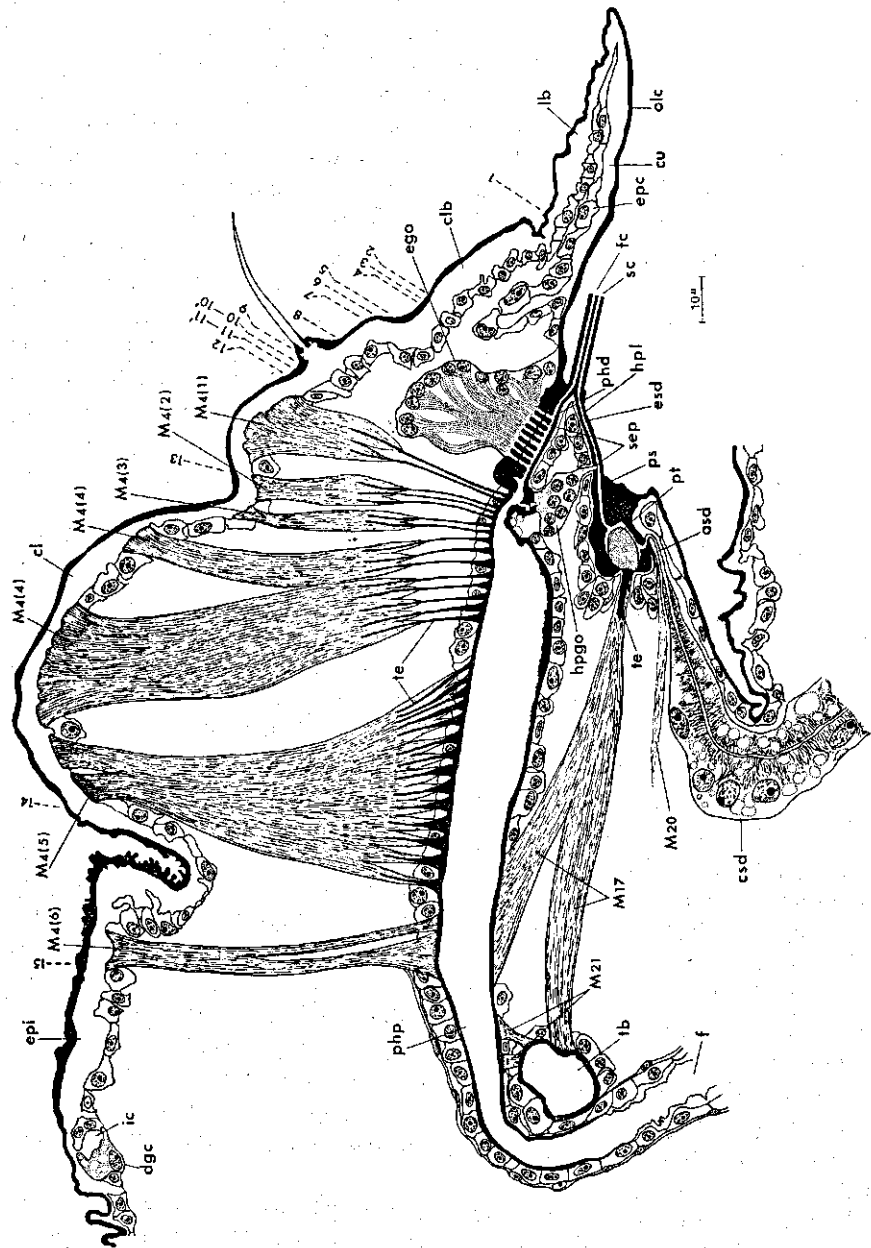FIG. 5. Sagittal section through the head of a seven days old *Myzus persicae* larva showing pharynx and salivary pump. The dotted lines indicate the plane through which the transverse serial sections 1–15 in Figs. 7 and 8 have passed. Note the situation of the sensillum pores (sep) in the epipharynx, pharyngeal duct, and on the foot of the pumpstem (ps). For explanation of abbreviations see pages 144–147.

vacuoles and strongly basophilic irregular-shaped globules peripherally in the apical part. Each cell contains a spherical nucleus which occupies a large part of the cell, with regular scattered basophilic chromatin and an eosinophilic nucleolus. The data obtained on the length and maximum width of the valve, and the just slight variation in cell numbers of the outer layer in each larval stage (Table 4) indicate that the cells increase in size during larval life (Fig. 10 A'). No cells were observed in division. The cells retain their shape and structure; their nuclei and nucleoli increase in size proportionally to that of the valve, and their optical composition remains similar. The two celltypes of the valve rest upon a basement membrane; this membrane is a continuation of that of the foregut and passes into the basement membrane of the stomach. The intima of the foregut continues in the region of the valve and terminates at the base of it (Fig. 9). It is a colourless mass without any structures.

MOERICKE and MITTLER (1966) reported the occurrence in the stomach of adult aphids (*M. persicae, Brevicoryne brassicae,* and *Lipaphis pseudobrassicae*; Table 9) of four chitinous cuticular linings of the foregut and oesophageal valve of each of the four larval instars. These four thread-like structures, which sometimes clearly have a distinct swelling at one end, pass back into the stomach at each moult and remain there throughout the life of the aphid.

Midgut. The midgut is differentiated into two regions, the first greatly enlarged and forming the stomach, the second tubular and forming the intestine (Fig. 11). The anterior part of the stomach is situated closely to the principal salivary glands above the metathoracic ganglion (Fig. 3). In the abdomen it extends nearly as far as the bridge of the mycetome. The second region of the midgut or intestine is coiled and terminates into the hindgut. The course of it is shown in Fig. 11. The epithelium of the entire midgut consists of a single layer of cells resting upon a basement membrane joining the epithelial cells of the valve. During larval life the midgut expands reaching at the ninth day a size of about twice the original value (Table 4). In the case of the stomach the growth is more in the width than in the length. Cell divisions are never observed in the midgut; this agrees with the fact that the total number of cells between the various ages is nearly equal. From this it can be concluded that the growth of the midgut is merely the result of increase in cell size. The stomach has relatively more cells than the intestine, although the length of the latter is about six times longer; the basal part of the intestinal cells is also more elongated than that of the stomach (Table 4). Each cell contains a nucleus with regular scattered basophilic chromatin and an eosinopholic nucleolus. The nuclei and nucleoli increase in size proportionally to the midgut size, and retain their structure; the nucleoli contain small vacuoles which increase in size and number.

In the stomach three regions may be distinguished which are well-defined histologically (Fig. 9). The anterior region (Fig. 9 A) consists of cuboidal epithelial cells which contain nonhomogeneous basophilic cytoplasm filled with very small vacuoles. The cells increase in size and become gradually flattened on their apical part during larval life (Fig. 10 A'). The spherical nuclei are situated in the centre of the cell and become transformed in oval-shaped
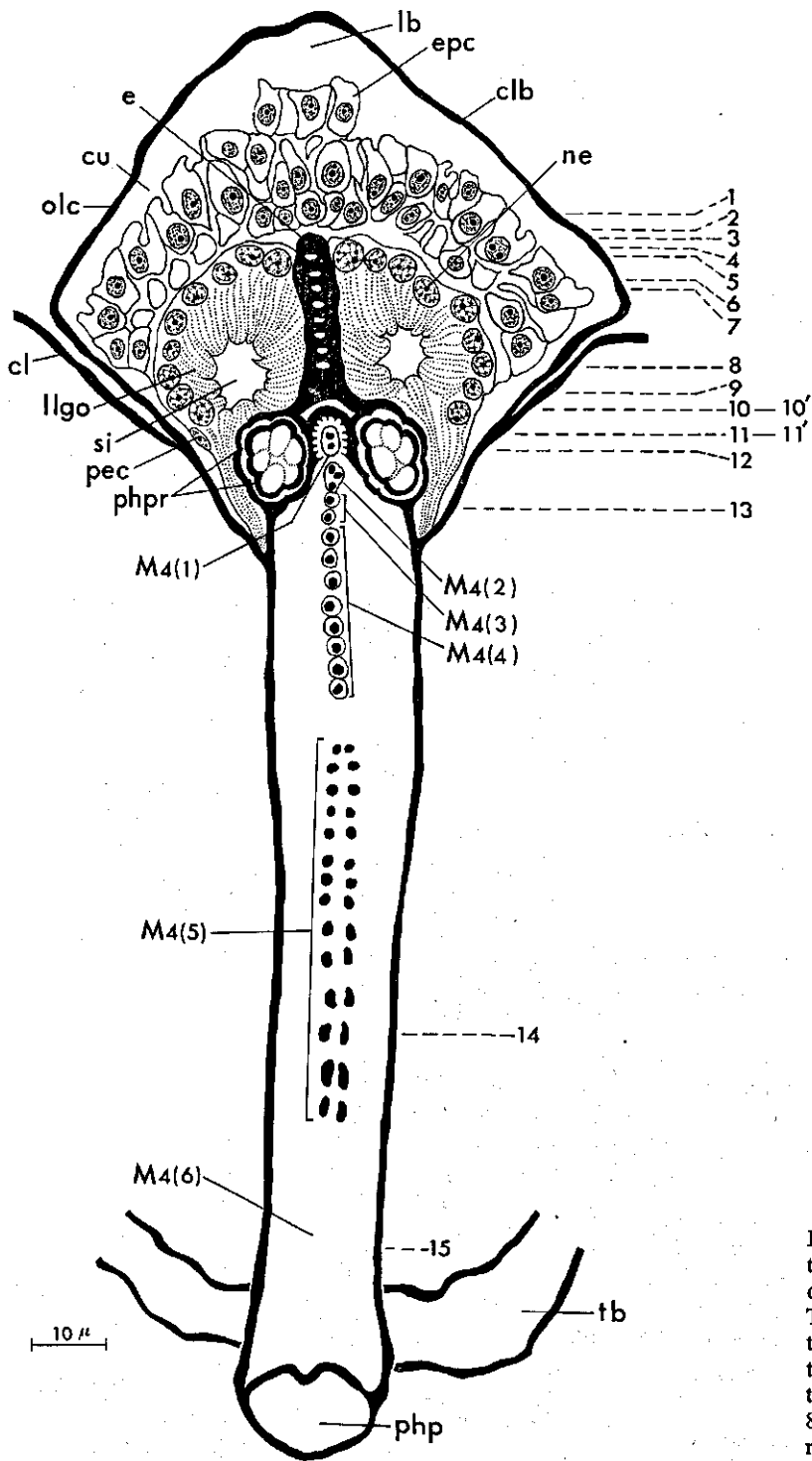
FIG. 6. Dorsal aspect of the pharynx of a five days old *Myzus persicae* larva. The dotted lines indicate the plane through which the transverse serial sections 1–15 in Figs. 7 and 8 have passed. For explanation of abbreviations see pages 144–147.

26

structures. The middle region of the stomach is occupied by tall, fingerlike columnar digestive cells, which protrude far into the lumen. They contain nonhomogeneous basophilic cytoplasm in which occur numerous vacuoles of various sizes and clusters of strongly basophilic granules, especially in the apical part of the cell (Fig. 9 B, 1). Subsequently the inner part of the cell constricts (2) by forming of a new striated border (3); the separated part surrounded by a striated zone is pushed off into the stomach lumen (4) and undergoes a gradual dissolution. The cell continues to increase in size (5) and during growth vacuoles and basophilic granules develop in the cytoplasm. This secretory cycle is repeated by all the cells in the middle region of the stomach during larval life and continues in the adult stage without degeneration of cells (Fig. 10 B'). Each cell contains a nucleus which retains its structure; the nuclei are situated mainly in the basal region of the cell. On the ninth day (just adult stage) in some nuclei strongly basophilic clusters of chromatin material develop (Fig. 10 B'). The epithelium of the posterior region of the stomach is cuboidal. These digestive cells contain nonhomogeneous basophilic cytoplasm in which occur some large vacuoles. The cells secrete material by forming buds which swell and extend between the rods of the striated border (Fig. 9 C). These small swellings become constricted at their base and dissolve into the lumen. The forming of buds continues during larval life and proceeds into the adult stage without degeneration of cells; the cells increase in size and retain their form and structure (Fig. 10 C'). The spherical nuclei in central position gradually develop into oval forms protruding into the stomach lumen.

The intestine is the tubular continuation of the stomach and can be divided histologically into two distinct parts. The first part is a small tube which runs from the stomach to the voluminous coil extending far into the abdomen (Fig. 11); from there it passes gradually into a broader one forming the second part of the intestine which terminates abruptly by constriction in the hindgut. The average diameter and length of both parts increases during larval life; the second part is about twice as long as the first part (Table 4). The large squamous cells of the first part protrude far into the lumen forming an irregular stellate narrow lumen (Fig. 11 D). They contain nonhomogeneous basophilic cytoplasm with some vacuoles and basophilic globules; their nuclei are spherical to ovoid. In the second part of the intestine (Fig. 11 E) the cells are situated around a wide lumen; they are strongly vacuolated and the cytoplasm is homogeneous basophilic. Their nuclei and nucleoli are large and ovoid.

The basal cytoplasm of the entire midgut epithelial cells is finely striated, and their free surface is lined by a striated border.

Hindgut. The hindgut starts in the mesothorax and runs caudad ventrally of the stomach and dorsally of the voluminous coil of the intestine towards a rectal canal (Fig. 11). The structure of the wall of the hindgut differs from that of the midgut. It is highly transparent and dilates gradually to reach its maximum size just behind the mycetome bridge (Fig. 3). The larval hindgut consists of a single layer of long squamous epithelial cells resting upon a basement membrane. During larval life a considerable part of the cells is occupied by vesicles sur-

FIG. 7. Transverse serial sections 1–8 taken from embryos in a nine days old larva and correspond with the numbers given in Figs. 5 and 6. For explanation of abbreviations see pages 144–147.

rounded by clusters of basophilic granules (Fig. 26 P). Inside the vesicles waxy droplets occur which increase in number by ageing of the larvae. The waxy droplets originating from degenerating fat cells are also present in the lumen and against the inner wall of the hindgut. Their optical image is similar to that present in degenerating fat cells and in the haemolymph. In the haemolymph they are scattered throughout the body cavity and a considerable number of
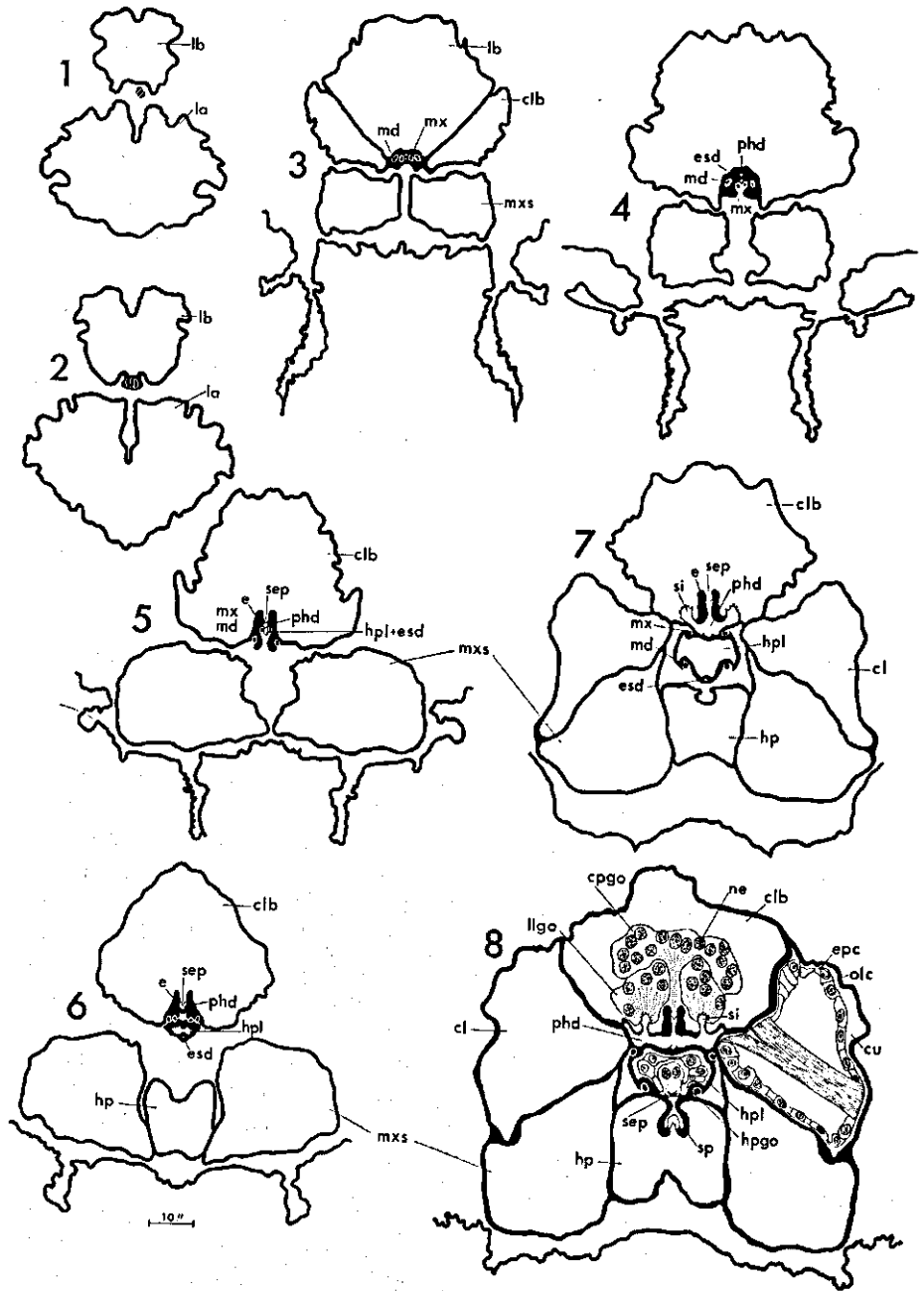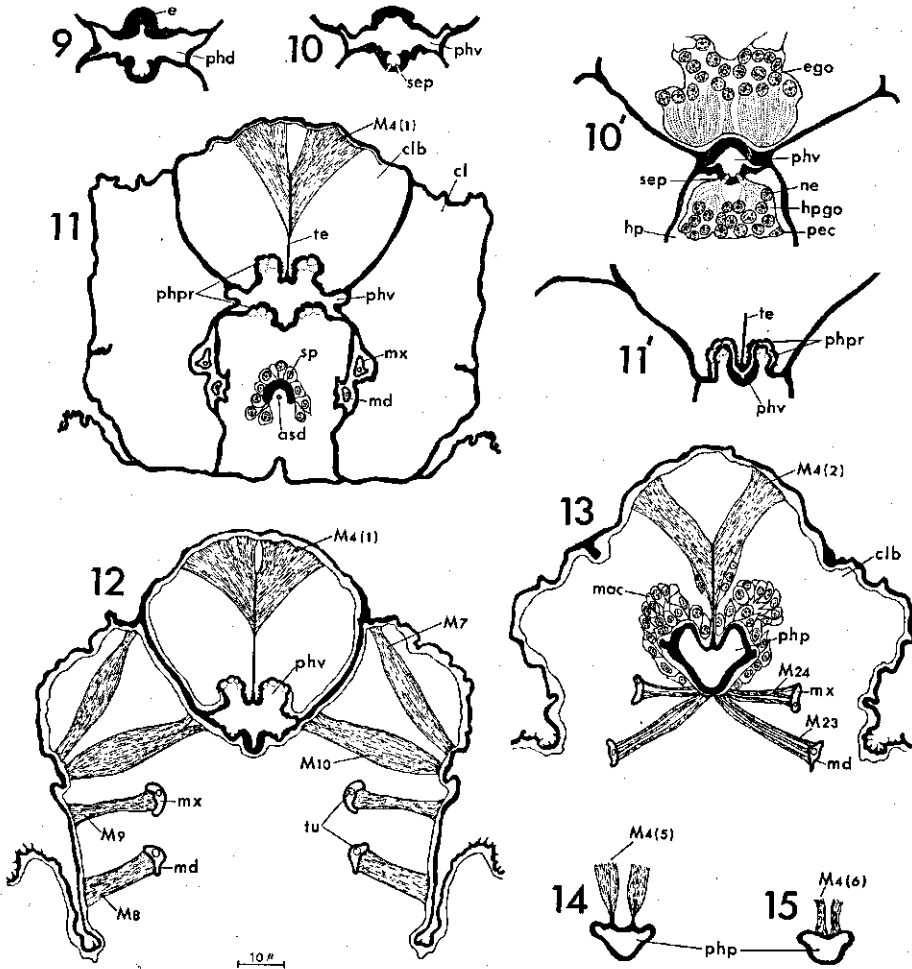

FIG. 8. Transverse serial sections 9–15 taken from embryos in a nine days old larva and correspond with the numbers given in Figs. 5 and 6. In the sections 10–12 the pharyngeal valve is in an open position, whereas in the transverse sections 10′ and 11′ it is in a closed position. These both sections are taken from a one day old larva. For explanation of abbreviations see pages 144–147.

TABLE 4. Actual sizes and cell numbers of the gut of *Myzus persicae* larvae bred on Chinese cabbage seedlings at 20 °C. The values are given in μ.

Age	One day old[1] larva	Three days old larva	Five days old larva	Seven days old larva	Nine days old larva
Larva					
length	865 (1)	1340 (1.5)	1320 (1.5)	1435 (1.7)	1750 (2.0)
maximum width	267 (1)	566.7 (2.1)	608.3 (2.3)	666.7 (2.5)	783.3 (2.9)
total length of gut	2425 (1)	4110 (1.7)	3805 (1.6)	4306 (1.8)	5465 (2.3)
length intestine/length stomach	4.6	5.4	5.6	4.6	5.7
length 2e part intestine/length 1e part intestine	1.9	2.2	2.1	2.0	2.1
length gut/length larva	2.8	3.1	2.9	3.0	3.1
Foregut[2]					
length	210 (1)	310 (1.5)	275 (1.3)	270 (1.3)	310 (1.5)
average diameter	16.2 ± 0.42 (1)	20.3 ± 0.38 (1.3)	22.0 ± 0.33 (1.4)	26.5 ± 0.62 (1.6)	28.0 ± 0.35 (1.7)
number of cells	82	78	80	77	75
average diameter nucleus	3.1 ± 0.08 (1)	3.4 ± 0.11 (1.1)	3.8 ± 0.11 (1.2)	4.7 ± 0.15 (1.5)	4.6 ± 0.16 (1.5)
average diameter nucleolus	0.9 ± 0.04 (1)	1.0 ± 0.07 (1.1)	1.1 ± 0.07 (1.2)	1.3 ± 0.06 (1.4)	1.3 ± 0.03 (1.4)
Oesophageal valve					
length	70 (1)	100 (1.4)	80 (1.1)	80 (1.1)	85 (1.2)
maximum width	23.3 (1)	35.0 (1.5)	33.3 (1.4)	47.5 (2.0)	47.5 (2.0)
number of cells[3]	104	114	104	112	109
average diameter nucleus[3]	3.1 ± 0.17 (1)	4.1 ± 0.14 (1.3)	4.6 ± 0.13 (1.5)	4.5 ± 0.06 (1.5)	4.6 ± 0.17 (1.5)
average diameter nucleolus[3]	0.9 ± 0.03 (1)	1.2 ± 0.07 (1.3)	1.3 ± 0.05 (1.4)	1.3 ± 0.06 (1.4)	1.3 ± 0.03 (1.4)
Midgut (stomach and intestine)					
length	1750 (1)	3030 (1.7)	2685 (1.5)	3096 (1.8)	3965 (2.3)
number of cells	611	605	598	600	605
Stomach					
length	310 (1)	470 (1.5)	405 (1.3)	555 (1.8)	590 (1.9)
maximum diameter	73.8 (1)	148.3 (2.0)	147.5 (2.0)	176.7 (2.4)	200.0 (2.7)
Anterior region of the stomach					
number of cells	79	81	95	75	79
average diameter nucleus	4.9 ± 0.12 (1)	6.3 ± 0.12 (1.3)	6.9 ± 0.18 (1.4)	7.3 ± 0.25 (1.5)	7.4 ± 0.19 (1.5)
average diameter nucleolus	2.1 ± 0.09 (1)	2.8 ± 0.09 (1.3)	3.1 ± 0.09 (1.5)	2.9 ± 0.12 (1.4)	3.1 ± 0.07 (1.5)

30

	200	205	211	213	212
Middle region of the stomach					
number of cells	200	205	211	213	212
average diameter nucleus	8.3 ± 0.16 (1)	10.7 ± 0.30 (1.3)	10.7 ± 0.12 (1.3)	15.5 ± 0.65 (1.9)	15.1 ± 0.45 (1.8)
average diameter nucleolus	3.9 ± 0.12 (1)	4.5 ± 0.15 (1.2)	4.2 ± 0.07 (1.1)	5.9 ± 0.35 (1.5)	5.5 ± 0.28 (1.4)
Posterior region of the stomach					
number of cells	52	51	47	52	49
average diameter nucleus	7.0 ± 0.11 (1)	9.3 ± 0.23 (1.3)	10.5 ± 0.21 (1.5)	13.5 ± 0.46 (1.9)	13.6 ± 0.41 (1.9)
average diameter nucleolus	3.1 ± 0.07 (1)	4.0 ± 0.09 (1.3)	4.6 ± 0.09 (1.5)	5.3 ± 0.15 (1.7)	5.3 ± 0.19 (1.7)
Intestine					
First part					
length	495	805	730	841	1095
average diameter	21.0 ± 0.47 (1)	32.5 ± 0.60 (1.5)	42.3 ± 1.35 (2.0)	47.2 ± 0.97 (2.2)	44.3 ± 1.93 (2.1)
number of cells	113	90	81	86	88
average diameter nucleus	6.8 ± 0.11 (1)	9.1 ± 0.16 (1.3)	9.4 ± 0.15 (1.4)	12.2 ± 0.46 (1.8)	12.6 ± 0.61 (1.9)
average diameter nucleolus	3.1 ± 0.08 (1)	3.9 ± 0.10 (1.3)	3.7 ± 0.15 (1.2)	5.1 ± 0.17 (1.6)	4.5 ± 0.13 (1.5)
Second part					
length	945	1755	1550	1700	2280
average diameter	29.7 ± 0.80 (1)	53.5 ± 0.63 (1.8)	50.2 ± 0.73 (1.7)	53.7 ± 1.45 (1.8)	64.8 ± 1.15 (2.2)
number of cells	164	178	164	174	177
average diameter nucleus	9.4 ± 0.19 (1)	12.8 ± 0.31 (1.4)	12.7 ± 0.48 (1.4)	13.3 ± 0.65 (1.4)	15.2 ± 0.53 (1.6)
average diameter nucleolus	4.1 ± 0.05 (1)	5.1 ± 0.17 (1.2)	4.6 ± 0.08 (1.1)	5.1 ± 0.22 (1.2)	5.4 ± 0.19 (1.3)
Hindgut					
length	425	690	765	850	1085
maximum diameter	68.3 (1)	82.5 (1.2)	103.3 (1.5)	103.3 (1.5)	101.7 (1.5)
number of cells	74	74	74	79	74
average diameter nucleus	9.7 ± 0.39 (1)	13.0 ± 0.32 (1.3)	14.0 ± 0.40 (1.4)	17.0 ± 0.58 (1.8)	17.4 ± 0.88 (1.8)
average diameter nucleolus	2.2 ± 0.07 (1)	2.8 ± 0.08 (1.3)	2.9 ± 0.07 (1.3)	3.0 ± 0.09 (1.4)	3.3 ± 0.13 (1.5)
Rectum					
length	40	80	80	90	105
number of cells	39	38	37	37	35
average diameter nucleus	3.7 ± 0.11 (1)	4.9 ± 0.19 (1.3)	5.2 ± 0.14 (1.4)	5.1 ± 0.20 (1.4)	5.6 ± 0.13 (1.5)
average diameter nucleolus	1.2 ± 0.05 (1)	1.5 ± 0.06 (1.3)	1.5 ± 0.06 (1.3)	1.4 ± 0.05 (1.2)	1.7 ± 0.09 (1.4)

[1] The data are obtained from one larva
[2] Foregut and first layer of oesophageal valve
[3] Outer layer of oesophageal valve
In brackets relative sizes on basis of size at 1 day as 1
The sign ± is followed by the standard deviation of the mean $(s/\sqrt{n}; n = 10)$
The length is measured to the number of sections (each section is 5 μ thick)

them are situated against the tunica propria of the hindgut. The inner wall of the cells is strongly folded and coated by a delicate intima which continues in that of the rectum. Some parts of the cells show a mass of minute projections into the lumen and it was found that some of them are enclosed by a waxy droplet (Fig. 26 P and P'). The growth of the larval hindgut is a result solely of increase in cell size and not of cell division (Table 4). The long ellipsoid-shaped nuclei which project into the lumen, retain their regular scattered basophilic chromatin and a proportionally very small spherical eosinophilic nucleolus.

The rectum starts ventrally of the posterior end of the dorsal vessel to terminate as a narrow tube into the anal opening (Fig. 3). At the ventral side the hindgut passes into the rectum, dorsally it continues its course, to terminate in a dorsal rectal sac (Fig. 12 F). During larval life the rectal sac gradually disappears to form, together with the posterior part of the rectum, a rather straight duct. The rectal sac is innervated by a nerve originating from the medio-dorsal nervecord which runs alongside the dorsal vessel.

Histologically the rectum can be divided into two distinct parts, each part being covered with a single layer of epithelial cells resting upon a basement membrane. The anterior part of the rectum is composed of columnar cells quite different from the epithelial cells of the hindgut. The cells contain homogeneous, strongly basophilic cytoplasm with a proportionally big nucleus. The ovoid-shaped nuclei contain regularly scattered basophilic chromatin and a spherical eosinophilic nucleolus (Fig. 12 F). By ageing of the larvae the cells increase in size more than the nuclei (Fig. 12 G and H). The cytoplasm, in which vacuoles and strongly basophilic globules arise, becomes less basophilic. The vacuoles increase in size and number, so that the nuclei become somewhat pressed. The basal part of these cells shows laminated structures. The inner wall is coated by a delicate intima similar to that of the hindgut. On their surface small vesicles or exudations arise, which are being liberated into the lumen; it is possible that these cells have a secretory function, releasing their product by way of minute projections. Cell divisions are never observed and the total number of these cells remains nearly constant (Table 4).

The posterior part of the rectum can be considered as an invagination of the epidermis (Fig. 12 F). These cells are covered by a distinct intima which is a continuation of the outer layer of the cuticula of the body wall. Near the anal opening muscle fibres are attached to the intima, originating laterally and dorsally from the wall of the ninth abdominal segment or cauda. During larval life of *M. persicae* the cauda is obtusely rounded, broader at the base than long; the adult cauda is longer than broad and sub-conical to spoon-shaped (SYLVESTER, 1954).

FIG. 9. Graphic reconstruction of the stomach and oesophageal valve of a one day old *Myzus persicae* larva. A. Transverse section of the anterior region of the stomach with the oesophageal valve. B. Transverse section of the middle region of the stomach showing secretory cells which secrete by constriction of their apical parts. C. Transverse section of the posterior region of the stomach showing cells which secrete by forming of buds. The letters A-C correspond with those given in Fig. 3. For explanation of abbreviations see pages 144–147.
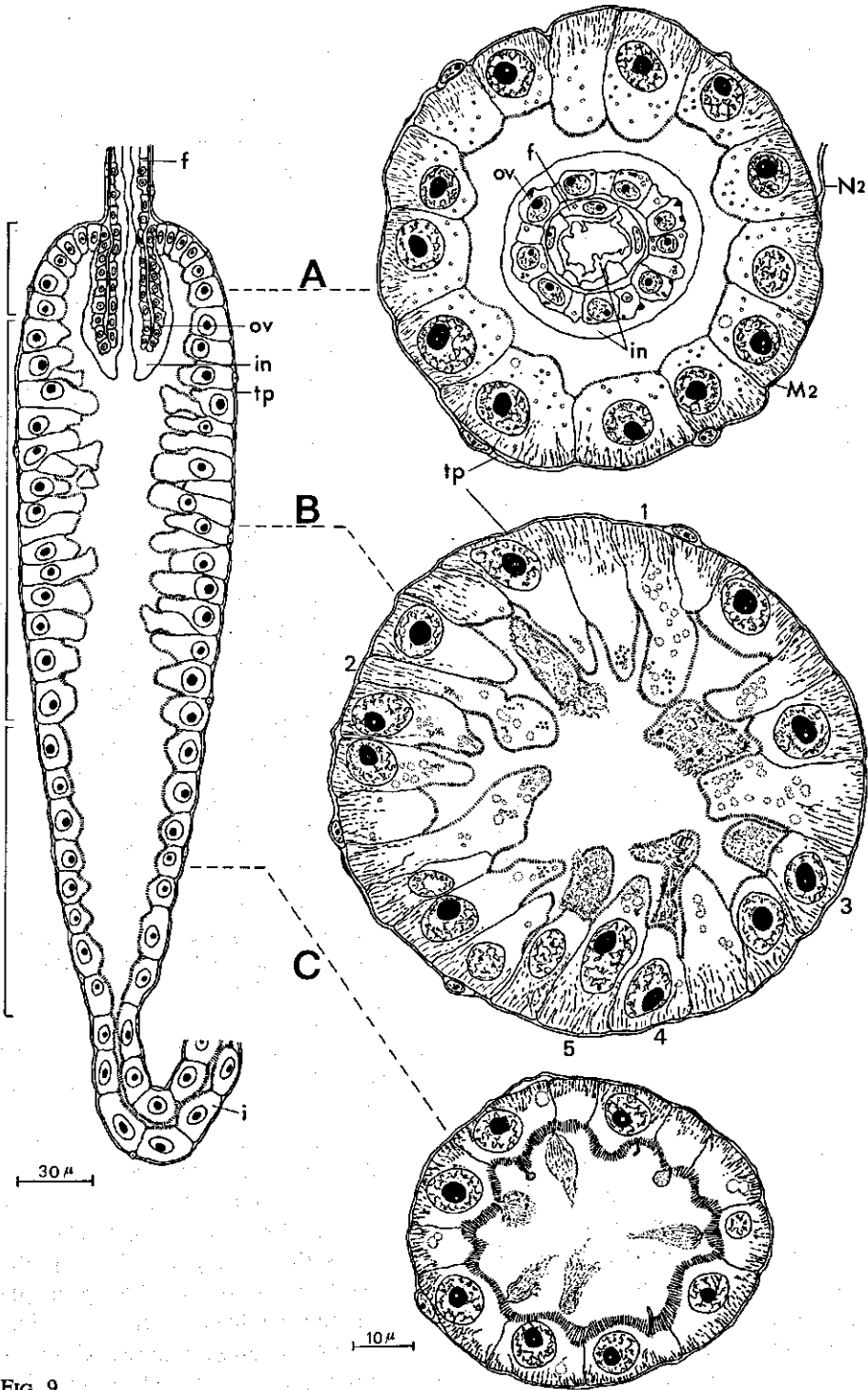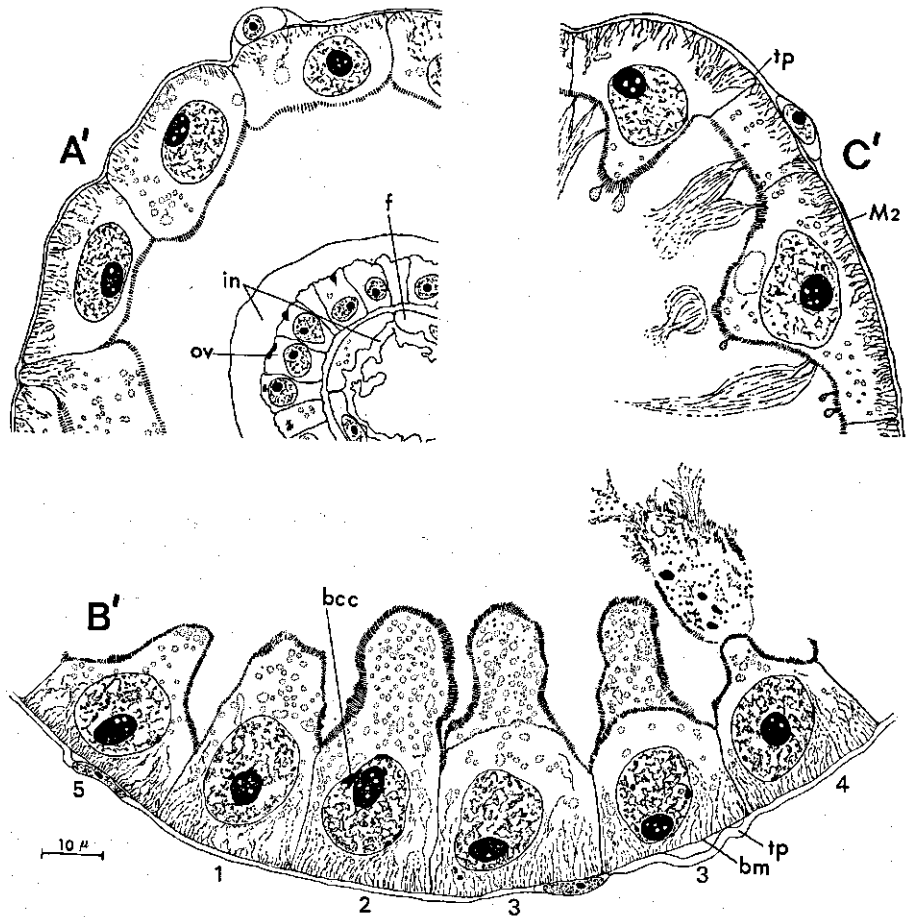
FIG. 9.

FIG. 10. Transverse sections of the stomach and oesophageal valve of a nine days old larva. The position of the sections A'-C' correspond with A-C given in Fig. 9. For explanation of abbreviations see pages 144–147.

Salivary gland

The salivary gland system is paired and situated at both sides of the foregut and dorsal vessel. Each lateral gland system is composed of a branched salivary duct which terminates in large acinoïd gland cells (Figs. 1 and 13). The two terminal parts are distinguished respectively as the principal gland and the accessory gland; the latter is situated anterior to the corresponding principal gland. The accessory glands lie dorsally of the suboesophageal ganglion, while the principal glands are situated above the first and second thoracic ganglion.

The ducts from both ipsilateral glands join to a single one which runs further

34

ventrally around the junction of the suboesophageal and the thoracic ganglion to form, together with the contralateral duct, a common salivary duct. The common duct runs forwards medio-rostrally beneath the suboesophageal ganglion, and turns downwards to the hypopharynx (Fig. 3). It becomes greatly reduced in diameter and passes into the afferent duct following the median line beneath the salivary pump. Then it turns upwards, forming an S-shaped flexure, and enters into the pump chamber at the ventral side of the pump cylinder (Fig. 5).

Accessory gland. This gland is composed of 3–4 cells of uniform size situated around the termination of the salivary duct (Fig. 13 A). The gland is enclosed by a thin basement membrane passing into that of the external salivary duct. The cells contain homogeneous acidophilic cytoplasm in which occur vacuoles and small areas of basophilic granules of about 0.35 μ. The basal part of these cells shows laminated structures. Moreover, in the cytoplasm many branching thread-like canaliculi occur, which are in connection with the termination of the chitinous lumen of the salivary duct; these intracellular canaliculi bear a delicate striation (Fig. 14). Each cell contains a nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus; in the latter occur sometimes one or more vacuoles. During larval life the cell, nucleus, and nucleolus enlarge (Fig. 14), while the optical composition does not change. The average diameter of the nuclei and nucleoli is given in Table 5.

A high concentration of waxy droplets are situated around the accessory glands; many of these droplets are in an intimate association with the basement membrane. However, they are never observed inside the cells as described for the hindgut.

Principal gland. The principal gland is composed of two lobes which anteriorly harbour the 'Deckzellen' and posteriorly the 'Hauptzellen' (Fig. 13 A). These german terms originate from WEBER (1928). In smear preparations the Deckzellen are clearly distinguished from the Hauptzellen; the latter stain more intensively than the Deckzellen, being translucent. This was already observed by WITLACZIL (1882) for different aphid species (Table 9).

The two lobes are enclosed by a thin basement membrane being a continuation of that of the external salivary duct. Each lobe is composed of six Deckzellen and fifteen Hauptzellen which are situated around the internal salivary duct. Considering the type of cytoplasm, the shape of the cell, the size of the nucleus and nucleolus, the six Deckzellen can be distinguished in two, and the fifteen Hauptzellen in six different types (Fig. 14).

Celltype 1 contains nonhomogeneous basophilic cytoplasm in which occur vacuoles of various size and strongly basophilic inclusions; the latter are mainly situated in the basal region of the cell.

Celltype 2 contains homogeneous acidophilic cytoplasm in which occur many vacuoles of various size, especially in the basal region, and areas of basophilic granules of about 0.70 μ scattered between the vacuoles; a compact mass of these granules is situated in the apical region of the cell.

Celltype 3 contains homogeneous strongly basophilic cytoplasm filled with

TABLE 5. The average diameter of nucleus and nucleolus of the several celltypes in the salivary glands of *Myzus persicæ* larvae bred on Chinese cabbage seedlings at 20 ° C. The values are given in μ.

Age		One day old larva[1]		Three days old larva	
Salivary gland	Celltype	Nucleus	Nucleolus	Nucleus	Nucleolus
Accessory gland		9.0 ± 0.52 [1]	3.3 ± 0.12 (7) [1]	9.8 ± 0.39 [1.1]	3.4 ± 0.14 (8) [1.1]
Principal gland 'Deckzellen'	1	5.8 ± 0.26 [1]	2.0 ± 0.02 (4) [1]	7.2 ± 0.27 [1.2]	2.2 ± 0.04 (4) [1.1]
	2	6.3 ± 0.22 [1]	2.1 ± 0.12 (10) [1]	8.2 ± 0.26 [1.3]	2.5 ± 0.06 (10) [1.2]
'Hauptzellen'	3	10.2 ± 0.37 [1]	4.9 ± 0.44 (4) [1]	12.3 ± 0.54 [1.2]	5.5 ± 0.32 (4) [1.1]
	4	8.3 ± 0.35 [1]	3.5 ± 0.25 (8) [1]	8.3 ± 0.34 [1.0]	3.7 ± 0.12 (8) [1.1]
	5	6.3 ± 0.38 [1]	2.2 ± 0.16 (10) [1]	7.7 ± 0.26 [1.2]	3.0 ± 0.12 (10) [1.4]
	6	5.2 ± 0.23 [1]	1.8 ± 0.16 (8) [1]	6.8 ± 0.30 [1.3]	2.0 ± 0.04 (8) [1.1]
	7	3.8 ± 0.09 [1]	1.1 ± 0.04 (8) [1]	4.5 ± 0.16 [1.2]	1.3 ± 0.02 (8) [1.2]
	8	9.7 ± 0.26 [1]	2.8 ± 0.11 (8) [1]	11.8 ± 0.35 [1.2]	2.8 ± 0.10 (8) [1.0]
Duct cells		4.4 ± 0.11 [1]	0.9 ± 0.05 (10) [1]	5.3 ± 0.07 [1.2]	1.4 ± 0.02 (10) [1.6]

[1]The data are obtained from one larva
The sign ± is followed by the standard deviation of the mean (s/√n)
( ) Number of nuclei with their nucleoli measured
[ ] Relative sizes on basis of size at 1 day as 1

clusters of basophilic granules varying between 0.35 and 2.70 μ; a compact mass of granules and some vacuoles occur in the apical region of the cell.

Celltype 4. The composition of this celltype is the same as described for celltype 3.

Celltype 5 contains nonhomogeneous acidophilic cytoplasm in which occur basophilic granules of about 0.70 μ and vacuoles; the granules are scattered in the cytoplasm or form a compact mass of clusters and thread-like structures.

Celltype 6. The cytoplasm of this celltype shows a polar feature, the basal region being basophilic with some vacuoles while the apical region is acidophilic; the whole cell is filled with a compact mass of granules of about 0.70 μ which are basophilic in the basal region and acidophilic in the apical region of the cell.

Celltype 7 contains homogeneous, strongly basophilic cytoplasm, filled with a compact mass of basophilic granules of about 0.70 μ and some vacuoles.

Celltype 8 consists of nonhomogeneous acidophilic cytoplasm strongly vacuolated with basophilic granules of about 0.50 μ.

Each of all these celltypes contains a nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus. The nucleus of celltype 3 is very large with a proportionally very big nucleolus provided with vacuoles. Although the composition of the cytoplasm of celltype 4 is the same as that of celltype 3, the nucleus of the former is smaller just like the nucleolus. On the other hand the big nucleus of celltype 8 contains one or sometimes two nucleoli, small in proportion to the nucleus. The average diameter of the nuclei and nucleoli are given in Table 5. This table shows that the size of the nuclei and nucleoli are

	Five days old larva		Seven days old larva		Nine days old larva	
	Nucleus	Nucleolus	Nucleus	Nucleolus	Nucleus	Nucleolus
	10.4 ± 0.43 [1.2]	3.5 ± 0.13 (8) [1.1]	10.6 ± 0.32 [1.2]	3.2 ± 0.15 (8) [1.0]	12.5 ± 0.50 [1.4]	3.8 ± 0.10 (8) [1.2]
	7.3 ± 0.25 [1.3]	2.2 ± 0.14 (4) [1.1]	8.0 ± 0.42 [1.4]	2.1 ± 0.06 (4) [1.1]	8.2 ± 0.34 [1.4]	2.4 ± 0.11 (4) [1.2]
	8.0 ± 0.26 [1.3]	2.3 ± 0.09 (10) [1.1]	8.7 ± 0.33 [1.4]	2.6 ± 0.08 (10) [1.2]	8.8 ± 0.29 [1.4]	2.5 ± 0.06 (10) [1.2]
	11.2 ± 0.26 [1.1]	4.7 ± 0.17 (4) [1.0]	15.7 ± 0.98 [1.5]	6.7 ± 0.41 (4) [1.4]	13.5 ± 0.38 [1.3]	5.7 ± 0.28 (4) [1.2]
	9.5 ± 0.31 [1.1]	4.4 ± 0.11 (8) [1.3]	10.9 ± 0.33 [1.3]	4.2 ± 0.14 (10) [1.2]	9.7 ± 0.27 [1.2]	4.0 ± 0.12 (8) [1.1]
	8.0 ± 0.26 [1.3]	3.2 ± 0.14 (10) [1.5]	8.3 ± 0.18 [1.3]	2.9 ± 0.11 (10) [1.3]	8.4 ± 0.28 [1.3]	2.7 ± 0.10 (10) [1.2]
	6.9 ± 0.26 [1.3]	2.3 ± 0.08 (8) [1.3]	6.8 ± 0.20 [1.3]	1.7 ± 0.09 (8) [0.9]	6.1 ± 0.14 [1.2]	1.9 ± 0.06 (8) [1.1]
	4.9 ± 0.15 [1.3]	1.5 ± 0.05 (8) [1.4]	5.6 ± 0.15 [1.5]	1.5 ± 0.05 (8) [1.4]	4.8 ± 0.11 [1.3]	1.4 ± 0.06 (8) [1.3]
	11.5 ± 0.23 [1.2]	3.3 ± 0.19 (8) [1.2]	12.7 ± 0.35 [1.3]	2.7 ± 0.11 (8) [1.0]	10.8 ± 0.41 [1.1]	2.8 ± 0.10 (8) [1.0]
	5.5 ± 0.20 [1.3]	1.4 ± 0.02 (10) [1.6]	5.5 ± 0.14 [1.3]	1.4 ± 0.02 (10) [1.6]	6.1 ± 0.17 [1.4]	1.4 ± 0.04 (10) [1.6]

correlated with the optical composition of the cytoplasm and cellsize of the different celltypes (Fig. 14). The difference between the average diameter of the nucleus and nucleolus of celltype 1 and 2 (Deckzellen) is very small; the division of the Deckzellen in two types is based on the composition of the cyto-plasm and on the size of the cells (celltype 1 is much smaller than those of the

TABLE 6. The total number of cells of the accessory and the principal glands of *Myzus persicae* during larval life.

Salivary gland	Celltype	Left salivary gland			Right salivary gland		
		Number of cells			Number of cells		
		Accessory gland	Principal gland		Accessory gland	Principal gland	
			Left lobe	Right lobe		Left lobe	Right lobe
Accessory gland		4			4		
Principal gland							
'Deckzellen'	1		1	1		1	1
	2		5	5		5	5
'Hauptzellen'	3		1	1		1	1
	4		2	2		2	2
	5		6	6		6	6
	6		2	2		2	2
	7		2	2		2	2
	8		2	2		2	2
Total number of of cells		4	21	21	4	21	21

remaining Deckzellen). During larval life the cell, nucleus, and nucleolus enlarge; the composition of the cytoplasm and that of the nucleus and nucleolus does not change. During this time the number of cells per lobe belonging to each celltype remains constant. Table 6 gives a survey of these numbers for all larval ages. In one case (seven days old larva) each of the two lobes of the left principal gland contained three cells of celltype 4 instead of two, and five cells of celltype 5 instead of six; the total number of each lobe remained constant. This implies the absence of cell divisions which have never been observed.

Notwithstanding the age of the larva during larval life, the topographical position of all the afore-mentioned celltypes is constant in each lobe of the principal gland (Fig. 13A). Each lobe consists of six Deckzellen of which celltype 1 lies adjacent partly around the entrance of the salivary duct; the remaining five Deckzellen (celltype 2) are situated successively around celltype 1. From the Hauptzellen celltype 3 is a very distinct large cell exteriorly of the lobe; this biggest cell of each lobe is at the top partly covered by the Deckzellen and from below by two cells of celltype 4. Partly interior each lobe is composed of two big cells (celltype 8). In the distal region of each lobe six cells (celltype 5) are situated around the salivary duct. The terminal portion of each salivary duct is bounded rostrally by the two smallest cells of each lobe (celltype 7), and distally by the two cells of celltype 6.

In each lobe of the principal gland the secretory cells are situated around the internal salivary duct. Each of them is connected with the chitinous lumen of the salivary duct by an intercellular secretory canaliculum. These canaliculi are already observed in the principal salivary glands of Schizoneura ulmi, Aphis sambuci, Chermes abietis (MARK, 1877), Aphis fabae (WEBER, 1928), Lachnus piceae (LEONHARDT, 1940), and Dactylosphaera vitifolii (RILLING, 1967) (Table 9). Electron microscopically the canaliculum terminates in an invagination of the apical cell surface of the gland cell. These invaginations bear a few short, irregularly oriented microvilli (Fig. 15). The release of the secretory granules from the gland cells into the secretory canaliculi takes place in the same way as described in detail for the pancreatic acinar cells (BLOOM and FAWCETT, 1969).

