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CHEMOTHERAPY OF RODENT MALARIA

ANNUAL REPORT

PART ONE

WALLACE PETERS MD DSc

OCTOBER 1987

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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Department of Medical Protozoology

London School of Hygiene and Tropical Medicine

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London, WC1E 7HT, UK

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

TABLE OF CONTENTS

1. INTRODUCTION

Until September 1987, no new compounds had been submitted during this second year of the contract. As a result of this, the main emphasis of the work has been on our continuing studies into drug resistance, involving further examinations of cross-resistance patterns in resistant lines. In addition, ultrastructural studies comparing treated and untreated parasites resistant to a wide range of antimalarials have been carried out. Some preliminary studies into the *in vivo* effect of calcium channel blockers on chloroquine resistant strains of rodent malaria have also been undertaken.

A problem which was encountered with a virus infection in our mosquito colony stimulated a series of studies into the action of some antiviral agents on polyhedrosis virus in *Anopheles stephensi*. Ultrastructural studies of treated and control mosquitoes are included in this report. Keywords: chemotherapy

In response to a request from Dr T Sweeney, definitive test data from all blood schizontocidal and causal prophylactic tests were assembled. Where necessary the test results were recalculated using our current procedures in order to provide data which could be validly compared. These data are appended to the Report as Appendices 5.3 and 5.4.

2. ADMINISTRATIVE EVENTS

Staff employed on US Army funds are as follows:

Senior Technologist/ Research Assistant	- Mr B L Robinson	100% Time
Technicians	- Ms A West	100% Time
	- Ms J R Cox	100% Time
Secretary	- Mrs B A Sargeaunt	25% Time

Other staff associated with the project but not financially

supported by USAMRDC are :

Professor W Peters (Principal Investigator)	20% Time
Dr D C Warhurst (Biologist)	20% Time
Dr D S Ellis (Electron Microscopist)	10% Time

Dr Ellis has now retired and, in future, advice and assistance with ultrastructural studies will be supplied by his successor, Dr S L Croft. Dr Croft has been associated with the Principal Investigator for many years, having obtained his PhD as a student in the Department of Parasitology at the Liverpool School of Tropical Medicine whilst Professor Peters was Professor of Parasitology there. Subsequently, Dr Croft has worked in the Department of Medical Protozoology in the London School of Hygiene and Tropical Medicine and at the Wellcome Research Laboratories, Beckenham, Kent. He has had extensive experience of protozoology, especially Leishmania, and electron microscopy.

3. CHEMOTHERAPY STUDIES

We have been increasingly of the opinion that antimalarial drugs exert their effects by relatively few different mechanisms. This notion has been induced by the observation that multiple drug resistance is both a common phenomenon and one in which resistance to one compound, e.g. chloroquine, can entail cross-resistance to other compounds in apparently quite divergent chemical classes. Moreover, once resistance has evolved to a particular compound, the resistance parasites appear to develop resistance to ther compounds with great rapidity. We therefore have followed two lines of investigation, using a wide battery of drugs - (1) the study of the ultrastructure of drug-resistant rodent Plasmodium and the effects of the compounds on them (as compared with the effects of the same drugs on drug sensitive

organisms) and (2) a careful study of cross-resistance patterns. This work has entailed a long and painstaking series of investigations that are not as yet complete. Some observations, e.g. on the ultrastructure of certain drug-resistant lines, are still being completed so that a final analysis of all our data must await next year's Annual Report.

In the meantime, it is becoming clear that antimalarials in many chemical classes induce basically similar ultrastructural changes in intraerythrocytic parasites, namely modifications in the structure of the malaria pigment, and damage to nuclear, mitochondrial and external parasite membranes. Moreover, once a moderately high level of resistance has been established to these types of compounds, the drugs themselves fail to produce these physical manifestations of their action. So far we have failed to find ultrastructural evidence for gross nuclear changes suggestive of gene amplification in any line. Details of the electron microscope studies and cross-resistance experiments carried out to the end of October 1987 are summarised in the following pages.

The chronic problem of viral contamination of Anopheles colonies that plagues all who work with experimental malaria has stimulated us both to investigate the biology and transmission of the contaminants and to seek ways of decontaminating the colonies. We have had promising preliminary results in both directions, in following the vertical transmission of polyhedrosis virus in A.stephensi and in identifying an antiviral agent that holds promise of containing its spread. Data on this work are presented in Section 3.4 below (p.89).

3.1 Cross-resistance studies

Data are contained in the detailed report sheets which are appended in Part 2 as Tables 5 through 119. Complete results of cross-resistance studies completed to date are shown in Tables 1 through 4.