Salivary duct. The entire salivary duct is formed by a cylinder of cuboidal epithelial cells surrounding a chitinous lumen (Figs. 3 and 13 A). The external part of the salivary duct is enclosed by a thin basement membrane which is continuous with that of the two glands. In the accessory gland the lumen of the internal duct splits at its termination into very delicate canaliculi. They run via the intercellular spaces of the duct cells into the accessory cells, in which they further branch (Fig. 14). After the entrance of the salivary duct in the principal

FIG. 11. Dorsal aspect of a graphic reconstruction of the alimentary canal of a one day old Myzus persicae larva. D. Transverse section of the first part of the intestine running from the stomach to the voluminous coil of intestine (vci) far in the abdomen. E. Transverse section of the second part of the intestine running from the voluminous coil of intestine (vci) to the hindgut (hg). The transverse sections D' and E' are those of a nine days old larva. The letters F, L, P, and R correspond with transverse sections given in Figs. 12 and 25–27. For explanation of abbreviations see pages 144–147.
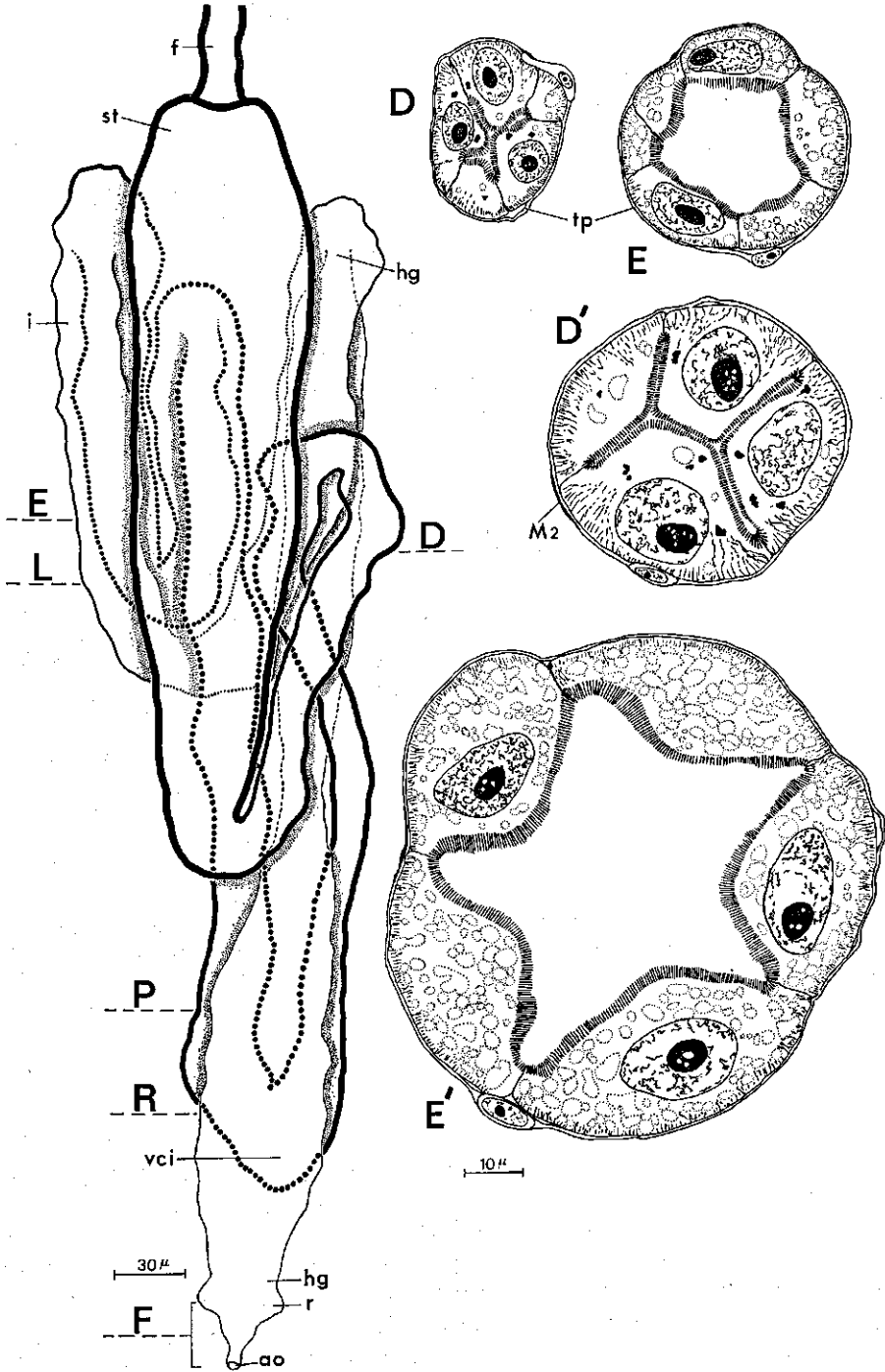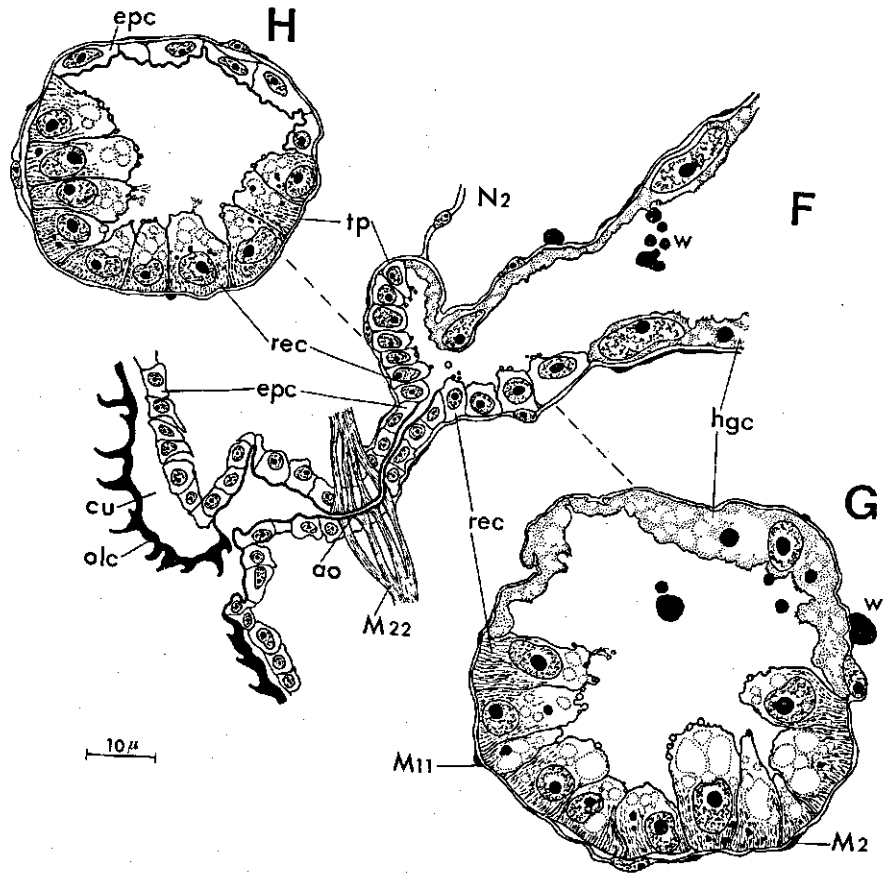
38

FIG. 11

FIG. 12. F. Sagittal section of the hindgut and rectum of a one day old *Myzus persicae* larva. G. Transverse section through the anterior part of the rectum showing hindgut cells (hgc) and secretory rectal cells (rec) of a nine days old larva. H. Transverse section through the posterior part of the rectum showing secretory rectal cells (rec) and epidermal cells (epc) of the integumental invagination of a nine days old larva. For explanation of abbreviations see pages 144–147.

gland it divides into two ducts. Each of them runs through the substance of the lobe, receiving throughout its course the intercellular canaliculi of the secretory gland cells (Figs. 13 A).

The duct cells of the entire salivary duct show numerous vacuoles of various size in a homogeneous acidophilic cytoplasm; some cells, however, contain basophilic cytoplasm, especially in the region of the Deckzellen. Cell boundaries are not clearly defined; their distinct nuclei contain regularly scattered basophilic chromatin and a spherical eosinophilic nucleolus. During larval life cell divisions are never observed. The nuclei and their nucleoli increase in size

40

(Table 5) and retain their optical composition just like that of the cytoplasm. The vacuoles in the cytoplasm increase in size and number. Granules are absent in duct cells.

Around the chitinous lumen the apical surface of the duct cells is finely striated. Similar striae are already observed by TÓTH (1936) and FORTIN (1958) for several other aphid species. Especially in the common salivary duct the striae are very long and penetrate one-half of the depth of the duct cells (Fig. 13 B). In this part of the salivary duct the duct cells are also bigger. Electron microscopically the duct cells have been studied by MOERICKE and WOHLFARTH-BOTTERMANN (1960b). The striae appear to be microvilli which are regularly oriented around a chitinous lumen (Fig. 16 B and C). In the external salivary duct the lining of the lumen is very thick, but after entering the principal gland it gradually becomes thinner. The substance of the wall has the same optical density as the outer layer of the cuticule. In the salivary glands of *Schizoneura ulmi*, *Aphis sambuci*, and *Chermes abietis* treated with KOH, MARK (1877) showed that the substance of the luminal wall of the entire salivary duct consists of chitin. A cuticular lining is also found in the aphids *Chermes lapponicus* (CHOLODKOSVKY, 1905), *Stomaphis graffii*, *Cinara pinihabitans*, *Megoura aconiti* (TÓTH, 1936), and *Macrosiphum solanifolii* (FORTIN, 1958) (Table 9).

Myoepithelioid cell. The distal parts of the two lobes of the principal gland are connected by a mass of thread-like structures with an eccentrically placed nucleus: a myoepithelioid cell. The distinct nucleus contains regularly scattered basophilic chromatin and an eosinophilic nucleolus (Fig. 13 A and D). The lumen of both salivary ducts terminates in this cell.

Electron microscopically the bulk of the cytoplasm is occupied by myofibrils of varying width (Fig. 15). Within these myofibrils contractile material is visible characterized by typical Z-lines and arrays of parallel arrangements of myofilaments (Fig. 16 A). Between the myofibrils a large number of small vesicles can be observed, presumably sarcoplasmic reticulum (Fig. 17 A). At the periphery of this contractile mass some mitochondria and many vacuoles occur. At the border of this mass axon terminals can be recognized, filled with large numbers of synaptic vesicles (Fig. 15).

The myofibrils are oriented in an interwoven pattern and it seems that the myofibrils branch into bundles of myofilaments. They are attached to the cell surface through which the myoepithelioid cell acquires an irregular form. The surface of the myoepithelioid cell, bordered by the four intercellular canaliculi of celltype 7 and the termination of both ducts, is strongly folded inward. These irregularly oriented processes are closely packed forming a labyrinthine system of clefts between them (Fig. 15, see arrows).

In each principal gland the myoepithelioid cell is innervated by a paired nerve of the medial dorsal nervous sytem. This sytem passes along the dorsal vessel (Fig. 13 A). One branch of the paired nerve enters the right lobe of the left principal gland, while the other one enters the left lobe of the right principal gland between the two gland cells of celltype 6. At the attachment to the gland the nerve contains a nucleus with regularly scattered basophilic chromatin and
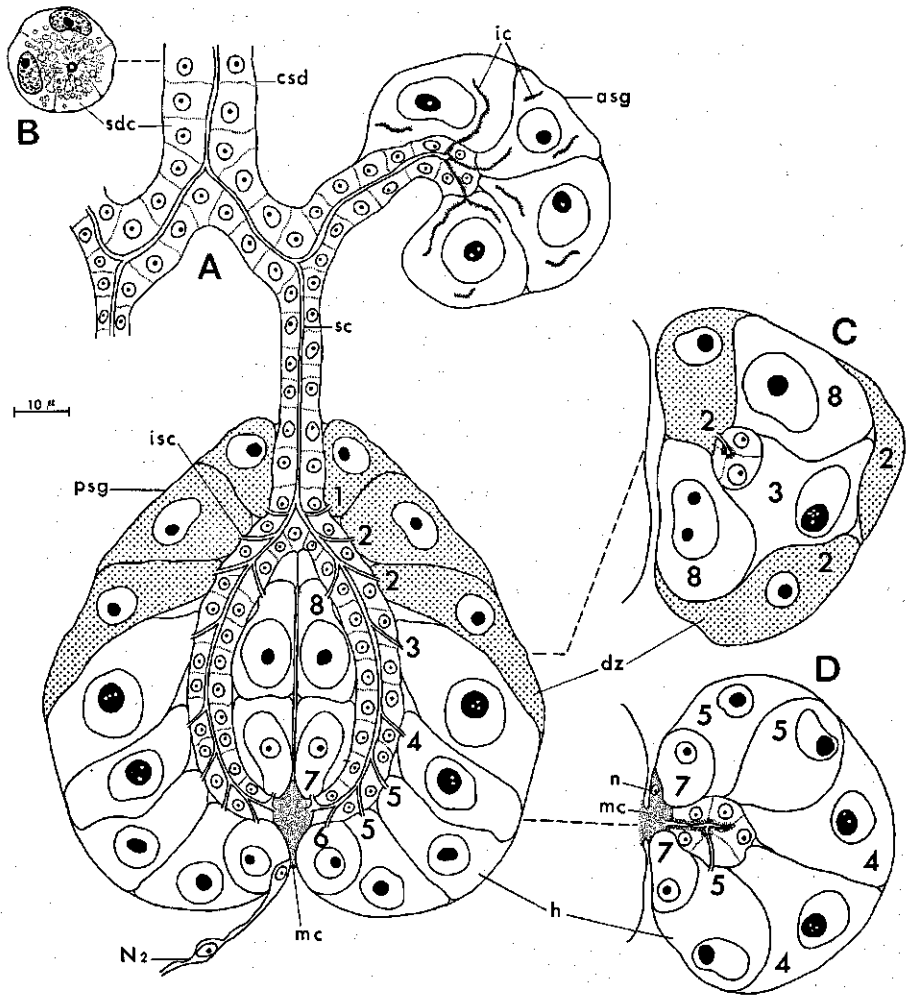
Fig. 13. A. Graphic reconstruction of the salivary gland from transverse sections of a five days old *Myzus persicae* larva illustrating the principal gland (psg), the accessory gland (asg), the salivary duct, and the common salivary duct (csd). Each lobe of the principal gland is composed of eight types of gland cells (1–8) situated around the internal salivary duct. Celltypes 1 and 2 represent the Deckzellen (dz) and celltypes 3–8 the Hauptzellen (h). Notice that the intracellular canaliculi (ic) in the accessory gland cells communicate with the lumen of the salivary duct. B. Transverse section of the common salivary duct (csd). C. Transverse section of the middle region of the principal gland showing the two separated lobes. D. Transverse section of the posterior region of the principal gland. The two lobes are connected with each other by the myoepithelioid cell (mc) with its eccentrically placed nucleus (n). For explanation of abbreviations see pages 144–147.
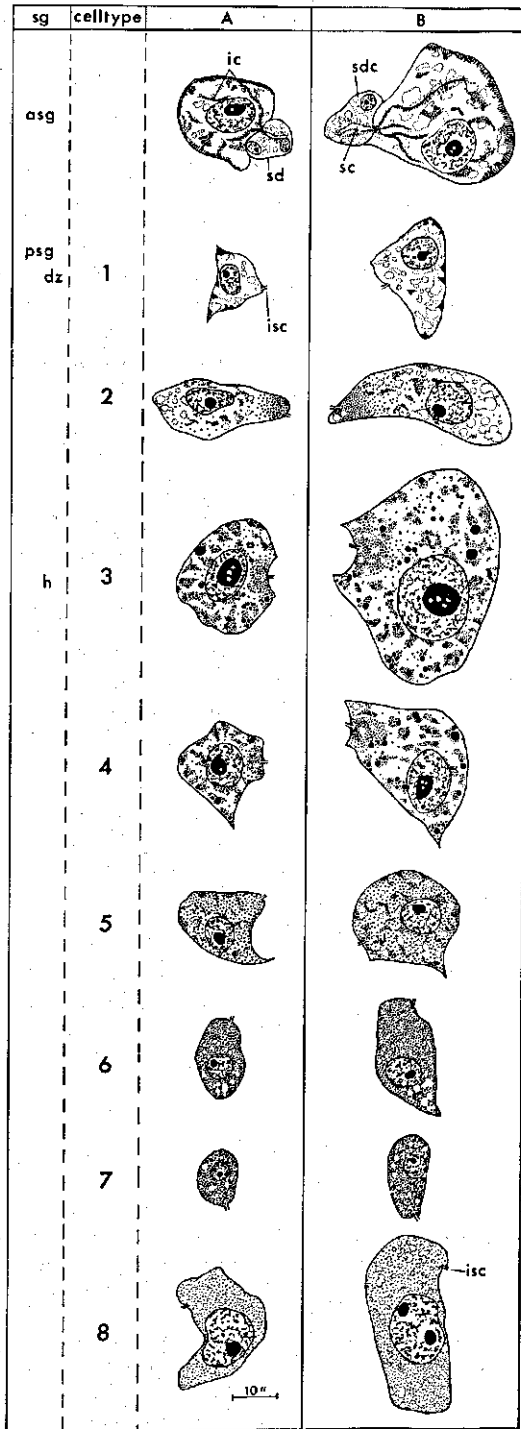
42

FIG. 14. Histological composition of the gland cells in the accessory (asg) and the principal salivary gland (psg) at the first (A) and last day (B) of the larval period of *Myzus persicae*. The celltypes correspond with those given in Fig. 13 A, C, and D. For explanation of abbreviations see pages 144–147.

an eosinophilic nucleolus. Axon terminals filled with synaptic vesicles, mitochondria, and microtubuli are visible in the nerve. The neurilemma passes into the basement membrane of the salivary gland (Figs. 17 B and 18).

The presence of a myoepithelioid cell between the terminal parts of both salivary ducts suggests, that haemolymph can be taken up by contraction of the myofibrils into the lumen of both ducts. Via the duct lumina it can be transported through the salivary pump into the plant. To test the value of this supposition, larval aphids were injected via the cornicles with 1.5% EVANS blue in 0.85% NaCl. After anaesthesia the aphids were allowed to feed during 16–24 hours on Chinese cabbage seedlings. Dissection showed that in the salivary glands only the myoepithelioid cell and both lumina of the salivary duct were stained intensively blue in contrast to the remaining part of the salivary glands being colourless. This result indicates that both lumina of each principal gland communicate with the haemolymph by means of the myoepithelioid cell. However, contractions of the myofibrils in the myoepithelioid cell of dissected aphids have so far never been observed.

Salivary pump. The salivary pump lies in the hypopharynx beneath the pharyngeal pump (Figs. 5 and 19). It consists of a chitinous tulip-shaped cylinder and a pumpstem both of which have the same optical density as the outer layer of the cuticle. In its open end there fits a U-shaped piston connecting with their lips on the edge of the cylinder. It differs optically from the chitin of the cylinder itself. The middle of the piston is provided with a tendon to which muscles are attached originating from the tentorial bar and the pharyngeal pump. The cylinder shows on both sides a chitinous projection on which muscles are attached; these muscles originate from a chitinous ridge (called ventral rods by DAVIDSON, 1914) leading from the hypopharynx wall to the tentorial bar. Similar muscles are also found in *Schizoneura americana* (MILLER, 1933) and *Aphis (Doralis) frangulae* (ROBERTI, 1946) as well as in several psyllids (WEBER, 1929) (Table 9).

On the ventral side the pumpcylinder receives the common afferent salivary duct as an S-shaped flexure (Fig. 3). At the place of entry the opening is controlled by two small bands of muscles originating from the chitinous ridges. A similar construction is described by DAVIDSON (1914) for *Schizoneura lanigera* and by RILLING (1960) for *Dactylosphaera vitifolii* (Table 9). In electron micro-

FIG. 15. Electron micrograph of the posterior region of the principal salivary gland of *Myzus persicae* larva showing two gland cells of celltype 7, two duct cells with their nuclei (sdc), two gland cells of celltype 6, and the myoepithelioid cell (mc) (see Fig. 13 A). The myoepithelioid cell is occupied by myofibrils which are oriented in an interwoven pattern. At its border axon terminals (at) filled with synaptic vesicles are visible. The surface of the myoepithelioid cell, bounded by the canaliculum (isc) of celltype 7 (left side) and both duct lumina (sc), shows irregularly oriented, closely packed processes forming a labyrinthine system of clefts between them (arrows). The intercellular canaliculi terminate in an invagination of the apical cell surface of the gland cells (celltypes 7, and on the right side celltype 6). The invaginations bear a few short, irregularly oriented microvilli. In the duct cells occur many mitochondria (m) and vacuoles (va). Magnification: × 10,500.
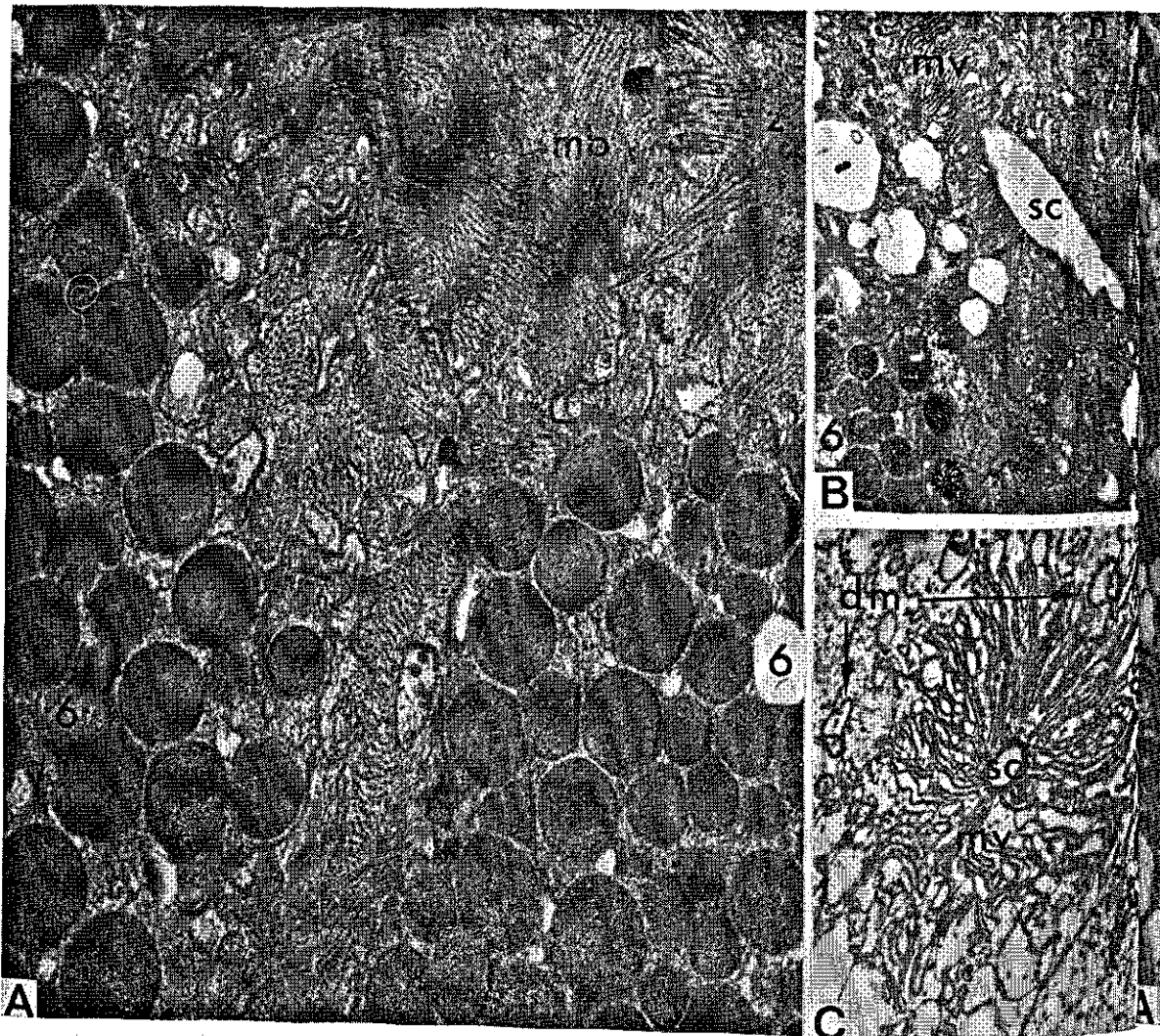
44
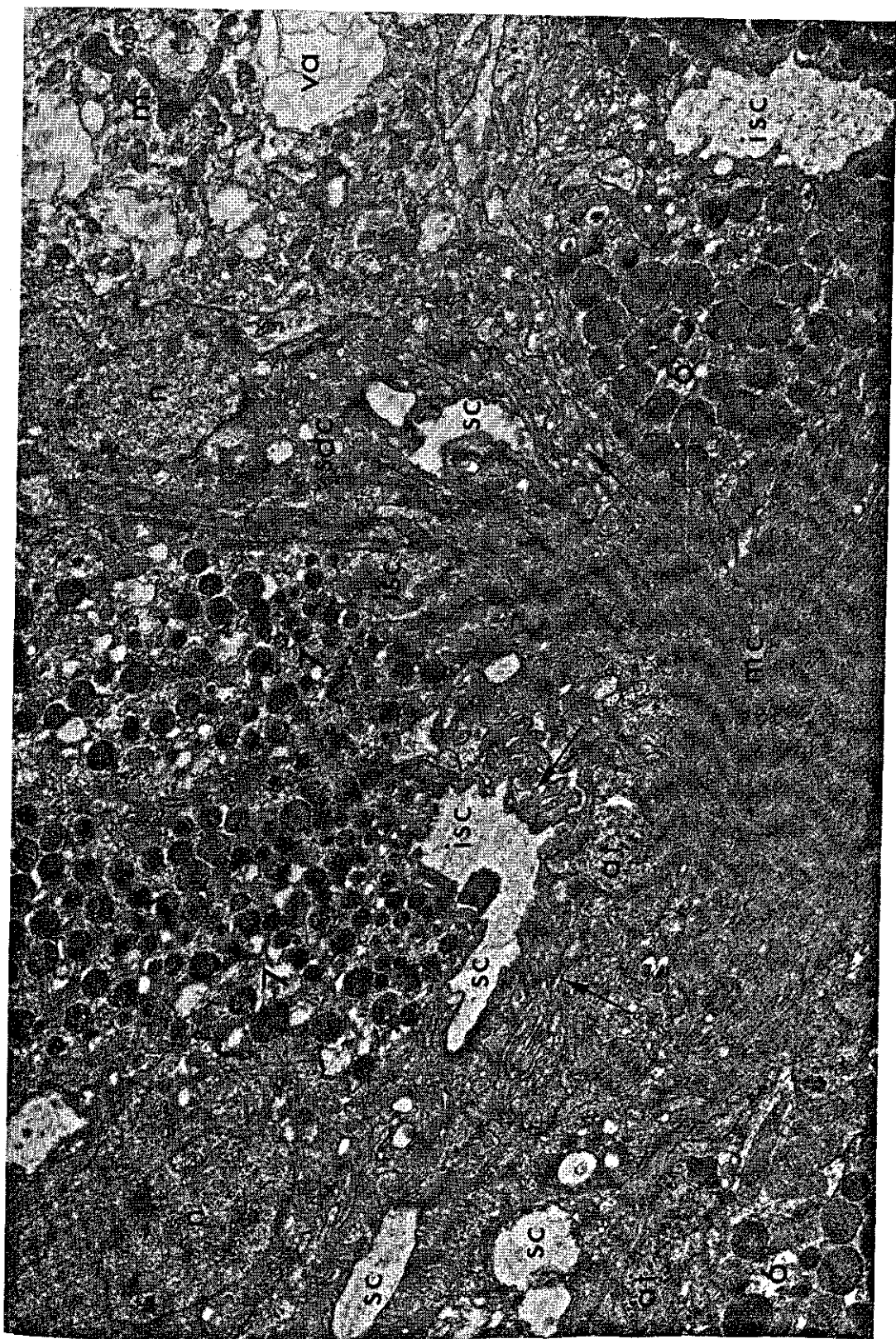
FIG. 16. A. Electron micrograph of an area of the myoepithelioid cell in which the myofibrils (mb) are oriented in an interwoven pattern and in transverse section are seen as small dots. It seems that the myofibrils branch into bundles of myofilaments. Note the closely arranged secretory granules in celltype 6. Magnification: × 22,750. B. Electron micrograph of a longitudinal section through a thin-walled chitinous lumen (sc) of the internal salivary duct in the posterior region of the principal gland. The lumen passes into an intercellular secretory canaliculum (isc) leading to celltype 7 (see Fig. 15 on the left side). Note the regularly oriented microvilli (mv) around the lumen while they are lacking around the intercellular canaliculum. Magnification : × 8,000. C. Electron micrograph of a transverse section through a thick-walled chitinous lumen (sc) of the internal salivary duct in the anterior region of the principal gland. The luminal surface of the duct cells has regularly oriented long infoldings (microvilli) which are situated around the chitinous lumen. Note the presence of desmosomes (dm). Magnification: × 10,000.
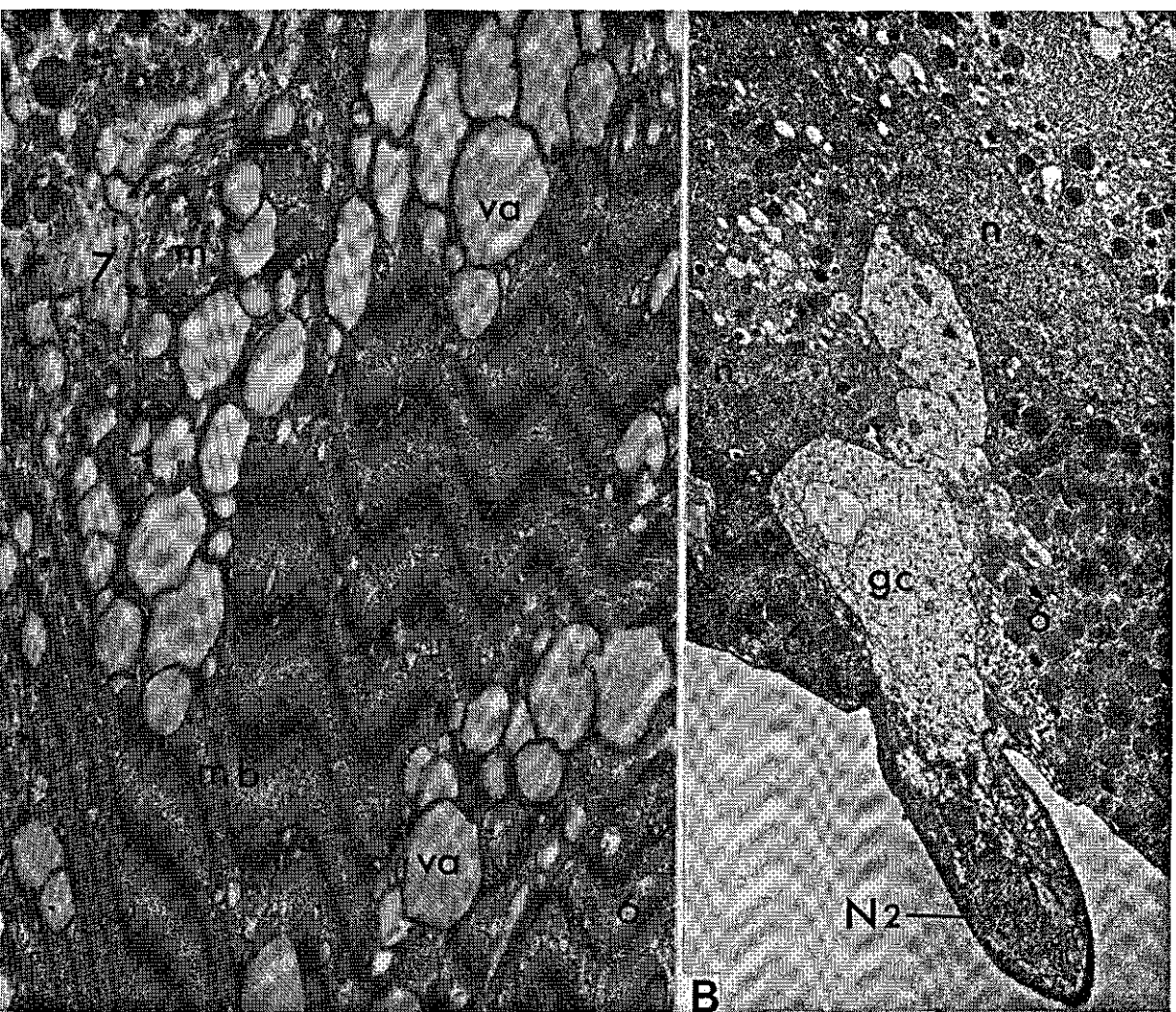
46

FIG. 17. A. Electron micrograph of an area of the myoepithelioid cell. Between the myofibrils (mb) is a large number of small vesicles (v) presumably sarcoplasmic reticulum. At the periphery of this contractile mass some mitochondria (m) and many large vacuoles (va) occur. Magnification: × 22,000. B. Electron micrograph of the entrance of a branch of the medial dorsal nerve (N2) between two gland cells (celltype 6) of the principal gland (see Fig. 13 A). Note the glial processes of a supporting cell (gc). Magnification: × 7,600.
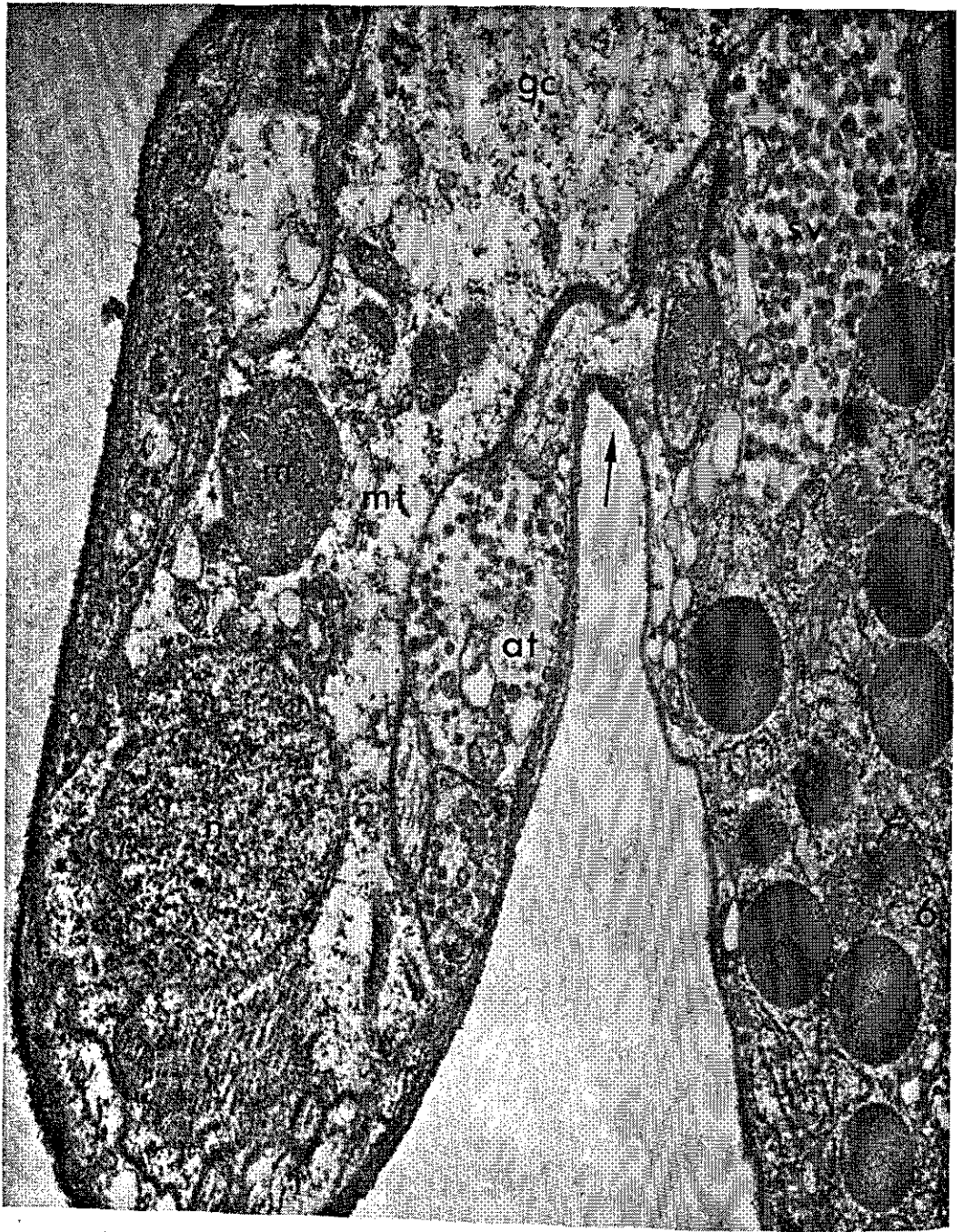
FIG. 18. The same nerve as in Fig. 17 B at a higher magnification, characterized by the presence of axon terminals (at) filled with synaptic vesicles (sv), mitochondria (m), microtubuli (mt), and glial processes. The neurilemma passes into the basement membrane of the principal salivary gland (arrow). Magnification: × 28,000.

graphs it appeared that in the leafhopper, *Macrosteles fascifrons* STÅL, the opening of the afferent duct is regulated by a flap valve. This valve allows the saliva to enter the pumpchamber and prevents it from flowing back (RAINE and FORBES, 1971).

The exit opening from the pumpchamber is situated on the distal end of the pumpcylinder, and leads into the pumpstem as a narrow duct. On the foot of the pumpstem the duct is somewhat dilated and is provided with two sensillum pores. The duct passes into the efferent salivary duct and runs in the median line on the ventral side of the hypopharynx lip to terminate in the salivary canal enclosed by the maxillary stylets (Figs. 5 and 7 no. 5–8).

Mesodermal derivates

The larval mesodermal tissue of *M. persicae* consists of a mixed cell population. It forms a continuous sheet situated in the body cavity against the body wall and drained by the haemolymph. In the head the sheet is a single layer of cells which enter between the various organs (Fig. 30). In the posterior region of the head the sheet acquires a thickness of about 2–3 cell layers (Fig. 32 Z) which extends further into the last abdominal segment. On the lateral sides of the thorax (Fig. 20) and on those of the anterior part of the abdomen it has a thickness of about 5 cell layers. The mesodermal tissue is present in all appendages of the aphid.

Histologically the mesodermal tissue shows three distinct types of cells (Figs. 20 and 32 Z). Most numerous are the fat cells, characterized by a strong vacuolization of their cytoplasm, the vacuoles being filled with a yellow oil-like substance. The second type of cells are evenly situated among the fat cells. They can be distinguished from the latter by their homogeneous basophilic cytoplasm without any structures. These cells are called basophilic mesodermal cells. Mainly distributed along the surface of the mesodermal tissue are the much smaller connective tissue cells. The visible processes of these cells seem to form a complicated network of delicate membranes supporting the mesodermal tissue Numerous finely branching tracheae concur with this membranous construction.

The first mesodermal cells are observed in the eldest embryo of a three days old larva in the area anterior of the future cornicles (Fig. 22, 1). During embryonic development the number of mesodermal cells continuously increases by cell divisions; the direction of cell divisions is from the site of origin both anteriad and posteriad (Fig. 22, 1–3). The somewhat spherical embryonic mesodermal cells contain homogeneous basophilic cytoplasm. Each of them consists of a conspicuous spherical nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus (Fig. 22, celltype A).

In the eldest embryo of a five days old larva the embryonic mesodermal cells lying in the area of origin start to develop into fat cells. These cells are characterized by the forming of 1–2 lipid droplets in their cytoplasm (Fig. 22, 2 B). Thereafter the fat cells increase in size and many lipid droplets arise in the cytoplasm (Fig. 22, 3C). At the same time some other cells of this tissue start to

increase in size and become the final basophilic mesodermal cells (Fig. 22, bmc). Their optical composition remains similar to that of the embryonic mesodermal cells.

During growth of the embryo all mesodermal cells gradually develop into fat cells, basophilic mesodermal cells, and connective tissue cells forming the final mixed mesodermal tissue. After the embryonic mesodermal cells are differentiated into their derivates, no cell divisions any more take place in these elements (Fig. 22, 4–7). In the eldest embryo of a nine days old larva the mesodermal tissue has attained its final composition (Fig. 22, 4).

Fat cells. After transformation of the embryonic mesodermal cells into fat cells, they start to increase in size just like their nuclei and nucleoli. During this process the lipid droplets increase both in number and in size (Fig. 22, 4 D). As a result of the pressure within the cell by the accumulation of lipid, the cell boundaries become more irregular in shape. The spherical nuclei gradually transform into various shapes from polygonal to stellate, or flattened and displaced to one side (Fig. 22, 4 E). Subsequently the lipid droplets gradually start to coalesce (Fig. 22, 4 F). The fat cells show a multitude of various-sized cavities lying in a very thin network of cytoplasmic strands. These cavities contain the lipid droplets which are dissolved during preparation of the paraffin sections.

During coalescence of the lipid droplets the first waxy droplets and rhomb-shaped crystals arise in the cytoplasm (Fig. 22, 4 G), gradually increasing in number and size. In phase microscopy the waxy droplets are recognizable by their white refractive appearance. They are not dissolved during the dehydration process of the aphids for serial sections. The nuclei again recover their original shape, presumably because the pressure inside the cell declines. The polygonal and/or stellate nuclei primarily appear in the eldest embryo of a nine days old larva (Fig. 22, 4), and occur mainly in the celltypes E–G during development of the aphid. The degeneration of the fat cells starts by the appearance of vacuoles in the karyoplasm and in the nucleolus (Fig. 22, 4 H). During vacuolization of the karyoplasm, the chromatin begins to clot. This process gradually continues till all the chromatin mass disintegrates into fragments (Fig. 22, 4 I-J). Simultaneously with the process of karyorhexis of the chromatin, many vacuoles arise in the nucleolus showing a sponge-like body. This body starts to dissolve peripherically resulting in a gradual decreasing of its size till it is completely disappeared. Sometimes the sponge-like nucleolus disintegrates in 2–3 parts which in turn disperse.

During the degeneration process of the nuclei and nucleoli a part of the cytoplasm starts to disintegrate. The cell membrane decomposes and the disintegrating cytoplasm gradually disappears into the haemolymph leaving a part of the cell membrane on which cytoplasmic remnants are fastened, containing some lipid and waxy droplets, and rhomb-shaped crystals (Fig. 22, J-L). Afterwards they completely disintegrate into unrecognizable substances. In the disintegrating cytoplasm eosinophilic bodies arise which become progressively larger and are clearly visible as irregular bodies especially in the degenerating
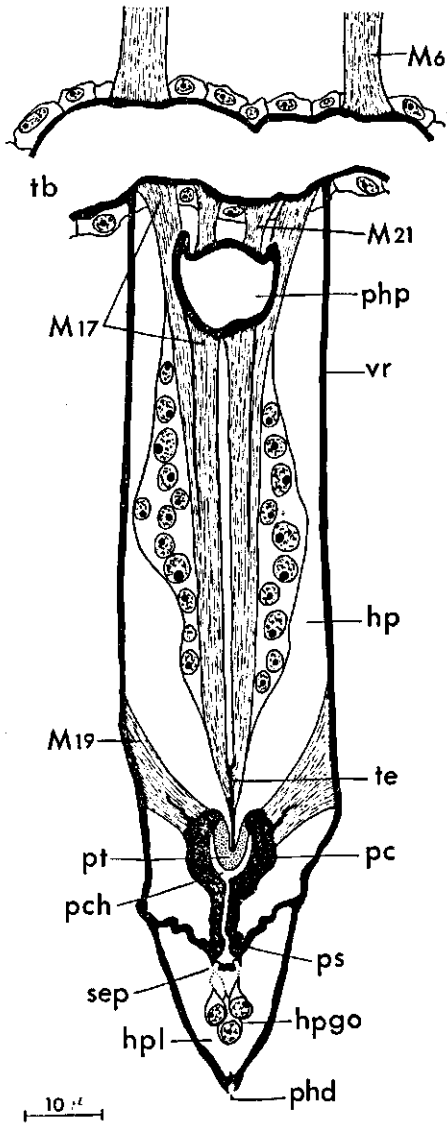
50

FIG. 19. Oblique section of the salivary pump and its muscles of a five days old *Myzus persicae* larva. Note the hypopharyngeal gustatory organ (hpgo) with two sensillum pores (sep) on the foot of the pumpstem (ps) (see Figs. 5 and 7 no. 8). For explanation of abbreviations see pages 144–147.

fat cells of celltype J-L.

On the other hand there occur fat cells of celltype J (Fig. 22) of which the cell membrane is still intact. These cells, which are clearly distinguished under the dissecting microscope as large yellow spheres under the cuticle of the larva, have besides a number of small lipid droplets a single very large one. In smear preparations of haemolymph or cornicle secretion these cells harden directly and are dissolvable in alcohol. They have an opaque white spherical appearance.

At the same time the nuclear membrane dissolves liberating the chromatin fragments into the cytoplasm and from there into the haemolymph (Fig. 22, 4 K). The chromatin fragments loose their basophilic nature to disintegrate further in eosinophilic granules of various sizes and shapes. In some cases it was found that after clotting of the chromatin the nuclear membrane remained intact. The chromatin fragments dissolve gradually to a delicate network of eosinophilic strands (karyolysis). Finally this network disappears remaining an optical empty nucleus, of which the membrane becomes ultimately dissolved. An other form of degeneration is the shrinkage (pyknosis) of the nuclear material. Pyknosis continues till all the nuclear material has completely disappeared, after which the nuclear membrane starts to disintegrate.

Numerous waxy droplets are visible throughout the body cavity of the aphid during its life. The droplets are released by the cell membrane of intact fat cells, or liberate into the haemolymph after dissolving of the cell membrane. They occur mainly against the visceral surface of the mesodermal tissue and especially in a high concentration around the hindgut and the accessory salivary glands. Many of them are distributed by the dorsal vessel.

During larval life of the aphid the enlargement and degeneration process of the fat cells gradually takes place in the mesodermal tissue, and is already in full course in the eldest embryo of a nine days old larva (Fig. 22, 4). In this embryo the entirely degenerated fat cells (celltypes J-L) occur in the vicinity of the cornicles. This agrees with the fact, that in this region the fat cells start to develop from the embryonic tissue of the embryo (Fig. 22, 1 and 2). Successively the celltypes H-I, G, and F (Fig. 22, 4) are situated both anteriorly and posteriorly of this area of origin. Further anteriad the mesodermal tissue contains a big mass of fat cells of celltype E, while celltype D is observed still only in the aphid's head. The transition from one celltype to the other is not so definite as represented in Fig. 22. In the frontier areas many intermediate forms occur.

In a one day old larva (Fig. 22, 5) celltype D does not occur anymore in the mesodermal tissue, since it changed into celltype E. Meanwhile celltypes E-G gradually increase in size, after which the nuclear material starts to degenerate (celltypes H-I). Subsequently the cell membrane dissolves, forming successively the celltypes J-L. During growth of the larva celltype F increases in size and develops into celltype G and H-I (Fig. 22, 6). After vacuolization of the nuclear material the celltypes H-I become disintegrated (celltypes J-L). The several developmental stages of the fat cells in the mesodermal tissue of a nine days old aphid (Fig. 22, 7) shows the same picture as that of a five days old larva (Fig. 22, 6). In the former aphid celltype G has further increased in size, while the degeneration of the other celltypes is still in progress. This process continues in the adult stage till all the fat cells are dissolved, although in this stage no enlargement of the fat cells takes place anymore. Concerning their size the celltypes J-L of a one day old larva are never observed in five and nine days old specimens. Likewise the celltypes J-L of a five days old larva do not occur in an aphid nine days old. This agrees with the fact that the fat cells completely disintegrate into unrecognizable substances.

52
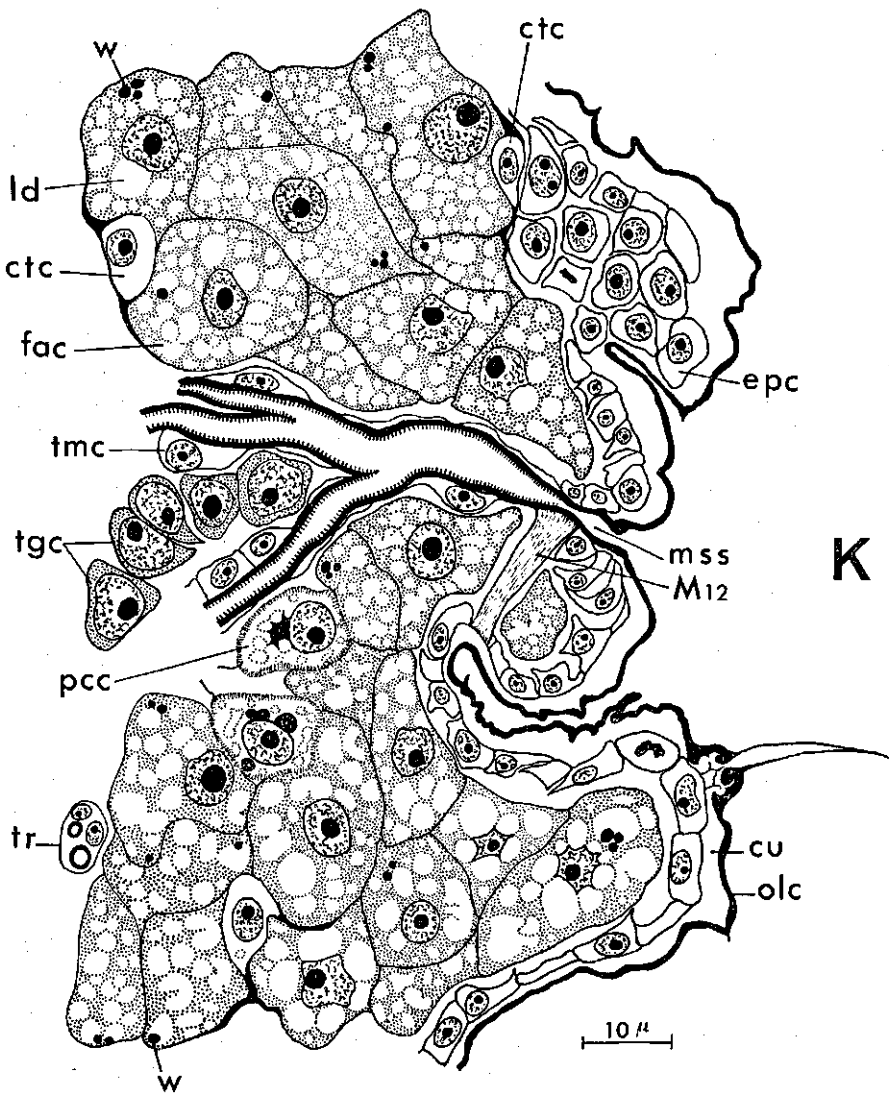
Fig. 20. Transverse section of the right half mesothoracic region of a one day old *Myzus persicae* larva showing arrangement of fat cells (fac), connective tissue cells (ctc), pericardial cells (pcc), and thoracic gland cells (tgc). The position of this section (K) is given in Figs. 1–3 and 24. For explanation of abbreviations see pages 144–147.

Basophilic mesodermal cells. During growth of the embryonic meso-
dermal tissue by cell divisions, some cells start to increase in size and become
the basophilic mesodermal cells (Fig. 22, bmc; Fig. 21, B-D). After this trans-
formation no further cell divisions are observed in these cells. This process con-
tinues till in the eldest embryo of a nine days old aphid the mesodermal tissue

has attained its final composition. In this embryo the total number of baso-philic mesodermal cells is then completed and remains during the rest of the larval life constant (Table 8). The polygonal basophilic mesodermal cells are even dispersed individually among the fat cells (Figs. 25, 27, and 32 V), and their topographical position during larval life is shown in Fig. 23. In the anterior region of the head and the appendages of the aphid the basophilic mesodermal cells are lacking.

As in the embryonic stage of the aphid the enlargement of the basophilic mesodermal cells and their nuclei and nucleoli continues during larval life (Fig. 21, E-I). The term basophilic mesodermal cells is introduced because of the presence of homogeneous basophilic cytoplasm in these cells during devel-opment of the embryo. Their centrally placed spherical nuclei contain regularly scattered basophilic chromatin and a large eosinophilic nucleolus. After larvi-position the cytoplasm of the basophilic mesodermal cells becomes gradually acidophilic, showing still basophilic parts at the periphery of the cytoplasm (Fig. 21, E). In the acidophilic area arise many minute vacuoles which increase rapidly in number (Fig. 21, F). At the seventh day some vacuoles appear in the karyoplasm after which the chromatin commences to clot (Fig. 21, H). At some places the nuclear membrane becomes somewhat irregular enclosed by cyto-plasmic vacuoles. In smear preparations of adults the nuclear membrane was found to dissolve. The clotting chromatin is now liberated into the vacuolized cytoplasm   followed by a complete disintegration of the basophilic mesoder-mal cells. The nucleolus starts to dissolve by the forming of vacuoles; its dis-solving process is further similar to that of nucleoli in fat cells.

The ageing process of the basophilic mesodermal cells is described for those cells situated among the degenerating fat cells (Fig. 22, celltypes J-L) in the area of origin. In this area, situated in the vicinity of the cornicles, the first basophilic mesodermal cells are developed from the embryonic mesodermal tissue. The intermediate forms of degenerating basophilic mesodermal cells occur in the rest of the mesodermal tissue and during the imaginal stage all the basophilic mesodermal cells gradually dissolve.

Connective tissue cells. Some embryonic mesodermal cells gradually change their character, elongating and stretching out along the surface of the mesodermal derivates to become the final spindle-shaped or stellate connec-tive tissue cells. Each cell contains a proportionally large, spherical or elliptical nucleus and a small nucleolus. During this process it seems that they increase somewhat in size, but in the larval stage of the aphid they retain their volume (Figs. 20, 25, 27, and 32 V). They are much smaller than the other derivates of the mesodermal tissue and contain homogeneous basophilic cytoplasm. Their tapering cell processes form a complicated network of delicate membranes which support the mesodermal tissue. Moreover, these extremely delicate membranes extend throughout the haemocoel and are connected with the various internal organs.

The connective tissue cells mainly occur at the periphery of the mesodermal tissue and near the muscles, although some of them are found in the haemocoel

54

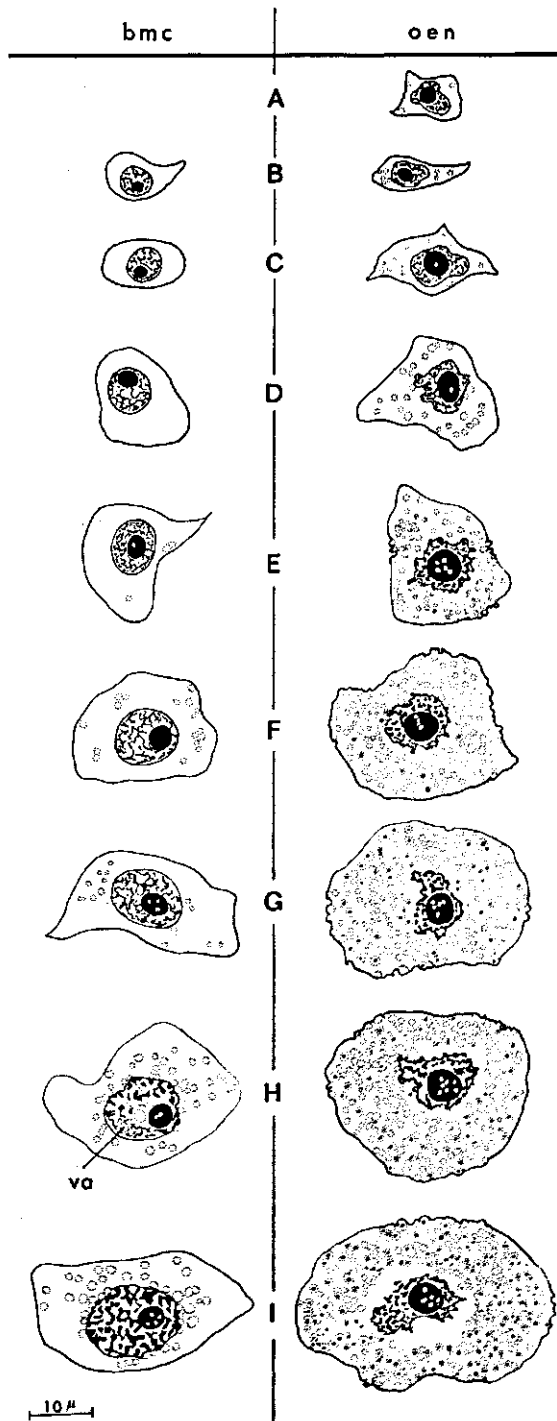bmc | oen

FIG. 21. Growth of basophilic mesodermal cells (bmc) and oenocytes (oen) of *Myzus persicae*. A-D represent basophilic mesodermal cells and oenocytes as found in the eldest embryo of a 3, 5, 7, and 9 days old larva respectively; E-I represent both celltypes in larvae of respectively 1, 3, 5, 7, and 9 days old. The topographical position of both celltypes are given in Figs. 23 and 1.

(Fig. 30). On the lateral sides of the thorax and on those of the anterior part of the abdomen, where the sheet has a thickness of about five cell layers, they are sparsely distributed among the fat cells and the basophilic mesodermal cells. Especially in the vicinity of the cornicles the mesodermal tissue shows a loose structure of delicate membranes as a result of the disintegration of the fat cells. In that region, at the end of the larval stage, the cytoplasm of the connective tissue cells starts to vacuolate.

Pericardial cells. The pericardial cells are derived directly from the embryonic mesodermal cells. In the earliest stages of development the pericardial cells are difficult to distinguish from the fat cells by the presence of small vacuoles. During growth of the embryo the pericardial cells and their nuclei and nucleoli increase in size. Meanwhile strongly basophilic globules arise in the perinuclear area of the basophilic cytoplasm. These globules enlarge and stretch out along the surface of the vacuoles to form stellate structures. Each cell contains a distinct spherical nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus.

The cell membrane of the pericardial cells is finely striated. Electron microscopically these cells of adult *M. persicae* are studied by BOWERS (1964). The striae appear to be infoldings which form narrow, extracellular channels. These infoldings are maintained by desmosomes which link the lips of the folds at frequent intervals.

In the eldest embryo of a nine days old larva the pericardial cells have attained their final structure and number. After larviposition the enlargement of these cells and their nuclei and nucleoli gradually continues, and the total number of cells remains constant (Table 8). During larval life they retain their original structure and persist unchanged into the adult stage (Fig. 32).

The pericardial cells are situated outside the mesodermal tissue, and their topographical position during larval life is shown in Fig. 24. The majority of them are evenly distributed along the entire dorsal vessel (Fig. 3). At the termination of the dorsal vessel above the corpus allatum some of them are arranged in a group of 7–9 cells (Fig. 32 V). Further there occur 2–3 pericardial cells on both lateral sides of each thoracic segment (Fig. 24; a, b, and c) where the legs enter the body cavity (Fig. 20), and 3–4 lateral pericardial cells in the meta-thoracic segment (Fig. 24; d). The pericardial cells which lie dorsally of the stomach and the mycetome bridge have an elongated form. Two or three of them may combine to a strand. The pericardial cells situated elsewhere, however, show a spherical or polygonal shape.

Along the dorsal vessel the pericardial cells receive a nerve from the medial dorsal nerve system. By injecting larval aphids with Florella blue, via the cornicles, the pericardial cells could be clearly traced as blue flags attached to the medial dorsal nerve. The other ones are likewise provided with nerve fibres like those along the dorsal vessel, but it was impossible to tracce from which part of the nervous system they arise.

Haemolymph. The haemolymph is characterized by the absence of circulating haemocytes. On the other hand, numerous waxy droplets are visible

56

throughout the body cavity of the aphid during its life. The waxy droplets are released by the cell membrane of intact fat cells, or liberated into the haemo-lymph after dissolving of the cell membrane. Further the blood fluid contains rhomb-shaped crystals originating from fat cells after dissolving of their cell membrane. In a nine days old larva the haemolymph is provided with intact or degenerating symbionts (Figs. 28 and 32 V'), which also are observed in the legs.

Mycetome

After birth of the larva the mycetome consists of two longitudinal masses of syncytial tissues which are situated in the body cavity of the thorax and the abdomen (Fig. 1). They partly surround the alimentary canal and run from the posterior part of the mesothorax, where the intestine passes into the hindgut, to half-way the fifth abdominal segment. In the fourth abdominal segment they join together forming a bridge dorsally of the hindgut (Fig. 26 P). In the posterior part of the thorax and the abdomen each half of the mycetome is bounded dorsally by the ovaries (Fig. 25). In dissected aphids the cells or mycetocytes of the mycetome reveal as spheres with a white opaque appearance.

The mycetome consisting of a compact mass of cells of uniform size, is enclosed by a nucleated membranous sheath. The total number of mycetocytes varies from 45–52 (Table 8). Each mycetocyte contains one nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus. The scanty cytoplasm of the mycetocytes is completely filled with spherical microorganisms or symbionts which divide like bacteria (Fig. 26 P). The symbionts have basophilic cytoplasm with strongly basophilic granulated thread-like structures in the centre. By the pressure of the microorganisms due to multiplication of them the nuclei have an irregular or stellate appearance. The much smaller nuclei of the coating sheath are projected into the mycetocytes. These last mentioned nuclei have the same optical characteristics as those of the mycetocytes.

During larval life the mycetocytes gradually increase in size as do the nuclei and nucleoli, whereas the microorganisms remain constant in size (Table 8). The optical composition of the cells and nuclei does not change, except in the nucleoli where vacuoles arise. In the various ages of the larva cell divisions are never observed in the mycetome. On the other hand the microorganisms are continuously multiplying which can be concluded from the several stages of divisions (Fig. 26). After the fifth day the larval mycetome starts to disintegrate in clusters of cells or in single mycetocytes. The nucleated sheath enveloping the mycetome breaks open, but a part of it remains connected with the separated mycetocytes.

Within the mycetocytes some irregularly shaped, strongly basophilic symbionts occur. These degenerating symbionts increase in number and they are easily observed in a nine days old larva (Fig. 26 P'). At the ninth day some mycetocytes start to degenerate and this process continues in the adult stage. Their cell membranes dissolve after which intact and degenerating symbionts are liberated into the haemolymph. They are distributed throughout the aphid's
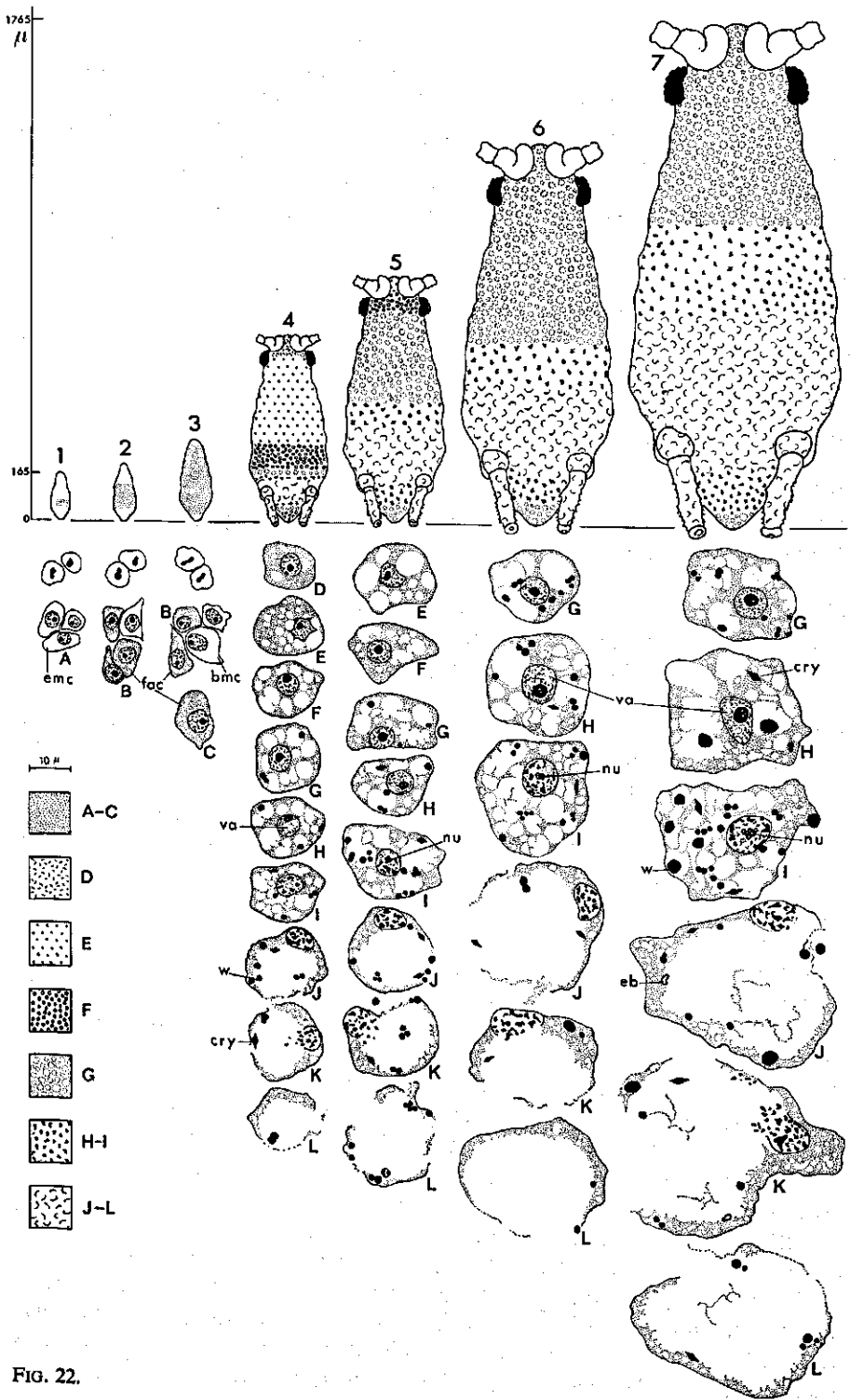
FIG. 22.

body cavity and occur in the mesodermal tissue between the degenerating fat cells as well as in the legs. Simultaneously with this process, the nuclei and nucleoli of the mycetocytes undergo several transformations similarly as described for the basophilic mesodermal cells (Fig. 21).

Oenocytes

The oenocytes are situated laterad on either side in the body cavity between the mesodermal tissue and the internal organs (Figs. 25 and 27) of the methathorax and the first five abdominal segments. They form a longitudinal row of 7–12 single cells, but in some cases 2–3 cells are found in intimate contact with each other. These polygonal cells are anchored by membranes which originate from the connective tissue cells, and which also connect them with tracheae or other internal organs. Their topographical position during larval life is shown in Fig. 1.

They are presumably of ectodermal origin because they are observed before the appearance of embryonic mesodermal cells. In embryos they are situated closely against the developing mycetome and during growth of the alimentary canal and the ovaries they partly become separated from the mycetome. Thereafter they are to be found between the mesodermal tissue and either the mycetome or the alimentary canal or the ovaries. In embryos the oenocytes are recognizable because of their somewhat irregular nuclei with basophilic granulated chromatin and a proportionally very large, eosinophilic, spherical or ovoïd nucleolus. The strongly basophilic cytoplasm contains some vacuoles (Fig. 21, A-D).

After larviposition the vacuolization process of the basophilic cytoplasm continues and the oenocytes resemble a sponge with numerous minute holes. Gradually a homogeneous acidophilic perinuclear zone develops after which the remaining cytoplasm becomes likewise acidophilic (Fig. 21, E-I). During this process some vacuoles arise around the nucleus containing 1–3 basophilic granules. These vacuoles progressively increase in number and disperse in the cytoplasm. At some places the cell membrane is irregular due to vacuoles which may either or not contain granules. The shape of the nucleus gradually becomes

FIG. 22. Development (histogenesis) of basophilic mesodermal cells (bmc) and fat cells (B-L) from embryonic mesodermal cells (A, emc) of *Myzus persicae*. The various stages in the development of the fat cells (B-L) are given in figured squares on the left side of the diagram, which correspond with the occurrence of these cells (A-L) in the successive stages of the larva (1–7). No. 1 represents the eldest embryo of a three days old larva, no. 2 that of a five days old larva, no. 3 that of a seven days old larva, and no. 4 that of a nine days old larva. Cell divisions of the embryonic mesodermal cells occur only in the first three mentioned embryos. The numbers 5–7 represent respectively the one day, five and nine days old larva. The size of the embryos and larvae are drawn to scale. Note the waxy droplets (w), rhomb-shaped crystals (cry), and eosinophilic bodies (eb) in the fat cells. For explanation of abbreviations see pages 144–147.

FIG. 23. Topographical position of the basophilic mesodermal cells of a one day old *Myzus persicae* larva. Graphic reconstruction. The cells of which the cytoplasm is dotted are to be found in the dorsal mesodermal tissue, those with white cytoplasm and unbroken cell membranes in the lateral mesodermal tissue, and the remaining ones with dashed membranes in the ventral mesodermal tissue.

60
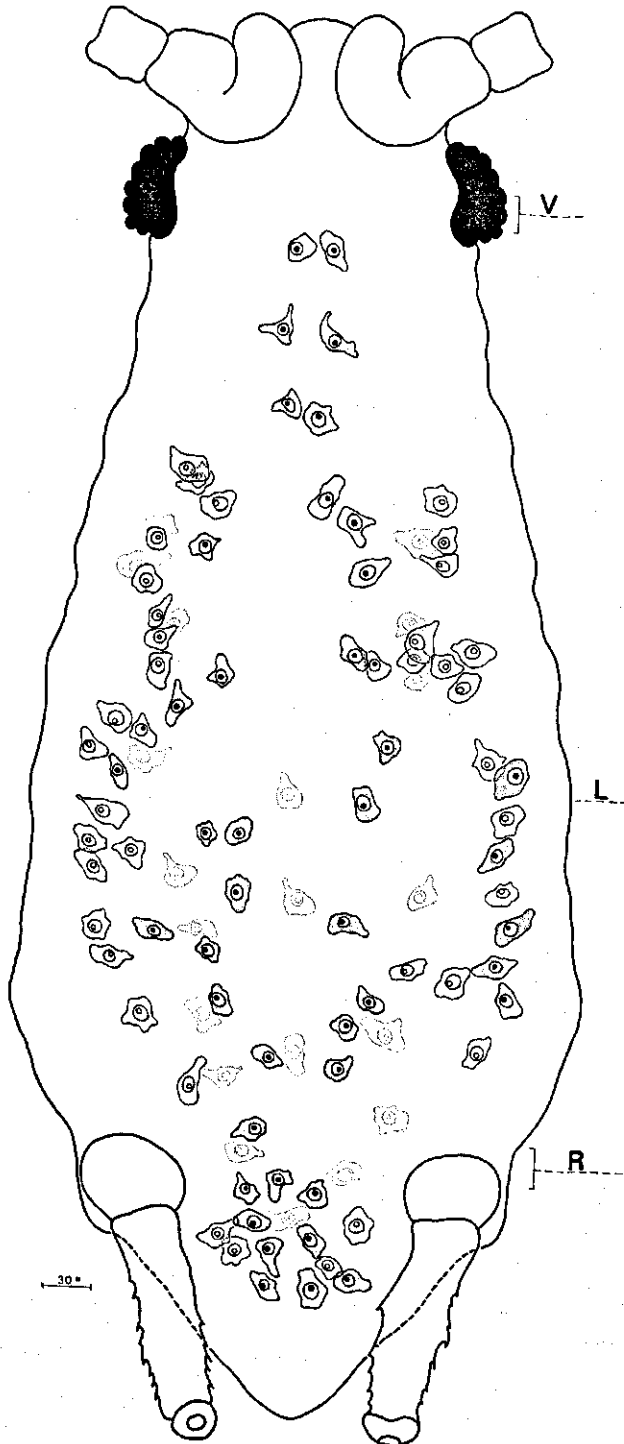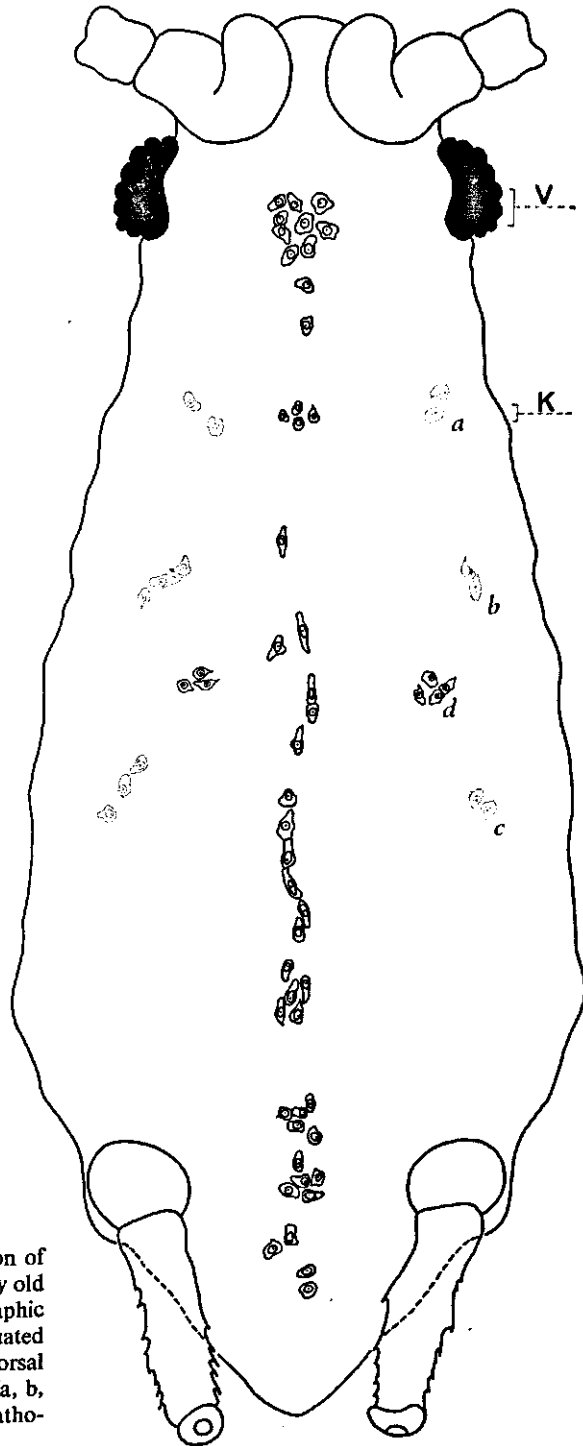
FIG. 24. Topographical position of the pericardial cells of a one day old *Myzus persicae* larva. Graphic reconstruction. They are situated laterally and ventrally of the dorsal vessel, in the coxae of all legs (a, b, and c), and laterad in the metathoracic segment (d).

more irregular or branched and at some places the nuclear membrane shows a granulated structure. It seems that granules are separated from the nucleus and are subsequently transported in cytoplasmic vacuoles to the cell membrane from which they are then released into the haemolymph. The extrusion of chromatin material probably makes that the nuclei of these cells hardly increase in size during larval life (Table 8). In the nucleolus minute vacuoles arise which sometimes fuse into big ones.

The enlargement of the oenocytes which started during the embryonic stage continues during larval life. Cell divisions are never observed and consequently the total number of cells remains constant (Table 8).

Circulatory system

The dorsal vessel lies in the median plane just beneath the dorsal mesodermal tissue (Figs. 25 and 27). It starts in the sixth abdominal segment dorsally at the termination of the hindgut, and extends forwards above the hindgut, mycetome bridge, stomach, foregut, and between the principal salivary glands discharging in the head (Fig. 3). The ventral wall of the funnel-shaped mouth terminates half-way above the corpus allatum, and is connected with its ventrolateral sides to this body (Fig. 32). The dorsal wall of the mouth extends further and is attached to the corpora cardiaca (Fig. 28). The dorsal vessel is gradually constricting caudad. Throughout its length the vessel pulsates vigorously and the anterior wider part is provided with three pairs of lateral ostia. Their presence was observed by injecting indian ink via the cornicles in the body cavity of the aphid. The indian ink entered the dorsal vessel via the ostia, rushed forwards as the result of pulsating movements and was discharged via the open end into the cavity of the head.

The posterior end of the dorsal vessel is closed, and bears the insertion of one pair of muscles. Each muscle runs lateroventrally and originates from the posterior edge of the seventh abdominal sternite. At the right and left side the entire vessel is attached to the longitudinal dorsal somatic muscles by groups of very thin muscle fibres (alary muscles, Fig. 27). The wall of the dorsal vessel is a thin sheath consisting of muscle fibres irregularly arranged in longitudinal and circular directions. The very small elipsoid-shaped nuclei are distributed in the sheath and protrude either in the lumen of the vessel or in the body cavity. During larval life the nuclei and their spherical nucleoli increase in size and cell divisions are never observed. This is in accordance with the increase in length of the dorsal vessel (Table 8). It does persist unchanged into the adult. Many waxy droplets are present in the lumen of the dorsal vessel which may be enter the vessel through the ostia.

The innervation of the dorsal vessel takes place by the medial dorsal nerve cord originating from the corpora cardiaca. It runs along the dorsal vessel giving off branches to the wall of this organ, the alary muscles, and the pericardial cells (Fig. 3); it terminates at the dorsal posterior end of the hindgut near the rectum (Fig. 12) and the gonopore.

In all coxae a small tubular organ occurs. This organ has a length of about

62

15 μ and shows a similar histological structure as that of the dorsal vessel. They are innervated by one nerve, and presumably represent the accessory pulsatile organs.

Cornicles

In *M. persicae* the cornicles are situated dorsolaterally on the fifth abdominal segment (Fig. 27). The length of the cornicles is about 1/3 of that of the aphid's body in all instars, and the average diameter is about 1/10 of the maximal abdominal width. In apterous viviparous females they are slender, nearly uniform in diameter throughout, slightly swollen on the inner margin near the distal end, at which point they curve slightly outward (GILLETTE, 1908a).

The epidermis of the cornicle is a continuation of that of the aphid's body. It is composed of a single layer of cells resting upon a basement membrane; the cells contain strongly basophilic cytoplasm without definite structures. A proportionally large nucleus with scattered basophilic chromatin and an eosinophilic small nucleolus is present in each cell. During larval life the cuboidal epidermal cells increase in size and become flattened. The presence of mitotic figures in these cells indicate that they also have to increase in number. The epidermis persists into the adult, and mitotic figures could no longer be observed in a nine days old larva. The cornicles are in open connection with the body cavity and are filled with degenerating fat cells. This mass forms a continuation

TABLE 7. Components of the cornicle secretion of *Myzus persicae* which were allowed to feed on Chinese cabbage seedlings at 20 °C. Each aphid produced 1–2 cornicle droplets.

Age of aphid	Number of aphids	Components				
		Degenerating fat cells[1]	Rhomb-shaped crystals	Waxy droplets	Symbionts	Basophilic mesodermal cells[2]
1 (larva)	2	+	+	+	—	—
2	2	+	+	+	—	—
3	2	+	+	+	—	—
4	4	+	+	+	—	1
5	3	+	+	+	—	1
6	4	+	+	+	—	—
7	2	+	+	+	—	—
8	4	+	+	+	—	—
9 (adult)	6	+	+	+	+	2
10	3	+	+	+	+	2
11	4	+	+	+	+	—
12	12	+	+	+	+	6
14	8	+	+	+	+	—
16	5	+	+	+	+	—
20	6	+	+	+	+	3

+ = present; — = absent
[1] These cells correspond with celltypes J-L in Fig. 22
[2] The figures refer to the number of cells found in the cornicle secretion of the total number of aphids examined

with that in the body cavity. Each cornicle is closed at its top with a valve-like flap. The flap is controlled by one muscle (valve retractor muscle) which runs throughout the length of the cornicle to the posterior edge of the sixth abdominal sternite. Anterior of the base of the cornicle is the insertion of another muscle (elevator muscle) originating from the anterior edge of the fifth abdominal sternite. The muscles are innervated by nerves arising from the main abdominal nerve (Figs. 3). Each cornicle is supplied by a trachea originating from the sixth abdominal spiracle (Fig. 2, e).

When the aphid shows a normal feeding attitude the cornicles are directed caudad nearly adjacent to the abdomen. Touching the aphid's body with a fine-pointed needle results in the elevation of the cornicles, which is followed immediately by droplet production. That is the only movement the cornicles can perform and droplets can be produced only after the cornicles are raised in this way. In general both cornicles excrete simultaneously, but in some cases it was observed that one of them produced the droplet, although both cornicles were erected. By continuous irritation the cornicles remained in this position without producing any droplet.

Cornicle secretion. Right after larviposition it is possible to trigger cornicle secretion. The droplets are very small in diameter, but during larval life they increase in size to remain approximately constant in the imaginal stage. Histologically the cornicle secretion is composed of degenerating fat cells, waxy droplets, and rhomb-shaped crystals (Table 7). The degenerating fat cells are all of celltype J-L (Fig. 22), although occasionally celltype H-I may occur. The nuclei which are partly or completely disintegrated, appear as basophilic nuclear fragments, and eosinophilic granules or irregularly shaped rods of various size. Moreover, there occur some fat cells of celltype J (Fig. 22) of which the cell membrane is still intact. These cells harden directly after having passed the cornicles and have an opaque white spherical appearance. They are dissolvable in alcohol. The waxy droplets, originating from degenerating fat cells, have a spherical or irregular shape with a milky appearance, and are dissolvable in ether or acetone, but not in alcohol. Especially in the second half of the larval period and during the adult stage the rhomb-shaped crystals occur in large quantities in the cornicle secretion. At the ninth day of the aphid's life the first intact and degenerating symbionts appear in the cornicle secretion (Table 7). These irregularly shaped, strongly basophilic symbionts increase continuously in number during the imaginal stage of the aphid. This agrees with the degeneration process of the mycetocytes (page 57). Incidentally an intact basophilic mesodermal cell was observed in the cornicle secretion of larval aphids. In the adult stage they are partly disintegrated showing the same structures as described on page 54. Connective tissue cells have never been observed in the cornicle secretion during aphid's life.

Nervous system

The cross picture of the central nervous system is shown in Figs. 3 and 29. The cortex of the protocerebral lobes encloses an irregularly formed neuropile
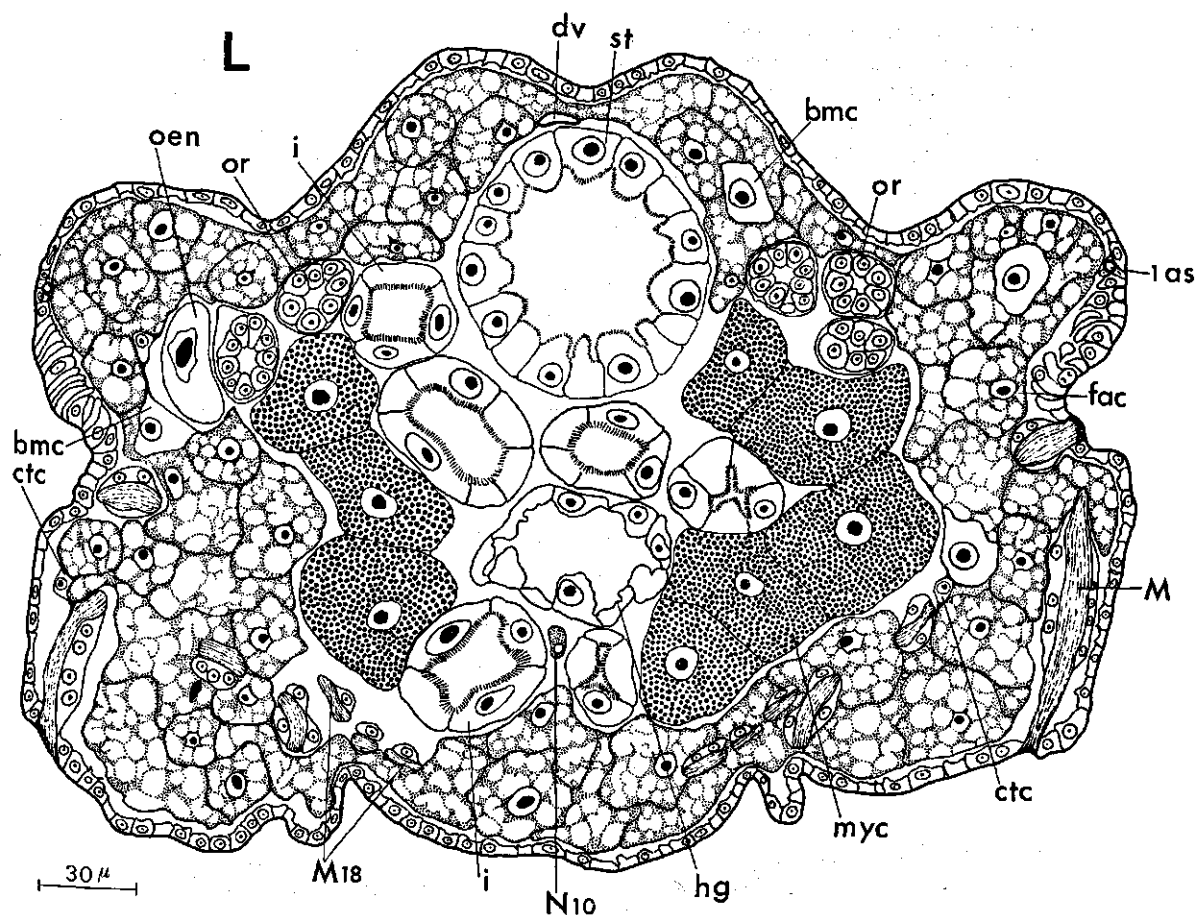
FIG. 25. Transverse section through the first abdominal segment of a one day old *Myzus persicae* larva showing position of stomach (st), intestine (i), hindgut (hg), fat cells (fac), basophilic mesodermal cells (bmc), connective tissue cells (ctc), mycetome (myc), oenocyte (oen), dorsal vessel (dv), main abdominal nerve (N10), and ovarioles (or). The position of this section (L) is given in Figs. 1–3, 11, and 23. For explanation of abbreviations see pages 144–147.

mass in which commissural tracts and a central body are clearly to be distinguished. As a result of the localisation of the elevator muscles of the tentorial bar the posterior part of each protocerebral lobe shows in the region of the pars intercerebralis a wedge-shaped impression by which a blunt protrusion is formed, viz. the dorsal lobe (Figs. 28, 30, and 31). The elevator muscles originate from the dorsal wall of the head and are attached to the tentorium on both sides of the posterior end of the pharynx. In some larvae it was observed that the dorsal lobes can vary considerably in form and size. Each dorsal lobe gives off laterally a nerve which runs to the corpora cardiaca (Fig. 28). Moreover, the latter is connected with the brain by another nerve branch. This nerve branch has a paired root and arises on the inner surface of each cerebral lobe where it

tapers into the circumoesophageal connective. The optic masses are composed of three optic centres: a proximal medulla interna, a medial medulla externa, and a distal lamina ganglionaris (Fig. 28). The latter is connected with the compound eye by optic fibres. Moreover, each optic lobe gives off a thin nerve to the tubercle situated ventrally of the compound eye and containing three optical elements. Between the proximal medulla interna and the medial medulla externa on each side of the optic lobe a centre of strongly basophilic embryonic cells occurs (neural imaginal discs) (Figs. 29 and 31). Their spherical nuclei have the same diameter as those of the neurons. These centra in which occasionally a mitotic figure occurs, are only visible in the first five days of the larval stage, and afterwards it is indistinguishable from the brain tissue.

The paired deutocerebrum is situated beneath the protocerebral lobes. Each lobe consists of two neuropile centres from which the antennal nerve arises (Figs. 3, 29, and 30). Both deutocerebral lobes are united by internal commissural tracts and each of them by connectives with various parts of the protocerebrum.

The tritocerebrum is the most ventral part of the brain and is divided into two widely separated lobes (Figs. 3, 29, and 30). Each tritocerebral lobe is connected by a connective tract with the protocerebrum which runs along the inner surface of the deutocerebral lobe. The central parts of the tritocerebral lobes are connected with the median frontal ganglion by a very short frontal nerve. Ventrolaterally each lobe gives off a paired nerve, which runs to the mandibular and maxillary retort-shaped organs. On each lateral side of the frontal ganglion a nerve arises which runs along the inner surface of the distal part of each tritocerebral lobe and fuses with a nerve from the tritocerebrum forming the labral nerve. The labral nerve is connected with the retractor muscles of the pharyngeal valve (M4(1), Fig. 5) and gives off branches to the muscles of the anterior part of the pharyngeal pump (M4(2) and M4(3)). From the most lateral distal part of each tritocerebral lobe a thick nerve thrunk arises which unites the pharyngeal ganglion with the brain (Figs. 3 and 30). This pharyngeal nerve gives off a branch to the lateral divaricator muscles of the pharyngeal valve (M10, Fig. 8 no. 12) and to the lateral muscles of clypeus (M7, Fig. 30).

The protocerebral, deutocerebral, and tritocerebral lobes taper into the circumoesophageal connectives that join the brain to the suboesophageal ganglion (Figs. 3, 29, and 32 V). In these connectives the fibrous neuropile mass of the deutocerebrum (deutocerebral longitudinal tract) and that of the tritocerebrum (tritocerebral longitudinal tract) are clearly seen. Just before the suboesophageal ganglion both tritocerebral longitudinal tracts are connected by transverse nerve fibres, presumably the main tritocerebral commissure (Fig. 32 V). In the same region each deutocerebral and tritocerebral longitudinal tract gives off at its lateral side a thick nerve trunk. The trunk divides into three nerves, of which two innervate the retractor and protractor muscles of the mandibular and maxillary stylets. The third one runs in the direction of the lateral mesodermal tissue. In the ventral part at each side there arises a nerve

from the tritocerebral tract which connects the basal part of the mandibular stylets (Figs. 4 and 32 V; N19). From the posterior part of the suboesophageal ganglion at the ventrolateral side two pairs of nerves arise, of which the anterior pair serves to innervate the muscles of the salivary pump, while the second pair runs beneath the common salivary duct into the labium (Fig. 29).

Internally, the thoracic ganglion complex shows a prothoracic, mesothoracic, and metathoracic subdivision (Figs. 3 and 29). The nerve trunks for the three pairs of legs arise ventrolaterally. The dorsolateral side of the meso and metathoracic regions gives off a nerve to innervate the muscles of the first two pairs of tracheal spiracles situated in the meso and metathoracic segment respectively. The most posterior part of the thoracic ganglion complex, situated behind the metathoracic region, is the abdominal region. From this region arises the main abdominal nerve trunk which extends far into the abdomen with terminal offshoots in the wall of the gonopore. A similar structure was found in *Siphonophora rosarum* (GROVE, 1909), *Schizoneura lanigera* (BAKER, 1915), *Drepanosiphum platanoides* (JOHNSON, 1963), and *Schizaphis graminum* (SAXENA and CHADA, 1971d) (Table 9). In the fourth abdominal segment it gives off two branches innervating the muscles of the cornicles, and in the fifth abdominal segment a branch to the hindgut (Fig. 3). In *Aphis fabae* and *Acyrthosiphum pisum*, two branches of the main abdominal nerve pass to the ventral surface of the hindgut, meeting it just posterior to the point of the voluminous coil of intestine (JOHNSON, 1963). The muscles of the seven pairs of abdominal spiracles are probably innervated from branches of the main abdominal nerve.

The cone-shaped frontal ganglion lies between the two separated lobes of the tritocerebrum; it is connected on both sides to the lobes by a very short nerve trunk (frontal nerve, Fig. 30). The recurrent nerve extends posteriad to the spindle-shaped hypocerebral ganglion, and from there to the corpus allatum. The hypocerebral ganglion is a very small body with a diameter of about 5 μ and lies between the deutocerebral lobes.

The paired pharyngeal ganglia are situated ventrally of the tritocerebral lobes and frontolaterally of the pharyngeal pump on both sides of their retractor muscles (Figs. 3 and 30). Each pharyngeal ganglion is bilobed of which the most laterally situated lobe is connected at its dorsal end with the tritocerebral lobe by the pharyngeal nerve. From the same point a second nerve arises which terminates in many branches to innervate the retractor muscles M4(4) and M4(5) (Fig. 5) of the pharyngeal pump. Before the pharyngeal valve the two bilobed pharyngeal ganglia fuse, and this mass extends ventrally as the epipharyngeal and the hypopharyngeal gustatory organ.

The epipharyngeal gustatory organ lies in the clypeo-labrum region (Figs. 3 and 5). In embryos it is composed of a mushroom-shaped central part enclosed left and right by a lateral lobe (Fig. 7 no. 8). The central part has on its periphery many neurons which are connected by nerve fibres with the pores of the eight sensilla. The pores communicate with the pharyngeal duct (Fig. 5). Centrally each lateral lobe is occupied by a stellate invagination of the pharyngeal duct (Figs. 6 and 7 no. 8). From the wall of this stellate invagination nerve

TABLE 8. Actual sizes and cell numbers of various organs of *Myzus persicae* larvae bred on Chinese cabbage seedlings at 20°C. The values are given in μ.

Age	One day old larva[1]	Three days old larva	Five days old larva	Seven days old larva	Nine days old larva
Basophilic mesodermal cells					
number of cells	100	97	90	95	89
Pericardial cells					
number of cells	67	62	68	63	68
average diameter cell	10.6 ± 0.40 (1)	13.4 ± 0.61 (1.3)	13.6 ± 0.71 (1.3)	15.7 ± 1.00 (1.5)	17.3 ± 1.07 (1.6)
average diameter nucleus	4.8 ± 0.09 (1)	6.6 ± 0.23 (1.4)	6.6 ± 0.17 (1.4)	7.0 ± 0.29 (1.5)	8.4 ± 0.51 (1.8)
average diameter nucleolus	1.4 ± 0.04 (1)	2.4 ± 0.10 (1.7)	2.3 ± 0.09 (1.6)	2.3 ± 0.12 (1.6)	2.4 ± 0.15 (1.7)
Mycetome					
length	340 (1)	770 (2.3)	—	—	—
maximum width	190	—	—	—	—
number of mycetocytes	49	45	48	50	52
average diameter nucleus	9.3 ± 0.23 (1)	12.3 ± 0.32 (1.3)	12.9 ± 0.49 (1.4)	12.9 ± 0.25 (1.4)	15.1 ± 0.37 (1.6)
average diameter nucleolus	4.1 ± 0.09 (1)	4.9 ± 0.12 (1.2)	5.3 ± 0.11 (1.3)	5.1 ± 0.09 (1.2)	5.1 ± 0.07 (1.2)
average diameter symbionts	2.7 ± 0.04 (1)	2.7 ± 0.04 (1)	2.7 ± 0.04 (1)	2.7 ± 0.04 (1)	2.7 ± 0.04 (1)
Oenocytes					
number of cells[2]	7–8	12–11	9–10	10–9	11–7
average diameter cell	21.3 ± 1.42 (1)	23.7 ± 1.02 (1.1)	25.3 ± 1.11 (1.2)	26.1 ± 0.75 (1.2)	30.2 ± 0.87 (1.4)
average diameter nucleus	8.0 ± 0.29 (1)	9.7 ± 0.28 (1.2)	9.0 ± 0.43 (1.1)	8.9 ± 0.46 (1.1)	9.1 ± 0.45 (1.1)
average diameter nucleolus	4.4 ± 0.21 (1)	5.0 ± 0.13 (1.1)	4.9 ± 0.17 (1.1)	4.8 ± 0.15 (1.1)	4.4 ± 0.14 (1.0)
Dorsal vessel					
length	660 (1)	1040 (1.6)	1010 (1.5)	1105 (1.7)	1420 (2.2)
average diameter nucleus	2.2 ± 0.09 (1)	2.5 ± 0.11 (1.1)	3.1 ± 0.09 (1.4)	3.5 ± 0.10 (1.6)	4.1 ± 0.19 (1.9)
average diameter nucleolus	0.5 ± 0.05 (1)	0.6 ± 0.05 (1.2)	0.9 ± 0.05 (1.8)	1.0 ± 0.04 (2.0)	1.1 ± 0.04 (2.2)
Nervous system					
length (without main abdominal nerve)	335 (1)	400 (1.2)	395 (1.2)	380 (1.1)	395 (1.2)
brain, maximum width	138.3 (1)	198.3 (1.4)	223.3 (1.6)	213.3 (1.5)	196.7 (1.4)
suboesophageal ganglion, maximum width	72.5 (1)	91.7 (1.3)	100.0 (1.4)	106.7 (1.5)	100.0 (1.4)
thoracal ganglion, maximum width	92.5 (1)	143.3 (1.5)	138.3 (1.5)	148.3 (1.6)	154.2 (1.7)

Perineurium					
average diameter nucleus	2.4 ± 0.08 (1)	2.5 ± 0.07 (1.0)	2.6 ± 0.11 (1.1)	3.0 ± 0.16 (1.3)	2.8 ± 0.13 (1.2)
average diameter nucleolus	0.5 ± 0.04 (1)	0.5 ± 0.04 (1.0)	0.5 ± 0.05 (1.0)	0.7 ± 0.03 (1.4)	0.7 ± 0.01 (1.4)
Neurons					
average diameter nucleus	3.8 ± 0.09 (1)	3.7 ± 0.11 (1)	3.9 ± 0.11 (1)	3.8 ± 0.09 (1)	3.8 ± 0.11 (1)
average diameter nucleolus	0.7 ± 0.02 (1)	0.7 ± 0.02 (1)	0.7 ± 0.01 (1)	0.7 ± 0.02 (1)	0.7 ± 0.02 (1)
Glial cells					
average diameter nucleus	2.1 ± 0.06 (1)	2.2 ± 0.07 (1.0)	2.3 ± 0.10 (1.1)	2.4 ± 0.09 (1.1)	2.7 ± 0.07 (1.3)
average diameter nucleolus	0.5 ± 0.03 (1)	0.5 ± 0.03 (1.0)	0.6 ± 0.01 (1.2)	0.7 ± 0.02 (1.4)	0.7 ± 0.02 (1.4)
Corpus cardiacum					
number of cells[3]	9–9	11–8	8–9	9–9	10–9
average diameter cell	5.1 ± 0.09 (1)	6.0 ± 0.23 (1.2)	5.9 ± 0.20 (1.2)	5.9 ± 0.22 (1.2)	6.5 ± 0.29 (1.3)
average diameter nucleus	3.0 ± 0.10 (1)	3.7 ± 0.15 (1.2)	4.0 ± 0.15 (1.3)	3.9 ± 0.16 (1.3)	4.0 ± 0.17 (1.3)
average diameter nucleolus	0.6 ± 0.03 (1)	0.7 ± 0.03 (1.2)	0.8 ± 0.05 (1.3)	0.7 ± 0.02 (1.2)	0.8 ± 0.05 (1.3)
Corpus allatum					
average diameter	16.7 (1)	31.0 (1.9)	26.7 (1.6)	34.3 (2.1)	38.8 (2.3)
number of cells	12	12	11	13	12
average diameter nucleus	3.9 ± 0.19 (1)	7.1 ± 0.28 (1.8)	7.1 ± 0.36 (1.8)	8.1 ± 0.31 (2.1)	9.2 ± 0.34 (2.4)
average diameter nucleolus	0.9 ± 0.09 (1)	1.8 ± 0.07 (2.0)	2.1 ± 0.16 (2.3)	2.0 ± 0.09 (2.2)	2.2 ± 0.07 (2.4)
Thoracic glands					
number of cells[4]	11–10	10–11	8–10	4–7	degenerated
average diameter cell	7.5 ± 0.33 (1)	10.9 ± 0.37 (1.5)	11.2 ± 0.57 (1.5)	10.7 ± 0.46 (1.4)	
average diameter nucleus	5.3 ± 0.36 (1)	7.8 ± 0.19 (1.5)	8.0 ± 0.41 (1.5)	8.3 ± 0.31 (1.6)	
average diameter nucleolus	1.5 ± 0.05 (1)	2.1 ± 0.07 (1.4)	2.4 ± 0.19 (1.6)	2.3 ± 0.13 (1.5)	

[1] The data are obtained from one larva
[2] Number of cells situated laterad on either side in the body cavity
[3] Number of cells in each corpus cardiacum
[4] Number of cells in each thoracic gland
The length is measured to the number of sections (each section is 5 µ thick)
The sign ± is followed by the standard deviation of the mean (s/√n; n = 10)
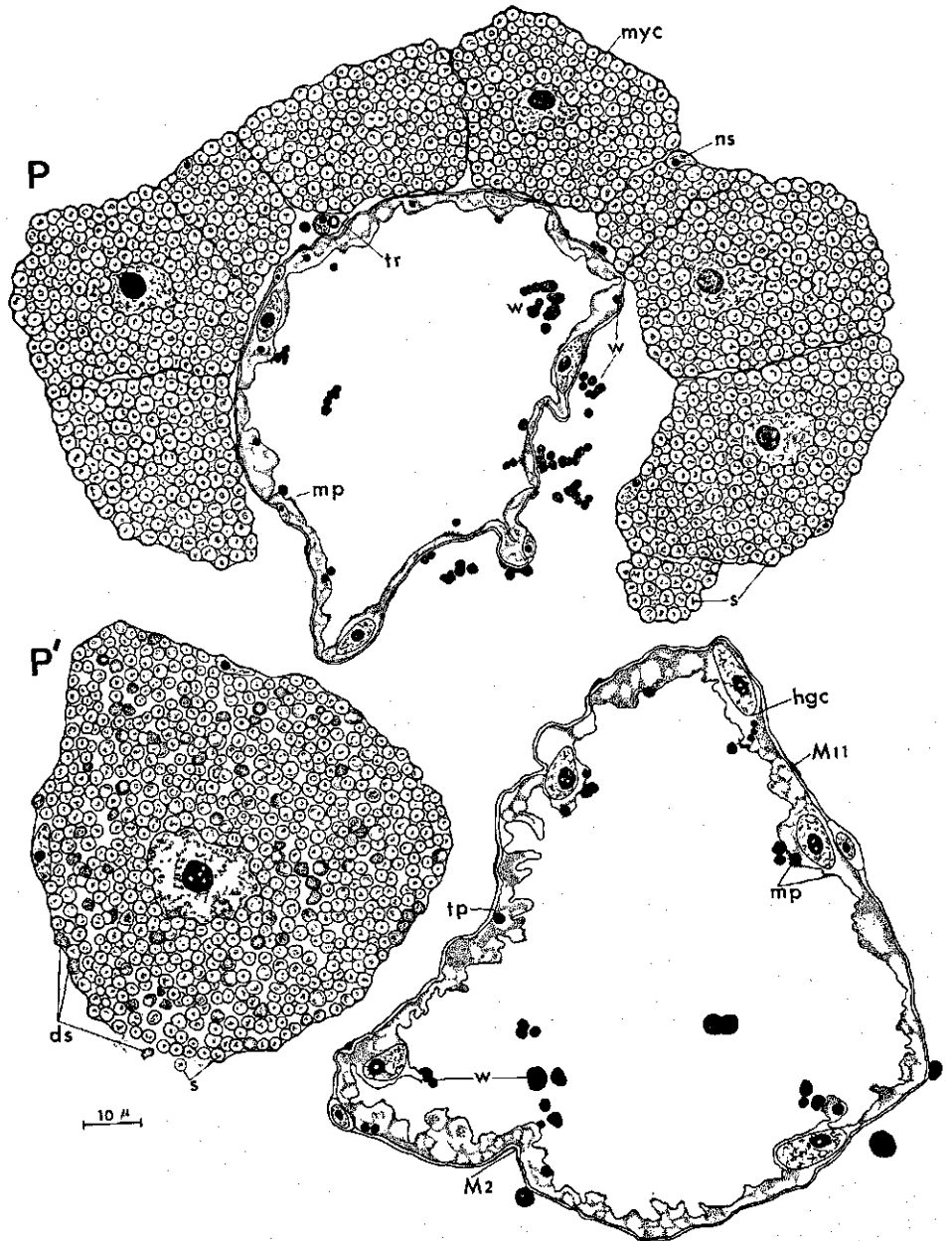In brackets relative sizes on basis of size at 1 day as 1

FIG. 26. P. Transverse section of the mycetome (myc) surrounding the hindgut of a one day old *Myzus persicae* larva showing nine mycetocytes with their spherical symbionts (s) and some degenerating ones. The position of this section is given in Figs. 1–3 and 11. P′. Transverse section of the hindgut and a mycetocyte of a nine days old larva. The mycetocyte harbours intact (s) and degenerating symbionts (ds) which liberate into the haemolymph. The degenerating symbionts are recognized by their irregular shape. Note in both figures the waxy droplets (w) in the haemolymph, inside the hindgut cells, and in the lumen of the hindgut which are of the same optical composition. For explanation of abbreviations see pages 144–147.

fibres arise running to neurons situated mainly on the periphery of the lateral lobes. Possibly this are sensory neurons of another type than those of the gustatory sensillae. This means that the gustatory organ is a complex taste organ. Some neurons in the gustatory organ have nerve fibres running in the direction of the clypeo-labrum wall, probably comunicating with receptor hairs placed on that part of the integument (Figs. 8 no. 10' and 31).