The activities of twenty-one antimalarial compounds are being compared in twenty-nine different sensitive and resistant strains of Plasmodium berghei and P.yoelii in order to define the patterns of cross-resistance which exist.

Other than sulfadoxine and the 3:1 combination of sulfadoxine and pyrimethamine (comparable with Fansidar), neither of which are currently favoured for use, almost all the compounds tested in this series showed marked lack of activity against at least some of the strains used. It should be noted that, for convenience, the 3:1 combination of sulfadoxine and pyrimethamine is described as Fansidar in this Report. The other exceptions to this were floxacrine and clindamycin, both of which were hyperactive against all of the strains which have been tested to date.

As a direct result of this series of investigations it has been realised that the practice of directly comparing resistant lines which have been derived from P.yoelii ssp. (NS strain) with P.berghei N strain in the four day test for blood schizontocidal activity will tend to produce exaggerated resistance factors (I_{90} values).

For example, the ED_{90} of chloroquine against the artemisinin resistant ART strain (derived from NS) was found to be 400 mg/kg. When resistance is calculated by comparison with N strain (ED_{90} = 3.1 mg/kg), an I_{90} value of 129 is obtained as opposed to an I_{90} of 7.1 when related to the ED_{90} of the parent NS strain.

Consequently, resistance factors for strains of this type will in future be obtained by comparison with the appropriate parent strain in order to avoid unrealistic results.

3.2 The effect of calcium channel blockers on chloroquine resistant strains of rodent malaria

Our preliminary studies on the effect of calcium channel blockers, which used Verapamil as a representative compound, were carried out on the highly chloroquine-resistant RC strain of P. berghei. No evidence of any activity was detected with Verapamil, neither alone nor in combination with chloroquine. However, when these experiments were repeated using P.yoelii ssp. NS strain, marked potentiation of the effect of chloroquine was noted. Verapamil itself still produced no reduction at all in the parasitaemia. Data from these tests are summarised in Tables 120 through 130. We have long felt that P.yoelii NS is a better model for chloroquine resistant P.falciparum than P.berghei RC (see Peters et al., 1975, Ann.trop.Med.Parasitol., 69 : 155 - 171).

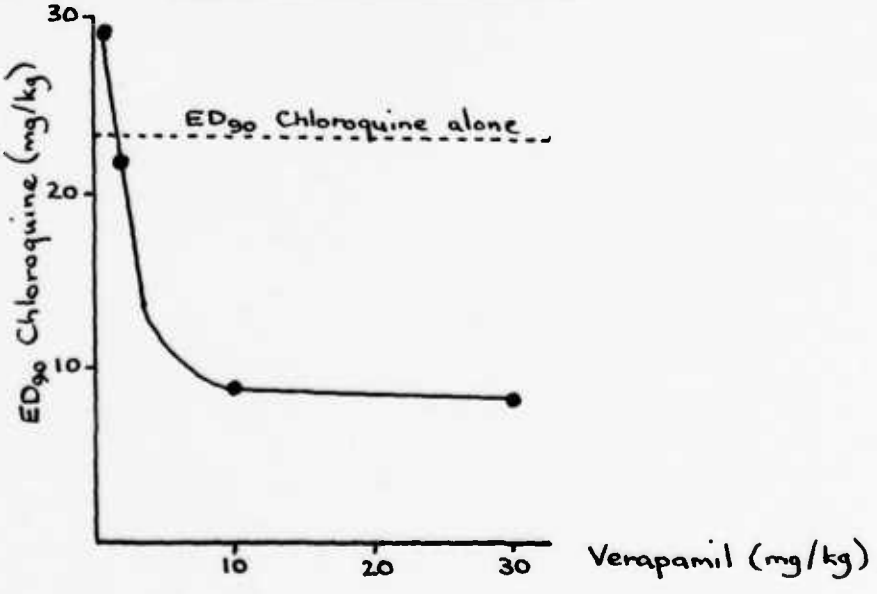

Figure 1. A graphic representation of the influence of increasing Verapamil dosage on the ED90 of chloroquine in the moderately chloroquine resistant P.yoelii ssp. NS strain.

Unfortunately, the absence of direct animalarial activity with Verapamil itself makes it impossible to produce an isobologram to demonstrate the synergistic interaction with chloroquine. However, this has been portrayed graphically in Figure 1 by plotting the ED_{90} values of chloroquine at differing levels of Verapamil doseage. The total lack of activity of chloroquine, when administered either alone or in combination with Verapamil against the RC strain, prevents the preparation of a similar graph for this strain. The lack of demonstrable synergistic activity between calcium channel blockers and chloroquine against the RC strain of P.berghei has been noted by us in other tests and is comparable with the lack of activity which was found in similar conditions with desferrioxamine. At this stage, it remains an open question whether this is a reflection of an essential difference between the two distinct species of parasite or an indication of two completely different mechanisms of chloroquine resistance. Further tests with Verapamil and other calcium channel blockers are in progress.

3.3 Ultrastructural studies on resistant strains

A series of ultrastructural studies has been carried out to compare thirty different rodent malaria parasites. These include cloned and uncloned P.berghei N and RC strain, P.yoelii yoelii 17X strain, P.y.nigeriensis, P.yoelii ssp. NS strain and a number of drug resistant lines derived from P.berghei and P.yoelii ssp. NS. It will be appreciated that we do not yet have ideal electron micrographs of each series of drug-parasite combinations. Those presented here should be viewed, in some cases, as preliminary material only. A further programme of experiments is to be performed in 1988 to complete these series and examine the

morphological effects of previously unreported compounds on drug-sensitive parasites.

3.3.1 P.berghei N strain.

This strain, which has come in a direct line from the original Keyberg 173 isolate of P.berghei, is sensitive to all the standard antimalarial drugs. As a result of many years of syringe passage this strain has lost the ability to produce gametocytes. Uncloned N strain, however, will occasionally show the presence of small numbers of gametocytes since the original isolate was a cryptic mixture of P.berghei and P.yoelii ssp.. This latter species (NS strain) has been separated from the mixture by cyclical transmission through A.stephensi and is discussed further in Section 3.21.

Plates 1 - 4 show the characteristic ultrastructure of the trophozoites of this strain. There are many large, but not swollen, mitochondria and many small dark crystals of pigment, which are not generally hard enough to have dropped out during processing. The overall state of the parasite, its nucleus and endoplasmic reticulum are normal.

Plates 5 - 7 are also of P.berghei N strain, but in this case the strain was a clone. The considerable degree of peripheral vacuolation and nuclear blebbing apparent in these preparations is artefactual.

Plate 1. Plasmodium berghei N strain trophozoite (X 20,000)

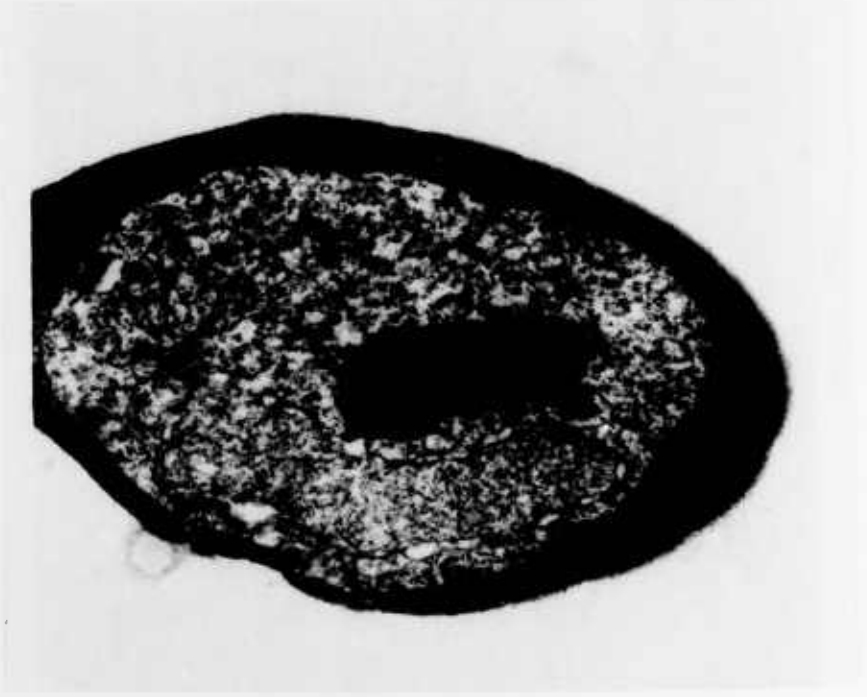

Plate 2. Plasmodium berghei N strain trophozoite (X 34,800)