The hypopharyngeal gustatory organ starts beneath the pharyngeal valve in the posterior part of the hypopharynx (Fig. 3) and extends into the anterior part of the hypopharynx lip (Fig. 5). The neurons in this organ communicate by nerve fibres with two sensillum pores which are situated in the cup-shaped part of the floor of the pharyngeal duct, close before the valve (Fig. 8 no 10 and 10'). Moreover, fibres from the anterior part of the hypopharyngeal gustatory organ communicate with two other sensillum pores located on the foot of the salivary pumpstem (Figs. 5, 7 no. 8, and 19).

The whole nervous system is enclosed by a very thin squamous epithelium (perineurium) covered by a neural lamella (neurilemma). Their elongated nuclei contain scattered basophilic chromatin and a small spherical nucleolus. The cortical region is a compact mass composed of neurons and glial cells without any histological differentiation (Fig. 28). The neurons contain basophilic cytoplasm characterized by the presence of a proportionally big spherical nucleus with basophilic granulated chromatin. In many nuclei the small spherical nucleoli are not distinguishable from the chromatin granules. One can distinguish the glial cells from the neurons only when they occur on the periphery of the neuropile where they penetrate into these centres with their strongly basophilic protoplasmic cell processes. Typical are their irregularly-shaped small nuclei with a spherical nucleolus. During larval life the nuclei of the neurons retain their original size, while those of the epithelial and glial cells have the tendency to increase somewhat in volume (Table 8). Moreover, many mitotic figures occur in the cortical region of the brain, suboesophageal, and thoracic ganglion of one day and three days old larvae. Thereafter these figures are found only in the optic lobes. After the ninth day (just adult stage), however, no more mitoses are found in these centra. Evidently, the growth of the nervous system is the result of both multiplication and increase in volume of some tissue elements such as glial cells. In the beginning of the larval stage the cortex and neuropile form a compact mass, but as the larval stage advances, the fibrous and cellular components of the nervous tissue show a looser relation.

Endocrine glands. The paired corpora cardiaca are situated between the dorsal lobes of the protocerebrum and the posterior part of the pharynx (Figs. 3 and 28). They are irregularly-shaped bodies which are attached to the inner side of the dorsal aortic wall and supplied by numerous finely branching tracheae originating from the antennal tracheal stem (Fig. 2, c). Each gland is composed of 8–11 cells arranged around a neuropile mass from which nerves arise. The cells contain strongly basophilic cytoplasm and a proportionally big nucleus of which the basophilic chromatin is mainly situated against the inner surface of the nuclear membrane. During larval life cell divisions are never

observed in the corpora cardiaca. The cells increase in size, while after the thirdth day the average diameter of their nuclei remains practically constant (Table 8). As a result of the increasing cell size the two glands show some degree of median fusion. The corpora cardiaca persist unchanged into the adult stage. From each body arise three nerves which are called by JOHNSON (1963) the medial, lateral, and ventral nerves (Fig. 28). Both medial nerves fuse together beneath the dorsal wall of the aorta forming a single nerve which runs between the corpus allatum and the ventral wall of the aorta (Fig. 32) extending
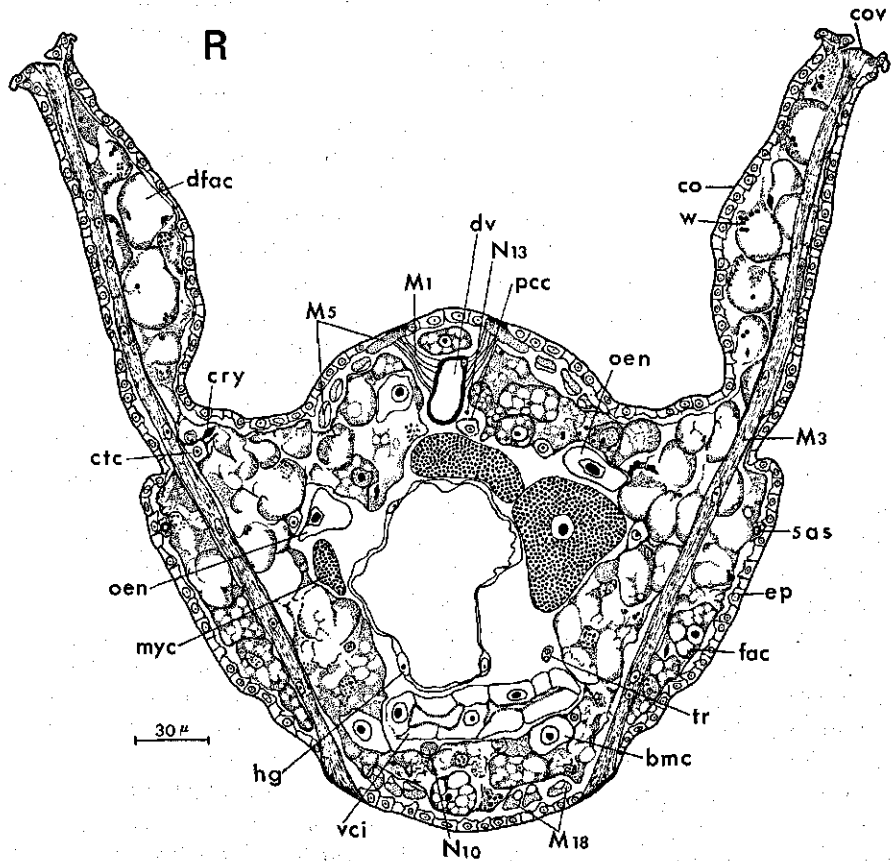
FIG. 27. Composition made from six successive transverse serial sections through the cornicle region of a one day old *Myzus persicae* larva showing position of voluminous coil of intestine (vci), hindgut (hg), intact (fac) and degenerating fat cells (dfac), basophilic mesodermal cells (bmc), connective tissue cells (ctc), pericardial cell (pcc), mycetome (myc), oenocytes (oen), dorsal vessel (dv), main abdominal nerve (N10), and medial dorsal nerve (N13). The dorsoventral and cornicle elevator muscles are omitted. The position of this section (R) is given in Figs. 1–3 and 11. For explanation of abbreviations see pages 144–147.

72

further along the dorsal vessel. This nerve crosses diagonally the funnel-shaped mouth of the aorta. In its course it gives off a branch to each principal salivary gland (Fig. 13A), the dorsal vessel and its alary muscles, the pericardial cells (Fig. 3), the alimentary canal (Fig. 9 A), and the dorsal posterior end of the hindgut near the rectum (Fig. 12 F). At the posterior end of the dorsal vessel the medial nerve divides in two branches to innervate the lateral muscles of the gonopore. The lateral and ventral nerves have not further been traced.

The corpus allatum is a single body lying between the foregut and dorsal vessel behind the corpora cardiaca (Figs. 3 and 32). The body is connected by a nerve to each lobe of the corpora cardiaca and to the hypocerebral ganglion. It contains 11–13 cells which increase in size during larval life (Table 8). The cells retain their basophilic cytoplasm with vacuoles of various size, mainly situated in the apical part. Each cell has a nucleus consisting of regularly scattered basophilic chromatin and an eosinophilic nucleolus. The corpus allatum is surrounded by a thin membrane-like sheath without any visible cellular material. In the centre of the corpus allatum the apical parts of the gland cells seem to be connected with a mass of nerve fibres. The corpus allatum persist unchanged into the adult stage (Fig. 32 V').

The paired thoracic glands are situated in the vicinity of the mesothoracic spiracles. Each structure consists of about 8–11 gland cells (Fig. 20) which ly closely together between the tracheal trunk running to the prothoracic ganglion and the ventral tracheal trunk which connects both mesothoracic spiracles (Fig. 2). They contain strongly basophilic cytoplasm without any structures. Each of them contains a proportionally very big nucleus with regularly scattered basophilic chromatin and an eosinophilic nucleolus. During larval life the cell, nucleus, and nucleolus increase in size; after the seventh day some cells start to degenerate, while on the ninth day (just adult stage) all the cells are disintegrated. The degenerated cells are to be seen as clusters of irregular spheres in the haemolymph.

DISCUSSION

Alimentary canal

In general the morphology of the alimentary canal of *Myzus persicae* (MORREN, 1836; BUCKTON, 1876; BRAMSTEDT, 1948; SCHMIDT, 1959; FORBES, 1946a) shows many similarities with that of other aphid species (RAMDOHR, 1811; DUFOUR, 1833; WITLACZIL, 1882; GROVE, 1909, 1910; DAVIDSON, 1913; BAKER, 1915; WEBER, 1928; PELTON, 1938; SMITH, 1939; ROBERTI, 1946; PESSON, 1951; MARTINI, 1958; SAXENA and CHADA, 1971c) (Table 9). Growth of the alimentary canal of *M. persicae* during larval life is due solely to enlargement of the epithelial cells. The length of the entire gut during larval life is approximately three times the length of the aphid's body, which was also observed for *M. persicae* (MORREN, 1836) and other aphid species of unknown age (RAMDOHR, 1811; DUFOUR, 1833; PELTON, 1938; SMITH, 1939).

The structure of the retort-shaped organs as described in many aphid species (METSCHNIKOW, 1866; WITLACZIL, 1882; FLÖGEL, 1905; DAVIDSON, 1913; BAKER, 1915; KNOWLTON, 1925; WEBER, 1928; ROBERTI, 1946; BREIDER, 1952, 'cephalen Maxillomandibular-Drüsen') agrees in general with that of *M. persicae*. The fact that the stylets are secreted by the retort-shaped organs, implies that after completion of the final moult these organs probably degenerate, as has been suggested by DAVIDSON (1913). Later it was found that these organs are absent in adult jassids (WILLIS, 1949).

On the other hand, SORIN (1966) and SAXENA and CHADA (1971b) reported the presence of mandibular and maxillary glands in the head of the aphids *Aphis craccivora* and *Schizaphis graminum*. According to these authors the common ducts of the mandibular and maxillary glands unite before opening into the salivary channel of the maxillary stylets forming a part of the salivary gland complex (TAKAOKA, 1969). Thus, there are two separate duct openings into the salivary channel, one by the common salivary duct, and the other by the union of common ducts from mandibular and maxillary glands. Moreover, SORIN (1966) reported that the mandibular and maxillary glands in *Aphis craccivora* secrete a highly viscous fluid which is used for stylet sheath formation. However, from the descriptions and photographs presented by these authors, it may be concluded that these glands are in fact the mandibular and maxillary retort-shaped organs. In *M. persicae* and other aphid species as well as in coccoids (MARK, 1877; PESSON, 1944, 1951), in Phylloxeridae (KRASSILSTSCHIK, 1893), in psyllids (WITLACZIL, 1885; WEBER, 1929), and in white flies (WEBER, 1935b), the glandular function of the cells inside the retort-shaped organs is responsible for the secretion of the new stylet during each larval moult. Moreover, SORIN (1966) and SAXENA and CHADA (1971a) do not mention at all the presence of retort-shaped organs in the aphids investigated. Each of the four stylets of *Schizaphis graminum* originates from a ringlike piece of tentorium located close to the clypeal region of the head (SAXENA and CHADA, 1971a). It is possible that the two sensillum pores which are located on the foot of the salivary pumpstem of *M. persicae* (Figs. 5, 7. no. 8, and 19) were mistaken by SORIN (1966) and SAXENA and CHADA (1971b) as the duct openings of both the common salivary duct as well as the fused ducts from the mandibular and maxillary glands of *Aphis craccivora* and *Schizaphis graminum*.

The study of the pharyngeal valve in the embryonic and larval stage of *M. persicae* allows to discuss its function. In embryos the valve is open, but in larvae it is always found in a closed position (Fig. 8 no. 10' and 11'). The impression is evoked that at the passage of the embryonic stage into the larval stage, with the start of the gut function, the locking of the valve takes place due to the activity of the divaricator muscles. In the embryonic stage food uptake does not take place, the valve is not in function and is found in open position. It is likely that the opened valve in the larvae has the same position as can be observed in embryos. The opening of the valve is a result of the action of the two pairs of dorsal divaricator muscles, closing by contraction of the lateral pharyngeal valve muscles (Fig. 8 no. 11 and 12).
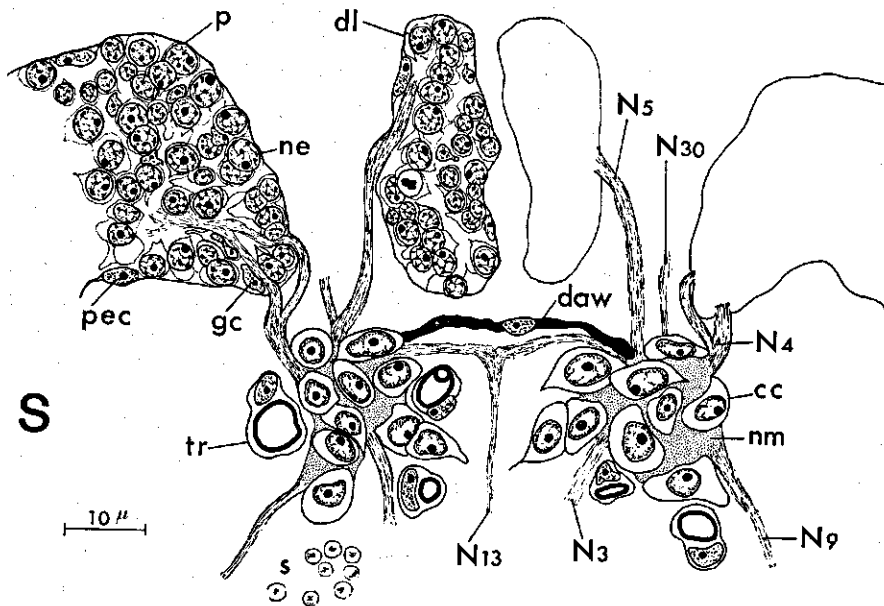
74

FIG. 28. Composition made from three successive transverse serial sections through the posterior region of the brain of a nine days old *Myzus persicae* larva (just adult stage) showing a part of the protocerebrum (p), dorsal lobes (dl), corpora cardiaca (cc), tracheae (tr), and dorsal aortic wall (daw). Note the intact symbionts (s) which are distributed throughout the aphid's body. This section (S) corresponds with those given in Figs. 1–3 and 29. For explanation of abbreviations see pages 144–147.

WEBER (1928) working with *Aphis fabae*, showed that all the epithelial cells of the stomach secrete by constricting of the apical parts. This was also observed for *Macrosiphum sanbornii* (MILLER, 1932), *Lachnus piceae* (LEONHARDT, 1940), *Lachnus roboris* (MICHEL, 1942), *M. persicae* (SCHMIDT, 1959), *Cryptomyzus ribis*, *Metopeurum fuscoviride*, *Pentatrichopus tetrarhodus* (MACKAUER, 1959), and *Megoura viciae* (EHRHARDT, 1963) (Table 9). According to FORBES (1964a) three cell forms are distinguishable in the stomach epithelium of adult *M. persicae*: replacement cells lying adjacent to the oesophageal valve, active digestive cells representing most of the epithelial cells, and resting digestive cells occurring in the posterior region of the stomach. In the stomach of adult *Schizaphis graminum* SAXENA and CHADA (1971c) reported the presence of two celltypes, viz. large columnar cells and small basal cells or regenerative cells. The secretion of digestive fluids is of the holocrine type, because the release of secretory material occurs mainly by detachment of complete cells from the epithelial layer. The cell wall, nucleus, and other cytoplasmic contents of the cells disin-

tegrate, and the secretory material is liberated into the stomach lumen. The regenerative cells form new cells to replace the detached columnar cells. This account, however, is in contradiction with our data of the larval stomach of *M. persicae*: (1) the digestive cells do not degenerate, (2) the number of stomach cells of the three celltypes remains practically constant during larval life (Table 4), and (3) the cells occurring in the posterior region of the stomach are really active digestive cells. Furthermore it was found that during larval life of *M. persicae* the digestive cells, which secrete material by constricting of the apical cell parts, only are observed in the middle region of the stomach. The digestive cells of the posterior region, however, secrete material by forming buds. The latter type of secretion was also observed in the midgut of the coccoid, *Pulvinaria mesembryanthemi* VALLOT (PESSON, 1944) and in the stomach of *Typhlocyba ulmi* L. (WILLIS, 1949).

Consequently the stomach can be considered as the secretorial part of the alimentary canal. Its lumen is filled with secretion products from the posterior stomach cells and cell fragments due to constricting of the fingerlike digestive cells. In the region of the intestine secretory cells, as found in the stomach, are absent. The epithelial cells of the intestine appear to be mainly resorbtive in function (WEBER, 1928; BRAMSTEDT, 1948; MACKAUER, 1959). This is supported by the fact that these cells have a large luminal surface (Table 4) and a well developed striated border (Fig. 11). It is furthermore observed that the material present in the stomach lumen, does not occur in the intestinal lumen after the stomach passes into the intestine. Similar observations are described for *Megoura viciae* by EHRHARDT (1963). In dissections of *M. persicae* the material found in the stomach lumen is flocculent (cell fragments and secretion material), while the lumen of the intestine and the hindgut is filled with a clear watery fluid.

BONNET (1745) was the first who stated that aphids did not possess an excretory system. Later on, RAMDOHR (1811) confirmed the absence of MALPIGHIAN tubes in *Aphis mali*, and MORREN (1836) in *M. persicae*. In connection with this absence several investigators mentioned that the excretory function of aphids is probably carried out by the gut (GOODCHILD, 1966), especially the hindgut (GERSCH, 1942), by the salivary glands (KLOFT, 1960; SCHÄLLER, 1961), especially the asccessory gland (BREIDER, 1952; WOHLFARTH-BOTTERMANN and MOERICKE, 1960; RILLING, 1967), by the mycetome (WITLACZIL, 1882), by the oenocytes (TÓTH, 1933), and by the cornicles (BONNET, 1778; WITLACZIL, 1884; LIU, 1956) (Table 9). The presence of waxy droplets inside the epithelial cells and in the lumen of the hindgut indicates that this part of the gut is able to excrete these droplets. The waxy droplets, being a product of degenerating fat cells, are scattered in the haemolymph throughout the aphid's body. They pass into the hindgut cells to be excreted subsequently in the lumen by minute projections situated on the apical membrane of the cells. The honeydew, which is emitted through the anus (VAN LEEUWENHOEK, 1696), likewise contains waxy droplets. It was suggested by FORBES (1964a) that the hindgut is excretory in function. His view is based on the fact that in some GOLGI complexes the area
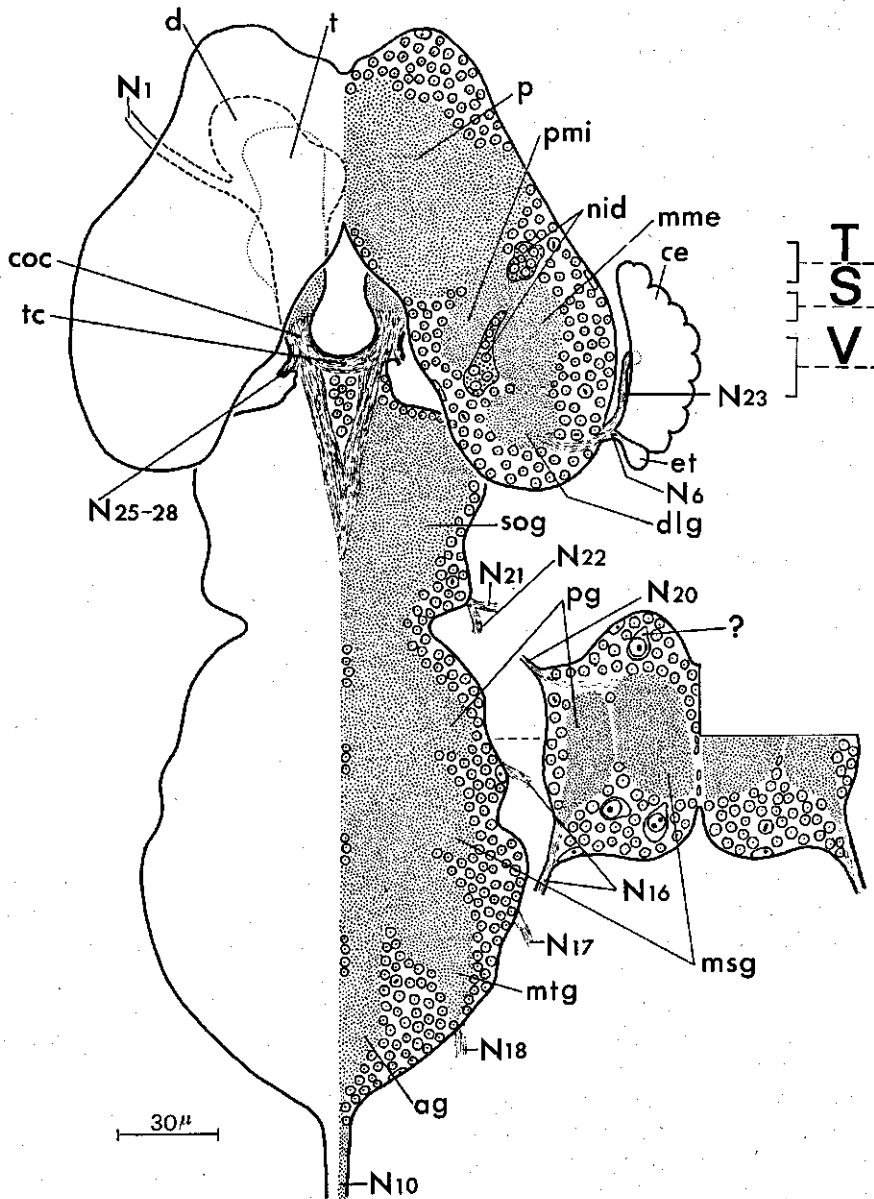
FIG. 29. Graphic reconstruction of the nervous system of a one day old *Myzus persicae* larva. The dorsal part of the right half of the nervous system is taken away as shown in the most right figure. Note the situation of the neural imaginal disc (nid) between the proximal medulla interna (pmi) and the medial medulla externa (mme) (see Fig. 31). The letters S, T, and V correspond with transverse sections given in Figs. 28, 30, and 32. For explanation of abbreviations see pages 144–147.

was fully expanded and occupied by small spheres and shells of electrondense material. Moreover, these materials were often seen at the free cell surface, as if they had just been ejected by the cell into the lumen.

Among all investigators who worked with *M. persicae* (MORREN, 1836; BUCKTON, 1876; BRAMSTEDT, 1948; SCHANDERL et al., 1949; SCHMIDT, 1959; KIKUMOTO and MATSUI, 1962; FORBES, 1964a) no one but TAKAOKA (1969) could demonstrate the presence of a filter chamber in this aphid. Possibly TAKAOKA (1969) worked with another aphid species than *M. persicae*, because in some aphid species a filter chamber is present as described by MORDWILKO (1895), KNOWLTON (1925), BÖRNER (1938), LEONHARDT (1940), MICHEL (1942), BRAMSTEDT (1948), and GOODCHILD (1966) (Table 9).

According to PAILLOT (1933) bacteria occur in the lumen and inside the epithelial cells of the midgut of some aphid species. On the other hand SCHOEL (1934) reported that the gut of *Cavariella aegopodii*, *Megoura viciae*, and *Doralis saliceti* are free of microorganisms. SCHANDERL et al. (1949) isolated yeast cells from the gut of *M. persicae* and other aphids (Table 9), which they described as vesicle-shaped granules. In the present study microorganisms were never observed either in the gut or in the honeydew in the larvae of *M. persicae*. OSSIANNILSSON (1961) and FORBES (1964a) likewise did not mention microorganisms in the gut of adult *M. persicae* in electron microscopical studies.

In *M. persicae* the anterior part of the rectum is occupied by epithelial cells with a secretory function. Till now in aphids the existence of similar cells in their rectum has never been reported. In coccoids analogous cells are found near the anal opening which secrete by means of minute pores (PESSON, 1944).

Salivary gland

The salivary gland of *M. persicae* has morphologically a similar structure as described for many aphid species (MARK, 1877; WITLACZIL, 1882; CHOLOD-KOVSKY, 1905; GROVE, 1909; DAVIDSON, 1913; BAKER, 1915; WEBER, 1928; TÓTH, 1936; SMITH, 1939; LEONHARDT, 1940; MICHEL, 1942; ROBERTI, 1946; FORTIN, 1958; SORIN, 1966; RILLING, 1967; TAKAOKA, 1969; ADAMS and WADE, 1970; SAXENA and CHADA, 1971b) (Table 9).

WEBER (1928) considered the principal salivary gland cells of *Aphis fabae* to be concerned with the production of secretory material. He found that in paraffin sections stained with purpurin - naphtolgreen or WEIGERT's haematoxylin-eosin the cytoplasm, the nuclei, and nucleoli of the gland cells within the group of 'Deckzellen' and 'Hauptzellen' of the principal gland of *Aphis fabae* reacted differently. Similar results were obtained with the salivary glands of *Lachnus piceae* (LEONHARDT, 1940), *Lachnus roboris* (MICHEL, 1942), *Cryptomyzus ribis*, *Metopeurum fuscoviride*, and *Pentatrichopus tetrarhodus* (MACKAUER, 1959) stained with haematoxylin-HEIDENHEIN or MALLORY-AZAN, and with the salivary glands of *Aphis frangulae* stained with haematoxylin-eosin (ROBERTI, 1946). They attributed the differences to the various stages of secrete production and release which occurred simultaneously in the principal gland. This could imply that cells with various secretory cycles would occur at random

78

in the two lobes of the principal gland. However, this view is in contradiction with the observations on the principal gland of *M. persicae*, since the optical composition of the different celltypes did not change during larval life. Moreover, the total number of cells within each celltype and the topographical position of these cells likewise remained constant.

WITLACZIL (1882) showed that the principal salivary glands of many aphid species (Table 9) are composed of two types of cells, which were named by WEBER (1928) the 'Deckzellen' and 'Hauptzellen.' Later TÓTH (1936) found in *Stomaphis graffii*, *Cinara pinihabitans*, and *Megoura aconiti* a third celltype, the 'central cells', situated between the cells of the above mentioned types. These central cells resemble somewhat celltype 8 in *M. persicae* as depicted in Fig. 13 A. In the principal salivary glands of *Macrosiphum solanifolii* FORTIN (1958) distinguished five different celltypes in each lobe. WEIDEMANN (1968) working with adult *M. persicae*, found in dissected principal salivary glands immersed in FEULGEN stain, nine different celltypes based on nuclear dimensions. In general his results were similar to those described in the present study for larval principal glands. WEIDEMANN (1968) distinguished, however, within the group of celltype 5 (Fig. 13 A) two different celltypes (A/F-nuclei) notwithstanding the average size of these nuclei was practical equally. In the larval gland the optical composition of the six gland cells of celltype 5 was identical as well the size of the nuclei and nucleoli (Fig. 14). Moreover, it appeared that (1) the A and F cells (corresponding with celltype 5, Fig. 13A) are histochemically identical (WEIDEMANN, 1970), (2) the A and F nuclei have the same volume, which may vary, however, with the host plant species on which the aphids were reared (WEIDEMANN, 1971a), and (3) only the A and F nuclei increased their size about twice when *M. persicae* was reared on PLRV diseased *P. floridana*, as compared to those grown on healthy *P. floridana* plants (WEIDEMANN, 1971b). From this it can be concluded that histologically each lobe of each principal gland consists of eight different celltypes (Figs. 13 A and 14). During larval life all these gland cells contain a mass of secretory granules which are released by the cell into the intercellular canaliculi. Presumably the substance secreted by the principal salivary gland is composed of eight different components. The ultrastructure of the principal gland cells of *M. persicae*, especially the presence of a well-developed rough-surfaced endoplasmic reticulum in them, also suggest that they are involved primarily in the synthesis of secretion proteins (MOERICKE and WOHLFARTH-BOTTERMANN, 1960a, 1963).

MOERICKE and WOHLFARTH-BOTTERMANN (1960a, c) reported the presence of myofibrils in the distal region of the principal salivary glands of *M. persicae*. They supposed that, by contracting, the myofibrils help to discharge the secretory material from the gland cells into the salivary duct. Such a mechanism takes place in the salivary, mammary, and sweat glands in mammals (BLOOM and FAWCETT, 1969). In these glands a discontinuous layer of processes of myoepithelial cells occurs lying on the epithelial side of the basement lamina. In *M. persicae*, however, the myoepithelioid cell lies only between the terminal portion of the two lobes. It is improbable that this cell should be acting as pump
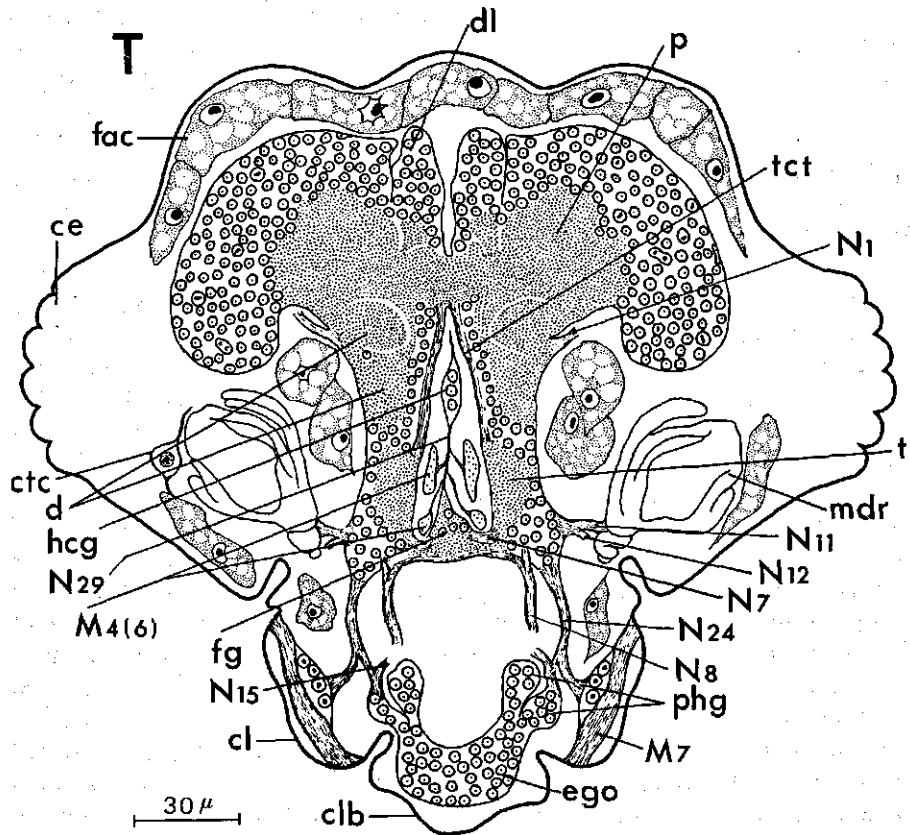
FIG. 30. Composition made from four successive transverse serial sections through the head of a one day old *Myzus persicae* larva showing position of protocerebral (p), deutocerebral (d), tritocerebral (t), and frontal ganglion (fg) with their nerves. Note the paired bilobed pharyngeal ganglion (phg) which passes into the epipharyngeal gustatory organ (ego). In the head the mesodermal tissue (fat cells, fac) is distributed between the various organs, connected with connective tissue cells (ctc). The position of this section (T) is given in Figs. 1–3 and 29. For explanation of abbreviations see pages 144–147.

mechanism for releasing secretory material from all the gland cells into the canaliculi.

On the other hand, it was found that the myoepithelioid cell has the opportunity to admit haemolymph into the lumen of both internal salivary ducts. This evidence is supported by the presence of (1) a labyrinthine system of irregularly oriented processes of the myoepithelioid cell membrane in the region of both ducts and the intercellular canaliculi of celltype 7 (Fig. 15), and (2) many vacuoles at the periphery of the contractile mass (Fig. 17 A). Similar structures have so far never been observed in myoepithelial cells (SMITH, 1968;

BLOOM and FAWCETT, 1969). Presumably the myoepithelioid cell functions as a sponge-like contractile filter mass possibly working selectively. The results with injection of EVANS blue in the haemolymph via the cornicles should indicate in that direction. The admission of haemolymph into the lumen of the salivary ducts presumably serves (1) to dissolve the granulated secretion products extruded by the principal gland cells, and/or (2) to remove waste material from the circulation with the saliva.

The innervation of the salivary gland of *M. persicae* appears to reach the principal salivary gland by a paired nerve of the dorsal nervous system originating from the paired corpus cardiacum mass. In *Heteroptera* the principal glands are innervated by a nervous plexus which is supplied by a nerve from the hypocerebral ganglion (BABTIST, 1941). In the coccoid, *Icerya purchasi* MASK, however, the accessory salivary glands are innervated by a nerve branch originating from the tritocerebrum (PESSON, 1944). SAXENA and CHADA (1971b, d) found a nerve plexus on the anterior end of the principal salivary glands of *Schizaphis graminum*, a caplike innervation formed by a short nerve arising from the posterior end of the suboesophageal ganglion, while the accessory glands are supplied with a nerve from the anterior region of the suboesophageal ganglion. The nerve which enters into the distal region of the principal salivary glands of *M. persicae*, has been mistaken for a trachea (MARK, 1877), or for a strand of fatty tissue (ADAMS and WADE, 1970) in several aphid species (Table 9).

The salivary glands of *M. persicae* alternately produce a watery secretion and a viscous one (BRADLEY, 1959). Similar secretions were observed in other aphid species (MILES, 1959, 1968; RILLING, 1967) (Table 9). According to MILES (1968) the nongelling watery secretion contains enzymes which serve to dissolve the layers of the mid-lamellae during penetration of the stylets into plant tissues (ZWEIGELT, 1915), while the viscous fluid forms the stylet sheath. The latter acts as a supporting structure for the stylets (BÜSGEN, 1891). From this it can be supposed that in *M. persicae* the watery secretion must originate from the secretory principal gland. The gland cells of the principal gland produce secretory granules, the substance of which probably is dissolved in the haemolymph pumped by the myoepithelioid cell into the lumen of the salivary duct. Moreover, BABTIST (1941) found that the accessory salivary glands of *Heteroptera* appear to produce only a watery secretion in which there are no enzymes.

The vertical orientation of mitochondria in slender compartments formed by infolding of the basal cell membrane of the accessoric cells (WOHLFARTH-BOTTERMANN and MOERICKE, 1960) indicates a rapid transport of water and ions (BLOOM and FAWCETT, 1969). Especially the extensive system of minute intracellular canaliculi lined with numerous closely aggregated, long microvilli points to a rapid discharge of excretory products to the chitinous lumen of the salivary duct. Presumably the accessory salivary gland excretes a watery suspension which, during its discharge to the salivary pump, is dehydrated by the duct cells to form the final viscous fluid. This implies that the salivary sheath is

an excretion product of the accessory glands. The viscous fluid, which coagulates rapidly in plant tissues forming the salivary sheath, presumably contains waxy droplets. This is suggested by the intimate association of numerous waxy droplets only with the accessory gland, although they are never observed inside their cells as described for the hindgut (page 29). The waxy droplets, originating from degenerating fat cells (page 50), presumably render the stylet sheath its waterproofing. It is interesting to note that the 'large particles' in the
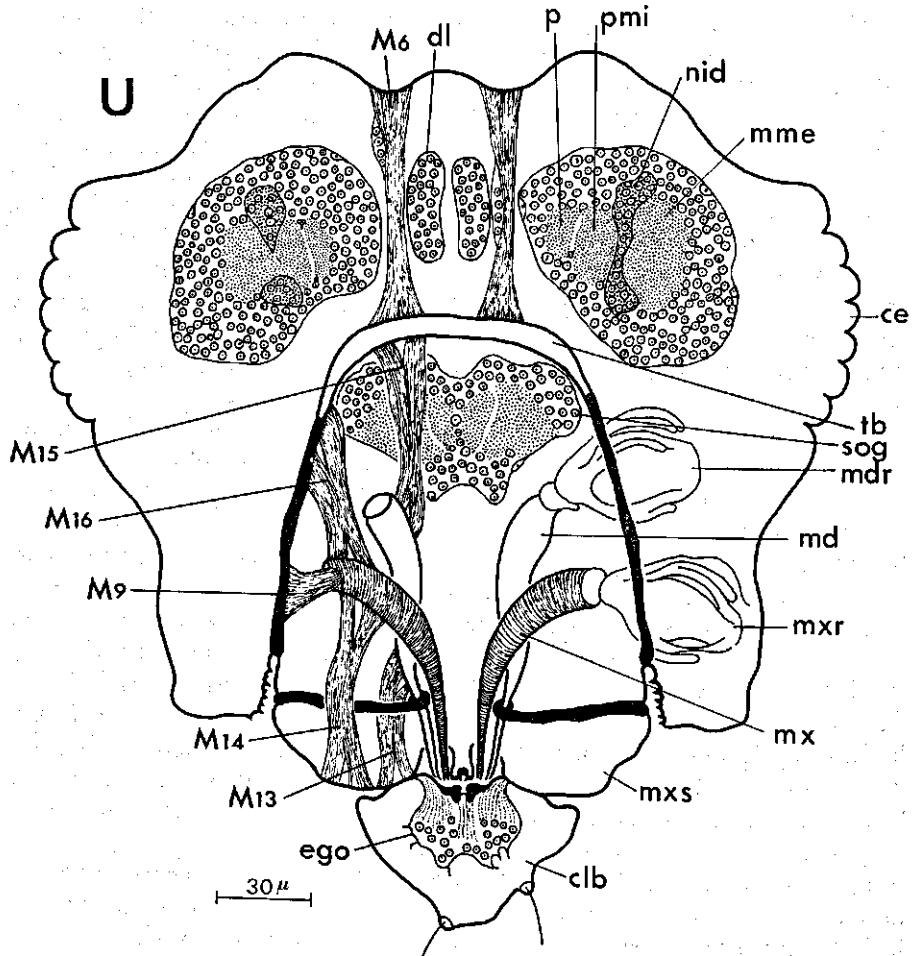
FIG. 31. Composition made from nine successive transverse serial sections through the head of a one day old *Myzus persicae* larva showing mandibular (mdr) and maxillary retort-shaped organs (mxr), and stylets (md; mx) with their muscles. Note the nerve fibres originating from the epipharyngeal gustatory organ (ego) which probably communicate with receptor hairs placed on the clypeo-labrum (clb). The position of this section (U) is given in Figs. 1 and 3. For explanation of abbreviations see pages 144–147.

cytoplasm of the accessory salivary gland cells (WOHLFARTH-BOTTERMANN and MOERICKE, 1960; Fig. 2), as well as the 'spheres and shells of electrondense material' in the epithelial cells of the hindgut (FORBES, 1964a; Figs. 63 and 64) show a similar structure as the waxy droplets in degenerating fat cells (Fig. 35 D) or in the haemolymph (Figs. 35 E and 36 C) of *M. persicae*. This suggests that the waxy droplets originating from degenerating fat cells may penetrate into the cells of the accessory glands.

The ultrastructure of the duct cells of *M. persicae* is described by MOERICKE and WOHLFARTH-BOTTERMANN (1960b). Similar structures are also found in the duct cells of the salivary gland of the orthopteron, *Schistocerca gregaria* FORSKAL (KENDALL, 1969), and the intermediate portion of the median salivary lobe of *Anopheles stephensi* LISTON (WRIGHT, 1969). These authors suppose that the fine structural organization of these cells is believed to be associated with an active transport mechanism of water and/or ions, but does not give an indication of the direction in which this transport occurs (SMITH, 1968). Presumably the duct cells of *M. persicae* function to regulate the 'water and ions content' of the secretion products extruded by the principal gland cells into the lumen of the salivary duct to which the myoepithelioid cell supplies an excess of haemolymph. Furthermore the duct cells presumably serve to dehydrate the watery suspension from the accessory gland cells to form the final viscous fluid for the salivary sheath.

Mesodermal derivates
Descriptions of the so-called 'fatbody' of several species of aphids are given by many authors. According to METSCHNIKOW (1866), working with *Aphis rosae* the first fat cells arise in the head of the embryo. Subsequently the small polygonal fat cells are distributed from the head via the thorax to the abdomen till the fatbody has attained its final composition. However, METSCHNIKOW's view is in contradiction with the findings in *M. persicae* where the area of origin of the mesodermal tissue is situated in the vicinity of each cornicle. The first fat and basophilic mesodermal cells start to degenerate in this region of the aphid, after differentiation of the embryonic mesodermal cells into their derivates. The degenerating fat cells in *M. persicae* (Fig. 22, 4–7; celltypes J-L) which are situated both inside the cornicles and in front of their bases are mistaken for gland cells by MORREN (1836). Similar cells were later on observed in many other aphids (Table 9) and described as air cells (KALTENBACH, 1843), oil-like globules (BUCKTON, 1876), sugar cells (WITLACZIL, 1882), excretion cells (WITLACZIL, 1884), wax producing gland cells (BÜSGEN, 1891; HORVATH, 1904), glandular wax cells (MORDWILKO, 1895; DAVIDSON, 1913; BAKER, 1915), glandular cells (FLÖGEL, 1905; HOTTES, 1928), wax cells (HOLLANDE, 1911; WEBER, 1933), fat cells (HOLLANDE, 1911; WYNN and BOUDREAUX, 1972), wax globules (ROBERTI, 1946), macronucleocytes, adipoleucocytes (LIU, 1956), modified oenocytes (EDWARDS, 1966), spherical bodies (STRONG, 1967), cornicle cells (LINDSAY, 1969), multicellular sacs, and glandular sacs (WYNN and BOUDREAUX, 1972). According to these authors these cells might be involved in

the function of the cornicles which were broadly classified as excretory (RÉAU-
MUR, 1742), respiratory (BONNET, 1776), protective (BÜSGEN, 1891), excretion
of non-nutritious resinous materials unavoidably ingested with the plant sap
(HOTTES, 1928), or removal of excess lipid metabolized from a diet high in
carbohydrates ((WYNN and BOUDREAUX, 1972).

In a study of the haemolymph of several species of aphids (Table 9), HOLLAN-
DE (1911) recognized five groups of cells, distinguished as proleucocytes, phago-
cytes, fat cells, wax cells, and oenocytoids. The phagocytes and fat cells are
derived from the proleucocytes, after which the fat cells develop into wax cells
by the forming of waxy droplets. Similar types of cells are also found in the hae-
molymph of the coccoids *Pseudococcus farinosus* de GEER, *Orthezia urticae* L.
(HOLLANDE, 1911), and *Pulvinaria mesembryanthemi* VALLOT (POISSON and
PESSON, 1939). In *M. persicae*, on the other hand, no circulating cells were ob-
served in the haemolymph. Its mesodermal tissue is composed of fat cells, baso-
philic mesodermal cells, and connective tissue cells, all of which are derived
from the embryonic mesodermal cells. The latter may be compared with the
proleucocytes of HOLLANDE (1911), while the wax cells represent the degener-
ating fat cells in the vicinity of the cornicles of *M. persicae*. In *Rhopalosiphum
nymphaeae* and *Aphis rosae* embryos the embryonic mesodermal cells are
mentioned as haemocytes by HIRSCHLER (1912).

Furthermore several celltypes are found in the haemolymph of other aphid
species and described as phagocytes, oenocytoids (HOLLANDE, 1911), and in the
coccoid *P. mesembryanthemi* as giant cells (POISSON and PESSON, 1939). In
M. persicae these cells correspond to stages in development, including the
degeneration process, of the basophilic mesodermal cells (Fig. 21). Moreover,
in contrast to the above mentioned suggestions it appears upon careful exam-
ination that the basophilic mesodermal cells do not occur in the haemolymph
of *M. persicae*, but they are evenly dispersed among the fat cells of the mesoder-
mal tissue. Similar cells are observed in the fat body of *Aphis pelargonii* (WILL,
1889), *Macrosiphum tanaceti* (UICHANCO, 1924), *Aphis gossypii* (SPENCER,
1926; LIU, 1956), *Macrosiphum solanifolii* (SPENCER, 1926; STILES, 1938),
Aphis pseudobrassicae (SPENCER, 1926), *Stomaphis quercus* (KLEVENHUSEN,
1927), *Hyalopterus pruni* (JANISZEWSKA, 1932), *Aphis pyraria* (PAILLOT, 1933),
Macrosiphoniella millefolii, Amphorophora aconiti (BOESE, 1936), *Sacchiphantes
viridis* (PROFFT, 1937), *Aphis frangulae* (ROBERTI, 1946), *Dactylosphaera vitifolii*
(BREIDER, 1952), *Metopeurum fuscoviride* (MACKAUER, 1959), *Aphis pomi*
(MACKAUER, 1965), *Aphis fabae* (EHRHARDT, 1966; TREMBLAY, 1966), *Brevicory-
ne brassicae* (TREMBLAY, 1966), *Amphorophora sonchi*, and *Monellia costalis*
(WYNN and BOUDREAUX, 1972), as well as in several white flies (WEBER, 1935a),
and called oenocytes, haemocytes, or unnamed by these authors (Table 9).
However, in *M. persicae* the oenocytes are situated in the body cavity and are
characterized by the presence of a large amount of cytoplasmic granules and an
irregular nucleus.

In *M. persicae* the connective tissue cells mainly occur at the periphery of the
mesodermal tissue although some of them are distributed among the mesoder-

84
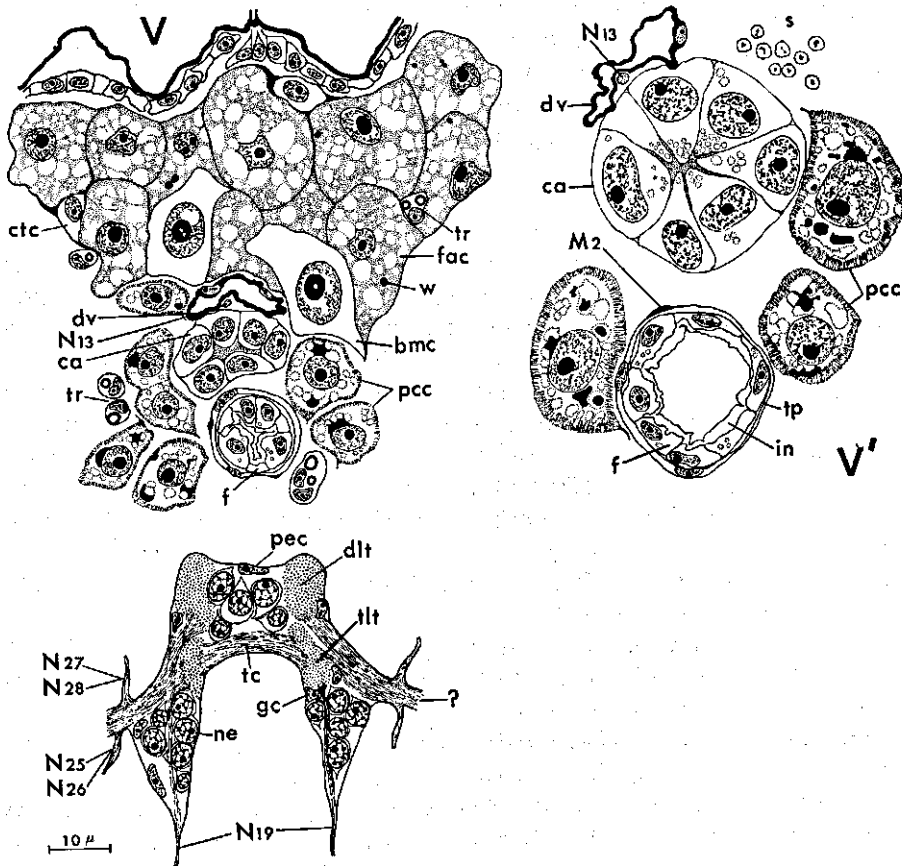
FIG. 32. V. Composition made from five successive transverse serial sections through the head of a one day old *Myzus persicae* larva showing arrangement of foregut (f), fat cells (fac), basophilic mesodermal cells (bmc), connective tissue cells (ctc), pericardial cells (pcc), dorsal vessel (dv), tracheae (tr), medial dorsal nerve (N13), corpus allatum (ca), and circumoesophageal connectives just before the suboesophageal ganglion. The position of this section (V) is given in Figs. 1–3, 23, 24, and 29. V'. Transverse section through the head of a nine days old larva (just adult stage). Note the intact symbionts (s) which are present in the haemolymph of adult aphids. For explanation of abbreviations see pages 144–147.

mal derivates as well as in the haemolymph (page 54). Similar spindle-shaped cells are found in the haemolymph of *Aphis gossypii* (LIU, 1956), *Metopeurum fuscoviride* (MACKAUER, 1959), *Aulacorthum circumflexum*, *Macrosiphum euphorbiae*, *Nasonovia ribis-nigri* (GRIFFITHS, 1961), *Brevicoryne brassicae*, *M. persicae*, and *Macrosiphum rosae* (HINDE, 1971a), and called phagocytes, lymphocytes, blood cells, or haemocytes by these authors (Table 9). However, in *M. persicae* the haemolymph is characterized by the absence of circulating haemocytes.

KLEVENHUSEN (1927) reported the presence of haemocytes in *Macrosiphum tanacetum* and *Macrosiphum tanaceticolum* (Table 9). From his pictures they appear to be fat cells of which the cytoplasm is filled with many vacuoles whereas the nucleus lies at the periphery against the cell membrane. According to this author these cells are capable to digest the rod-shaped symbionts which occur in these aphids.

In smear preparations of haemolymph of adult *M. persicae* and *Acyrthosiphon pisi* SCHMIDT (1959) observed two types of haemocytes. From his description of these celltypes, however, it may be deduced that they are intact nuclei from degenerated fat cells and intact symbionts which liberate after disintegration of the mycetocytes. The chromatin of these intact nuclei is partly or completely dissolved (karyolysis) showing a delicate network of eosinophilic strands (page 52). YEAGER and KNIGHT (1933) showed that the haemolymph of *M. persicae* and *Aphis maidis*, obtained by severing one or more appendages in a drop of oil (Nujol), exhibited no coagulation as well as the cells which occurred in the haemolymph (Table 9).

The existence of pericardial cells in aphids has already been reported by MORDWILKO (1895) from *Trama troglodytes*, by FLÖGEL (1905) from *Aphis ribis* and *Aphis platanoides*, by BREIDER (1952) from *Dactylosphaera vitifolii*, and by HOLMAN (1958) from 29 species of aphids including *M. persicae* (Table 9). Later on it was found that in *Drepanosiphum platanoides* the pericardial cells are connected by a thin nerve to the medial dorsal nerve (JOHNSON, 1963). Similar results were observed in *M. persicae* (BOWERS, 1964). Further it was found that some pericardial cells (Fig. 24, d) occur in the vicinity of the wing muscles of *Trama troglodytes* (MORDWILKO, 1895), and of some aphid species including *M. persicae* (HOLMAN, 1958). In *M. persicae* (Figs. 20 and 24 a, b, c), *Neomyzus circumflexus, Macrosiphon solanifolii*, and *Cinara pini* (Table 9) 2–3 pericardial cells are observed in both ventrolateral regions of each thoracic segment (HOLMAN, 1958). They presumably are closely associated with the presence of pulsatile organs, which are found in the legs of *M. persicae* (RICHARDSON, 1918).

Mycetome

The mycetome (pseudovitellus; HUXLEY, 1858), named by SULC (1910) is a complex organ composed of mycetocytes harbouring microorganisms in their cytoplasm. These microorganisms are commonly considered to be symbionts. In spite of the many studies which have been summarized by SULC (1910), UICHANCO (1924), BUCHNER (1953), and HINDE (1971a) their nature is not clearly understood.

WITLACZIL (1882) who has studied many aphid species (Table 9) reported that the mycetome is enclosed by a nucleated sheath. FLÖGEL (1905) doubted the presence of a sheath because he found the mycetocytes scattered throughout the body cavity of *Aphis ribis*. In the aphids *Macrosiphum tanaceti* (UICHANCO, 1924), *Cavariella aegopodii, Megoura viciae, Doralis saliceti* (SCHOEL, 1934), *Hormaphis hamamelidis* (LEWIS and WALTON, 1958), and *M. persicae* (Figs. 1

86

and 26) the mycetome is enclosed within a nucleated sheath and grows solely by enlargement of the mycetocytes during larval life to disintegrate from the fourth day onwards into groups of one or more mycetocytes. These results are supported by LeBlanc and Musgrave (1963) who showed that the mycetocytes are present as single cells in the abdominal region of adult *Aphis fabae* and *Macrosiphum pisi*, whereas in aphid embryos inside the adults the mycetocytes still form a mycetome.

Uichanco (1924) reported that soon after reaching the adult stage of *Macrosiphum tanaceti*, the mycetocytes and their symbionts begin to degenerate, whereas in *Aphis fabae* this process starts when the adults have reached the maximum reproductive stage until in very old adults there are no intact microorganisms present (Ehrhardt, 1966). In the present study it was found that during larval life of *M. persicae* symbionts degenerate within the mycetocytes, and that at the ninth day the cell membrane of some mycetocytes starts to dissolve after which intact and degenerating symbionts are liberated into the haemolymph. This process continues in the adult stage and it agrees with the occurrence of intact and degenerating symbionts in the cornicle secretion of adults (Table 7). According to Tóth (1933, 1938) the symbionts of *Stomaphis longirostris*, *Brachycaudus cardui*, and *Pemphigus spirothecae* become phagocytized or dissolved by oenocytes. The oenocytes move towards to the mycetome, contact it and next enter this organ. The symbionts then dissolve after which the oenocyte itself degenerates. During larval life of *M. persicae*, however, such process is never observed as well as in all developmental stages of *Brevicoryne brassicae* (Lamb and Hinde, 1967). Electron microscopically Hinde (1971a) showed that at all stages of development of *Brevicoryne brassicae*, *M. persicae*, and *Macrosiphum rosae*, degenerating symbionts occur within their mycetocytes, and that their destruction appears to be brought about by the action of lysosomes. Furthermore, Hinde (1971a) noted that symbionts released into the haemolymph are engulfed and digested by phagocytic, spindle-shaped haemocytes. These spindle-shaped cells with a relative large nucleus are the connective tissue cells, because in *M. persicae* the haemocytes are lacking.

In many species of aphids the mycetome harbours 1–3 species of microorganisms (Buchner, 1953). The mycetocytes in *M. persicae* contain only spherical symbionts dividing like bacteria. The average diameter of the microorganisms is 2.7 μ which is about equal to that of the spherical bodies found in the mycetomes of other aphids measuring about 1–4 μ (Koch, 1930; Wall, 1933; Tóth, 1933; Schoel, 1934; Roberti, 1946; Lewis and Walton, 1958; Vago and Laporte, 1965; Ehrhardt, 1966; Lamb and Hinde, 1967; Hinde, 1971b) (Table 9). Granulated structures in the centre of spherical symbionts as in *M. persicae* (Fig. 26) have been previously observed in *Aphis rumicis* (Koch, 1930), *Cavariella aegopodii*, *Megoura viciae*, *Doralis saliceti* (Schoel, 1934), and many other aphid species (Paillot, 1933) (Table 9). It was demonstrated that these substances which occur likewise in the thread-like symbionts of several insect species (Kolb, 1959), as well as in the spherical symbionts of *Brevicoryne brassicae* (Lamb and Hinde, 1967), represent nuclear material containing DNA.

In ultrathin sections HINDE (1971b) showed the presence of fibrillar DNA inside the symbionts of *Brevicoryne brassicae, M. persicae,* and *Macrosiphum rosae.*

Many investigators have considered the symbionts as yeasts (KONINGSBERGER and ZIMMERMANN, 1901), saccharomycetes (SULC, 1910), bacteria (PAILLOT, 1933), and cell units (LANHAM, 1952). VAGO and LAPORTE (1965) demonstrated that the spherical symbionts occurring in the mycetome of *Macrosiphum rosae,* are distinguished from free bacteria by the absence of a wall. They suggest that the symbionts which are only enclosed by a plasma membrane originate from bacteria in which the intracytoplasmic medium of the mycetocytes has induced an adaptation. On the other hand, LAMB and HINDE (1967) and HINDE (1971b) found that the symbionts of *Brevicoryne brassicae, M. persicae,* and *Macrosiphum rosae* are enclosed by a cell wall in addition to a plasma membrane. From their electron microscopical observations they concluded that the symbionts of aphids are rickettsialike microorganisms.

The disintegration of the mycetocytes has nothing to do with the transfer of the microorganisms to the developing embryos. The transfer of microorganisms to the progeny occurs very early. In the new adult we can find two generations of embryos which are already provided with a mycetome. A detailed study of the migration of symbionts from embryo to embryo is given by UICHANCO (1924) for *Macrosiphum tanaceti* and by EHRHARDT (1966) for *Aphis fabae.*

Oenocytes

WIELOWIEJSKI (1886) observed in the body cavity of several insect species cells, which he called oenocytes because of their wine-yellow colour in fresh preparations. According to this author these cells are easily distinguished from the fat and pericardial cells by the presence of numerous dispersed granules or accumulations of them in the cytoplasm. WHEELER (1892) concluded that the oenocytes are derived from the ectoderm and after their differentiation they never divide but gradually increase in size. The ectodermal origin of oenocytes indicates a glandular function and gland cells are usually distinguished by their large and often irregular or branched nuclei (SNODGRASS, 1935). GLASER (1912), working with larvae of several insect species, reported that oenocytes have a secretory function because of the presence of microscopical exudations around the periphery of the cytoplasm, especially at periods when the nucleus is greatly ramified. In the insect species studied by HOLLANDE (1914), the larval oenocytes possess a homogeneous cytoplasm with a spherical nucleus, whereas the cytoplasm of the imaginal oenocytes is vacuolated and contains a branched nucleus.

The cells which are found in the body cavity of *M. persicae* (Fig. 1), are presumably of ectodermal origin because they are observed before the appearance of embryonic mesodermal cells. In young embryos they lie against the developing mycetome, which is also reported to occur in some other aphid species by HOLLANDE (1911). Moreover, in *M. persicae* the cells contain granules in their cytoplasm and an irregular or branched nucleus (Fig. 21 F-I). The basophilic granules which occur in vacuoles in the cytoplasm, are presumably

released by the nucleus after which they are transported to the cell membrane and subsequently liberated into the haemolymph. Based on the above mentioned observations these cells have been named oenocytes. Oenocytes have been shown in *Stomaphis longirostris, Brachycaudus cardui, Pemphigus spirothecae* (TÓTH, 1933, 1938), and *Dactylosphaera vitifolii* (BREIDER, 1952), but in contrast to those of *M. persicae* these cells possess a spherical nucleus. Probably DAVIDSON (1913), PAILLOT (1934, 1938), and SCHOEL (1934) have also observed oenocytes in aphids, which may be concluded from their descriptions or figures of what they called large cells with coarse granular contents occurring in *Schizoneura lanigera*, giant cells in *Chaitophorus aceris* and *Chaitophorus lyropictus*, or phagocytes in *Cavariella aegopodii, Megoura viciae*, and *Doralis saliceti*. LAMPEL (1959, 1962) reports the presence of oenocytes in *Pemphigus populinigrae* and *Pemphigus bursarius*, which increase considerably in size to become giant cells when these aphids are parasitized by *Polynema schmitzi* SOYKA (Table 9).

Circulatory system

The presence of a dorsal vessel has been described for *Drepanosiphum platanoides* (BUCKTON, 1876; JOHNSON, 1963), *Aphis pelargonii, Pemphigus spirothecae* (WITLACZIL, 1882; WILL, 1889), *Trama troglodytes* (MORDWILKO, 1895), *Aphis platanoides, Aphis ribis* (FLÖGEL, 1905), *Pemphigus bursarius* (PFLUG-FELDER, 1936), *Aphis frangulae* (ROBERTI, 1946), and *Dactylosphaera vitifolii* (BREIDER, 1952) (Table 9). According to WITLACZIL (1884) a pair of ostia occur on the junction of each two abdominal segments. MORDWILKO (1895) refers to the presence of a chambered dorsal vessel of which each chamber is provided with a pair of ostia situating laterally in the middle of them. In *Dactylosphaera vitifolii* the dorsal vessel contains one pair of ostia whereas its posterior end is open (BREIDER, 1952). In paraffin sections of *M. persicae* it was not possible to detect chambers and ostia; by iniecting with indian ink via the cornicles, however, three pairs of ostia could be observed (Fig. 3).

According to PFLUGFELDER (1936) and JOHNSON (1962) the dorsal vessel is attached with its ventral wall to the corpus allatum. In *M. persicae* it was observed that only the lateral sides of the ventral aortic wall are connected with the corpus allatum, so that the fused medial nerve can run free between the aorta and the corpus allatum (Fig. 32). The ventral aortic wall terminates half-way the corpus allatum, while the dorsal aortic wall is attached to the corpora cardiaca (Fig. 28).

BUCKTON (1876) observed a rapid pulsation in the legs of *Drepanosiphum platanoides*. At the knee-joint of each tibia he found a pulsating sac which may be responsible for the propulsion of haemolymph into the leg. Similar observations are reported for *M. persicae* (RICHARDSON, 1918) and for *Aphis* sp. (HENKE, 1924). They found a delicate tubular organ in the tibia of each leg just below its juncture with the femur. It is possible that the tubular pulsatile organs, which in the present study of *M. persicae* were traced in the coxa, continue in the tibia of each leg. It was shown that the tubular pulsatile organs are inner-

vated by nerves. Presumably these nerves are branches from the lateral nerve. According to JOHNSON (1963) the lateral pair of nerves arising from the corpora cardiaca follow tracheae to the vicinity of the thoracic spiracles and then pass downward and posteriad into the abdomen.

Cornicles

The most characteristic morphological features associated with the family *Aphididae* are the cornicles. Apparently the first author who published on these structures was SWAMMERDAM (1669), characterizing them as 'pointed hairs.' VAN LEEUWENHOEK (1696), in an extensive study on the biology of aphids, observed that 'aphids possess two erected apparatus on the posterior dorsal end of their abdomen, from which a drop of clear liquid comes out'. Later on it was reported that in many aphid species the cornicles are placed dorsolaterally on the sixth abdominal segment (KALTENBACH, 1843; HUXLEY, 1858; BUCKTON, 1876; THEOBALD, 1926; HOTTES, 1928). This was contradicted by many authors (WITLACZIL, 1882; HORVATH, 1904; DAVIDSON, 1913; WEBER, 1928; ROBERTI, 1946; REMAUDIÈRE, 1964; LINDSAY, 1969; and others) who stated that they are outgrowths of the fifth abdominal tergite. Actually there are cases in very elongated aphids in which cornicles have shifted to the sixth tergite (BÖRNER, 1930: *Laingia*; HILLE RIS LAMBERS, 1939: *Holcaphis*).

The elevation of each cornicle, reported by WITLACZIL (1882), is controlled by a muscle originating from the ventral surface of the sixth abdominal sternite and radially connected with the tip of the cornicle. FLÖGEL (1905) found that this muscle is inserted at the valve-like flap situated on the tip of each cornicle, which serves to discharge the secretion products. The erection of the cornicles is controlled by a second muscle. HOTTES (1928) who has studied all types of cornicles within the family *Aphididae*, ascertained that the erection of the cornicles and the opening of the valve takes place by contraction of the valve retractor muscle. However, in a study on the cornicles of *Schizoneura americana* (MILLER, 1933), *Aphis frangulae* (ROBERTI, 1946), *Aphis gossypii* (LIU, 1956), and *Acyrthosiphon pisum* (LINDSAY, 1969) two muscles (the valve retractor muscle and the cornicle elevator muscle) are found, which have the same function as was suggested by FLÖGEL (1905) for *Aphis ribis*. Moreover, MILLER (1933) found a third muscle which effects the backward movement of the cornicle. This muscle arises on the posterior wall of the fifth abdominal segment, ventrally, and has its insertion on the posterior region of the base of the cornicle. In *M. persicae* each cornicle is supplied with two muscles, of which the cornicle elevator muscle presumably serves for the erection of the cornicle, while the opening of the valve takes place by contraction of the valve retractor muscle. This is supported by the fact that the cornicles can only secrete when they are in an erected position.

The cornicle secretion is composed of the same cellular material as was found in the haemolymph and mesodermal tissue, namely degenerating fat cells, disintegrated nuclear material, waxy droplets, rhomb-shaped crystals, intact and degenerating symbionts, and degenerating basophilic mesodermal cells. The

90

last two products occur in the secretion of adult *M. persicae* (Table 7). Following Büsgen (1891) many investigators have reported that the hardening process of the cornicle secretion is due to a waxy-like substance. According to Mordwilko (1895) the waxy substances in the cornicle secretion of *Lachnus viminalis* is identical to the fatty substance occurring in fat cells. In the present study it was found that the fat cells of celltype J (Fig. 22), of which the cell membrane is still intact and contain lipid, are responsible for the hardening of the secretion of *M. persicae* after it has passed the cornicles. This view is supported by Strong (1967) who showed that the hardened droplets in the cornicle secretion of *M. persicae*, *Acyrthosiphon pisum*, and *Chaitophorus* sp. were composed primarily of triglycerides. Moreover, the lipid found in the cornicle liquid of many other species of aphids (Table 9), appeared to be triglyceride (Wynn and Boudreaux, 1972).

Although cornicle droplets occasionally are observed on plants colonized by *M. persicae*, it remains an open question whether aphids continually expel this material via the cornicles. Buckton (1876) supposed that the 'oily globules', which are stored around the bases of the cornicles, must leave the aphid's body, since otherwise the accumulation of these globules would cause its death. Liu (1956) showed a reduction in longevity, reproductive period, and fecundity of *Aphis gossypii* with their cornicles being cut or sealed. On the other hand, Strong (1967) ascertained that there was no difference in the production of progeny and life span between *Acyrthosiphon pisum*, of which the tips of the cornicles were plugged 24 hours after birth with nontoxic silver paint, and untreated pea aphids. This suggests that it is not necessary for aphids to void the above mentioned substances via the cornicles. It is possible that these substances, of which the degenerating fat and basophilic mesodermal cells completely disintegrated into unrecognizable substances, are excreted via specialized organs as was found for the waxy droplets in *M. persicae*. These droplets are excreted by the hindgut and presumably also by the accessory salivary glands. That the cornicles might act as an excretory organ is suggested by Réaumur (1742, urinary organ) and supported by many investigators, especially in connection with the absence of the Malpighian tubes (Bonnet, 1745; Ramdohr, 1811; Morren, 1836; Buckton, 1876; Witlaczil, 1884; Liu, 1956). On the other hand, Hamilton (1935) supposed, that much of the material excreted from the haemolymph is not passed out of the body, but is removed to the epidermis, deposited in the cuticle, and cast off with the exuvium, or in adult *M. persicae* becomes accumulated there. Moreover, Holman (1958) found that in *Neomyzus circumflexus* the injected indian ink accumulated on the surface of the dorsal vessel and some other internal organs (ovarial ligaments, basement membrane of hindgut), but also beneath the epidermis and in the epidermal cells lying under abdominal sclerotised patterns. Eastop and Banks (1970) demonstrated that in populations of *M. persicae* resistent to organophosphorus insecticides the cornicles are distinctly longer than in susceptible aphids. According to them the selection for cornicle length might be due to increased excretory function, in casu excretion of insecticides.

According to BÜSGEN (1891) the rapidly solidified, waxy-like substance produced by the cornicles, serves to glue up the mouthparts of predacious and parasitic enemies. Although BÜSGEN's defense theory found many supporters (HORVATH, 1904; FLÖGEL, 1905; DE LA TORRE-BUENO, 1907; DE FLUITER, 1931; WEBER, 1933; COBBEN, 1958; EVENHUIS, 1958; DIXON, 1958; EDWARDS, 1966), GILLETTE (1908b) and HOTTES (1928) questioned this theory since the cornicles are not well adapted to perform effectively the function of protection. Within the family *Aphididae* the cornicles vary much in size and form, namely from long and cylindrical tube-like structures to pore-like ones which are practically on the level with the integument (HOTTES, 1928). Recently KISLOW and EDWARDS (1972) found that aphids are repelled by the odour of cornicle droplets. The aphids stopped feeding and rapidly moved away from the droplets by leaving the leaf or moving to the other side. When the odour was placed near aphids feeding on the underside of leaves they fell off.

Nervous system

Many authors have described the nervous sytem of several aphid species (WITLACZIL, 1882; MORDWILKO, 1895; GROVE, 1909, 1910; DAVIDSON, 1913; BAKER, 1915; WEBER, 1933; BREIDER, 1952; TAKAOKA, 1969; SAXENA and CHADA, 1971d). A more detailed study is given for the aphids *Pemphigus bursarius* (PFLUGFELDER, 1936) and *Aphis frangulae* (ROBERTI, 1946), as well as for coccoids by PESSON (1944).

The first report on the presence of a gustatory organ in aphids is that by DAVIDSON (1914) for *Schizoneura lanigera*. According to him it is an organ of taste, which enables the aphid to test the nature of the sap drawn into the pharyngeal duct before it enters into the pump. This organ is situated above the epipharynx which consists of a median row of eight sensillum pores. Since then a similar structure is found in *Aphis fabae* (WEBER, 1928), *Pemphigus bursarius* (PFLUGFELDER, 1936; buccal ganglion), *Aphis frangulae* (ROBERTI, 1946; buccal ganglion), *Hormaphis hamamelidis* (LEWIS and WALTON, 1958), *Megoura viciae* (EHRHARDT, 1963), *Brevicoryne brassicae*, and *Tuberolachnus salignus* (WENSLER and FILSHIE, 1969). Moreover, WENSLER and FILSHIE (1969) showed six lateral sensillum pores between the two protuberances, three on each side of the medial cuticular tendon. However, these pores are not observed in *M. persicae*, but a stellate invagination situated in each lateral lobe of the epipharyngeal gustatory organ which communicates with the pharyngeal duct. From the wall of this invagination nerve fibres arise running to neurons situated mainly on the periphery of the lateral lobes. Presumably these stellate invaginations can be compared with the lateral sensillum pores of *Brevicoryne brassicae* and *Tuberolachnus salignus* found by WENSLER and FILSHIE (1969).

The hypopharyngeal gustatory organ of *M. persicae* contains four sensillum pores; two of them are situated in the cup-shaped part of the floor of the pharyngeal duct (Figs. 5 and 8 no. 9–10), and the two other ones are located on the foot of the salivary pumpstem (Figs. 5 and 19). This arrangement agrees with that of the coccoids studied by PESSON (1944). The first mentioned sensillum

pores occur also in the aphids *Brevicoryne brassicae* and *Tuberolachnus salignus* (WENSLER and FILSHIE, 1969).

Electron microscopical studies have revealed the presence of two dendrites in the mandibular stylets of *M. persicae*; each dendrite has a distinct plasma membrane enclosing two neurofilaments (FORBES, 1966). Similar results were observed in *Aphis maidis* (PARRISH, 1967) and *Schizaphis graminum* (SAXENA and CHADA, 1971a). Minute tubes were found earlier in the mandibular stylets (GROVE, 1909), although they were stated to be empty (WEBER, 1928; VAN HOOF, 1957). In coccoids these minute tubes are observed in the maxillary stylets too (PESSON, 1944). In *M. persicae* embryos, however, minute tubes are observed both in the mandibular and in the maxillary stylets (Fig. 8 no. 11–13). The tubes in the mandibular stylets can be traced throughout its length, while those in the maxillary ones only in their basal part. More distally it was impossible to detect them. It is interesting to note that both mandibular and maxillary retort-shaped organs are supplied by a nerve originating from the tritocerebral lobe (Fig. 30), while a nerve originating from the tritocerebral longitudinal tract terminates in the basal part of the mandibular stylets (Figs. 4 and 32 V).

In *Trama troglodytes*, *Lachnus viminalis*, and *Lachnus pineus*, MORDWILKO (1895) described the presence of two ganglions which are connected by a nerve with an unpaired organ lying under the dorsal vessel. Now today these organs are called the corpora cardiaca and the corpus allatum; they have been found in many aphid species (PFLUGFELDER, 1936; ROBERTI, 1946; CAZAL, 1948; JOHNSON, 1963; TAKAOKA, 1969). The total number (8–11) of cells in each corpus cardiacum of *M. persicae* agrees approximately with that found in the aphids studied by JOHNSON (1963). The corpus allatum of *M. persicae* contains 11–13 cells, but in the aphids *Macrosiphum rosae*, *Aphis* sp., and *Pemphigus* sp. it was about 20 (CAZAL, 1948). From each corpus cardiacum three nerves arise, namely the medial, lateral, and ventral nerve. According to JOHNSON (1963) the lateral nerves in *Drepanosiphum platanoides* follow tracheae to the vicinity of the thoracic spiracles and then pass downward and backward into the abdomen. The ventral nerve passes straight downwards and branches, the branches going to the muscles connecting the stylets to the tentorium.

JOHNSON (1963) found that in adult aphids the thoracic gland cells have disappeared completely. From this data it can be concluded that the nine days old *M. persicae* used in the present study, was just in the adult stage. *M. persicae* moults very irregularly and the average duration of its larval stage on Chinese cabbage plants was 9–10 days at 20°C (PONSEN, 1969). That is the reason that throughout this study the aphids were fixed at appointed days. The degeneration process of the thoracic gland cells starts in a seven days old larva after which they were completely disintegrated in clusters of irregular spheres.

The thoracic gland cells are situated in the vicinity of the mesothoracic spiracles (Figs. 2 and 20), and not near the prothoracic spiracles as suggested by JOHNSON (1963). The first pair of thoracic spiracles are situated in the anterolateral part of the mesothorax, and the second pair in the metathorax. The seven abdominal stigmata are found in the first seven abdominal segments.

Their position and total number agrees with that found in *Macrosiphum tanaceti* (UICHANCO, 1924), *Aphis fabae* (WEBER, 1928), and *Eriosoma lanigerum* (DE FLUITER, 1931). The supposition that the muscles of the seven abdominal spiracles are innervated by branches originating from the main abdominal nerve is based on the fact that in one case this connection was clearly seen, namely that of the first abdominal spiracle. In *Schizaphis graminum* the first pair of abdominal spiracles is innervated by a nerve originating from the abdominal ganglion, while the remaining six abdominal spiracles are supplied by nerve branches from the main abdominal nerve (ventral nerve cord; SAXENA and CHADA, 1971d).

TABLE 9. Aphid names used by various quoted authors, corrected by D. HILLE RIS LAMBERS.

Acaudinum centaureae HOLMAN, 1958 = *Acaudinum dolychosiphon* MORDV.
Acyrthosiphon caraganae ADAMS and WADE, 1970 = *Acyrthosiphon caraganae* CHOL.
Acyrthosiphon pisi SCHMIDT, 1959 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisi PARRISH, 1967 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum MOERICKE and MITTLER, 1965 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum RICHARDSON and SYLVESTER, 1965 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum SHIKATA et al., 1966 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum STRONG, 1967 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum LINDSAY, 1969 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon pisum KISLOW and EDWARDS, 1972 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon sp. HOTTES, 1928 = *Acyrthosiphon* sp.
Acyrthosiphum pisum JOHNSON, 1963 = *Acyrthosiphon pisum* HARRIS
Acyrthosiphon onobrychis HOLMAN, 1958 = *Acyrthosiphon pisum* HARRIS
Amphorophora aconiti BOESE, 1936 = *Delphiniobium junackianum* KARSCH
Amphorophora agathonica KISLOW and EDWARDS, 1972 = *Amphorophora agathonica* HOTTES
Amphorophora sonchi WYNN and BOUDREAUX, 1972 = *Hyperomyzus lactucae* L.
Anoecia corni HOLMAN, 1958 = *Anoecia corni* F.
Anoecia oenotherae HOTTES, 1928 = *Anoecia oenotherae* WILSON
Aphis aceris PFLUGFELDER, 1936 = *Periphyllus aceris* L.
Aphis cardui BÜSGEN, 1891 = *Brachycaudus cardui* L.
Aphis cardui HOLLANDE, 1911 = *Brachycaudus cardui* L.
Aphis craccivora MILES, 1959 = *Aphis craccivora* KOCH
Aphis craccivora JOHNSON, 1963 = *Aphis craccivora* KOCH
Aphis craccivora SORIN, 1966 = *Aphis craccivora* KOCH
Aphis craccivora WYNN and BOUDREAUX, 1972 = *Aphis craccivora* KOCH
Aphis fabae BÜSGEN, 1891 = *Aphis fabae* SCOP.
Aphis fabae WEBER, 1928 = *Aphis fabae* SCOP.
Aphis fabae MARTINI, 1958 = *Aphis fabae* SCOP.
Aphis fabae JOHNSON, 1962, 1963 = *Aphis fabae* SCOP.
Aphis fabae LEBLANC and MUSGRAVE, 1963 = *Aphis fabae* SCOP.
Aphis fabae EDWARDS, 1966 = *Aphis fabae* SCOP.
Aphis fabae EHRHARDT, 1966 = *Aphis fabae* SCOP.
Aphis fabae TREMBLAY, 1966 = *Aphis fabae* SCOP.
Aphis fabae ADAMS and WADE, 1970 = *Aphis fabae* SCOP.
Aphis fabae WYNN and BOUDREAUX, 1972 = *Aphis fabae* SCOP.
Aphis (Doralis) frangulae ROBERTI, 1946 = *Aphis frangulae* KLTB. complex
Aphis gossypii SPENCER, 1926 = *Aphis gossypii* GLOVER
Aphis gossypii WALL, 1933 = *Aphis gossypii* GLOVER

Aphis gossypii Liu, 1956 = *Aphis gossypii* Glover
Aphis gossypii Wynn and Boudreaux, 1972 = *Aphis gossypii* Glover
Aphis grossulariae Zweigelt, 1915 = *Aphis schneideri* Börner
Aphis maidis Yeager and Knight, 1933 = *Rhopalosiphum maidis* Fitch
Aphis maidis Parrish, 1967 = *Rhopalosiphum maidis* Fitch
Aphis mali Ramdohr, 1811 = *Aphis pomi* de Geer
Aphis mali Paillot, 1933 = *Aphis pomi* de Geer
Aphis papaveris Dufour, 1833 = *Aphis fabae* Scop.
Aphis papaveris Schanderl et al., 1949 = *Aphis fabae* Scop.
Aphis pelargonii Huxley, 1858 = ? *Acyrthosiphon malvae* Mosley
Aphis pelargonii Witlaczil, 1882 = ? *Acyrthosiphon malvae* Mosley
Aphis pelargonii Will, 1889 = ? *Acyrthosiphon malvae* Mosley
Aphis persicae Morren, 1836 = *Myzus persicae* Sulz.
Aphis pioridis Büsgen, 1891 = *Uroleucon picridis* F.
Aphis plantaginis Büsgen, 1891 = *Aphis plantaginis* Goeze
Aphis platanoides Witlaczil, 1882 = *Drepanosiphum platanoides* Schrank
Aphis platanoides Flögel, 1905 = *Drepanosiphum platanoides* Schrank
Aphis pomi Schäller, 1961 = *Aphis pomi* de Geer
Aphis pomi Mackauer, 1965 = *Aphis pomi* de Geer
Aphis pomi Adams and Wade, 1970 = *Aphis pomi de* Geer
Aphis pseudobrassicae Spencer, 1926 = *Lipaphis erysimi* Kltb.
Aphis pyraria Paillot, 1933 = ? *Dysaphis pyri* Fonsc. or? *Melanaphis pyraria* Pass.
Aphis ribis Flögel, 1905 = *Cryptomyzus ribis* L.
Aphis rosae Dufour, 1833 = *Macrosiphum rosae* L.
Aphis rosae Metschnikow, 1866 = *Macrosiphum rosae* L.
Aphis rosae Büsgen, 1891 = *Macrosiphum rosae* L.
Aphis rosae Hirschler, 1912 = *Macrosiphum rosae* L.
Aphis rumicis Zweigelt, 1915 = *Aphis fabae* Scop.
Aphis rumicis Koch, 1930 = *Aphis fabae* Scop.
Aphis sambuci Buckton, 1876 = *Aphis sambuci* L.
Aphis sambuci Mark, 1877 = *Aphis sambuci* L.
Aphis sambuci Witlaczil, 1882 = *Aphis sambuci* L.
Aphis sambuci Büsgen, 1891 = *Aphis sambuci* L.
Aphis sambuci Zweigelt, 1915 = *Aphis sambuci* L.
Aphis sambuci Gersch, 1942 = *Aphis sambuci* L.
Aphis solidaginis Büsgen, 1891 = *Uroleucon* (*Uromelan*) *solidaginis* F.
Aphis spiraecola Wynn and Boudreaux, 1972 = *Aphis spiraecola* Patch
Aphis verbasci Schanderl et al., 1949 = *Aphis verbasci* Schrank
Aphis viburni Büsgen, 1891 = *Aphis virburni* Scop.
Aulacorthum circumflexum Griffiths, 1961 = *Neomyzus circumflexus* Buckt.
Betacallis gigantea Holman, 1958 = *Clethrobius comes* Wlk.
Brachycaudus cardui Tóth, 1933 = *Brachycaudus cardui* L.
Brachycaudus cardui Holman, 1958 = *Brachycaudus cardui* L.
Brachycaudus helichrysi Schanderl et al., 1949 = *Brachycaudus helichrysi* Kltb.
Brevicoryne brassicae Schanderl et al., 1949 = *Brevicoryne brassicae* L.
Brevicoryne brassicae Moericke and Mittler, 1966 = *Brevicoryne brassicae* L.
Brevicoryne brassicae Tremblay, 1966 = *Brevicoryne brassicae* L.
Brevicoryne brassicae Lamb and Hinde, 1967 = *Brevicoryne brassicae* L.
Brevicoryne brassicae Wensler and Filshie, 1969 = *Brevicoryne brassicae* L.
Brevicoryne brassicae Hinde, 1971a, b = *Brevicoryne brassicae* L.
Brevicoryne brassicae Kislow and Edwards, 1972 = *Brevicoryne brassicae* L.
Byrsocrypta ulmi Holman, 1958 = *Tetraneura ulmi* L.
Cachryphora serotinae Wynn and Boudreaux, 1972 = *Cachryphora serotinae* Oestl.
Calaphis betulella Hottes, 1928 = *Calaphis betulella* Walsh
Callaphis juglandis Holman, 1958 = *Callaphis juglandis* Goeze

Callipterinella betulaecolens Hottes, 1928 = *Calaphis betulaecolens* Fitch
Callipterus coryli Buckton, 1876 = *Myzocallis coryli* Goeze
Callipterus quercus Witlaczil, 1882 = *Tuberculoides annulatus* Htg.
Capitophorus sp. Holman, 1958 = *Capitophorus* sp.
Catamergus sp. Hottes, 1928 = *Catamergus* sp.
Cavariella aegopodii Schoel, 1934 = *Cavariella aegopodii* Scop.
Chaetophorella aceris Holman, 1958 = *Periphyllus aceris* L.
Chaetosiphon fragaefolii Kislow and Edwards, 1972 = *Chaetosiphon (Pentatrichopus)*
    fragaefolii Cock.
Chaitophorus aceris Paillot, 1933, 1934 = *Periphyllus aceris* L.
Chaitophorus lyropictus Paillot, 1933, 1938 = *Periphyllus lyropictus* Kessler
Chaitophorus populeti Holman, 1958 = *Chaitophorus populeti* Pz.
Chaitophorus populi Witlaczil, 1882 = *Chaitophorus populeti* Pz.
Chaitophorus sp. Strong, 1967 = *Chaitophorus* sp.
Chermes abietis Mark, 1877 = *Adelges abietis* L.
Chermes lapponicus Cholodkovsky, 1905 = *Adelges lapponicus* Chol.
Cinara carolina Wynn and Boudreaux, 1972 = *Cinara carolina* Tissot
Cinara pini Holman, 1958 = *Cinara pini* L.
Cinara pinihabitans Tóth, 1936 = ? *Cinara pinea* Mordv.
Cinara watsoni Wynn and Boudreaux, 1972 = *Cinara watsoni* Tissot
Cinaria nuda Holman, 1958 = *Cinara nuda* Mordv.
Cryptomyzus ribis Mackauer, 1959 = *Cryptomyzus ribis* L.
Dactylosphaera vitifolii Breider, 1952 = *Viteus vitifoliae* Fitch
Dactylosphaera vitifolii Rilling, 1960, 1967 = *Viteus vitifoliae* Fitch
Doralis fabae Gersch, 1942 = *Aphis fabae* Scop.
Doralis mali Schanderl et al., 1949 = *Aphis pomi* de Geer
Doralis saliceti Schoel, 1934 = *Aphis farinosa* Gmelin
Drepanaphis acerifolii Hottes, 1928 = *Drepanaphis acerifolii* Thos.
Drepanosiphon platanoidis Holman, 1958 = *Drepanosiphum platanoides* Schrank
Drepanosiphum platanoides Buckton, 1876 = *Drepanosiphum platanoides* Schrank
Drepanosiphum platanoides Gersch, 1942 = *Drepanosiphum platanoides* Schrank
Drepanosiphum platanoides Johnson, 1962, 1963 = *Drepanosiphum platanoides* Schrank
Dysaphis anthrisci Holman, 1958 = *Dysaphis anthrisci* Börner
Dysaphis devecta Forrest and Noordink, 1971 = *Dysaphis devecta* Wlk.
Elatobium abietinum Kloft, 1960 = *Elatobium abietinum* Wlk.
Eucallipterus tiliae Holman, 1958 = *Eucallipterus tiliae* L.
Euceraphis punctipennis Holman, 1958 = *Euceraphis punctipennis* Zett.
Eulachnus agilis Hottes, 1928 = *Eulachnus agilis* Kltb.
Eriosoma lanigerum De Fluiter, 1931 = *Eriosoma lanigerum* Hsmnn.
Hormaphis hamamelidis Lewis and Walton, 1958 = *Hormaphis hamamelidis* Fitch
Hyalopterus pruni Janiszewska, 1932 = *Hyalopterus pruni* Geoffr.
Hyalopterus pruni Holman, 1958 = *Hyalopterus pruni* Geoffr.
Hyalopterus pruni Wynn and Boudreaux, 1972 = *Hyalopterus pruni* Geoffr.
Hyperomyzus lactucae Holman, 1958 = *Hyperomyzus lactucae* L.
Hyperomyzus lactucae O'Loughlin and Chambers, 1967 = *Hyperomyzus lactucae* L.
Hyperomyzus lactucae Sylvester and Richardson, 1970 = *Hyperomyzus lactucae* L.
Lachnus coloradensis Hottes, 1928 = *Cinara coloradensis* Gill.
Lachnus fasciatus Hollande, 1911 = *Lachniella costata* Zett.
Lachnus grossus Hollande, 1911 = *Cinara abieticola* Chol.
Lachnus piceae Leonhardt, 1940 = *Cinara piceae* Pz.
Lachnus pineus Mordwilko, 1895 = *Cinara pinea* Mordv.
Lachnus (Pterochlorus) roboris Michel, 1942 = *Lachnus roboris* L.
Lachnus roboris Holman, 1958 = *Lachnus roboris* L.
Lachnus salicis Hollande, 1911 = *Tuberolachnus salignus* Gmelin
Lachnus saligna Buckton, 1876 = *Tuberolachnus salignus* Gmelin

Lachnus viminalis MORDWILKO, 1895 = *Tuberolachnus salignus* GMELIN
Lachnus viminalis HOLLANDE, 1911 = *Tuberolachnus salignus* GMELIN
Lachnus viminalis WENSLER and FILSHIE, 1969 = *Tuberolachnus salignus* GMELIN
Lipaphis pseudobrassicae MOERICKE and MITTLER, 1966 = *Lipaphis erysimi* KLTB.
Longistigma caryae KNOWLTON, 1925 = *Longistigma caryae* HARRIS
Longistigma caryae HOTTES, 1928 = *Longistigma caryae* HARRIS
Longistigma caryae WYNN and BOUDREAUX, 1972 = *Longistigma caryae* HARRIS
Macrosiphoniella millefolii BOESE, 1936 = *Macrosiphoniella millefolii* de GEER
Macrosiphoniella sp. HOTTES, 1928 = *Macrosiphoniella* sp.
Macrosiphon solanifolii HOLMAN, 1958 = *Macrosiphum euphorbiae* THOS.
Macrosiphum albifrons ADAMS and WADE, 1970 = *Macrosiphum albifrons* ESSIG
Macrosiphum avenae PALIWAL and SINHA, 1970 = *Macrosiphum (Sitobion) avenae* F.
Macrosiphum euphorbiae GRIFFITHS, 1961 = *Macrosiphum euphorbiae* THOS.
Macrosiphum euphorbiae ADAMS and WADE, 1970 = *Macrosiphum euphorbiae* THOS.
Macrosiphum euphorbiae WYNN and BOUDREAUX, 1972 = *Macrosiphum euphorbiae* THOS.
Macrosiphum granarium RUTSCHKY and CAMPBELL, 1964 = *Macrosiphum (Sitobion) avenae* F.
Macrosiphum pisi LEBLANC and MUSGRAVE, 1963 = *Acyrthosiphon pisum* HARRIS
Macrosiphum rosae CAZAL, 1948 = *Macrosiphum rosae* L.
Macrosiphum rosae VAGO and LAPORTE, 1965 = *Macrosiphum rosae* L.
Macrosiphum rosae HINDE, 1971a, b = *Macrosiphum rosae* L.
Macrosiphum rudbeckiae EDWARDS, 1966 = *Uroleucon rudbeckiae* FITCH
Macrosiphum sanbornii MILLER, 1932 = *Macrosiphoniella sanborni* GILL.
Macrosiphum solanifolii SPENCER, 1926 = *Macrosiphum euphorbiae* THOS.
Macrosiphum solanifolii STILES, 1938 = *Macrosiphum euphorbiae* THOS.
Macrosiphum solanifolii SMITH, 1939 = *Macrosiphum euphorbiae* THOS.
Macrosiphum solanifolii LANHAM, 1952 = *Macrosiphum euphorbiae* THOS.
Macrosiphum solanifolii FORTIN, 1958 = *Macrosiphum euphorbiae* THOS.
Macrosiphum tanaceti UICHANCO, 1924 = *Metopeurum fuscoviride* STROYAN
Macrosiphum tanaceticolum KLEVENHUSEN, 1927 = *Uroleucon tanaceti* L.
Macrosiphum tanacetum KLEVENHUSEN, 1927 = *Metopeurum fuscoviride* STROYAN
Megoura aconiti TÓTH, 1936 = *Delphiniobium junackianum* KARSCH.
Megoura viciae SCHOEL, 1934 = *Megoura viciae* BUCKT.
Megoura viciae HOLMAN, 1958 = *Megoura viciae* BUCKT.
Megoura viciae EHRHARDT, 1963 = *Megoura viciae* BUCKT.
Megoura viciae SCHMUTTERER, 1969 = *Megoura viciae* BUCKT.
Melanoxantherium salicis HOTTES, 1928 = *Pterocomma salicis* L.
Melanoxantherium smithiae HOTTES, 1928 = *Pterocomma smithiae* MONELL
Melaphis rhois WYNN and BOUDREAUX, 1972 = *Melaphis rhois* FITCH
Metopeurum fuscoviride MACKAUER, 1959 = *Metopeurum fuscoviride* STROYAN
Microlophium evansi DIXON, 1958 = *Microlophium carnosum* BUCKT.
Microsiphum artemisiae HOTTES, 1928 = *Microsiphum artemisiae* GILL.
Monellia costalis WYN and BOUDREAUX, 1972 = *Monellia costalis* FITCH
Monellia caryella HOTTES, 1928 = *Monellia caryella* FITCH
Morvilkoja vagabunda ADAMS and WADE, 1970 = *Mordvilkoja vagabunda* WALSH
Myzocallis bellus HOTTES, 1928 = *Myzocallis bellus* WALSH
Myzodes persicae HOLMAN, 1958 = *Myzus persicae* SULZ.
Myzus ascalonicus KLOFT, 1959, 1960 = *Myzus ascalonicus* DONC.
Myzus ornatus HOLMAN, 1958 = *Myzus ornatus* LAING
Nasonovia ribis-nigri GRIFFITHS, 1961 = *Nasonovia ribis-nigri* MOSLEY
Neomyzus circumflexus HOLMAN, 1958 = *Neomyzus circumflexus* BUCKT.
Neophyllaphis podocarpi WYN and BOUDREAUX, 1972 = *Neophyllaphis podocarpi* TAKAHASHI
Neothomasia abditus HOTTES, 1928 = *Chaitophorus abditus* HOTTES
Neothomasia populicola HOTTES, 1928 = *Chaitophorus populicola* THOS.
Paraprociphilus tessellata ADAMS and WADE, 1970 = *Paraprociphilus tessellatus* FITCH
Pemphigus balsamiferae ADAMS and WADE, 1970 = *Pemphigus balsamiferae* WILLIAMS

Pemphigus bursarius WITLACZIL, 1882 = *Pemphigus bursarius* L.
Pemphigus bursarius PFLUGFELDER, 1936 = *Pemphigus bursarius* L.
Pemphigus bursarius LAMPEL, 1959, 1962 = *Pemphigus bursarius* L.
Pemphigus bursarius JOHNSON, 1963 = *Pemphigus bursarius* L.
Pemphigus populi-nigrae LAMPEL, 1959, 1962 = *Pemphigus populi-nigrae* SCHRANK
Pemphigus populicaulis ADAMS and WADE, 1970 = *Pemphigus populicaulis* FITCH
Pemphigus populitransversus ADAMS and WADE, 1970 = *Pemphigus populitransversus* RILEY
Pemphigus spirothecae WITLACZIL, 1882 = *Pemphigus spyrothecae* PASS.
Pemphigus spirothecae WILL, 1889 = *Pemphigus spyrothecae* PASS.
Pemphigus spirothecae TÓTH, 1938 = *Pemphigus spyrothecae* PASS.
Pemphigus sp. CAZAL, 1948 = *Pemphigus* sp.
Pentatrichopus tetrarhodus MACKAUER, 1959 = *Pentatrichopus tetrarhodus* WLK.
Phylloxera vastatrix KRASSILSTSCHIK, 1893 = *Viteus vitifoliae* FITCH
Prociphilus tesselata PELTON, 1938 = *Paraprociphilus tessellatus* FITCH
Pterocallis alni GERSCH, 1942 = *Pterocallis alni* de GEER
Rhopalosiphoninus tulipaellus MARTINI, 1958 = *Rhopalosiphoninus tulipaellus* THEOB.
Rhopalosiphum nymphaeae HIRSCHLER, 1912 = *Rhopalosiphum nymphaeae* L.
Rhopalosiphum padi MOREAU and VAN LOON, 1966 = *Rhopalosiphum padi* L.
Rhopalosiphum padi ADAMS and WADE, 1970 = *Rhopalosiphum padi* L.
Rhopalosiphum sp. ADAMS and WADE, 1970 = *Rhopalosiphum* sp.
Sacchiphantes viridis PROFFT, 1937 = *Adelges viridis* RATZ.
Schizaphis graminum SAXENA and CHADA, 1971a, b, c, d = *Schizaphis graminum* ROND.
Schizolachnus pineti HOLMAN, 1958 = *Schizolachnus pineti* F.
Schizoneura americana MILLER, 1933 = *Eriosoma americanum* RILEY
Schizoneura lanigera DAVIDSON, 1913, 1914 = *Eriosoma lanigerum* HSMNN.
Schizoneura lanigera BAKER, 1915 = *Eriosoma lanigerum* HSMNN.
Schizoneura lanigera HOTTES, 1928 = *Eriosoma lanigerum* HSMNN.
Schizoneura ulmi MARK, 1877 = *Eriosoma ulmi* L.
Siphonophora absinthii ZWEIGELT, 1915 = *Macrosiphoniella absinthii* L.
Siphonophora circumflexa BUCKTON, 1876 = *Neomyzus circumflexus* BUCKT.
Siphonophora pisi BUCKTON, 1876 = *Acyrthosiphon pisum* HARRIS
Siphonophora rosae ZWEIGELT, 1915 = *Macrosiphum rosae* L.
Siphonophora rosarum BUCKTON, 1876 = *Pentatrichopus tetrarhodus* WLK.
Siphonophora rosarum GROVE, 1909, 1910 = *Pentatrichopus tetrarhodus* WLK.
Stagona xylostei HOLMAN, 1958 = *Stagona xylostei* de GEER
Stegophylla sp. WYNN and BOUDREAUX, 1972 = *Stegophylla* sp.
Stomaphis graffii TÓTH, 1936 = *Stomaphis graffii* CHOL.
Stomaphis longirostris TÓTH, 1933 = *Stomaphis quercus* L.
Stomaphis quercus KLEVENHUSEN, 1927 = *Stomaphis quercus* L.
Symydobius americanus HOTTES, 1928 = *Symydobius americanus* BAKER
Thargelia albipes HOTTES, 1928 = *Aphthargelia symphoricarpi* THOS.
Thecabius populiconduplifolius ADAMS and WADE, 1970 = *Thecabius populiconduplifolius* COWEN
Trama rara ADAMS and WADE, 1970 = *Trama rara* MORDV.
Trama troglodytes MORDWILKO, 1895 = *Trama troglodytes* v. HEYD.
Trama sp. HOLMAN, 1958 = *Trama* sp.
Tuberolachnus salignus WENSLER and FILSHIE, 1969 = *Tuberolachnus salignus* GMELIN
Tuberolachnus salignus ADAMS and WADE, 1970 = *Tuberolachnus salignus* GMELIN
Tuberolachnus salignus KISLOW and EDWARDS, 1972 = *Tuberolachnus salignus* GMELIN
Tritogenaphis sp. HOTTES, 1928 = *Uroleucon* sp.
Uroleucon nigrotuberculatus WYNN and BOUDREAUX, 1972 = *Uroleucon nigrotuberculatus* OLIVE
Uroleucon tissoti WYNN and BOUDREAUX, 1972 = *Uroleucon tissoti* BOUDREAUX
Uroleucon verbesinae WYNN and BOUDREAUX, 1972 = *Uroleucon verbesinae* BOUDREAUX
Uromelan aeneus HOLMAN, 1958 = *Uroleucon aeneus* H. R. L.

5. SITE OF VIRUS MULTIPLICATION IN THE APHID

INTRODUCTION

Although it has been shown beyond doubt, that PLRV can multiply in the aphid vector, *M. persicae*, the site of virus multiplication remains unknown. The distribution of animal and plant viruses in arthropod vectors has been examined in recent years by many investigators, whose results are summarized in Table 10.

The object of this part of our study was to obtain information concerning the fate of the ingested virus in the aphid vector by trying to recover PLRV from dissected organs of larvae, shortly after being fed on a leafroll-diseased host plant.

MATERIALS AND METHODS

The culture of *P. floridana* and Chinese cabbage plants used in this study is described in chapter 3.

Dissections

For dissecting the various organs new-born larvae were selected from a non-viruliferous colony on Chinese cabbage and were allowed to feed for six days on leafroll diseased *P. floridana* plants of about five weeks old. Thereafter the larvae were fixed on self-adhesive tape which was fixed to a glass-plate (ARAGAO and DA COSTA LIMA, 1929). Under a dissecting microscope each larva was quenched with a drop of cold buffer which did not spread on the glue surface. In this drop the larva was dissected for preparation of the various organs. As dissecting fluid 0.15 M SÖRENSEN phosphate buffer (pH 7.2) was used.

The various organs were prepared out of the larvae with a very fine sharpened watchmakers forceps and a fine glass needle. With the forceps both compound eyes of the larva were seized, and the head was separated from the thorax by pulling. As a result the complete gut, the salivary glands, and the nervous system were separated in intact form together with the head. By removing the cauda, the intact reproductive organs were obtained in a similar way. By dissecting the larva with this method the mycetocytes, which contain the symbionts, were liberated in the buffer. To obtain guts free of contents the intestine was transsected which resulted in a flow of flocculent contents from the stomach, and a clear watery fluid from the intestine and hindgut into the buffer.

After amputation of the head and cauda the body (thorax and abdomen) was vigorously washed in buffer solution to remove the still remaining fat cells and basophilic mesodermal cells. Aphids treated in this way contain only

muscles, tracheae, the dorsal vessel and its pericardial cells, connective tissue cells, and integument. In routine dissections, as was done for each organ separately, it was very difficult to collect dorsal vessels. This organ is attached to the dorsal body wall by muscles and to the dorsal nerve by nerve branches; the pericardial cells are connected to branches of the dorsal nerve (page 56).

The prepared organs were sucked up with a fine pipette and transferred into a glass micro-mortar with a glass holder standing in a box with ice (Fig. 33). The organs were washed three times with fresh cold buffer and after the third time the superfluous buffer was sucked up out of the micro-mortar. To homogenate the organs they were ground with a glass pestle fitting in the mortar.

The cornicle secretion was produced by slightly pinching the head of the aphid with forceps. This results in the elevation of the cornicles, followed immediately by the production of a droplet. Secretion of about 10 larvae was caught in a drop of buffer and then homogenated. The honeydew was obtained by fixing a glass-plate about 3 cm under the leaves of leafroll diseased *P. floridana* plants colonized with aphids. After about 16 hours the honeydew droplets on the glass-plate were dissolved with a paint brush wetted with buffer.

About 200 viruliferous larvae were fixed in groups of 20 very close together on self-adhesive tape and each group was quenched with a drop of cold buffer. In this drop the heads of the larvae were separated from the thoraxes by which haemolymph, fat cells (intact and degenerating ones), basophilic mesodermal cells, and mycetocytes were liberated in the buffer. In the dissecting fluid the intact fat cells are visible as spherical translucent cells characterized by the presence of fat droplets with a yellow refractive appearance. The mycetocytes appear as opaque white spheres. These suspensions were collected in a serum-tube (6 × 46 mm) with a fine pipette. The total quantity amounted to about 1 ml from 200 larvae. The suspension was centrifuged at 3000 rpm during 20

A   B

1 CM.

Fig. 33. Longitudinal view of a glass micro-mortar (A) with glass pestle (B).

minutes. The supernatant was collected, carefully avoiding the small amount of fat floating at the top of the tube, and filtered through a Jena G4 glass filter. The pellet was suspended in 1 ml of buffer and sedimented again by centrifugation at 3000 rpm. The washing of the pellet was repeated once more. The obtained pellet was suspended in about 0.02 ml of buffer.

Injections

Infectivity of suspensions of various parts from larval viruliferous aphids was tested by injecting about 0.003 µl of the preparations into nonviruliferous larvae. The injections were carried out with fine glass needles (Worst, 1954); our technique for injecting and handling the aphids has been described by Stegwee and Ponsen (1958) and Peters (1967b). Before injection the larvae were anaesthetized with gaseous carbon dioxide. In general the larvae were injected intra-abdominally on the dorsolateral side of the aphid. Working with dye solutions (e.g. Indian ink, Evans blue, or Florella blue), the aphids were injected via one of the two cornicles, which are in open connection with the body cavity. The injected larvae were placed on filterpaper in a petridish until they were recovered from the anaesthesia. Only those aphids which were still alive after 3 hours were put on *P. floridana* seedlings. Two-leaf-stage test plants received 5 injected larvae, whereas only one larva was put on a one-leaf-stage plant.

LOCALISATION OF PLRV IN THE APHID

The results concerning the localisation of PLRV in the various organs and tissues of viruliferous larvae are presented in Table 11. The presence of virus could be demonstrated in the intact gut, honeydew, haemolymph, and cornicle secretion. Negative results were obtained with the gut without contents, salivary glands, mycetocytes, nervous system, ovaries, and integument including connective tissue cells, pericardial cells, dorsal vessel, muscles, and tracheae from viruliferous larvae. Moreover, it appeared that the intact gut and the honeydew of viruliferous larvae had lost their infectivity after the larvae were allowed to feed for 1–2 days on immune Chinese cabbage seedlings. On the other hand, the haemolymph and cornicle secretion retained their infectivity. The haemolymph was obtained directly from a viruliferous aphid with an injection needle and injected into a nonviruliferous one.

Although in the aphid no circulating cells occur in the haemolymph, smear preparations of haemolymph obtained with an injection needle showed an almost identical histological picture as compared with that of the cornicle secretion (Table 7), i.e. fat cells (intact and degenerating ones), nuclear material from degenerating fat cells, waxy droplets, rhomb-shaped crystals, and occasionally a basophilic mesodermal cell. The presence of both celltypes, especially the intact fat cells, is solely due to the injection technique, which involves the

101

TABLE 10. The presence of circulative and propagative animal and plant viruses in various organs, tissues, and liquids of viruliferous insect vectors.

Virus	Insect vector	Method	Gut epithelium	Gut lumen	Malpighian tubules	Fecula	Salivary gland	Saliva	Haemolymph
A Yellow fever	*Aëdes aegypti* (L.)[1]	DO	+			+	+		+
Yellow fever	*Aëdes aegypti* (L.)	EM					+		
Dengue	*Aëdes aegypti* (L.)	DO	+				+		+
Western equine encephalitis	*Aëdes aegypti* (L.)	DO							+
Western equine encephalitis	*Culex tarsalis* COQUILLETT	DO					+		
West Nile	*Aëdes aegypti* (L.)	DO	+				+		
West Nile	*Anopheles quadrimaculatus* SAY	DO	+				+		
Murray Valley encephalitis	*Culex annulirostris* SKUSE	DO	+				+		+
Murray Valley encephalitis	*Aëdes queenslandis* STRICKLAND	DO	+				+		+
Japanese B encephalitis	*Culex pipiens pipiens* L.	DO	+		+		+		+
Japanese encephalitis	*Culex tritaeniorhynchus* GILES							+	
Semliki Forest	*Anopheles albimonus* WIEDEMANN							+	
St. Louis encephalitis	*Culex pipiens pipiens* L.							+	
Eastern equine encephalitis	*Aëdes triseriatus* SAY	EM					+		
Venezuelan equine encephalitis	*Aëdes aegypti* (L.)	DO					+		
Bluetongue	*Culicoides variipennis* COQUILLETT[2]	EM					+		
Russian spring summer encephalitis[7]	*Ixodes persulcatus* SCHULZE[3]	DO	+		+		+		
Western equine encephalitis	*Triatoma sanguisuga* LECONTE	DO				+			
Western equine encephalitis	*Dermanyssus gallinae* de GEER[4]					+			
P Maize streak	*Cicadulina mbila* NAUDÉ[5]	DO				+			+
Curly top	*Eutettix tenellus* BAKER	DO	+				+	+	+
Rice dwarf[7]	*Nephotettix cincticeps* UHL.								+
Rice dwarf[7]	*Nephotettix cincticeps* UHL.	EM	+		+		+		+
Wound tumor[7]	*Agallia constricta* van DUZ.	UV	+		+		+		haemocytes
Wound tumor[7]	*Agallia constricta* van DUZ.	EM	+		+		+		haemocytes
Wound tumor	*Agalliopsis novella* SAY	EM	+		−		−		haemocytes
Potato yellow dwarf[7]	*Agallia constricta* van DUZ.	DO	+		+		+		+
Maize mosaic	*Peregrinus maidis* ASH.	EM	+				+		
Maize rough dwarf	*Laodelphax striatellus* FALL.	EM	+				+		
Wheat striate mosaic	*Endria inimica* SAY	DO	+		−		+		haemocytes
Rice hoja blanca	*Sogatodes oryzicolus* MUIR	EM	+	+					
Pea enation mosaic	*Acyrthosiphon pisum* HARRIS[6]	DO				+			+
Pea enation mosaic	*Acyrthosiphon pisum* HARRIS	EM		+					
Pea enation mosaic	*Megoura viciae* BUCKT.					+			+
Lettuce necrotic yellows	*Hyperomyzus lactucae* L.	EM	+	−			+		
Sowthistle yellow vein[7]	*Hyperomyzus lactucae* L.	EM	+				+		
Barley yellow dwarf	*Macrosiphum* (*Sitobion*) *avenae* F.	DO	+				+		+

[1]mosquito
[2]fly
[3]tick
[4]mite
[5]leafhopper
[6]aphid
[7]transovarial passage of virus to progeny

Fatbody	Mycetome	Muscles	Nervous system	Female reproductive organs	Male reproductive organs	Tracheoblasts	Integument	Author
				+				HINDLE (1929, 1930); ARAGAO and DA COSTA LIMA (1929); DAVIS and SHANNON (1930); WHITMAN (1952)
								BERGOLD and WEIBEL (1962)
				+				HOLT and KINTNER (1931)
								MERRILL and TenBROECK (1934)
								THOMAS (1963)
								WHITMAN (1952)
								WHITMAN (1952)
				+				McLEAN (1955)
				+				McLEAN (1955)
			+	+				LaMOTTE (1960)
								HURLBUT (1964)
								COLLINS (1963)
								HURLBUT (1966)
								WHITFIELD et al. (1971)
								GAIDAMOVICH et al. (1971)
								BOWNE and JONES (1966)
			+		+		—	PAVLOVSKY and SOLOVIEV (1940)
				+				GRUNDMANN et al. (1943)
								CHAMBERLAIN and SIKES (1955)
								STOREY (1933)
								BENNETT and WALLACE (1938)
				eggs +				FUKUSHI and KIMURA (1959)
+				+				FUKUSHI et al. (1962); FUKUSHI and SHIKATA (1963)
+	+		+	+	—			SINHA (1965a)
+	+	+	+	+	—	+	+	SHIKATA and MARAMOROSCH (1965, 1967a, b); HIRUMI et al. (1967); GRANADOS et al. (1968)
+	—	+	+			+		GRANADOS et al. (1967)
+	+		+	+	+			SINHA (1965b)
								HEROLD and MUNZ (1965)
+	+	+	+	+		+	+	VIDANO and BASSI (1966); VIDANO (1968)
+	+		+	—	—			SINHA and CHIYKOWSKI (1969)
								SHIKATA and GÁLVEZ (1969)
								NAULT et al. (1964); SCHMUTTERER and EHRHARDT (1964); RICHARDSON and SYLVESTER (1965)
+								SHIKATA et al. (1966)
								SCHMUTTERER (1969)
+	+	+	+	embryos —		+	+	O'LOUGHLIN and CHAMBERS (1967)
+	+	+	+	embryos —				RICHARDSON and SYLVESTER (1968); SYLVESTER and RICHARDSON (1970)
				ovaries +				
				—				PALIWAL and SINHA (1970)

A = animal virus
P = plant virus
DO = dissected organs tested for their infectivity
EM = electron microscopy
UV = UV microscopy (immunofluorescent technique)
+ = individual organs which were shown to contain virus
— = organs in which no virus could be detected

introduction of a needle via the mesodermal tissue into the body cavity in order to obtain the haemolymph sample.

In another experiment the haemolymph was acquired by amputation of the aphid's head. After centrifugation both the pellet as well as the supernatant appeared to be infective. The histological composition of the pellet was nearly similar to that of the above mentioned preparations, though in this case more basophilic mesodermal cells as well as symbionts were observed. As a consequence of centrifugation the symbionts present in the mycetocytes were liberated into the dissecting fluid.

In all preparations the fat cells were in the majority. The connective tissue cells, another derivate of the mesodermal tissue, remain inside the aphid's body where they have a supporting function. Neither have connective tissue cells been found in the cornicle secretion (Table 7).

DISCUSSION

Many investigators have demonstrated the presence of circulative and propagative animal and plant viruses in various dissected organs and tissues from viruliferous insect vectors. From their results (Table 10) it can be concluded that these viruses are ingested with the food by the insect vectors and are partly egested with the fecula. After penetrating the gut wall the virus enters into the haemolymph after which this tissue plays an important role in the distribution of virus towards the various internal organs including the salivary glands. The virus may subsequently be secreted again with the saliva into the host. Regarding the propagative viruses the process of multiplication occurs somewhere during this circulation. By means of electron and UV microscopy (immunofluorescent technique) several investigators revealed the presence of animal and plant viruses in the various organs and tissues of their insect vectors. It seems unlikely that such a quantity of particles could be accumulated in cells by a process other than multiplication.

In the present study it is found that it is impossible to recover PLRV either from the intact gut of viruliferous *M. persicae* larvae after 1–2 days feeding period on immune Chinese cabbage seedlings, or in the gut from which the contents have been removed (Table 11). From these results it may be concluded that only the gut contents contains the infectious material which the aphid acquires by feeding on a leafroll diseased *P. floridana* plant. This agrees with the observed presence of PLRV in the honeydew only when the aphids actually feed on the source of leafroll virus. Similar results with the leafhopper, *Cicadulina mbila* NAUDÉ, were obtained by STOREY (1933) who investigated maize streak virus. When viruliferous individuals fed on an immune plant the virus could not be demonstrated anymore in the fecula. Pea enation mosaic virus (RICHARDSON and SYLVESTER, 1965) was still infectious in honeydew from *Acyrthosiphon pisum* up to 3 days after viruliferous aphids had been transferred to healthy sweetpea (*Lathyrus odoratus* L.) seedlings. The same virus was also

104

present in the honeydew of *Megoura viciae* maintained on virus diseased *V. faba*, which could be proven by injecting this honeydew into larvae of the same species (SCHMUTTERER, 1969) (Table 9).

The finding that no virus could be recovered from the gut epithelium of viruliferous larvae indicates that PLRV does not multiply in this organ. This conclusion is supported by the fact that MOERICKE (1963) and FORBES (1964b) in their detailed electron microscopical studies of the gut epithelium of viruliferous *M. persicae* adults have never found spherical particles characteristic for PLRV (i.e. about 23 nm in diameter and with a hexagonal outline; PETERS, 1967a, b; KOJIMA et al., 1968, 1969).

Several investigators were interested in the mechanism of PLRV transport from the gut lumen to the haemolymph. According to SCHMIDT (1959) this process takes place presumably in the stomach because in viruliferous *M. persicae* adults the nuclei in the epithelial cells of this organ are bigger than those of nonviruliferous ones. Moreover, in these enlarged nuclei the chromatin is lacking and in addition to the nucleolus, several small partly dark-coloured, partly refractive bodies containing vacuoles, are observed. On the other hand, such bodies were also frequently found by FORBES (1964a) in nuclei of stomach cells of nonviruliferous *M. persicae* adults. In the present study (page 27) it has been shown that strongly basophilic clusters of chromatin develop in some nuclei of digestive cells situated in the middle region of the stomach, after nonviruliferous larvae have reached the imaginal stage (Fig. 10 B'). Presumably the number of these clusters increase during the imaginal stage till all chromatin has disappeared as seems to be reflected in the pictures given by SCHMIDT (1959) and FORBES (1964a). In work on the ultra-structure of the gut of *M. persicae*, OSSIANNILSSON (1961) concluded that intact plant virus particles cannot penetrate the gut wall because of its very compact structure. He presumes that the virus material entering the body cavity consists of smaller units. According to MOERICKE (1963) plant viruses might possibly enter the body cavity as intact particles intercellulary through the stomach wall. This view is based on the presence of rod-shaped particles (30 × 200 nm) in the enlarged intercellular spaces of the basement labyrinth, between two cell membranes, in vesicles of the 'Vesikelnester,' and in the basement membrane itself of leafroll infected as well as nonviruliferous adults of *M. persicae*. Another explanation is given by FORBES (1964a), who suggests that transport of virus particles through the gut wall to the haemolymph takes place by pinocytosis. This process is restricted to the midgut, because membrane vesicles are only observed in these epithelial cells. We have furthermore demonstrated that the ingested virus is partly excreted with the honeydew, and has partly penetrated the gut wall after which it can be demonstrated in the haemolymph (PONSEN, 1970). Consequently the gut wall does not function as an appreciable barrier for PLRV. Already after an acquisition feeding period of 8 hours on a leafroll diseased *P. floridana* plant a demonstrable amount of virus was present in the haemolymph (STEGWEE, 1960). This also points to a rapid penetration. Presumably PLRV undergoes no change during passage through the gut wall because nonviruliferous aphids

105

TABLE 11. Recovery of potato leafroll virus from homogenized larvae, organs, tissues, and liquids of viruliferous *Myzus persicae* larvae. New-born larvae selected from Chinese cabbage were allowed to feed for 6 days on leafroll diseased *Physalis floridana* plants and then immediately (A), or after a stay for 1 or 2 days on Chinese cabbage seedlings (B), used for the preparation of inoculum. Infection feeding period 5 days.

		Number of tests	Number of sphids or dissected organs per test[1]	Number of injected larvae per test plant	Number of diseased plants/ number of test plants
Larva	A	2	20	5	6/6
	A	2	20	1	24/26
Intact gut	A	3	10	5	3/8
	A	3	10	1	18/45
	B	3	10	5	(1) 0/14
	B	3	10	1	(1) 0/58
	B	4	10	1	(2) 0/63
Guts without contents	A	2	20	5	0/12
	A	3	20	1	0/57
Honeydew	A	3		1	6/43
	B	3		1	(1) 0/52
Salivary glands	A	2	ca. 40	5	0/7
	A	2	ca. 40	1	0/28
Head including salivary glands, foregut, and nervous system	A	5	30	1	0/74
Integument including connective tissue cells, pericardial cells, dorsal vessel, muscles, and tracheae	A	5	30 larvae	1	0/76
Mycetocytes	A	3	many	1	0/47
Ovaries and embryos	A	3	20	5	0/9
	A	3	20	1	0/49
Cornicle secretion	A	3		1	12/27
	B	3		1	(2) 19/43
Haemolymph[2]	A	5		1	57/63
	B	5		1	(2) 50/58
Haemolymph[3] pellet	A	3	200 larvae	1	9/45
supernatant				1	10/43

[1]Whole aphids or dissected organs were homogenized
[2]Haemolymph obtained with an injection needle from the aphid's body
[3]Haemolymph obtained after decapitation of the larvae
In brackets number of days on Chinese cabbage seedlings before viruliferous larvae were dissected

were found to transmit virus after injection with phloem sap (HILLE RIS LAMBERS, 1959; PONSEN and STEGWEE, 1960) or with extracts from leafroll diseased *P. floridana* (MURAYAMA and KOJIMA, 1965) into the haemocoel. PALIWAL and SINHA (1970) reported that barley yellow dwarf virus was not

detected in the gut of *Macrosiphum avenae* (Table 9) which were injected with a massive dose of virus, an observation suggesting that it does not pass from the haemolymph into the gut.

Since *M. persicae* does not regurgitate the ingested food (DAY and IRZYKIE-WICZ, 1953) and haemolymph contains a demonstrable amount of virus (Table 11), it may be concluded that the virus has to penetrate the salivary glands before the aphid is capable of virus transmission (SMITH, 1926, 1931; ELZE, 1931). The negative results obtained for the salivary glands suggest that the multiplication of virus does not occur in this organ. As in the case of the gut, this supposition is supported by detailed electron microscopical studies of MOERICKE and WOHLFAHRT-BOTTERMANN (1962) and MOERICKE (1961) who never mentioned any evidence for the presence of spherical particles in the salivary glands of viruliferous *M. persicae* adults. Moreover, a gradual virus accumulation in the salivary glands during acquisition feeding of 6 days on the leafroll source cannot be considered in view of the negative results. The transportation of PLRV by the myoepithelioid cell from the haemolymph (page 41) seems to provide the most attractive explanation for the virus translocation. This cell is situated in the distal region of each principal salivary gland (Fig. 13 A), and regulates haemolymph movement into the lumen of both internal salivary ducts.

BLATTNY (1931) reported that the salivary glands of nonviruliferous *M. persicae* showed a clear zone around their nuclei, which was lacking in some viruliferous aphids. In the present study these zones were never found in salivary glands of nonviruliferous larvae. Possibly this discrepancy is due to differences in age. In that case the clear zones are limited to the imaginal stage. BLATTNY, however, did not mention the age of his aphids.

It has been observed that the phloem-feeding aphids, *M. persicae* (VAN HOOF, 1957; POLLARD, 1971), *Aphis fabae* (VAN HOOF, 1957), and *Rhopalosiphum padi* (MOREAU and VAN LOON, 1966), secrete a saliva sheath which encloses the path of the stylets, beginning with an external collar of sheath material on the leaf cuticle (ZWEIGELT, 1915). With *Myzus ascalonicus* KLOFT (1959) found saliva secretion 6–8 minutes after the penetration of the stylets through the cuticle. With the method used by KLOFT the presence of a salivary sheath could not be ascertained, but only the presence of salivary material secreted into the sieve elements. Moreover, KLOFT (1959) showed that this saliva dispersed directly in the fluid of the sieve elements. FORREST and NOORDINK (1971) demonstrated that when radioactive (^{32}P) apple aphids (*Dysaphis devecta*) fed below the cotyledons, saliva was not only transported throughout the plant, including the roots, but taken up by groups of *Dysaphis devecta* and *M. persicae* feeding at the shoot tip. From this it can be concluded that the saliva injected in the sieve elements must be a watery secretion in contrast to the saliva which hardens rapidly and thus forms the stylet sheath. Many authors (page 3) have found that *M. persicae* needs at least 15 minutes to reach the phloem which agrees with the minimum feeding period necessary for PLRV transmission. It is tempting to suppose that the watery saliva which is injected in the sieve tubes ori-

107

ginates from the principal salivary glands (page 81). In these glands the fore-mentioned myoepithelioid cell regulates the transport of PLRV from the hae-molymph via the lumen of both internal salivary ducts into the sieve tubes.

It cannot be excluded that a fraction of PLRV present in the haemolymph is excreted via the accessory salivary glands of *M. persicae*, since SYLVESTER and RICHARDSON (1970) have observed electron microscopically sowthistle yellow vein virus in the accessory salivary glands of *Hyperomyzus lactucae*, in addition to its presence in the principal salivary glands. Although the excretion products of the accessory glands form the salivary sheath, the question remains whether the virus in the stylet sheath is responsible for the induction of virus disease in the plant.

When watery saliva is injected into the sieve elements some of it must be sucked back into the alimentary canal of the aphid during the actual feeding process. As a consequence it cannot be excluded that also some of the injected PLRV is ingested again. This will be excreted, at least partially, with the honey-dew. It should be noticed that MOERICKE and MITTLER (1965) found that *M. persicae* and *Acyrthosiphon pisum* could ingest their own saliva when they were fed via a parafilm membrane.

It is interesting to mention that WEDDE (1885) already presumed that the saliva of aphids stimulates the sap stream in plants, whereas GROVE (1909) sup-posed that the salivary secretion, besides preventing the coagulation of the sap, may contain some digestive ferment to assist in digestion. According to ZWEI-GELT (1915) the saliva serves for extra intestinal digestion.

ELZE (1927) showed that PLRV could neither pass via the eggs of viruliferous oviparous parents to the offspring, nor to the embryos in viviparous *M. persicae* (SMITH, 1929, 1931; DAVIES, 1932; MACCARTHY, 1954; DAY, 1955). On the other hand, MIYAMOTO and MIYAMOTO (1966, 1971) found that only during the winter season a small proportion of the offspring from viruliferous apterous mothers in the second and even in the fifth generation could transmit PLRV. This implies virus penetration through the ovarian wall. In view of the fact that second and fifth generation aphids still carry quantities of virus large enough to ensure a successfull virus infection it must be assumed that virus multiplica-tion has occurred in the meantime. The negative results in this study suggest that no virus was present in the ovaries and embryos (Table 11). No evidence for the presence of spherical particles in ultra-thin sections of follicle epithe-lium from viruliferous adults has been reported by MOERICKE (1963). As shown in Table 10 virus could be recovered from female reproductive organs of several viruliferous insect vectors, although these viruses were in general not transmitted to the offspring. Exceptions are the viruses causing Russian spring-summer encephalitis (PAVLOVSKY and SOLOVIEV, 1940), wound tumor (BLACK, 1953; SINHA and SHELLEY, 1965), rice dwarf (FUKUSHI et al., 1962), and potato yellow dwarf (SINHA, 1965a) which were demonstrated in the female reproductive organs and which did pass transovarially to the offspring.

NASU (1965) demonstrated by electron microscopy that symbionts in *Nepho-tettix cincticeps* UHL., along with mycetocytes, play a role in the transovarial

transmission of rice dwarf virus. The transmission of PLRV to the viviparous offspring of *M. persicae*, certainly does not occur via the symbionts during the larval stage, because PLRV cannot be found in the symbionts (Table 11) and moreover, because the symbionts are not liberated into the haemolymph before the end of the larval stage. On the contrary, it is conceivable that the virus is transmitted via the symbionts from the first to the second embryo generation.

From all dissected organs (Table 11), only the fat cells and presumably the basophilic mesodermal cells have been shown to contain a demonstrable amount of PLRV. This indicates that these cells can be considered as sites of PLRV multiplication since this virus multiplies in its vector, *M. persicae* (STEGWEE and PONSEN, 1958). Moreover, the infectivity of the supernatant suggests the presence of infectious material in the haemolymph. In the procedure of decapitation the gut remained completely intact which prevents contamination of the obtained suspension with the infectious contents of the gut. The presence of symbionts in the pellet, which were liberated from the mycetocytes due to centrifugation, are probably not responsible for the infectivity of the pellet because the lack of activity in this respect of mycetocyte homogenates. During the lifetime of an aphid all fat cells gradually degenerate, in the process of which the cell membranes decompose and the contents are released into the haemolymph in the form of waxy droplets, rhomb-shaped crystals, nuclear material, and unrecognizable cytoplasmic substances (page 50). Consequently the virus, which multiplies in fat cells, is liberated into the haemolymph. It is then transported via both myoepithelioid cells and subsequently injected, together with the saliva of the principal salivary gland, into the sieve elements of the plant. On the other hand, in the basophilic mesodermal cells the cell membranes start to degenerate only in the adult stage (page 54).

The infectivity of homogenates of viruliferous larvae (Table 11) and aphids (HARRISON, 1958; STEGWEE and PONSEN, 1958; PETERS, 1965, 1967a,b; MURA-YAMA and KOJIMA, 1965) may be attributed to the presence of infectious material in the contents of the gut, haemolymph, fat cells, and presumably basophilic mesodermal cells. The haemolymph and fat cells (intact and degenerating ones) are responsible for the infectivity of the cornicle secretion.

6. ELECTRON MICROSCOPY OF FAT AND BASOPHILIC MESODERMAL CELLS OF NONVIRULIFEROUS AND VIRULIFEROUS LARVAE

In the preceding chapter it was concluded that the multiplication of PLRV presumably takes place in fat and basophilic mesodermal cells. An electron microscopical study of both celltypes was undertaken to verify this finding.

MATERIALS AND METHODS

New-born larvae of *M. persicae* were selected from a virus-free colony on Chinese cabbage and transferred either to Chinese cabbage seedlings in the one-leaf-stage or to leafroll diseased *P. floridana* plants about 5 weeks old. The technique of cultivating *P. floridana* and Chinese cabbage plants is described in chapter 3. After an acquisition period of 24 hours on the leafroll source these aphids were allowed to feed on one-leaf-stage Chinese cabbage plants. Each seedling received 5–10 larvae. After 5 days the larvae were removed from the plants to prepare them for electron microscopical investigation of fat and basophilic mesodermal cells.

To collect these cells the larvae were fixed on self-adhesive tape stuck to a glass-plate. Under a dissecting microscope each larva was quenched with a drop of warm 2% Difco agar in 0.1 M SöRENSEN phosphate buffer (pH 6.8) after which the cornicles were immediately removed from the aphid's abdomen. As a result of this procedure fat cells, basophilic mesodermal cells, and mycetocytes were liberated in the warm buffered agar. After cooling the agar was cut into small blocks and treated for electron microscopical sections in the same way as described for the salivary glands in chapter 4.

NONVIRULIFEROUS LARVAE

The mesodermal tissue of *M. persicae* is represented by a cell layer below the epidermis. The majority of this tissue is occupied by fat cells between which basophilic mesodermal and connective tissue cells are evenly distributed. The latter cells were observed to keep the mesodermal tissue on its place with their processes forming a network of delicate membranes (Figs. 20, 25, and 32 V). A phase microscopical study of the developmental of these three celltypes is described in chapter 4 of which the results are presented in Figs. 21 and 22.

Fat cells

In electron micrographs the cytoplasm of a fat cell is composed of cisternal rough-surfaced endoplasmic reticulum, ribosomes, mitochondria, and GOLGI bodies. The nucleus is enveloped by a double-membrane possessing occasional

nuclear pores. The nucleolus contains translucent vacuoles with a· distinct electron-dense membrane (Fig. 34 A). The cell is ensheathed by a delicate plasma membrane which shows minute extrusions and sometimes invaginations. During growth of the cell many lipid droplets are set off by the surrounding cytoplasm and become progressively larger to coalesce subsequently into big ones. They are surrounded by a membranelike structure. Due to the preparation of the ultrathin sections the lipid vacuoles appear empty, but in some of them, especially at their periphery, traces of filamentous material occur. Coalescence of the various-sized droplets starts with the resolution of their membranelike structures on those sides where they contact each other. Simultaneously with the development and coalescence of the lipid droplets, glycogen-like particles, protein granules, waxy droplets, and rhomb-shaped crystals arise in the cytoplasm (Fig. 35, A-E). The waxy droplets are visible as small spherical drops consisting of amorphous material not enclosed by a membrane (Fig. 36 C). They increase gradually in size and some of them stretch out between the lipid vacuoles. During growth they retain their original texture and many of them fuse into big ones forming an irregular outline (Fig. 35 D). In phase microscopy it was found that in intact fat cells these droplets diffuse through the plasma membrane into the haemolymph, but in electron microscopy this process could not be traced. The crystals arise from minute needles arranged in a rhomb-shaped pattern (Figs. 35, B-C, and 36 H).

The degeneration process of the fat cells starts with the appearance of vacuoles in the karyoplasm after which the chromatin begins to condense (Fig. 22, H-I). The nuclear envelope loses its double-membrane structure and appears as a granulated, irregular outline with minute vesicles (Fig. 35 H). When the condensation of the nuclear material advances the nucleus fragments, the remains of which can be observed scattered within the cell and in the haemolymph as well. The nucleolus vacuolates and usually open spaces occur between areas of dense material (Fig. 34 A). This material dissolves peripherally so that the nucleolus decreases in size after which it disappears completely. When the degeneration process of the fat cells proceeds the plasma membrane gradually decomposes by vesiculation and fragmentation (Fig. 36 E). The various-sized membrane limited vesicles disengage and appear in diverse forms in the haemolymph. The disintegrating cells now contain numerous translucent cavities which originated from the lipid vacuoles. The cavities become larger as a result of the disintegration of their membranelike structures. Moreover, some parts of the cells show a multitude of irregular-shaped vacuoles and vesicles (Fig. 35 F and 37 B). The former are derived from degenerating endoplasmic reticulum, while the latter presumably arise from disintegrating GOLGI bodies. Similar structures were described by WRISCHER (1965) in plant and animal cells during necrobiosis induced by means of various physical and chemical agents. The cytoplasmic material from the degenerating fat cells is gradually released into the haemolymph.
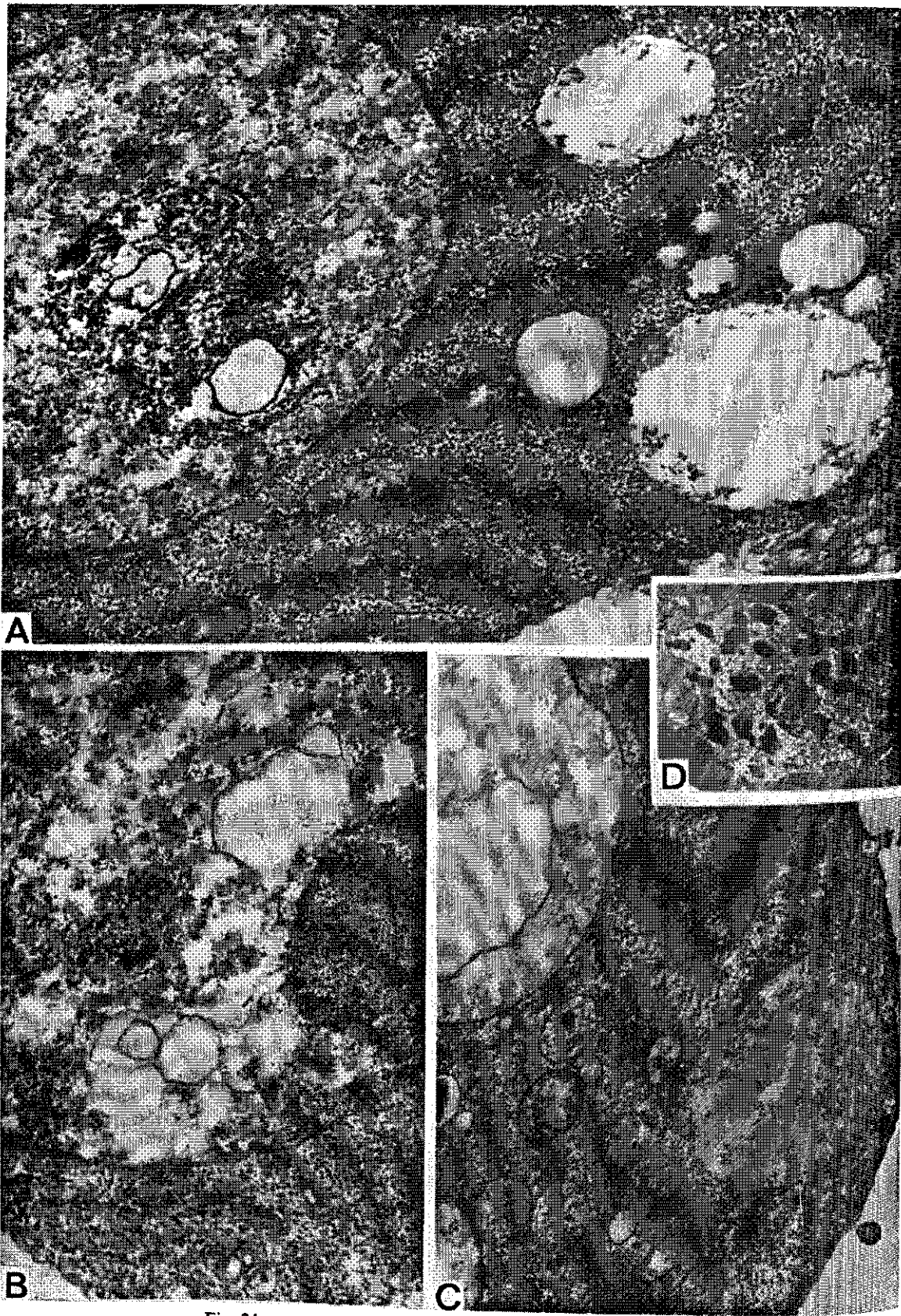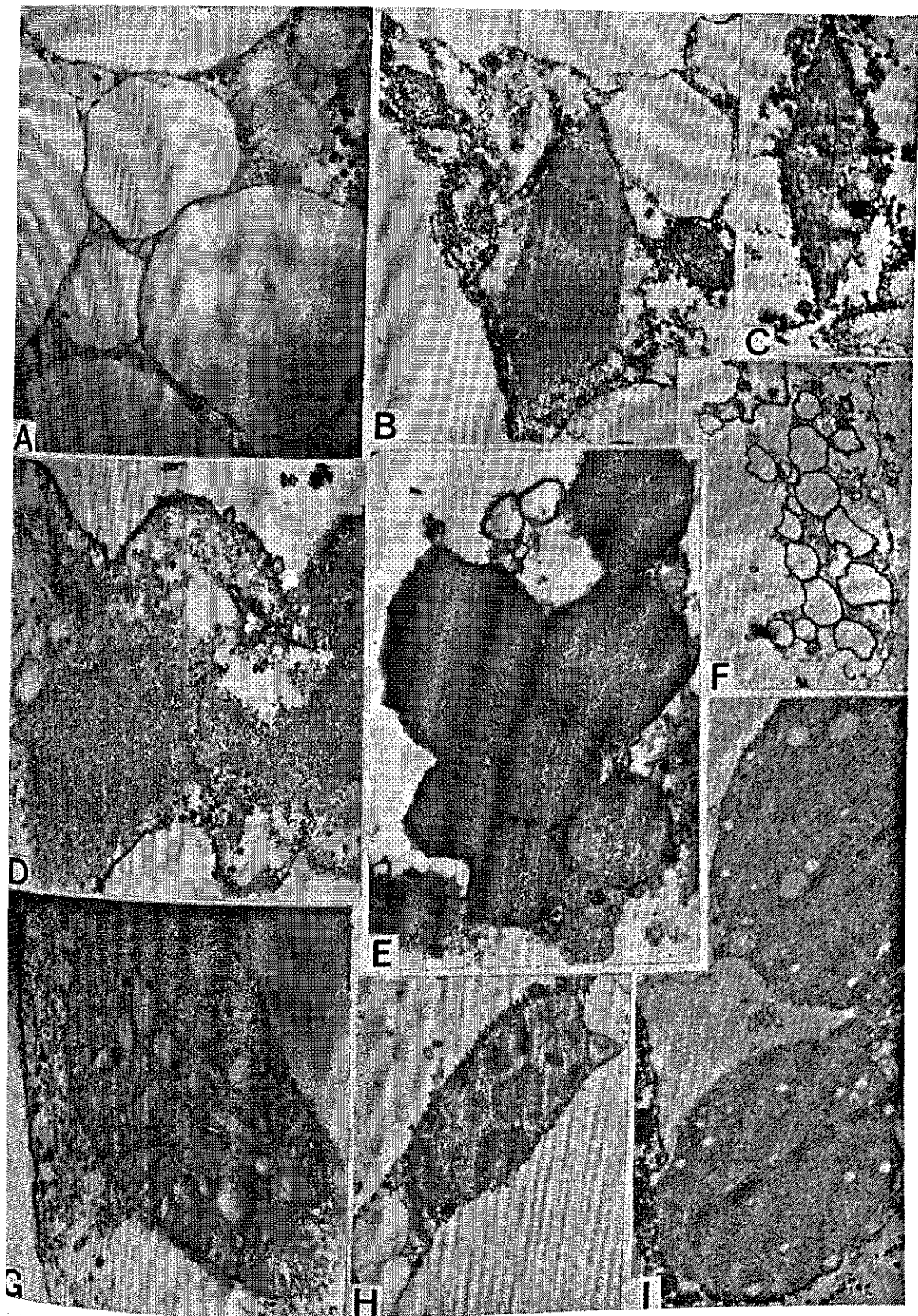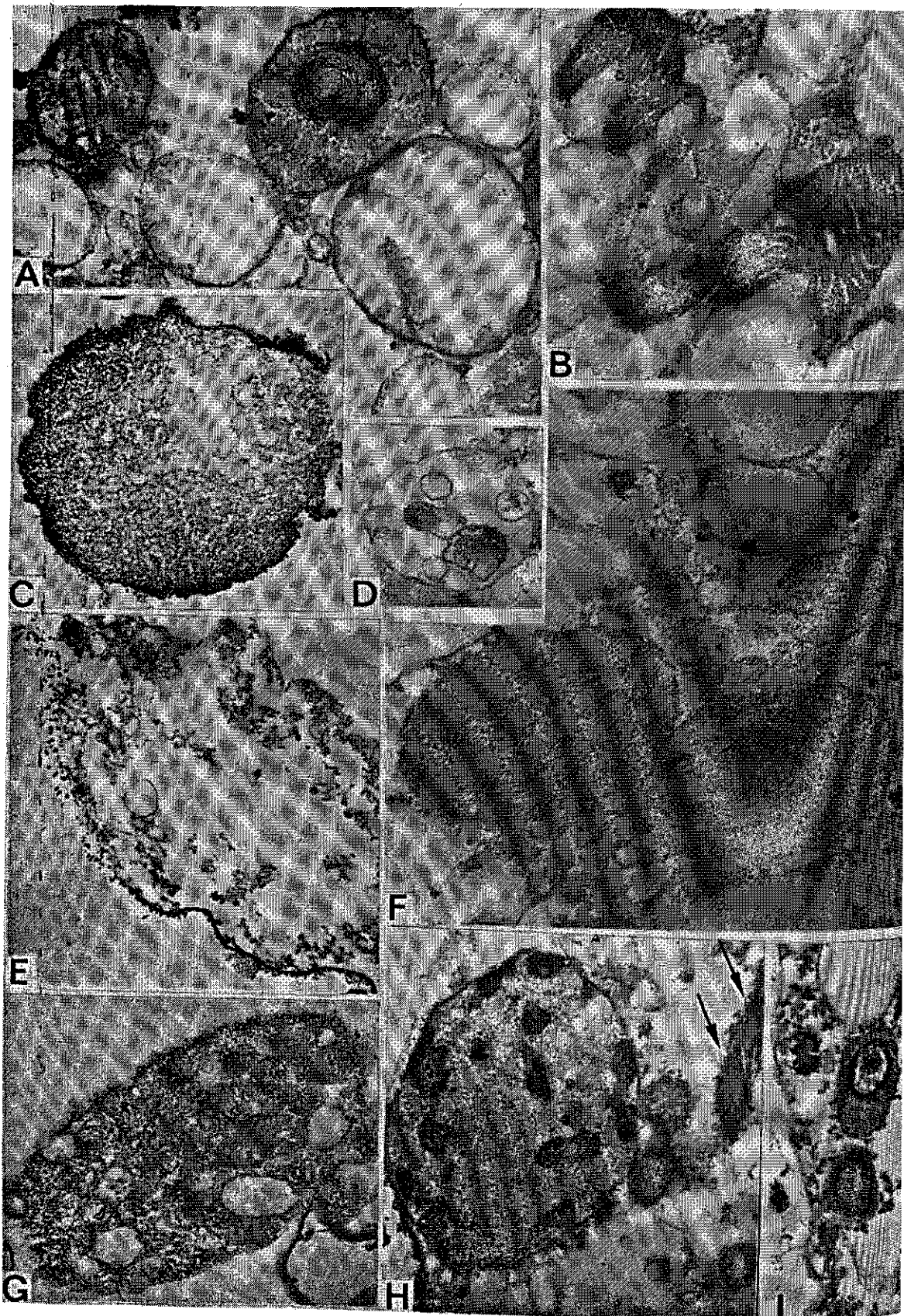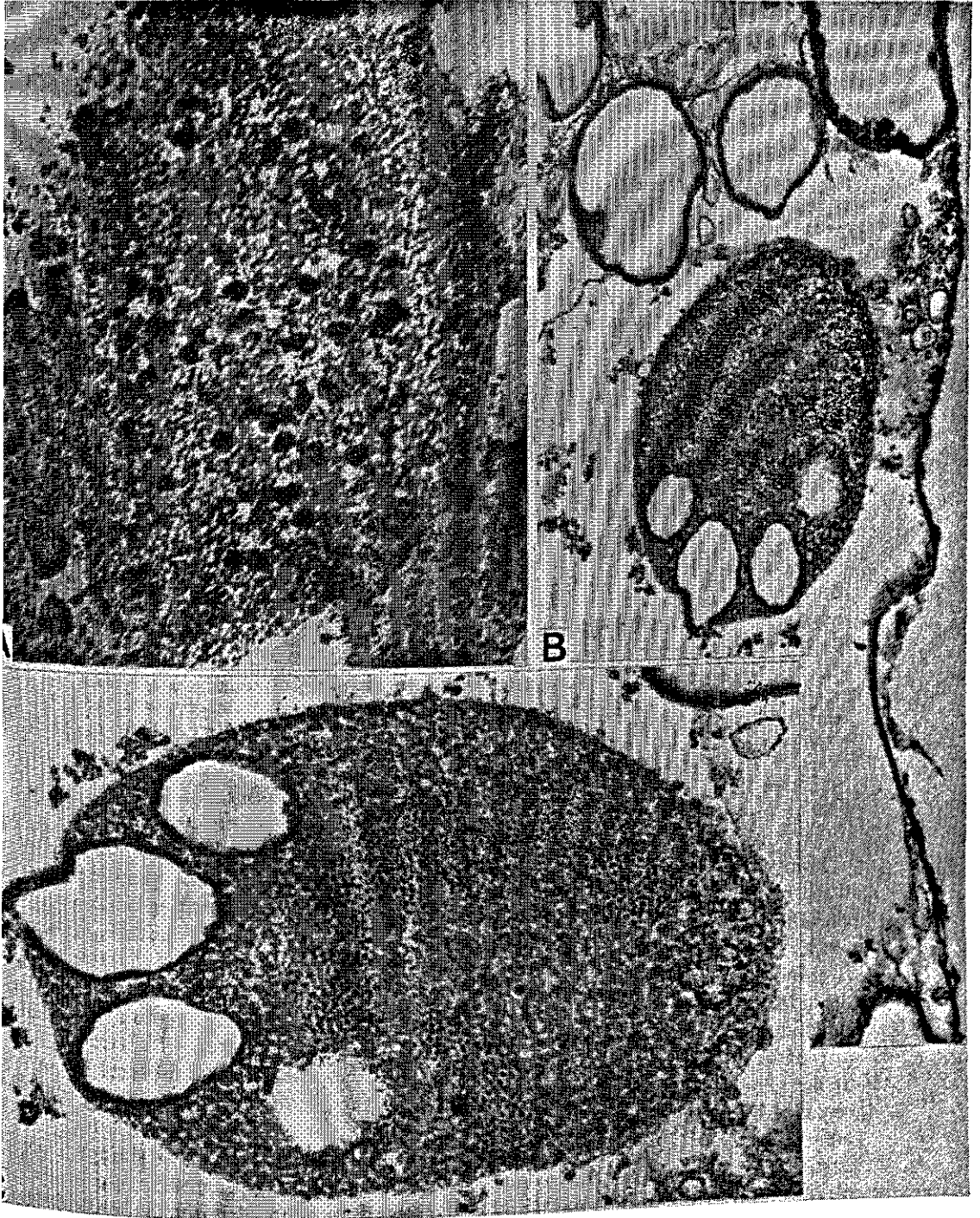
Fig. 34

Fig. 35

Fig. 36

Fig. 37

FIG. 34. A. Electron micrograph of a young fat cell of *Myzus persicae* showing endoplasmic reticulum, ribosomes, mitochondria, and lipid droplets. The nucleolus has membrane limited vacuoles. Magnification: × 25,500. B. Basophilic mesodermal cell of which the nucleus contains vacuolarlike structures. Magnification: × 36,000. C. Basophilic mesodermal cell showing endoplasmic reticulum, mitochondria, and vacuoles. Magnification: × 16,500. D. An invaginated nucleus with condensed chromatin of a degenerating basophilic mesodermal cell. Magnification: × 7,500.

FIG. 35. Electron micrographs of several organelles and inclusions of a degenerating fat cell of *Myzus persicae*. A. Glycogenlike particles situated among lipid droplets. B. Rhomb-shaped crystal bounded by degenerating cytoplasmic material and mitochondria in which many various-sized particles are formed. C. Rhomb-shaped crystal. D. Three waxy droplets which are fused forming an irregular outline. Magnification: × 20,000. E. A conglomerate of eight waxy droplets lying in the haemolymph. Magnification: × 16,000. F. Vacuolized cytoplasmic material at the periphery of a degenerating fat cell. Magnification: × 10,000. G. Giant mitochondrion with partly dissolved cristae. Magnification: × 30,000. H. Fragment of a disintegrating nucleus containing condensed chromatin. Magnification : × 10,000. I. Multivesicular bodies lying in the haemolymph. Magnification: × 8,000.

FIG. 36. Electron micrographs of several organelles and inclusions of a degenerating fat cell of *Myzus persicae*. A. Mitochondria with completely dissolved cristae and a protein granule with in its centre a myelin figure lying in the haemolymph. B. Partly dissolved mitochondria with swollen cristae. C. Waxy droplet lying in the haemolymph. Magnification: × 40,000. D. Dissolving mitochondrion containing concentric granular structures. E. Plasma membrane which decomposes by vesiculation and fragmentation. Magnification: × 20,000. F. Protein granules with myelin figures are bounded by vacuolated cytoplasmic material. Magnification: × 45,000. G. Cytolysome with disintegrating mitochondria and other cytoplasmic material. H. Cytolysome in a final stage of cytolysomic activity and two rhomb-shaped crystals (see arrow). Magnification: × 10,000. I. Protein granule with in its centre a thick concentric figure. Magnification: × 38,750.

FIG. 37. Electron micrographs of inclusions situated at the periphery of degenerating fat cells of celltype J-L (Fig. 22) of viruliferous *Myzus persicae* larvae. A. Inclusion containing dense (PLRV?) particles. Magnification: × 140,000. B. A presumably immature inclusion in which numerous dense (PLRV?) particles seem to develop. Magnification: × 38,750. C. The same inclusion at a magnification of 77,500 x.

Many bodies of varied structure are found in degenerating fat cells and in the haemolymph. They clearly show the different stages of degenerating mitochondria containing nearly normal cristae, or completely dissolved ones (Fig. 36 A). These empty membrane limited bodies transform into various shapes from polygonal to erratic after which the inner and outer membranes start to decompose. Some of these empty bodies are provided with concentric granular structures (Fig. 36 D), which are also found in the fat body of starving *Blaberus discoidalis* SERV. (cockroach; WALKER, 1965). In other ones, located especially at the periphery of the degenerating cell, the cristae become swollen and are replaced by irregular loosely packed whorls of membrane material. In a more advanced stage of degeneration numerous various-sized, darkly staining particles develop in the matrix which are released into the degenerating cytoplasm or into the haemolymph (Fig. 35 B). The formation of mitochondrial granules from lysis of mitochondria has been described in the fat body of meta-

116

morphosing *Calpodes ethlius* CRAMER (Lepidoptera; LOCKE and COLLINS, 1965), *Philosamia cynthia ricini* BOISD. (Lepidoptera; WALKER, 1966), and in starving *Rhodnius prolixus* STÅL (Hemiptera; WIGGLESWORTH, 1967). Another type of mitochondrial degeneration is the swelling and dissolution of the cristae which is accompanied by a partial disintegration of the inner and outer membrane and the matrix as well (Fig. 36 B).

The protein granules have a granular texture and are enclosed by a single membrane. They become larger and myelin figures arise in many of them (Fig. 36 F). In some cases the protein material dissolves leaving empty membrane limited bodies with a myelin figure inside (Fig. 36 A). In other ones the membrane dissolves after which the granular material is liberated into the haemolymph. In this stage many separated myelin figures can be observed inside or outside the degenerating cell in a more or less dissolved state. Furthermore a wide variation in size of granules without myelin figures are surrounded by a vesiculated membranous structure. They contain a variable number of micro vesicles embedded in a texture similar to the protein granules (Fig. 35 I). These granules have the characteristic appearance of the multivesicular bodies described by LOCKE and COLLINS (1968) in the fat cells of *C. ethlius*. They assume that protein granules become multivesicular bodies by accumulation of microvesicles which are involved in the transport of material from the cytoplasm into the granule. Due to the decomposition of the plasma membrane the multivesicular bodies are released into the haemolymph. The latter lose their contents, which results in the appearance of empty bodies in which, however, microvesicles still occur. Occasionally membrane limited bodies are found in degenerating fat cells showing a fibrous or bristle texture. According to LOCKE and COLLINS (1968) these fibrous bodies presumably represent an intermediate form in the degeneration process of the multivesicular bodies (Fig. 36 I).

The eosinophilic bodies which are observed in degenerating fat cells (Fig. 22, celltype H-L) appear to be cytolysomes (Fig. 36 G). It is suggested from electron microscopical pictures that the cytolysomes originate from condensed cytoplasmic material after which it becomes enveloped by a membrane. They become progressively larger by encapsulating mitochondria and other cytoplasmic organelles such as nuclear fragments, myelin figures, vesicles, and membranes. In an advanced state of digestion the mitochondria show several stages of breakdown after which they transform into structureless darkly staining structures embedded in an amorphous mass (Fig. 36 H). These cytolysomes represent the final stage of cytolysomic activity followed by a solution in the haemolymph leaving giant vacuoles.

Basophilic mesodermal cells

The ultrastructural organization of a basophilic mesodermal cell is quite similar to that of a young fat cell (Fig. 34 B, C). They become larger and many membrane limited vacuoles arise in the cytoplasm. Vacuolarlike structures develop in the nucleus and in an advanced stage the chromatin starts to condense showing irregular, darkly staining structures. During the intranuclear condensation of chromatin the nuclear membrane becomes lobed with small

117

invaginations (Fig. 34 D). In smear preparations of adults it was found that the nuclear membrane dissolves after which the clotting chromatin liberates into the vacuolized cytoplasm after which the basophilic mesodermal cells completely disintegrate. Presumably the infolded nucleus is the initial stage of the disintegration process of the nuclear envelop followed by the release of condensed chromatin material into the cytoplasm. The nucleolus at the same time becomes vacuolated and decreases in volume and then disappears completely. The first degenerating basophilic mesodermal cells are only observed in a nine days old larva in the vicinity of the cornicles (Fig. 21 I).

VIRULIFEROUS LARVAE

The growth and degeneration of fat and basophilic mesodermal cells in viruliferous larvae show the same picture as that described for nonviruliferous ones (Figs. 21 and 22). In electron micrographs there appears to be no difference between nonviruliferous and viruliferous larvae as far as the ultrastructural aspect of both these celltypes is concerned. In one instance a fat cell was found in a leafroll virus carrying larva containing an inclusion with dense particles having a hexagonal outline and a diameter of about 23 nm (Fig. 37 A). Presumably they represent PLRV. They are loosely embedded in an amorphous material enveloped by a partly dissolved membrane. Another inclusion body was found to contain some large vacuoles as well as numerous small dense particles surrounded by vesiculated material, which might be an intermediate stage in the development of PLRV. Both these inclusions are found in the degenerating fat cells of celltype J-L (Fig. 22), especially at its periphery (Fig. 37 B, C).

DISCUSSION

An investigation of the ultrastructural organization of young fat cells of *M. persicae* reveals that the organization of these cells is similar as compared with those described for various other insect orders (BISHOP, 1958; VON GAUDECKER, 1963; ISHIZAKI, 1965; WALKER, 1965, 1966; ODHIAMBO, 1967; WIGGLESWORTH, 1967; LOCKE and COLLINS, 1965, 1968; DE LOOF and LAGASSE, 1970). This was also the case with the fat cells of the pea aphid, *Acyrthosiphon pisum* (LINDSAY, 1969). Moreover, LINDSAY distinguished a second type of cells to which he referred as cornicle cells because of their presence in the cornicles and vicinity. In *M. persicae*, however, these cells which were described in detail in chapter 4 appeared to be degenerating fat cells (Figs. 22). Of the five types of vacuoles which according to LINDSAY are present in his 'cornicle cells', the first type represents, in our opinion, the lipid vacuolar structures, while the other types presumably are degenerating cell organelles and inclusions.

In one instance a degenerating fat cell of a leafroll virus carrying larva an inclusion was observed containing dense particles of about 23 nm in diameter

118

and with a hexagonal profile (Fig. 37 A). These particles might represent PLRV, resulting from the multiplication process in the cytoplasm of fat cells, as they are similar to those of purified extracts of leafroll virus carrying aphids (*M. persicae*; PETERS, 1967a, b) and extracts of leafroll diseased *P. floridana* and *D. stramonium* plants (KOJIMA et al., 1968, 1969). The particles were identical in size and shape as compared with those found in ultrathin sections of the phloem elements of both plant species (KOJIMA et al., 1968, 1969); they were neither found in leafroll virus-free aphids nor in healthy plants. PETERS (1967a, b) demonstrated their infectious nature by injecting purified aphid extracts in nonviruliferous aphids. However, it is feasible that the particles seen in Fig. 37 A are virus-like particles, described by PETERS (1965, 1967b), who found such particles in purified extracts from both leafroll virus carrying and leafroll virus-free aphids. These particles had a diameter equal to that of PLRV particles. In the present electron microscopical study it was impossible to distinguish the virus-like particles and those of PLRV among the many various-sized particles originating from degenerating fat cells.

VAGO (1958) reported the presence of hypertrophied fat cells which coincided with deformations of the nuclei in viruliferous *M. persicae*. These phenomena cannot be attributed to PLRV multiplication because in the present study they were also observed in nonviruliferous larvae and represent a phase in the degeneration process of the fat cells as depicted in Fig. 22. Moreover, VAGO (1958) found that in electron microscopical sections 'plages' occurred in fat cells of viruliferous aphids which could not be demonstrated in those of nonviruliferous ones. Although VAGO did not mention anything about the structure of these plages, it is possible that these plages represent intermediate forms (Fig. 37 C) or inclusions possibly containing PLRV particles (Fig. 37 A).

On the other hand, SCHMIDT (1959) observed more stellate nuclei in the fat-body of viruliferous *M. persicae* adults than in that of nonviruliferous ones. In the present study it appeared that stellate nuclei occur mainly in celltypes E-G (Fig. 22) during development of both nonviruliferous aphids and viruliferous ones, while in celltypes H-L (Fig. 22) occasionally a stellate nucleus was observed. Since the degeneration process of the fat cells continues in the imaginal stage (page 52), and SCHMIDT (1959) did not mention anything about the age of his investigated adults, it is possible that the nonviruliferous adults were older than the viruliferous ones. This supposition is supported by the photographs given by SCHMIDT (1959, 1960). However, RUTSCHKY and CAMPBELL (1964) found no difference in the total number of stellate nuclei in the fatbody of nonviruliferous *Macrosiphum granarium* and those of the same age infected with barley yellow dwarf virus.

Similar cytoplasmic inclusions containing pea enation mosaic virus particles have been described in the fat cells of the aphid vector, *Acyrthosiphon pisum* (SHIKATA et al., 1966). In the cytoplasm of the various organs including haemocytes and fat cells of the leafhopper, *Agallia constricta* van DUZ., infected with wound tumor virus, virus particles were found embedded in inclusions, which were described by SHIKATA and MARAMOROSCH (1967a, b) as viroplasms.

119

in which virus assembly takes place. Viroplasms have been demonstrated for wound tumor virus (SHIKATA et al., 1964; SHIKATA and MARAMOROSCH, 1965; HIRUMI et al., 1967; GRANADOS et al., 1967, 1968), rice dwarf virus (FUKUSHI et al., 1960, 1962; FUKUSHI and SHIKATA, 1963; NASU, 1965; SHIKATA, 1966), and maize rough dwarf virus (VIDANO, 1970) in several organs of their leafhopper vectors (Table 10). Presumably the inclusion depicted in Fig. 37 C, is an immature viroplasm in which PLRV particles are developing .

In chapter 5 it was already shown that in the preparations obtained with the adapted techniques the basophilic mesodermal cells form a minority in contrast to the fat cells. This is not surprising because *M. persicae* larvae possess about 94 basophilic mesodermal cells which are evenly dispersed among the numerous fat cells. Similar results were obtained with the ultrathin sections. In the few basophilic mesodermal cells which were found, no accumulations of virus particles or inclusions were observed. From this it was assumed that the fat cells are the only sites of virus multiplication. This conclusion is supported by the fact that in ultrathin sections VAGO (1958) could not demonstrate any difference between the 'haemocytes' of viruliferous and nonviruliferous *M. persicae*. However, since in *M. persicae* haemocytes do not occur, the cells mentioned by VAGO, in our opinion, represent the basophilic mesodermal and/or connective tissue cells.

During the degeneration process of the fat cells in *M. persicae* the inclusion bodies likewise disintegrate and with decomposition of the plasma membrane the virus is released into the haemolymph. Now the PLRV particles are dispersed followed by a transportation via the myoepithelioid cells. It was found that after a six-hours acquisition feeding period on a leafroll source the efficiency of PLRV transmission by *M. persicae* increased till about the ninth day after birth, then decreased gradually in the imaginal stage during the time the aphids remained on Chinese cabbage (PONSEN, 1970). This finding corresponds with the fact that as a result of the degeneration process of the fat cells in the larval stage and its continuation during the imaginal stage, the number of fat cells, susceptible to virus multiplication, decreases. The finding that the propagative PLRV is not pathogenic to its vector (PONSEN, 1969) may be due to the fact that the fat cells degenerate in any case. For the animal and plant viruses which multiply in the various organs of their insect vectors (Table 10) nothing is reported about possible degeneration symptoms of the cells concerned. On the other hand, MIMS et al. (1966) found a disintegration of the salivary glands of *Aëdes aegypti* three weeks after infection with Semliki Forest virus. However, this pathogen did not shorten the life span of the mosquito. Eastern equine encephalitus virus which multiplies in the salivary glands of *Aëdes triseriatus* SAY (Table 10), has no cytopathic effect in virus-infected salivary gland cells during the experimental period of 31 days (WHITFIELD et al., 1971).

SUMMARY

In search of the site of PLRV multiplication in its vector a detailed study was made of the anatomy of the aphid, *Myzus persicae* SULZ. The findings are summarized in the following lines:

Alimentary canal

The most anterior part of the alimentary canal is the food canal which is firmly interlocked by the maxillary stylets. From the stylets it passes into the pharyngeal duct which subsequently leads into the pharyngeal valve, pharyngeal pump, foregut, oesophageal valve, stomach, intestine, hindgut, and rectum to terminate into the anal opening (Figs. 3, 5, and 11).

Each stylet originates from a retort-shaped organ (Fig. 4) and is attached by muscles to the tentorial bar, the maxillary sclerite (Fig. 31), and the pharynx floor (Fig. 8 no. 12–13). The further pathway of the stylets is described and figured (Figs. 7 no. 1, 2 and 3 no. 1–4). The stylet bundle shows in its way through the longitudinal labial groove a torsion of 180°.

The pharyngeal duct is formed by the epipharynx and the hypopharynx lip (Fig. 7 no. 2–8). The epipharynx is marked by a thick sclerotized plate and reveals a median row of eight sensillum pores (Fig. 5). On each side of the epipharynx is an invagination (Fig. 7 no. 7–8), which runs as an arch from the third pore to the valve (Fig. 3). The cup-shaped part of the floor before the valve is provided with two sensillum pores (Figs. 5 and 8 no. 9–10).

The pharyngeal duct is separated from the pharyngeal pump by a valve, of which both the dorsal wall and ventral wall are marked by two cuticular dome-shaped prominences, the pharynx protuberances. The dorsal wall of the valve is controlled by two pairs of muscles, and each lateral side by only one muscle. In embryos the valve is open (Fig. 8 no. 10–12), but in larvae it is always found in a closed position (Fig. 8 no. 10'–11'). In the latter situation the opposite pharynx protuberances and the valve itself fit closely together. It is logical to suppose that the opened valve in the larvae has the same position as can be observed in embryos.

The pharyngeal pump is controlled by 29 pairs of dorsal elevator muscles (Figs. 5, 6, and 8 no. 13–15). Close to the tentorial bar the floor of the pump is attached by muscles, one pair originating from the tentorium, and one pair from the piston of the salivary pump.

The foregut consists of a single layer of squamous epithelial cells which are lined with an intima forming a stellate narrow lumen (Fig. 32).

The oesophageal valve marks the junction of the foregut and the stomach (Fig. 9). The inner layer is the continuation of the foregut into the stomach. The outer layer consists of cuboidal epithelial cells. The intima of the foregut continues in the region of the valve and terminates at the base of it.

121

The anterior and posterior region of the stomach consists of cuboidal epithelial cells, while the middle region is occupied by tall, fingerlike columnar digestive cells (Figs. 9 and 10). The latter secrete material by constricting of apical cell parts. In some nuclei strongly basophilic clusters of chromatin material develop on the ninth day of the larva (Fig. 10 B'). The cells of the posterior region secrete material by forming of buds (Figs. 9 C and 10 C'). Both processes continue during larval life without any degeneration or multiplication of cells.

The first part of the intestine is a narrow tube which passes into a broader one to terminate abruptly by constriction in the hindgut. In the first part the squamous cells form a stellate narrow lumen, while in the second division the strongly vacuolated cells are situated around a wide lumen (Fig. 11). It is suggested that the intestine is resorbtive in function in contrast to the stomach which is the secretory part of the midgut.

The hindgut consists of long squamous epithelial cells containing many vesicles in which waxy droplets occur. These droplets, originating from degenerating fat cells, also occur in the lumen where they are released by minute projections situated at the apical surface of the cells (Fig. 26).

On the ventral side the hindgut passes into the rectum whereas it continues dorsally a distance to terminate in a dorsal rectal sac (Fig. 12). During larval life the rectal sac gradually disappears to form with the posterior part of the rectum an almost straight duct. The rectum is built up of columnar cells having small vesicles liberating secretion into the lumen.

During larval life the growth of the entire gut is due solely to enlargement of the epithelial cells. They retain their optical composition and persist unchanged into the adult stage. Cell divisions were never observed (Table 4).

Salivary gland

Each half of the system is composed of the principal and the accessory gland (Figs. 1 and 13). The common afferent duct forms a S-shaped flexure before entering the salivary pump (Figs. 5 and 19). At the place of entry the opening is controlled by two small muscles originating from the chitinous ridges. The exit opening from the pump chamber leads into the pumpstem which on its foot is provided with two sensillum pores. The pathway of the efferent salivary duct follows from Fig. 7 no. 5–8. As well the cylinder as the piston are supplied with muscles.

The accessory gland is composed of 3–4 cells of uniform size (Fig. 13 A). The basal part of these cells shows laminated structures and in the cytoplasm many branching canaliculi occur which cross the cuticular lining of the salivary duct (Fig. 14). It is suggested that the accessory glands excrete a watery suspension that during its discharge to the salivary pump is dehydrated by the duct cells to form the final viscous fluid. The viscous fluid, forming the salivary sheath, contains presumably waxy droplets. These droplets presumably render the stylet sheath its waterproofing.

The principal gland is bilobed and each lobe contains six Deckzellen and

fifteen Hauptzellen which are situated around the salivary duct. Considering the optical composition of the cytoplasm, the shape of the cell (Fig. 14), the size of the nucleus and nucleolus (Table 5), the six Deckzellen can be distinguished in two, and the fifteen Hauptzellen in six different types. During larval life the topographical position of all the gland celltypes is constant in each lobe (Table 6). Each cell has a canaliculum which traverses the cuticular lining and the lumen of the salivary duct. The eight different celltypes (Fig. 13 A) contain a mass of secretory granules which are released by the cell into the canaliculi. It is supposed that the watery saliva originates from the principal gland.

The distal part of the two lobes of each principal gland is connected by a myoepithelioid cell (Fig. 13 A and D). The bulk of the cytoplasm is occupied by myofibrils oriented in an interwoven pattern (Figs. 15, 16 A, and 17 A). At the periphery of this contractile mass some mitochondria and many vacuoles occur. The surface of this cell, bounded by the four intercellular canaliculi of celltype 7 and the termination of both ducts, is strongly infolded. Injections with Evans blue via the cornicles in the larvae gave the evidence that haemolymph is pumped into the lumen of both ducts by pulsations of the myoepithelioid cell. Via the duct lumina it becomes transported through the salivary pump into the plant.

In each principal gland the myoepithelioid cell is innervated by a paired nerve of the mediodorsal nervous system (Figs. 13 A, 17 B, and 18).

The salivary duct is lined with endocuticle throughout its length. The apical surface of the duct epithelial cells bears regularly oriented microvilli (Fig. 16 B and C).

During larval life cell divisions are never observed in the salivary gland system. However, the nuclei and their nucleoli increase in size (Table 5) as well as the cells (Fig. 14).

Mesodermal derivates

The mesodermal tissue is represented by a cell layer beneath the epidermis. It is composed of numerous fat cells between which basophilic mesodermal cells and connective tissue cells are evenly distributed (Figs. 20, 25, and 32 V).

The first embryonic mesodermal cells are observed in the eldest embryo of a three days old larva in the area anterior of the future cornicles (Fig. 22, 1). During embryonic development the number of mesodermal cells continuously increases by cell divisions; the direction of cell divisions is from the site of origin both anteriad and posteriad (Fig. 22, 1–3). In the eldest embryo of a five days old larva the mesodermal cells lying in the area of origin start to develop into fat cells by the forming of lipid droplets in their cytoplasm (Fig. 22, 2). At the same time other embryonic mesodermal cells start to increase in size and become the final basophilic mesodermal cells (Fig. 22, bmc). During growth of the embryo all embryonic mesodermal cells develop into fat, basophilic mesodermal, and connective tissue cells. In the eldest embryo of a nine days old larva the mesodermal tissue has attained its final composition (Fig. 22, 4). After the embryonic mesodermal cells are differentiated into their derivates,

123

no cell divisions take place anymore in these products (Fig. 22, 4–7).

After transformation of the embryonic mesodermal cells into fat cells, they start to increase in size just like their nuclei and nucleoli. Lipid droplets increase both in number and in size (Fig. 22, 4 D). During coalescence of the lipid droplets (Fig. 22, 4 F) the first waxy droplets and rhomb-shaped crystals arise in the cytoplasm (Fig. 22, 4 G). The degeneration process of the fat cells is revealed by the appearance of vacuoles in the karyoplasm (Fig. 22, 4 H), after which the chromatin condenses and disintegrates into fragments (karyo-rhexis), granules (karyolysis), or becomes pyknotic. The nucleolus vacuolizes and dissolves peripherally and disappears completely. In the cytoplasm of the degenerating cells eosinophilic bodies arise. After breakup of the cell membrane the cell contents release into the haemolymph (Fig. 22, J-L). The degeneartion process described is already in full existence in the eldest embryo of a nine days old larva and proceeds during larval life.

The enlargement of the basophilic mesodermal cells and their nuclei and nucleoli continues during larval life (Fig. 21). The cytoplasm becomes gradually acidophilic and many minute vacuoles now arise. After the seventh day the chromatin starts to condense and the nuclear membrane becomes somewhat irregular to dissolve subsequently in the adult stage. The clotting chromatin is liberated into the vacuolized cytoplasm followed by a complete disintegration of the basophilic mesodermal cells.

Some embryonic mesodermal cells change into the final connective tissue cells. They are much smaller than the other mesodermal derivates, and retain their original volume in the larval stage (Figs. 20 and 32 V). Their delicate cell extensions form a complicated network which support the mesodermal tissue. Moreover, these membranous filaments extend throughout the haemocoel and are connected with the various internal organs.

The pericardial cells are derived from embryonic mesodermal cells. In the eldest embryo of a nine days old larva these cells have attained a structure and number, which persists during whole further life (Fig. 32). After larviposition these cells and their nuclei and nucleoli increase in size, but the total number of cells remains constant (Table 8). They are distributed along the entire dorsal vessel and connected with a branch of the medial dorsal nerve (Fig. 3). Further-more there occur 2–3 pericardial cells on both lateral sides of each thoracic segment, and 3–4 lateral pericardial cells in the metathoracic segment (Figs. 20 and 24).

The haemolymph is characterized by the absence of circulating haemocytes. On the other hand, the body cavity contains quite a number of waxy droplets during aphid's life. The droplets are released by the cell membrane of intact fat cells, or are liberated into the haemolymph after dissolving of the cell membrane. They are predominantly close to the visceral surface of the mesodermal tissue and especially in a high concentration around the hindgut and the accessory salivary glands. Many of them are distributed by the dorsal vessel.

Mycetome

The mycetome consists of two longitudinal bodies of syncytial tissues (Fig. 1) linked together dorsally of the hindgut (Fig. 26 P). The mycetocytes are completely filled with spherical microorganisms or symbionts which divide like bacteria. After the fifth day the larval mycetome starts to disintegrate in clusters of cells or in single mycetocytes. During larval life symbionts degenerate within the mycetocytes, and at the ninth day the cell membrane of some mycetocytes starts to dissolve after which intact and degenerating symbionts are liberated into the haemolymph (Fig. 26 P'). They are distributed throughout the body cavity and occur in the mesodermal tissue among the degenerating fat cells as well as in the legs.

Oenocytes

The oenocytes are situated laterad at either side in the body cavity between the mesodermal tissue and the internal organs of the metathorax and the first five abdominal segments (Figs. 1, 25, and 27). They form a longitudinal row of 7–12 single cells and are anchored by membranes which originate from the connective tissue cells. They are recognizable because of their irregular or branched nuclei with granulated chromatin and a proportionally very big nucleolus. The cytoplasm is strongly vacuolated and many vacuoles contain granules (Fig. 21).

Circulatory system

The dorsal vessel lies just beneath the dorsal mesodermal tissue (Figs. 3, 25, and 27). The ventral wall of the funnel-shaped mouth is stretched transversally over the corpus allatum to which it is connected along the ventrolateral lines (Fig. 32). The dorsal wall of the mouth extends further anteriad and is attached to the corpora cardiaca (Fig. 28). Throughout its length the vessel pulsates vigorously and the anterior wider part is provided with three pairs of lateral ostia. In all coxae a tubular accessory pulsatile organ occurs.

Cornicles

The cornicles are in open connection with the body cavity and are filled with degenerating fat cells (Fig. 27). Each cornicle is closed at its top with a valve-like flap, which is controlled by the valve retractor muscle. Only in an erected position due to contraction of the elevator muscle, the cornicles can produce excretory material. This material is composed of degenerating fat cells, disintegrated nuclear material, waxy droplets, rhomb-shaped crystals, and incidently a basophilic mesodermal cell, while connective tissue cells have been never observed (Table 7). At the ninth day of the aphid's life the first intact and degenerating symbionts appear in the cornicle secretion. The fat cells of celltype J (Fig. 22), of which the cell membrane is still intact, are responsible for the hardening of the secretion after it has passed the cornicles.

125

Nervous system

The nervous system is described and figured (Figs. 3, 5, 28–31, and 32 P). The paired pharyngeal ganglions are situated ventrally of the tritocerebral lobes and frontolaterally of the pharyngeal pump on both sides of their retractor muscles (Figs. 3 and 30). Before the pharyngeal valve the two bilobed pharyngeal ganglions fuse together and this mass extends ventrally as the epipharyngeal and the hypopharyngeal gustatory organ. The central part of the epipharyngeal gustatory organ has on its periphery many neurons whose dendrites are connected with the pores of the eight sensilla in the pharyngeal roof (Fig. 5). Centrally each lateral lobe is occupied by a stellate invagination of the pharyngeal duct (Figs. 6 and 7 no. 8). From the wall of this invagination nerve fibres arise running to neurons situated mainly on the periphery of the lateral lobes. The hypopharyngeal gustatory organ lies beneath the pharyngeal valve. The neurons in this ganglion communicate with four sensillum pores, of which two of them are situated in the cup-shaped part of the floor of the pharyngeal duct (Fig. 8 no. 10 and 10'), while the other two are located on the foot of the salivary pumpstem (Fig. 7 no. 8 and Fig. 19).

Endocrine glands. The paired corpora cardiaca are irregular- shaped bodies which are attached to the inner side of the dorsal aortic wall. Each gland is composed of 8–11 cells arranged around a neuropile mass from which nerves arise (Fig. 28).

The corpus allatum is a single body containing 11–13 cells (Fig. 32). It is situated between the foregut and dorsal vessel behind the corpora cardiaca (Fig. 3).

The paired thoracic glands consisting each of 8–11 cells, are situated in the vicinity of the tracheal trunks of the mesothoracic spiracle (Figs. 2 and 20). After the seventh day of larval life some cells start to degenerate, while on the ninth day all the cells are disintegrated into clusters of irregular spheres.

The second part of the study deals with experimental and electron microscopic evidence on transport, multiplication, and release of PLRV.

An attempt has been made to obtain information concerning the fate of the ingested virus in the aphid vector by recovering PLRV from dissected organs of viruliferous *M. persicae* larvae. The various organs were removed from the larvae and, after homogenization, tested for infectivity by injecting them into nonviruliferous aphids. As is shown in Table 11, the presence of PLRV could be demonstrated only in the contents of the alimentary canal, honeydew, haemolymph, fat cells presumably including basophilic mesodermal cells, and cornicle secretion. It may be concluded that a part of the ingested virus is transported from the gut's lumen into the haemolymph. After being circulated it multiplies in the fat cells. The remaining part of the ingested virus is egested with the honeydew. The negative results obtained with the salivary glands presented the evidence that the myoepithelioid cell, situated in the distal region of each principal salivary gland (Fig. 13 A), regulates the transport of PLRV

from the haemolymph via the lumen of both internal salivary ducts into the sieve tubes.

An electron microscopical investigation of the fat and basophilic mesodermal cells is given (Figs. 34–37). The cytoplasm of a fat cell is composed of cisternal rough-surfaced endoplasmic reticulum, ribosomes, mitochondria, and GOLGI bodies. During growth of the cell many lipid droplets are set off by the surrounding cytoplasm and become larger to coalesce subsequently into big ones. Simultaneously with the development and coalescence of the lipid droplets, glycogenlike particles, protein granules, multivesicular bodies, waxy droplets, and rhomb-shaped crystals arise in the cytoplasm. The degeneration process of the fat cells starts with the appearance of vacuoles in the karyoplasm after which the chromatin condenses and the nuclear envelope loses its double-membrane structure. In a more advanced stage of degeneration the cytoplasmic material and the cell membrane disintegrates. In the disintegrating area of the cytoplasm arise cytolysomes which become larger by encapsulating mitochondria and other cytoplasmic organelles. After decomposition of the cell membrane the disintegrating cytoplasm is released into the haemolymph in which it dissolves completely.

The ultrastructural organization of a basophilic mesodermal cell is quite similar to that of a young fat cell. They become larger and many membrane limited vacuoles are found in the cytoplasm. In the nucleus vacuolarlike structures develop, which is followed by condensation of the chromatin. During this process the nucleus becomes lobed with deep invaginations after which the nuclear envelope is dissolved leading to the release of the granulated chromatin material into the cytoplasm. The first degenerating basophilic mesodermal cells are observed only in a nine days old larva in the vicinity of the cornicles.

The growth and degeneration of fat and basophilic mesodermal cells in viruliferous larvae show the same picture as that described for nonviruliferous ones (Figs. 21 and 22). In one instance a fat cell was found in a leafroll virus carrying larva containing an inclusion with dense particles measuring 23 nm in diameter with a hexagonal profile, and which presumably represent PLRV (Fig. 37 A).

ACKNOWLEDGEMENTS

SAMENVATTING

Nadat QUANJER in 1916 had gepostuleerd dat het aardappelbladrol door een virus wordt veroorzaakt, kon OORTWIJN BOTJES (1920) de overdracht van dit pathogeen door de groene perzikluis, *Myzus persicae*, aantonen. ELZE (1927) stelde vast dat wanneer de luizen een zekere tijd hadden gezogen op een bladrolzieke plant, deze niet in staat waren het aardappelbladrol virus over te brengen gedurende een bepaalde periode. Na deze latente periode bleken de luizen gedurende een zeer lange tijd van hun leven infectieus te zijn. Dit feit schreef ELZE (1931) toe aan een mogelijke vermeerdering van dit virus in zijn vector. In 1958 konden STEGWEE en PONSEN deze veronderstelling bevestigen. De vraag deed zich nu voor in welk orgaan van de bladluis het virus zich vermeerdert. Hiertoe was het noodzakelijk om eerst de anatomie van de luis gedurende zijn larvale stadium te bestuderen. Hoewel verscheidene onderzoekers de anatomie van luizen hadden beschreven en de verschillende afwijkingen in virusbevattende luizen toeschreven aan virusvermeerdering, bleken hun resultaten in vele opzichten tegenstrijdig te zijn. Een overzicht van wat er uit de literatuur bekend is over de relaties van het bladrolvirus en zijn vector, is in het tweede hoofdstuk uitvoerig beschreven.

In het derde hoofdstuk wordt beschreven hoe de voor dit onderzoek gebruikte bladluizen en planten, *Physalis floridana* en Chinese kool, werden opgekweekt.

In het vierde hoofdstuk wordt een uitvoerige histologische beschrijving gegeven van het spijsverterings kanaal, de speekselklieren, het mesodermale weefsel, het mycetoom, de oenocyten, het circulatie apparaat, de sifonen en het zenuwstelsel (Figs. 1–32). Uit dit onderzoek kwamen gegevens naar voren welke van belang waren voor het onderzoek naar het transport en de eventuele vermeerdering van het virus in de luis wat in de twee volgende hoofdstukken wordt behandeld.

In hoofdstuk 5 is een poging ondernomen om informatie te verkrijgen omtrent het lot van het ingenomen virus in de luis. Hiertoe werden de verschillende organen van virusbevattende luizen uitgeprepareerd en in een buffer gehomogeniseerd om vervolgens de infectiositeit van deze preparaten te toetsen door injectie van kleine porties in virusvrije luizen met een fijn glazen naaldje. Het bleek (Tabel 11) dat de aanwezigheid van het virus alleen in de darminhoud, honingdauw, hemolymfe, vetcellen, en waarschijnlijk de basofiele mesodermale cellen, en sifonen-secreet kon worden aangetoond. Hieruit kon worden geconcludeerd, dat een deel van het ingenomen virus vanuit het darmlumen naar de hemolymfe wordt getransporteerd waar het circuleert om zich vervolgens in de vetcellen te vermeerderen. Het overige deel van het ingenomen virus wordt met de honingdauw afgescheiden. De negatieve resultaten die zijn verkregen met de speekselklieren, gaven het bewijs dat de myo-epitheelachtige cel, gelegen in het distale deel van de hoofd-speekselklier (Figs. 1 and 13 A), het

129

transport van het virus vanuit de hemolymfe via de beide speekselkanalen naar de zeefvaten van de plant regelt.

Hoofdstuk 6 geeft een beschrijving van de ultrastructuur van de vetcellen en basofiele mesodermale cellen van zowel virusvrije als virusbevattende luizen (Figs. 34–36). In een geval werd in het cytoplasma van een vetcel van een bladrolvirusbevattende luis een insluitsel waargenomen met deeltjes die aan het bladrolvirus deden denken (Fig. 37). Deze deeltjes hebben een diameter van 23 nm en een hexagonaal profiel, hetgeen overeenkomt met de deeltjes die door andere auteurs in gezuiverde preparaten van bladrolvirus zijn waargenomen alsook in het floeem van met dit virus geïnfecteerde planten. De mogelijkheid is echter niet uitgesloten dat het hier gaat om de 'viruslike particles', die door PETERS (1965, 1967a, b) zijn beschreven. Gedurende het leven van de luis degenereren alle vetcellen geleidelijk aan, waarbij het virus in de hemolymfe vrij zou kunnen komen.

REFERENCES

(Abbreviations according to World list of scientific Periodicals).

ADAMS, J. B. and C. V. WADE (1970). The size and shape of the salivary glands in some aphid species. Can. J. Zool. 48: 965–968.

APPEL, O. (1906). Neuere Untersuchungen über Kartoffel und Tomaten-Erkrankungen. Jber. Verein. angew. Bot. 3: 130–136.

ARAGÃO, H. B. de and A. da COSTA LIMA (1929). On the contamination of haemolymph in mosquitoes infected by the yellow fever virus. Mems Inst. Oswaldo Cruz 10: 253–255.

BABTIST, B. A. (1941). The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. Q. Jl microsc. Sci. 83: 91–139.

BAKER, A. C. (1915). The woolly apple aphis. Rep. U.S. Dep. Agric. 101: 1–55.

BAWDEN, F. C. and H. L. NIXON (1951). The application of electron microscopy to the study of plant viruses in unpurified plant extracts. J. gen. Microbiol. 5: 104–109.

BEEMSTER, A. B. R. (1960). Translocation of leaf roll and virus Y in the potato. Proc. Conf. Potato Virus Dis., Braunschweig, 60–67.

BENNETT, C. W. and H. E. WALLACE (1938). Relation of the curly top virus to the vector, Eutettix tenellus. J. agric. Res. 56: 31–52.

BERGOLD, G. H. and J. WEIBEL (1962). Demonstration of yellow fever virus with the electron microscope. Virology 17: 554–562.

BINDRA, O. S. and E. S. SYLVESTER (1961). Effect of insect numbers on aphid transmission of potato leafroll virus. Hilgardia 31: 279–325.

BISHOP, G. H. (1958). Nuclear and cytoplasmic changes in fat body cells of the queen bee during metamorphosis. J. exp. Zool. 137: 501–516.

BJÖRLING, K. and F. OSSIANNILSSON (1958). Investigations on individual variations in the virus-transmitting ability of different aphid species. Socker 14: 1–13.

BLACK, L. M. (1953). Occasional transmission of some plant viruses through eggs of their insect vectors. Phytopathology 43: 9–10.

BLACK, L. M. (1959). Biological cycles of plant viruses in insect vectors. In: The viruses. (F. M. BURNET and W. M. STANLEY, eds), New York and London, vol. 2: 157–185.

BLATTNY, C. (1931). Is it possible to find out the presence of virus causing some diseases of potatoes in their carriers, plant-lice? Mém. Soc. r. Sci. Bohème, 1–7.

BLOOM, W. and D. W. FAWCETT (1969). A textbook of Histology. Philadelphia, 858 pp.

BOESE, G. (1936). Der Einfluss tierischer Parasiten auf den Organismus der Insekten. Z. ParasitKde 8: 241–284.

BONNET, C. (1745). Oevres d'histoire naturelle et de philosophie. Observations sur les pucerons. Neuchatel, Tome 1: 22–25

BONNET, C. (1776). Considérations sur les corps organisés. Amsterdam, Tome 2: 112–116.

BONNET, C. (1778). Traité d'insectologie. Observations sur les pucerons. Amsterdam, Tome 1: 3–4.

BÖRNER, C. (1930). Beiträge zu einem neuen System der Blattläuse. Arch. klassif. phylogen. Ent. 1: 115–194.

BÖRNER, C. (1938). Neuer Beitrag zur Systematik und Stammesgeschichte der Blattläuse. Abh. naturw. Ver. Bremen 30: 167–179.

BOWERS, B. (1964). Coated vesicles in the pericardial cells of the aphid (Myzus persicae SULZ.). Protoplasma 59: 351–367.

BOWNE, J. G. and R. H. JONES (1966). Observations on bluetongue virus in the salivary glands of an insect vector, Culicoides variipennis. Virology 30: 127–133.

BRADLEY, R. H. E. (1952). Studies on the aphid transmission of a strain of henbane mosaic virus. Ann. appl. Biol. 39: 78–97.

BRADLEY, R. H. E. (1953). Infectivity of aphids after several hours on tobacco infected with

131

potato virus Y. Nature, Lond. **171**: 755–757.

BRADLEY, R. H. E. (1959). Loss of virus from the stylets of aphids. Virology **8**: 308–318.

BRAMSTEDT, F. (1948). Über die Verdauungsphysiologie der Aphiden. Z. Naturf. **3**: 14–24.

BREIDER, H. (1952). Beiträge zur Morphologie und Biologie der Reblaus *Dactylosphaera viti-folii* SHIM. Z. angew. Ent. **33**: 517–543.

BUCHNER, P. (1953). Endosymbiose der Tiere mit pflanzlichen Mikroorganismen. Basel, 771 pp.

BUCKTON, G. B. (1876). Monograph of the British Aphides. Rep. Ray Soc. **1**: 1–180.

BÜSGEN, M. (1891). Der Honigtau. Eine biologische Studie an Pflanzen und Pflanzenläusen. Jena. Z. Naturw. **25**: 339–428.

CADMAN, C. H. (1957). Transmission of leaf roll virus. A. Rep. Scott. hort. Res. Inst. **4**: 30.

CADMAN, C. H. and B. D. HARRISON (1956). Transmission of leaf roll virus. A. Rep. Scott. hort. Res. Inst. **3**: 23–24.

CARLETON, H. M. and R. A. B. DRURY (1957). Histological technique. London, 343 pp.

CAZAL, P. (1948). Les glandes endocrines rétro-cérébrales des insectes. Bull. biol. Fr. Belg. **32**: 1–227.

CHAMBERLAIN, R. W. and R. K. SIKES (1955). Laboratory investigations on the role of bird mites in the transmission of eastern and western equine encephalitis. Am. J. trop. Med. Hyg. **4**: 106–118.

CHOLODKOVSKY, N. (1905). Über die Speicheldrüsen von *Chermes*. Z. wiss. InsektBiol. **1**: 167–169.

CLARK, M. F. and F. A. Ross (1964). Variation among clones of *Myzus persicae* in ability to transmit potato leafroll virus acquired by injection. Phytopathology **54**: 199–204.

COBBEN, R. H. (1958). Biotaxonomische einzelheiten über Niederländische Wanzen (Hemiptera, Heteroptera). Tijdschr. Ent. **101**: 1–46.

COLLINS, W. E. (1963). Transmission of Semliki Forest virus by *Anopheles albimanus* using membrane feeding techniques. Mosquito News **23**: 96–99.

DAVIDSON, J. (1913). The structure and biology of *Schizoneura lanigera* HAUSMANN or Woolly Aphis of the apple tree. I. The apterous viviparous female. Q. Jl microsc. Sci. **58**: 653–702.

DAVIDSON, J. (1914). On the mouthparts and mechanism of suction in *Schizoneura lanigera*. J. Linn. Soc. **32**: 307–330.

DAVIES, W. M. (1932). Ecological studies on aphides infesting the potatocrop. Bull. ent. Res. **23**: 535–548.

DAVIS, N. C. and R. C. SHANNON (1930). The location of yellow fever virus in infected mosquitoes and the possibility of hereditary transmission. Am. J. Hyg. **11**: 335–344.

DAY, M. F. (1955). The mechanism of the transmission of potato leaf roll virus by aphids. Aust. J. biol. Sci. **8**: 498–513.

DAY, M. F. and H. IRZYKIEWICZ (1953). Feeding behavior of the aphids *Myzus persicae* and *Brevicoryne brassicae*, studied with radiophosphorus. Aust. J. biol. Sci. **6**: 98–108.

DAY, M. F. and M. ZAITLIN (1958). Infectivity and electron microscopy of extracts of *Physalis floridana* plants infected with potato leafroll virus. Phytopath. Z. **34**: 83–85.

DIXON, A. F. G. (1958). The protective function of the siphunculi of the nettle aphid, *Microlophium evansi* (THEOB.) (Hem., Aphididae). Entomologist's mon. Mag. **94**: 8.

DUFOUR, M. L. (1833). Recherches anatomiques et physiologiques sur les Hémiptères. Paris, Tome 4: 333 pp.

DYKSTRA, T. P. and W. C. WHITAKER (1938). Experiments on the transmission of potato viruses by vectors. J. agric. Res. **57**: 319–334.

EASTOP, V. F. and C. J. BANKS (1970). Suspected insecticide resistance mechanism in the peach-potato aphid. Nature, Lond. **225**: 970–971.

EDWARDS, J. S. (1966). Defence by smear: supercooling in the cornicle wax of aphids. Nature, Lond. **211**: 73–74.

EHRHARDT, P. (1963). Untersuchungen über bau und function des Verdauungstraktes von *Megoura viciae* BUCKT. (Aphidae, Homoptera) unter besonderer Berücksichtigung der Nahrungsaufnahme und der Honigtauabgabe. Z. Morph. Ökol. Tiere **52**: 597–677.

EHRHARDT, P. (1966). Entwicklung und Symbionten Geflügelter und Ungeflügelter Virgines

von *Aphis fabae* Scop. unter dem Einfluss künstlicher Ernährung. Z. Morph. Ökol. Tiere **57**: 295–319.

ELZE, D. L. (1927). De verspreiding van virusziekten van den aardappel (*Solanum tuberosum* L.) door insecten. Meded. LandbHoogesch. Wageningen **31**: 1–90.

ELZE, D. L. (1931). The relation between insect and virus as shown in potato leaf roll, and a classification of viroses based on this relation. Phytopathology **21**: 675–686.

ESAU, K., R. NAMBA and E. A. RASA (1961). Studies on penetration of sugar beet leaves by stylets of *Myzus persicae*. Hilgardia **30**: 517–529.

EVENHUIS, H. H. (1958). Een oecologisch onderzoek over de appelbloedluis, *Eriosoma lanigerum* (HAUSM.), en haar parasiet *Aphelinus mali* (HALD.) in Nederland. Tijdschr. PlZiekt. **64**: 1–103.

FLÖGEL, J. H. L. (1905). Monographie der Johannisbeeren-Blattlaus, *Aphis ribis* L. Z. wiss. InsektBiol. **1**: 49–235.

FLUITER, H. J. de (1931). De bloedluis *Eriosoma lanigerum* (HAUSM.) in Nederland. Wageningen, 126 pp.

FORBES, A. R. (1964a). The morphology, and fine structure of the gut of the green peach aphid, *Myzus persicae* (SULZER) (Homoptera: Aphididae). Mem. ent. Soc. Can. **36**: 1–74.

FORBES, A. R. (1964b). The morphology, histology, and fine structure of the gut of the green peach aphid, *Myzus persicae* (SULZER). Res. Rep. Dep. agric. Can., 14.

FORBES, A. R. (1966). Electron microscope evidence for nerves in the mandibular stylets of the green peach aphid. Nature, Lond. **212**: 726.

FORBES, A. R. (1969). The stylets of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). Can. Ent. **101**: 31–41.

FORREST, J. M. S. and J. Ph. W. NOORDINK (1971). Translocation and subsequent uptake by aphids of ^{32}P introduced into plants by radioactive aphids. Entomologia exp. appl. **14**: 133–134.

FORTIN, B. (1958). Etude préliminaire des glandes salivaires de *Macrosiphum solanifolii* ASHM. (Homoptera: Aphididae): anatomie et histologie. Proc. Int. Congr. Ent. Montreal, 1956, **1**: 601–603.

FUKUSHI, T. and I. KIMURA (1959). On some properties of the rice dwarf virus. Proc. Japan Acad. **35**: 482–484.

FUKUSHI, T. and E. SHIKATA (1963). Localization of rice dwarf virus in its insect vector. Virology **21**: 503–505.

FUKUSHI, T., E. SHIKATA and I. KIMURA (1962). Some morfological characters of rice dwarf virus. Virology **18**: 192–205.

FUKUSHI, T., E. SHIKATA, I. KIMURA and M. NEMOTO (1960). Electron microscopic studies on the rice dwarf virus. Proc. Japan Acad. **36**: 352–357.

GAIDAMOVICH, S. Y. A., YA.YA. TSILINSKY, A. I. LVOVA and N.V. KHUTORETSKAYA (1971). *Aëdes aegypti* mosquitoes as an experimental model for studies on the ecology and genetics of Venezuelan Equine encephalomyelitis virus. Acta Virologica **15**: 301–308.

GAUDECKER, B. von (1963). Über den Formwechsel einiger Zellorganelle bei der Bildung der Reservestoffe im Fettkörper von *Drosophila* Larven. Z. Zellforsch. **61**: 56–95.

GERSCH, M. (1942). Verteilung und Ausscheidung von Fluorescein bei Aphiden. II. Beitrag zur Exkretion bei Insekten. Z. vergl. Physiol. **29**: 506–531.

GILLETTE, C. P. (1908a). Notes and descriptions of some orchard plant lice of the family Aphididae. J. econ. Ent. **1**: 359–369.

GILLETTE, C. P. (1908b). Honeydew and the cornicles of the Aphididae. Can. Ent. **40**: 235–236.

GLASER, R. W. (1912). A contribution to our knowledge of the function of the oenocytes of insects. Biol. Bull. mar. biol. Lab., Woods Hole **23**: 213–224.

GOODCHILD, A. J. P. (1966). Evolution of the alimentary canal in the hemiptera. Biol. Rev. **41**: 97–140.

GRANADOS, R. R., H. HIRUMI and K. MARAMOROSCH (1967). Electron microscopic evidence for wound-tumor virus accumulation in various organs of an inefficient leafhopper vector, *Agalliopsis novella*. J. Invertebr. Pathol. **9**: 147–159.

GRANADOS, R. R., L. S. WARD and K. MARAMOROSCH (1968). Insect viremia caused by a

plant-pathogenic virus: electron microscopy of vector hemocytes. Virology **34**: 790–796.

GRIFFITHS, D. C. (1961). The development of *Monoctonus paludum* MARSHALL (Hym., Braconidae) in *Nasonovia ribis-nigri* on lettuce, and immunity reactions in other lettuce aphids. Bull. ent. Res. **52**: 147–163.

GROVE, A. J. (1909). The anatomy of *Siphonophora rosarum*, WALK., the green-fly pest of the rose-tree. I. The apterous viviparous stage. Parasitology **2**: 1–28.

GROVE, A. J. (1910). The anatomy of *Siphonophora rosarum*, WALK., the green fly pest of the rose-tree. II. The winged viviparous stage compared with the apterous viviparous stage. Parasitology **3**: 1–16.

GRUNDMANN, A. W., C. M. KITSELMAN, L. M. RODERICK and R. C. SMITH (1943). Studies on the transmission of the western strain virus of equine encephalomyelitis by the American dog tick, *Dermacentor variabilis* SAY and by *Triatoma sanguisuga* (LeConte). J. infect. Dis. **72**: 163–171.

GYTHRIE, F. E., W. V. CAMPBELL and R. L. BARON (1962). Feeding sites of the green peach aphid with respect to its adaptation to tobacco. Ann. ent. Soc. Am. **55**: 42–46.

HAMILTON, M. A. (1935). Further experiments on the artificial feeding of *Myzus persicae* (SULZ.). Ann. appl. Biol. **22**: 243–258.

HARRISON, B. D. (1957). Transmission of leaf roll virus. A. Rep. Scott. hort. Res. Inst. **4**: 30.

HARRISON, B. D. (1958). Studies on the behaviour of potato leaf roll and other viruses in the body of their aphid vector, *Myzus persicae*. Virology **6**: 265–278.

HEINZE, K. (1955). Versuche zur Übertragung des Blattrollvirus der Kartoffel in den Überträger (*Myzodes persicae* SULZ.) mit Injektionsverfahren. Phytopath. Z. **25**: 103–108.

HENKE, K. (1924). Die Färbung und Zeichnung der Feuerwanze (*Pyrrhocoris apterus* L.). und ihre experimentelle Beeinflussbarkeit. Z. vergl. Physiol. **1**: 297–499.

HEROLD, F. and K. MUNZ (1965). Electron microscopic demonstration of viruslike particles in *Peregrinus maidis* following acquisition of maize mosaic virus. Virology **25**: 412–417.

HILLE RIS LAMBERS, D. (1939). On some western european aphids. Zoöl. Meded., Leiden **22**: 79–119.

HILLE RIS LAMBERS, D. (1959). Onderzoekingen over bladluizen. Versl. landbouwk. Onderz. Ned., 90–91.

HINDE, R. (1971a). The control of the mycetome symbiotes of the aphids *Brevicoryne brassicae*, *Myzus persicae*, and *Macrosiphum rosae*. J. Insect Physiol. **17**: 1791–1800.

HINDE, R. (1971b). The fine structure of the mycetome symbiotes of the aphids *Brevicoryne brassicae*, *Myzus persicae*, and *Macrosiphum rosae*. J. Insect Physiol. **17**: 2035–2050.

HINDLE, E. (1929). An experimental study of yellow fever. Trans. R. Soc. trop. Med. Hyg. **22**: 405–434.

HINDLE, E. (1930). The transmission of yellow fever. Lancet **219**: 835–842.

HIRSCHLER, J. (1912). Embryologische Untersuchungen an Aphiden nebst theoretischen Erwägungen über den morphologischen Wert der Dotterelemente (Dotterzellen, Vitellophagen, Dotterepithel, Merocyten, Parablast) im allgemeinen. Z. wiss. Zool. **100**: 393–446.

HIRUMI, H., R. R. GRANADOS and K. MARAMOROSCH (1967). Electron microscopy of a plant-pathogenic virus in the nervous system of its insect vector. J. Virol. **1**: 430–444.

HOLLANDE, A. Ch. (1911). Étude histologique comparée du sang des insectes a hémorrhée et des insectes sans hémorrhée. Archs Zool. exp. gén. **6**: 283–323.

HOLLANDE, A. Ch. (1914). Les cérodécytes ou oenocytes des insectes considérés au point de vue biochemique. Archs Anat. microsc. **16**: 1–66.

HOLMAN, J. (1958). The nephrocyte cells in aphids. Čas. čsl. Spol. ent. **55**: 58–66.

HOLT, R. L. and J. H. KINTNER (1931). Location of dengue virus in the body of mosquitoes. Am. J. trop. Med. **11**: 103–111.

HOOF, H. A. van (1957). An investigation of the biological transmission of a non-persistent virus. Meded. Inst. plziektenk. Onderz. (Wageningen) **161**: 1–96.

HORSFALL, J. L. (1923). The effects of feeding punctures of aphids on certain plant tissues. Bull. Pa agric. Exp. Stn **182**: 1–22.

HORVATH, G. (1904). Sur les cornicules ou nectaires des Aphidiens. C. r. Congr. Int. Zool., Berne, 421–424.

134   *Meded. Landbouwhogeschool Wageningen 72-16 (1972)*

HOTTES, F. C. (1928). Concerning the structure, function, and origin of the cornicles of the family Aphididae. Proc. biol. Soc. Wash. **41**: 71–84.

HOVEY, C. and R. BONDE (1948). Physalis angulata, a test plant for the potato leafroll virus. Phytopathology **38**: 505–507.

HURLBUT, H. S. (1964). The pig-mosquito cycle of Japanese encephalitis virus in Taiwan. J. Med. Entomol. **1**: 301–307.

HURLBUT, H. S. (1966). Mosquito salivation and virus transmission. Am. J. trop. Med. Hyg. **15**: 989–993.

HUXLEY, T. H. (1858). On the agamic reproduction and morphology of aphids. Part I. Trans. Linn. Soc. Lond. **22**: 193–219.

ISHIZAKI, H. (1965). Electron microscopic study of changes in the subcellular organization during metamorphosis of the fat-body cell of *Philosamia cynthia ricini* (Lepidoptera). J. Insect Physiol. **11**: 845–855.

JANISZEWSKA, J. (1932). Untersuchungen über die Hymenoptera *Aphidius* sp., Parasiten der Blattlaus *Hyalopterus pruni* FABR. Bull. int. Acad. pol. Sci. Lett. 7 B II: 277–293.

JOHNSON, B. (1962). Neurosecretion and the transport of secretory material from the corpora cardiaca in aphids. Nature, Lond. **195**: 1338–1339.

JOHNSON, B. (1963). A histological study of neurosecretion in aphids. J. Insect Physiol. **9**: 727–739.

KALTENBACH, J. H. (1843). Monographie der Familien der Pflanzenläuse. Teil I. Die Blatt- und Erdläuse. Aachen, 222 pp.

KASSANIS, B. (1949). Potato tubers free from leafroll virus by heat. Nature, Lond. **164**: 881.

KASSANIS, B. (1952). Some factors affecting the transmission of leafroll virus by aphids. Ann. appl. Biol. **39**: 157–165.

KENDALL, M. D. (1969). The fine structure of the salivary glands of the desert locust *Schistocerca gregaria* FORSKÅL. Z. Zellforsch. **98**: 399–420.

KENNEDY, J. S., M. F. DAY and V. F. EASTOP (1962). A conspectus of aphids as vectors of plant viruses. Commonw. Inst. Ent. Lond., 114 pp.

KIKUMOTO, T. and C. MATSUI (1962). Electron microscopy of plant viruses in aphid midguts. Virology **16**: 509–510.

KIRKPATRICK, H. C. (1948). Indicator plants for studies with the leafroll virus of potatoes. Am. Potato J. **25**: 283–290.

KIRKPATRICK, H. C. and A. F. ROSS (1952). Aphid-transmission of potato leafroll virus to solanaceous species. Phytopathology **42**: 540–546.

KISLOW, C. J. and L. J. EDWARDS (1972). Repellent odour in aphids. Nature, Lond. **235**: 108–109.

KISLOW, C. J., P. BARBOSA and L. J. EDWARDS (1971). An embedding procedure for the study of aphid feeding and insect tissues. Ann. ent. Soc. Am. **64**: 296–297.

KLEVENHUSEN, F. (1927). Beiträge zur Kenntnis der Aphidensymbiose. Z. Morph. Ökol. Tiere **9**: 97–165.

KLOFT, W. (1959). Wechselwirkungen zwischen pflanzensaugenden Insekten und den von ihnen besogenen Pflanzengeweben. Z. angew. Ent. **45**: 337–381.

KLOFT, W. (1960). Nachweis freier aminosäuren als Phytopathologisch wirksame stoffe im Speichel Plantisugar insekten. Proc. Int. Congr. Ent., Vienna, vol. 3: 141–144.

KLOSTERMEYER, E. C. (1953). Entomological aspects of the potato leafroll problem in central Washington. Tech. Bull. agric. Exp. Stn Wash. St. **9**: 1–42.

KNOWLTON, G. F. (1925). The digestive tract of *Longistigma caryae* (HARRIS). Ohio J. Sci. **25**: 244–252.

KOCH, A. (1930). Über das Vorkommen von Mitochondrien in Mycetocyten. Z. Morph. Ökol. Tiere **19**: 259–290.

KOJIMA, M., E. SHIKATA, M. SUGAWARA and D. MURAYAMA (1968). Isolation and electron microscopy of potato leafroll virus from plants. Virology **35**: 612–615.

KOJIMA, M., E. SHIKATA, M. SUGAWARA and D. MURAYAMA (1969). Purification and electron microscopy of potato leafroll virus. Virology **39**: 162–174.

KOLB, G. (1959). Untersuchungen über die Kernverhältnisse und morphologischen Eigen-

135

schaften Symbiontischer Mikroorganismen bei verschiedenen insekten. Z. Morph. Ökol.
Tiere 48: 1–71.

KONINGSBERGER, J. C. and A. ZIMMERMANN (1901). De dierlijke vijanden der koffiecultuur op
Java. Meded. LdsPLTuin, Batavia 44: 1–125.

KRASSILSTSCHIK, I. (1893). Zur Anatomie und Histologie der *Phylloxera vastatrix* PLANCHON.
Horae Soc. ent. rossicae 27: 1–37.

KUSHIDA, H. (1961). A new embedding method for ultra thin sectioning using a methacrylate
resin with three dimensional polymer structure. J. Electron Microsc., Chiba Cy 10: 194–197

KUSHIDA, H. and K. FUJITA (1966). Simultaneous double staining. Int. Congr. Electr. Micr.,
Kyoto, vol. II: 39.

LAMB, K. P. and R. HINDE (1967). Structure and development of the mycetome in the cabbage
aphid, *Brevicoryne brassicae*. J. Invertebr. Pathol. 9: 3–11.

LAMOTTE, L. C. (1960). Japanese B encephalitis virus in the organs of infected mosquitoes.
Am. J. Hyg. 72: 73–87.

LAMPEL, G. (1959). Über die Entstehung von Riesenzellen im Abdomen von *Pemphiginen* bei
Schlupfwespenbefall. Z. angew. Ent. 45: 204–211.

LAMPEL, G. (1962). Zum Problem der Riesenzellbildung in parasitierten Blattläusen. Natur-
wissenschaften 49: 45–46.

LANHAM, U. N. (1952). Observation on the supposed intracellulair microorganisms of aphids.
Science, N. Y. 115: 459–460.

LEBLANC, N. N. and A. J. MUSGRAVE (1963). Some microbiological studies of two species of
aphids, *Aphis fabae* SCOP. and *Macrosiphum pisi* (HARRIS) (Hemiptera, Homoptera:
Aphididae). Can. J. Microbiol. 9: 65–73.

LEEUWENHOEK, A. van (1696). Ondekte onsigtbaarheeden. Leiden, Tome 4: 87–293.

LEONHARDT, H. (1940). Beiträge zur Kenntnis der Lachniden, der wichtigsten Tannenhonig-
tauerzeuger. Z. angew. Ent. 27: 208–272.

LEWIS, I. F. and L. WALTON (1958). Gall-formation on *Hamamelis virginiana* resulting from
material injected by the aphid *Hormaphis hamamelidis*. Trans. Am. microsc. Soc. 77:
146–200.

LINDSAY, K. L. (1969). Cornicles of the pea aphid, *Acyrthosiphon pisum*; their structure and
function. A light and electron microscope study. Ann. ent. Soc. Am. 62: 1015–1021.

LIU, Yu-swu (1956). A histological study of the cornicles of the cotton aphid, *Aphis gossypii*
GLOVER, with some experiments concerning its function. Acta zool. sin. 8: 17–28.

LOCKE, M. and J. V. COLLINS (1965). The structure and formation of protein granules in the
fat body of an insect. J. Cell Biol. 26: 857–884.

LOCKE, M. and J. V. COLLINS (1968). Protein uptake into multivesicular bodies and storage
granules in the fat body of an insect. J. Cell. Biol. 36: 453–483.

LOOF, A. de and A. LAGASSE (1970). Juvenile hormone and the ultrastructural properties of
the fat body of the adult colorado beetle, *Leptinotarsa decemlineata* SAY. Z. Zellforsch.
106: 439–450.

LOUGHNANE, J. B. (1943). *Aphis rhamni* BOYER; its occurrence in Ireland and its efficiency as a
vector of potato viruses. J. Dep. Agric. Repub. Ire. 40: 291–298.

LOWE, H. J. B. (1967). Interspecific differences in the biology of aphids (Homoptera: Aphidi-
dae) on leaves of *Vicia faba*. Entomologia exp. appl. 10: 347–357.

MACCARTHY, H. R. (1954). Aphid transmission of potato leafroll virus. Phytopathology 44:
167–174.

MACKAUER, M. (1959). Histologische Untersuchungen an parasitierten Blattläusen. Z. Para-
sitKde. 19: 322–352.

MACKAUER, M. (1965). Histologische und karyometrische Untersuchungen der Oenocyten
von *Aphis pomi* de GEER (Hom., Aphididae). Naturwissenschaften 52: 351–352.

MACKINNON, J. P. (1962). Comparative studies between the aphid transmission of potato leaf
roll and turnip latent viruses. Can. J. Bot. 40: 525–531.

MACKINNON, J. P. (1963a). The availability to aphids of virus in different parts of leaves. Can.
J. Bot. 41: 1597–1598.

MACKINNON, J. P. (1963b). Some factors that affect the aphid transmission of two viruses that

136

persist in the vector. Virology 20: 281–287.

MacKinnon, J. P. (1964). Some effects of virus source and acquisition feeding on the aphid transmission of potato leaf roll and turnip latent viruses. Can. J. Bot. 42: 1159–1165.

Mark, E. L. (1877). Beiträge zur Anatomie und Histologie der Pflanzenläuse, insbesondere der Cocciden. Arch. mikrosk. Anat. 13: 31–86.

Martini, Chr. (1958). Beobachtungen über das Saugen bei Blattläusen. Z. PflKrankh. PflPath. PflSchutz 65: 90–92.

McLean, D. M. (1955). Multiplication of viruses in mosquitoes following feeding and injection into the body cavity. Aust. J. exp. Biol. med. Sci. 33: 53–65.

Meester-Manger Cats, V. de (1956). Korte overdrachttijd van bladrolvirus. Tijdschr. PLZiekt. 62: 174–176.

Merrill, M. H. and C. TenBroeck (1934). Multiplication of equine encephalomyelitis virus in mosquitoes. Proc. Soc. exp. Biol. Med. 32: 421–422.

Metschnikow, E. (1866). Embryologische Studien an Insecten. Die Entwicklung der viviparen Aphiden. Z. wiss. Zool. 16: 49–80.

Meyer, K. F. (1953). Vectors and reservoirs of virus diseases. Am. J. trop. Med. Hyg. 2: 757–770.

Michel, E. (1942). Beiträge zur Kenntnis von *Lachnus (Pterochlorus) roboris* L., einer wichtigen Honigtauerzeugerin an der Eiche. Z. angew. Ent. 29: 243–281.

Miles, P. W. (1959). Secretion of two types of saliva by an aphid. Nature, Lond. 183: 756.

Miles, P. W. (1968). Insect secretions in plants. A. Rev. Phytopathol. 6: 137–164.

Miller, F. W. (1932). The digestive epithelium of the aphid, *Macrosiphum sanbornii*. Proc. Pa Acad. Sci. 6: 148–151. In: Forbes, A. R. (1964a).

Miller, F. W. (1933). The musculature of the woolly aphid of the elm (*Schizoneura americana*). Ann. ent. Soc. Am. 26: 473–489.

Mims, C. A., M. F. Day and I. D. Marshall (1966). Cytopathic effect of Semliki Forest virus in the mosquito *Aëdes aegypti*. Am. J. trop. Med. Hyg. 15: 775–784.

Mitchell, B. I. M. (1937). Ph. D. Thesis, Univ. of London. In: Watson, M. A. and F. M. Roberts (1939).

Miyamoto, S. and Y. Miyamoto (1966). Notes on aphid-transmission of potato leafroll virus. Sci. Rep. Hyogo Univ. Agric. 7: 51–56.

Miyamoto, S. and Y. Miyamoto (1971). Notes on aphid-transmission of potato leafroll virus. II. Transference of the virus to nymphs from viruliferous adults of *Myzus persicae* Sulz. Sci. Rep. Kobe Univ. Agric. 9: 59–70.

Moericke, V. (1961). Virusartige Körper in Speicheldrüsen von Kartoffelblattrollinfektiösen *Myzus persicae* (Sulz.). Z. PflKrankh. PflPath. PflSchutz 68: 581–587.

Moericke, V. (1963). Über Virusartige Körper in Organen von *Myzus persicae* (Sulz.). Z. PflKrankh. PflPath. PflSchutz 70: 464–470.

Moericke, V. and T. E. Mittler (1965). Schlucken Blattläuse ihren eigenen Speichel? Z. PflKrankh. PflPath. PflSchutz 72: 513–515.

Moericke, V. and T. E. Mittler (1966). Oesophageal and stomach inclusions of aphids feeding on various cruciferae. Entomologia exp. appl. 9: 287–297.

Moericke, V. and K. E. Wohlfarth-Bottermann (1960a). Zur funktionellen Morphologie der Speicheldrüsen von Homopteren. I. Die Hauptzellen der Hauptdrüsen von *Myzus persicae* (Sulz.), Aphididae. Z. Zellforsch. 51: 157–184.

Moericke, V. and K. E. Wohlfarth-Bottermann (1960b). Zur funktionellen Morphologie der Speicheldrüsen von Homopteren. IV. Die Ausführgänge der Speicheldrüsen von *Myzus persicae* (Sulz.), Aphididae. Z. Zellforsch. 53: 25–49.

Moericke, V. and K. E. Wohlfarth-Bottermann (1960c). Über die Feinstructur der Speicheldrüse von Blattläusen (Aphididae). Zool. Anz. Suppl. 23: 437–448.

Moericke, V. and K. E. Wohlfarth-Bottermann (1962). Nachweis von virusartigen Partikeln in Prosekretgranula von Speicheldrüsen der Pfirsichblattlaus (*Myzus persicae*). Protoplasma 54: 345–354.

Moericke, V. and K. E. Wohlfarth-Bottermann (1963). Zur funktionellen Morphologie der Speicheldrüse von Homopteren. II. Die Deck- und die Zentralzellen der Speicheldrüse

von *Myzus persicae* (SULZ.), Aphididae. Z. Zellforsch. **59**: 165–183.

MOHR, W. P. and E. C. COCKING (1968). A method of preparing highly vacuolated, senescent, or damaged plant tissue for ultrastructural study. J. Ultrastruct. Res. **21**: 171–181.

MORDWILKO. A. (1895). Zur Anatomie der Pflanzenläuse, Aphiden. Zool. Anz. **18**: 345–364.

MOREAU, J. P. and L. C. van LOON (1966). Le comportement de piqûre chez *Rhopalosiphum padi* L. (Homoptères, Aphididés). C. r. hebd. Séanc. Acad. Sci., Paris **262**: 904–907.

MORREN, M. Ch. (1836). Mémoire sur l'émigration du puceron du pêcher (*Aphis persicae*), et sur les caractères et l'anatomie de cette espèce. Annls Sci. nat. Zool. **6**: 65–93.

MUELLER, W. C. and A. F. ROSS (1961). Difficulties encountered in the use of an aphid-injection method for the transmission of potato leafroll virus. Am. Potato J. **38**: 249–258.

MURAYAMA, D. and M. KOJIMA (1965). Studies on the properties of potato leafroll virus by the aphid-injection method. Ann. phytopath. Soc. Japan **30**: 209–215.

NASU, S. (1965). Electron microscopic studies on transovarial passage of rice dwarf virus. Jap. J. appl. Ent. Zool. **9**: 225–237.

NAULT, L. R., G. G. GYRISCO and W. F. ROCHOW (1964). Biological relationship between pea enation mosaic virus and its vector, the pea aphid. Phytopathology **54**: 1269–1272.

ODHIAMBO, T. R. (1967). The fine structure and histochemistry of the fat body in the locust, *Schistocerca gregaria*. J. Cell Sci. **2**: 235–242.

O'LOUGHLIN, G. T. and T. C. CHAMBERS (1967). The systemic infection of an aphid by a plant virus. Virology **33**: 262–271.

OORTWIJN-BOTJES, J. G. (1920). De bladrolziekte van de aardappelplant. Diss., Wageningen, 136 pp.

OSSIANNILSSON, F. (1961). Negative evidence against passage of whole plant virus particles through gut wall in aphid vectors. Nature, Lond. **190**: 1222.

PAILLOT, A. (1933). L'infection chez les insectes. Immunité et symbiose. Paris, 535 pp.

PAILLOT, A. (1934). Modifications cytologiques et organiques engendrées chez les pucerons par les Hyménoptères parasites. C. r. hebd. Séanc. Acad. Sci., Paris **199**: 1450–1452.

PAILLOT, A. (1938). Sur l'origine des cellules géantes des chenilles de *Pieris brassicae* et des aphides parasités par Hyménoptères. C. r. Séanc. Soc. Biol. **127**: 1504–1506.

PALIWAL, Y. C. and R. C. SINHA (1970). On the mechanism of persistence and distribution of barley yellow dwarf virus in an aphid vector. Virology **42**: 668–680.

PARRISH, W. B. (1967). The origin, morphology, and innervation of aphid stylets (Homoptera). Ann. ent. Soc. Am. **60**: 273–276.

PAVLOVSKY, E. N. and V. D. SOLOVIEV (1940). Experimental study of the circulation of the encephalitis virus within the organism of the tick *Ixodes persulcatus*. Archs Sci. biol., St. Pétersb. **59**: 111–115. In: MEYER, K. F. (1953).

PELTON, J. Z. (1938). The alimentary canal of the aphid *Prociphilus tesselata* FITCH. Ohio J. Sci. **38**: 164–169.

PESSON, P. (1944). Contribution à l'étude morphologique et fonctionnelle de la tête, de l'appareil buccal et du tube digestif des femelles de Coccides. Paris, 266 pp.

PESSON, P. (1951). Ordre des Homoptères. In: GRASSÉ's Traité de Zoologie **10**: 1390–1656.

PETERS, D. (1965). The purification of viruslike particles from the aphid *Myzus persicae*. Virology **26**: 159–161.

PETERS, D. (1967a). The purification of potato leafroll virus from its vector *Myzus persicae*. Virology **31**: 46–54.

PETERS, D. (1967b). Potato leafroll virus, its purification from its vector *Myzus persicae*. Meded. Fonds Landb. Exp. Bur. **45**: 1–100.

PFLUGFELDER, O. (1936). Vergleichend-anatomische, experimentelle und embryologische Untersuchungen über das Nervensystem und die Sinnesorgane der Rhynchoten. Zoologica, Stuttg. **93**: 1–102.

POISSON, R. and P. PESSON (1939). Contribution a l'étude du sang des coccides (Hémiptères Homoptères Sternorhyncha). III. Le sang de *Pulvinaria mesembryanthemi* VALLOT. Archs Zool. exp. gén. **81**: 23–32.

POLLARD, D. G. (1971). Some aspects of plant penetration by *Myzus persicae* (SULZ.) nymphs (Homoptera, Aphididae). Bull. ent. Res. **61**: 315–324.

138

PONSEN, M. B. (1969). The effect of potato leafroll virus on the biology of *Myzus persicae*. Neth. J. Pl. Path. **75**: 360–368.

PONSEN, M. B. (1970). The biological transmission of potato leafroll virus by *Myzus persicae*. Neth. J. Pl. Path. **76**: 234–239.

PONSEN, M. B. and D. STEGWEE (1960). Enkele eigenschappen van het aardappelbladrolvirus in *Physalis floridana* RYDB. Tijdschr. PlZiekt. **66**: 121–122.

PROFFT, J. (1937). Beiträge zur Symbiose der Aphiden und Psylliden. Z. Morph. Ökol. Tiere **32**: 289–326.

QUANJER, H. M. (1913). Die nekrose des Phloëms der Kartoffelpflanze, die Ursache der Blattrollkrankheit. Meded. LandbHoogesch. Wageningen **6**: 41–80.

QUANJER, H. M. (1916). Aard, verspreidingswijze en bestrijding van phloeemnecrose (bladrol) en verwante ziekten. Meded. LandbHoogesch. Wageningen **10**: 1–90.

RAINE, J. and A. R. FORBES (1971). The salivary syringe of the leafhopper *Macrosteles fascifrons* (Homoptera: Cicadellidae) and the occurrence of mycoplasma-like organisms in its ducts. Can. Ent. **103**: 110–116.

RAMDOHR, K. A. (1811). Abhandlung über die Verdauungswerkzeuge der Insecten. Halle, 221 pp.

RÉAUMUR, R. A. F. de (1742). Memoires pour servir a l'histoire des insectes. Tome 9. Histoire des Pucerons. Paris, 281–350.

REED, W. (1902). Recent researches concerning the etiology, propagation and prevention of yellow fever by the United States Army Commission. J. Hyg. **2**: 101–119.

REMAUDIÈRE, G. (1964). Sur la présence de cornicules surnuméraires chez un aphis (Hom. Aphididae). Revue Path. vég. Ent. agric. Fr. **43**: 31–35.

RICHARDSON, C. H. (1918). The pulsatile vessels in the legs of Aphididae. Psyche, Camb. **25**: 15–17.

RICHARDSON, J. and E. S. SYLVESTER (1965). Aphid honeydew as inoculum for the injection of pea aphids with pea-enation mosaic virus. Virology **25**: 473–475.

RICHARDSON, J. and E. S. SYLVESTER (1968). Further evidence of multiplication of sowthistle yellow vein virus in its aphid vector, *Hyperomyzus lactucae*. Virology **35**: 347–355.

RILLING, G. (1960). Das Skelettmuskelsystem der ungeflügelten Reblaus (*Dactylosphaera vitifolii* SHIMER). Vitis **2**: 222–240.

RILLING, G. (1967). Die Speicheldrüsen der Reblaus (*Dactylosphaera vitifolii* SHIMER). Vitis **6**: 136–150.

ROBERT, Y., Y. MAURY and J. QUÉMÉNER (1969). Transmission du virus de l'enroulement de la pomme de terre par différentes formes et stades d'une souche de *Myzus persicae* (SULZ.) (Homoptera: Aphididae): résultats comparés sur *Physalis floridana* RYDBERG et *Solanum tuberosum* L. var. Claudia. Annls Phytopathol. **1**: 167–179.

ROBERTI, D. (1946). Monografia dell '*Aphis (Dorsalis) frangulae* KOCH. I. Morfologia, anatomia, istologia. Boll. R. Lab. Ent. agr. Portici **6**: 127–312.

ROBERTS, F. M. (1940). Studies on the feeding methods and penetration rates of *Myzus persicae* SULZ., *Myzus circumflexus* BUCKT., and *Macrosiphum gei* KOCH. Ann. appl. Biol. **27**: 348–358.

ROZENDAAL, A. (1946). Virusziekten van aardappelplanten. Landbouwk. Tijdschr., 's-Grav. **58**: 533–543.

RUTSCHKY, C. W. and R. L. CAMPBELL (1964). Fat body morphology of english grain aphids infected with barley yellow dwarf virus. Ann. ent. Soc. Am. **57**: 53–56.

SAXENA, P. N. and H. L. CHADA (1971a). The greenbug, *Schizaphis graminum*. I. Mouth parts and feeding habits. Ann. ent. Soc. Am. **64**: 897–904.

SAXENA, P. N. and H. L. CHADA (1971b). The greenbug, *Schizaphis graminum*. II. The salivary gland complex. Ann. ent. Soc. Am. **64**: 904–912.

SAXENA, P. N. and H. L. CHADA (1971c). The greenbug, *Schizaphis graminum*. III. Digestive system. Ann. ent. Soc. Am. **64**: 1031–1038.

SAXENA P. N. and H. L. CHADA (1971d). The greenbug, *Schizaphis graminum*. IV. Central and stomatogastric nervous systems. Ann. ent. Soc. Am. **64**: 1038–1044.

SCHÄLLER, G. (1961). Aminosäuren im Speichel und Honigtau der Grünen Apfelblattlaus

Aphis pomi DEG. Entomologia exp. appl. **4**: 73–85.

SCHANDERL, H., G. LAUFF and H. BECKER (1949). Studien über die Mycetom- und Darm-symbionten der Aphiden. Z. Naturf. **4**: 50–53.

SCHMIDT, H. B. (1959). Beiträge zur Kenntnis der Übertragung pflanzlicher Viren durch Aphiden. Biol. Zbl. **78**: 889–936.

SCHMIDT, H. B. (1960). Die Übertragung pflanzlicher Viren durch Insekten. Mber. dt. Akad. Wiss. Berl. **2**: 214–223.

SCHMUTTERER, H. (1969). Versuche zur Übertragung des Erbsenenationenvirus durch Injek-tion virushaltiger Hämolymphe von einer Blattlausart oder -form in die andere. Z. Pfl-Krankh. PflPath. PflSchutz **76**: 257–263.

SCHMUTTERER, H. and P. EHRHARDT (1964). Untersuchungen über die Beziehungen zwischen dem Enationenvirus der Erbse und seinen Vektoren. II. Versuche zur Übertragung des Virus durch Injektion von Haemolymphe in verschiedene Aphidenarten. Phytopath. Z. **50**: 80–85.

SCHOEL, W. (1934). Beitrag zur Kenntnis der Aphidensymbiose. Bot. Arch. **36**: 152–190.

SCHULTZ, E. S. and D. FOLSOM (1921). Leafroll, netnecrosis and spindling-sprout of the irish potato. J. agric. Res. **21**: 47–80.

SHIKATA, E. (1966). Electron microscopic studies on plant viruses. J. Fac. Agric. Hokkaido Univ. **55**: 1–110.

SHIKATA, E. and G. E. GÁLVEZ (1969). Fine flexuous threadlike particles in cells of plants and insect hosts infected with rice hoja blanca virus. Virology **39**: 635–641.

SHIKATA, E. and K. MARAMOROSCH (1965). Electron microscopic evidence for the systemic invasion of an insect host by a plant pathogenic virus. Virology **27**: 461–475.

SHIKATA, E. and K MARAMOROSCH (1967a). Electron microscopy of wound tumor virus assembly sites in insect vectors and plants. Virology **32**: 363–377.

SHIKATA, E. and K. MARAMOROSCH (1967b). Electron microscopy of the formation of wound tumor virus in abdominally inoculated insect vectors. J. Virol. **1**: 1052–1073.

SHIKATA, E., K. MARAMOROSCH and R. R. GRANADOS (1966). Electron microscopy of pea enation mosaic virus in plants and aphid vectors. Virology **29**: 426–436.

SHIKATA, E., S. W. ORENSKI, H. HIRUMI, J. MITSUHASHI and K. MARAMOROSCH (1964). Electron micrographs of wound-tumor virus in an animal host and in a plant tumor. Virology **23**: 441–444.

SINHA, R. C. (1965a). Sequential infection and distribution of wound-tumor virus in the internal organs of a vector after ingestion of virus. Virology **26**: 673–686.

SINHA, R. C. (1965b). Recovery of potato yellow dwarf virus from hemolymph and internal organs of an insect vector. Virology **27**: 118–119.

SINHA, R. C. and L. N. CHIYKOWSKI (1969). Synthesis, distribution and some multiplication sites of wheat striate mosaic virus in a leafhopper vector. Virology **38**: 679–684.

SINHA, R. C. and S. SHELLEY (1965). The transovarial transmission of wound-tumor virus. Phytopathology **25**: 324–327.

SMITH, C. F. (1939). The digestive system of *Macrosiphum solanifolii* (ASH.) (Aphidae: Homoptera). Ohio J. Sci. **39**: 57–59.

SMITH, D. S. (1968). Insect cells, their structure and function. Edinburgh, 372 pp.

SMITH, K. M. (1926). Comparative study of the feeding methods of certain Hemiptera and of the resulting effects upon the plant tissue with special reference to the potatoplant. Ann. appl. Biol. **13**: 109–139.

SMITH, K. M. (1929). Studies on potato virus diseases V; Insect transmission of potato leaf roll. Ann. appl. Biol. **16**: 209–230.

SMITH, K. M. (1931). Studies on potato virus diseases IX. Some further experiments on the insect transmission of potato leaf roll. Ann. appl. Biol. **18**: 141–157.

SNODGRASS, R. E. (1935). Principles of insect morphology. New York, 667 pp.

SORIN, M. (1960). Insertion of stylets of aphids and plant tissues. Jap. J. appl. Ent. Zool. **4**: 38–44.

SORIN, M. (1966). Physiological and morphological studies on the suction mechanism of plant juice by aphids. Bull. Univ. Osaka Prefect. Ser. B. **18**: 95–137.

SPENCER, H. (1926). Biology of the parasites and hyperparasites of aphids. Ann. ent. Soc. Am. **19**: 119–157.

SPRAU, F. (1952). Elektronenoptische Darstellung des Blattrollvirus der Kartoffel mit einem kurzen Überblick über die Elektronenmikroskopie in der Virusforschung. Z. PflBau PflSchutz *3*: 213–221.

STEGWEE, D. (1960). Further investigations on the relation between *Myzus persicae* and the potato leaf-roll virus. Proc. Conf. Potato Virus Dis., Braunschweig, 106–110.

STEGWEE, D. (1961). Multiplication of plant viruses in their aphid vectors. Recent Adv. Bot. **2**: 533–535.

STEGWEE, D. and D. PETERS (1961). The isolation of potato leafroll virus from its vector. Virology **15**: 202–203.

STEGWEE, D. and M. B. PONSEN (1958). Multiplication of potato leafroll virus in the aphid *Myzus persicae* (SULZ.). Entomologia exp. appl. **1**: 291–300.

STILES, K. A. (1938). Intermediate-winged aphids and the time of determination theory. Biol. Bull. mar. biol. Lab., Woods Hole **74**: 430–460.

STOREY, H. H. (1933). Investigations of the mechanism of the transmission of plant viruses by insect vectors. Proc. R. Soc. Lond. **113**: 463–485.

STRONG, F. E. (1967). Observations on aphid cornicle secretions. Ann. ent. Soc. Am. **60**: 668–673.

SULC, K. (1910). Pseudovitellus und ähnlische Gewebe der Homopteren sind Wohnstätten symbiotischer Saccharomyceten. Sber. K. böhm. Ges. Wiss. **18**: 1–39.

SWAMMERDAM, J. (1669). Bijbel der Natuure. Historie der insecten. Leyden, 910 pp.

SYLVESTER, E. S. (1954). Insectary life history and apterous instar morphology of *Myzus persicae* (SULZER) (Homoptera, Aphidae). Ann. ent. Soc. Am. **47**: 397–406.

SYLVESTER, E. S. and J. RICHARDSON (1970). Infection of *Hyperomyzus lactucae* by sowthistle yellow vein virus. Virology **42**: 1023–1042.

TAKAOKA, I. (1969). Studies on the morphology and histology of the digestive system, nervous system and endocrine organ of the green peach aphid, *Myzus persicae* (SULZER), (Aphididae: Homoptera). Bull. Hatano Tob. Exp. Stn **65**: 93–124.

TATE, H. D. (1936). Method of penetration, formation of stylet sheaths and source of food supply of aphids. Iowa St. Coll. J. Sci. **11**: 185–206.

THEOBALD, F. V. (1926). The plant lice or Aphididae of Great Britain. London, vol. I, 372 pp.

THOMAS, L. A. (1963). Distribution of the virus of western equine encephalomyelitis in the mosquito vector, *Culex tarsalis*. Am. J. Hyg. **78**: 150–165.

TORRE-BUENO, J. R. de la (1907). On the cornicles of the Aphidae. Can. Ent. **39**: 99–100.

TÓTH, L. (1933). Über die frühembryonale Entwicklung der viviparen Aphiden. Z. Morph. Ökol. Tiere **27**: 692–731.

TÓTH, L. (1936). Beiträge zur Kenntnis der Aphidenspeicheldrüse. Z. Morph. Ökol. Tiere **30**: 496–505.

TÓTH, L. (1938). Entwicklungszyklus und symbiose von *Pemphigus spirothecae* PASS. (Aphidina). Z. Morph. Ökol. Tiere **33**: 412–437.

TREMBLAY, E. (1966). Ricerche sugli imenotteri parassiti. II. Osservazioni sull' origine e sul destino dell' involucro embrionale degli Afidiini (Hymenoptera: Braconidae: Aphidiinae) e considerazioni sul significato generale delle membrane embrionali. Boll. Lab. Ent. agr. Portici **24**: 119–166.

UICHANCO, L. B. (1924). Studies on the embryogeny and postnatal development of the aphididae with special reference to the history of the symbiotic organ, or mycetom. Philipp. J. Sci. **24**: 143–247.

VAGO, C. (1958). Les signes cytologiques du passage des virus végétaux dans l'organisme de leurs insectes vecteurs. Revue Path. gén. Physiol. clin. **703**: 1837–1847.

VAGO, C. and M. LAPORTE (1965). Microscopie electronique des symbiontes globuleux des aphides (Hom. Aphidoidea). Annls Soc. ent. Fr. **1**: 181–196.

VIDANO, C. (1968). Microtubuli granulari in *Laodelphax* infettato con MRDV. Atti Accad. Sci., Torino **102**: 641–648.

VIDANO, C. (1970). Phases of maize rough dwarf virus multiplication in the vector *Laodelphax*

striatellus (FALLÉN). Virology **41**: 218–232.

VIDANO, C. and M. BASSI (1966). Dimostrazione al microscopio elettronico di particelle del virus del nanismo ruvido del mais (MRDV) nel vettore *Laodelphax striatellus* FALLÉN. Atti Accad. Sci., Torino **100**: 73–78.

WALKER, P. A. (1965). The structure of the fat body in normal and starved cockroaches as seen with the electron microscope. J. Insect Physiol. **11**: 1625–1631.

WALKER, P. A. (1966). An electron microscope study of the fat body of the moth *Philosamia* during growth and metamorphosis. J. Insect Physiol. **12**: 1009–1018.

WALL, R. E. (1933). A study of color and color-variation in *Aphis gossypii* GLOVER. Ann. ent. Soc. Am. **26**: 425–463.

WATSON, M. A. (1938). Further studies on the relationship between Hyoscyamus virus 3 and the aphid *Myzus persicae* with special reference to the effects of fasting. Proc. R. Soc. Lond. **125**: 144–170.

WATSON, M. A. and H. L. NIXON (1953). Studies on the feeding of *Myzus persicae* (SULZ.) on radioactive plants. Ann. appl. Biol. **40**: 537–545.

WATSON, M. A. and F. M. ROBERTS (1939). A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (SULZ.), *M. circumflexus* (BUCKTON) and *Macrosiphum gei* (KOCH). Proc. R. Soc. Lond. **127**: 543–576.

WEBB, R. E., R. H. LARSON and J. C. WALKER (1952). Relationships of potato leaf roll virus strains. Res. Bull. agric. Exp. Stn Univ. Wis. **178**: 1–40.

WEBER, H. (1928). Skelett, Muskulatur und Darm der schwarzen Blattlaus *Aphis fabae* SCOP. Zoologica, Stuttg. **28**: 1–118.

WEBER, H. (1929). Kopf und Thorax von *Psylla mali* SCHMIDB. (Hemiptera – Homoptera). Eine morphogenetische studie. Z. Morph. Ökol. Tiere **14**: 59–165.

WEBER, H. (1933). Lehrbuch der Entomologie. Jena, 698 pp.

WEBER, H. (1935a). Die postembryonale Entwicklung der Aleurodinen (Hemiptera – Homoptera). Ein Beitrag zur Kenntnis der Metamorphasen der Insekten. Z. Morph. Ökol. Tiere **29**: 268–305.

WEBER, H. (1935b). Der Bau der Imago der Aleurodinen. Zoologica, Stuttg. **89**: 1–71.

WEDDE, H. (1885). Beiträge zur Kenntnis des Rhynchotenrüssels. Berlin, 31 pp. In: ZWEIGELT, F. (1915).

WEIDEMANN, H. L. (1968). Zur Morphologie der Hauptspeicheldrüse von *Myzus persicae* (SULZ.). Entomologia exp. appl. **11**: 450–454.

WEIDEMANN, H. L. (1970). Histochemische Differenzierung verschiedener Zellen in der Hauptspeicheldrüse von *Myzus persicae*. Entomologia exp. appl. **13**: 153–161.

WEIDEMANN, H. L. (1971a). Karyometrische Untersuchungen an der Hauptspeicheldrüse von *Myzus persicae*. I. Abhängigkeit der Zellkerngrösze von verschiedenen Wirtspflanzen. Entomologia exp. appl. **14**: 107–114.

WEIDEMANN, H. L. (1971b). Karyometrische Untersuchungen an der Hauptspeicheldrüse von *Myzus persicae*. II. Kerngröszenänderungen nach Saugen auf *Physalis floridana*-Pflanzen, die mit dem Blattrollvirus der Kartoffel infiziert waren. Entomologia exp. appl. **14**: 161–174.

WENSLER, R. J. and B. K. FILSHIE (1969). Gustatory sense organs in the food canal of aphids. J. Morph. **129**: 473–492.

WHEELER, W. M. (1892). Concerning the blood-tissue of the insecta. III. Psyche, Camb. **6**: 253–258.

WHITFIELD, S. G., F. A. MURPHY and W. D. SUDIA (1971). Eastern equine encephalomyelitis virus: an electron microscopic study of *Aëdes triseriatus* (SAY) salivary gland infection. Virology **43**: 110–122.

WHITMAN, L. (1952). Virus behaviour in mosquitoes. A. Rep. Rockefeller Fdn **39**: 52–62.

WIELOWIEJSKI, H. R. von (1886). Über das Blutgewebe der Insekten. Z. wiss. Zool. **43**: 512–536.

WIGGLESWORTH, V. B. (1967). Cytological changes in the fat body of *Rhodnius* during starvation, feeding and oxygen want. J. Cell Sci. **2**: 243–256.

WILL, L. (1889). Entwicklungsgeschichte der viviparen Aphiden. Zool. Jb. **3**: 201–286.

WILLIAMS, W. L. and A. F. Ross (1957a). Acquisition and test feeding periods required on *Physalis floridana* and *Datura stramonium* for aphid transmission of potato leaf roll virus. Phytopathology **47**: 538.

WILLIAMS, W. L. and A. F. Ross (1957b). Aphid transmission of potato leaf roll virus as effected by the feeding of nonviruliferous aphids on the test plants and by vector variability. Phytopathology **47**: 538.

WILLIS, D. M. (1949). The anatomy and histology of the head, gut and associated structures of *Typhlocyba ulmi*. Proc. zool. Soc. Lond. **118**: 984–1001.

WITLACZIL, E. (1882). Zur Anatomie der Aphiden. Arb. zool. Inst. Univ. Wien **4**: 397–441.

WITLACZIL, E. (1884). Entwicklungsgeschichte der Aphiden. Z. wiss. Zool. **40**: 559–696.

WITLACZIL, E. (1885). Die Anatomie der Psylliden. Z. wiss. Zool. **42**: 569–638.

WOHLFARTH-BOTTERMANN, K. E. and V. MOERICKE (1959). Gesetzmäsziges Vorkommen cytoplasmatischer Lamellensysteme in Abhängigkeit vom Funktionsrhythmus einer Zelle. Z. Naturf. **14**: 446–450.

WOHLFARTH-BOTTERMANN, K. E. and V. MOERICKE (1960). Zur funktionellen Morphologie der Speicheldrüsen von Homopteren. III. Die Nebendrüse von *Myzus persicae* (SULZ.), Aphididae. Z. Zellforsch. **52**: 346–361.

WORST, J. (1954). A micro-injection system. Q. Jl microsc. Sci. **95**: 469–472.

WRIGHT, K. A. (1969). The anatomy of salivary glands of *Anopheles stephensi* LISTON. Can. J. Zool. **47**: 579–587.

WRISCHER, M. (1965). Elektronenmikroskopische Untersuchungen der Zellnekrobiose. Protoplasma **60**: 355–400.

WYNN, G. G. and H. B. BOUDREAUX (1972). Structure and function of aphid cornicles. Ann. ent. Soc. Am. **65**: 157–166.

YEAGER, J. F. and H. H. KNIGHT (1933). Microscopic observations on blood coagulation in several different species of insects. Ann. ent. Soc. Am. **26**: 591–602.

ZWEIGELT, F. (1915). Beiträge zur Kenntnis des Saugphänomens der Blattläuse und der Reaktionen der Pflanzenzellen. Zentbl. Bakt. ParasitKde **42**: 265–335.

ABBREVIATIONS USED IN FIGURES

a̍	antenna
ag	abdominal ganglion
ao	anal opening
as	abdominal spiracle
asd	afferent salivary duct
asg	accessory salivary gland
at	axon terminal
bcc	basophilic chromatin cluster
bm	basement membrane
bmc	basophilic mesodermal cell
ca	corpus allatum
cb	central body
cc	corpus cardiacum
ce	compound eye
cl	clypeus
clb	clypeo-labrum
co	cornicle
coc	circumoesophageal connective
cov	cornicle valve
cpgo	central part of epipharyngeal gustatory organ
cry	crystal
csd	common salivary duct
ctc	connective tissue cell
cu	cuticule
d	deutocerebrum
daw	dorsal aortic wall
dfac	degenerating fat cell
dg	dorsal groove
dgc	dermal gland cell
dl	dorsal lobe
dlg	distal lamina ganglionaris
dlt	deutocerebral longitudinal tract
dm	desmosome
ds	degenerating symbiont
dv	dorsal vessel
dz	Deckzell
e	epipharynx
eb	eosinophilic body
ego	epipharyngeal gustatory organ
emc	embryonic mesodermal cell
ep	epidermis
epc	epidermal cell
epi	epicranium
esd	efferent salivary duct
et	eye-tubercle
f	foregut
fac	fat cell
fc	food canal
fg	frontal ganglion
gc	glial cell

144

go	gonopore
h	Hauptzell
hcg	hypocerebral ganglion
hg	hindgut
hgc	hindgut cell
hp	hypopharynx
hpgo	hypopharyngeal gustatory organ
hpl	hypopharynx lip
i	intestine
ic	intracellular canaliculum
in	intima
isc	intercellular secretory canaliculum
la	labium
lb	labrum
ld	lipid droplet
llgo	lateral lobe of epipharyngeal gustatory organ
m	mitochondrion
mac	matrix cell
mb	myofibril
mc	myoepithelioid cell
md	mandibular stylet
mdr	mandibular retort-shaped organ
mme	medial medulla externa
mp	minute projections
msg	mesothoracic ganglion
mss	mesothoracic spiracle
mt	microtubuli
mtg	metathoracic ganglion
mv	microvilli
mx	maxillary stylet
mxr	maxillary retort-shaped organ
mxs	maxillary sclerite
my	mycetome
myc	mycetocyte
M1	alary muscle
M2	circular muscle fibres
M3	cornicle valve retractor muscle
M4	divaricator muscle 1, 2, 3, 4, 5, and 6
M5	dorsolongitudinal somatic muscle
M6	elevator muscle of tentorial bar
M7	lateral muscle of clypeus
M8	lateral muscle of mandibular stylet
M9	lateral muscle of maxillary stylet
M10	lateral pharyngeal valve muscle
M11	longitudinal muscle fibres
M12	mesothoracic spiracle muscle
M13	protractor muscle of mandibular stylet
M14	protractor muscle of maxillary stylet
M15	retractor muscle of mandibular stylet
M16	retractor muscle of maxillary stylet
M17	retractor muscle of salivary pump piston
M18	ventrolongitudinal somatic muscle
M19	retractor muscle of salivary pump wall
M20	retractor muscle of afferent salivary duct opening
M21	ventral pharyngeal pump muscle

M22	retractor muscle fibres of anal opening
M23	mandibular muscle fibres to pharyngeal pump floor
M24	maxillary muscle fibres to pharyngeal pump floor
n	nucleus
ne	neuron
nid	neural imaginal disc
nm	neuropile mass
ns	nucleated membrane-like sheath
nu	nucleolus
N1	antennal nerve
N2	branch of medial dorsal nerve
N3	corpus allatum nerve
N4	corpus cardiacum nerve
N5	dorsal lobe nerve to corpus cardiacum
N6	eye-tubercle nerve
N7	frontal nerve
N8	labral nerve
N9	lateral nerve
N10	main abdominal nerve
N11	mandibular retort-shaped organ nerve
N12	maxillary retort-shaped organ nerve
N13	medial dorsal nerve
N14	nerve to cornicle muscles
N15	nerve to divaricator muscles 4 and 5
N16	nerve to leg 1
N17	nerve to leg 2
N18	nerve to leg 3
N19	nerve to mandibular stylet
N20	nerve to mesothoracic spiracle muscle
N21	nerve to proboscis
N22	nerve to salivary pump muscles
N23	optic nerve
N24	pharyngeal nerve
N25	protractor muscle nerve of mandibular stylet
N26	protractor muscle nerve of maxillary stylet
N27	retractor muscle nerve of mandibular stylet
N28	retractor muscle nerve of maxillary stylet
N29	recurrent nerve
N30	ventral nerve
o	ostium
oen	oenocyte
olc	outer layer of cuticule
or	ovariole
ov	oesophageal valve
p	protocerebrum
pb	proboscis
pc	pumpcylinder
pcc	pericardial cell
pch	pumpchamber
pec	perineurium cell
pg	prothoracic ganglion
phd	pharyngeal duct
phg	pharyngeal ganglion
php	pharyngeal pump
phpr	pharynx protuberance

146

phv	pharyngeal valve
pmi	proximal medulla interna
ps	pumpstem
psg	principal salivary gland
pt	piston
r	rectum
rec	rectal cell
s	symbiont
sc	salivary canal
sd	salivary duct
sdc	salivary duct cell
sep	sensillum pore
sg	salivary gland
si	stellate invagination
sog	suboesophageal ganglion
sp	salivary pump
st	stomach
sv	synaptic vesicles
t	tritocerebrum
tb	tentorial bar
tc	tritocerebral commissure
tct	tritocerebral connective tract
te	tendon
tgc	thoracic gland cell
tlt	tritocerebral longitudinal tract
tmc	tracheal matrix cell
tp	tunica propria
tr	trachea
tu	tube
v	vesicles
va	vacuole
vci	voluminous coil of intestine
vr	ventral rod (chitinous ridge)
w	waxy droplet
z	Z-line