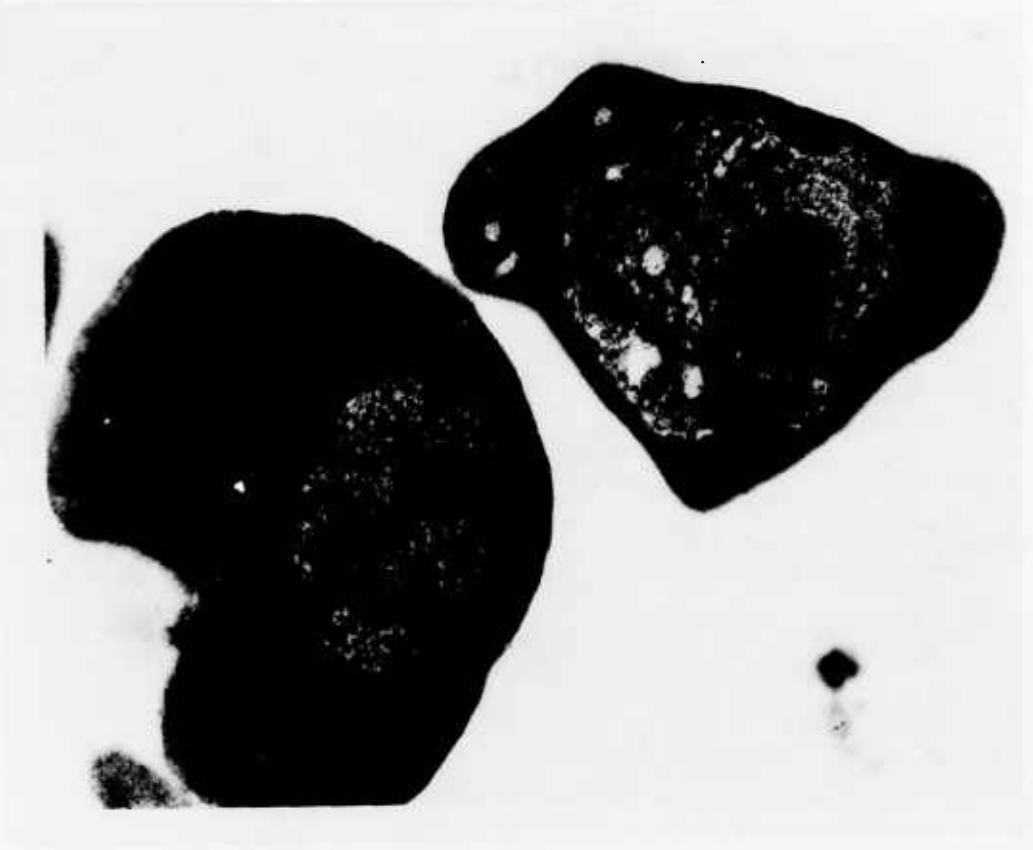

Plate 3. Plasmodium berghei N strain trophozoite (X 20,000)

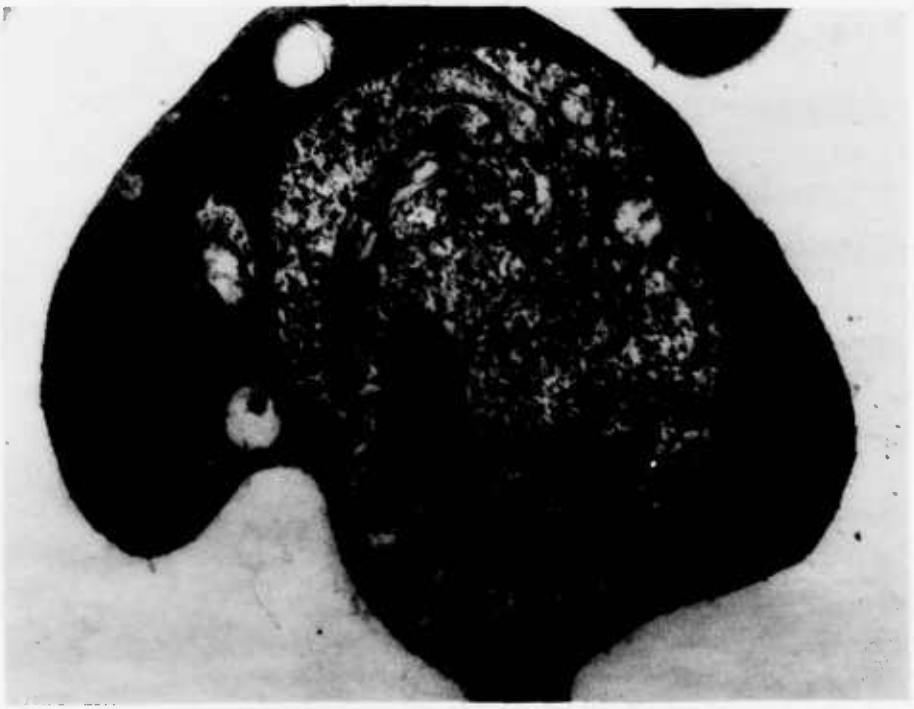

Plate 4. Plasmodium berghei N strain trophozoite (X 32,000)

Plate 5. Plasmodium berghei N strain clone trophozoite (X 40,000)

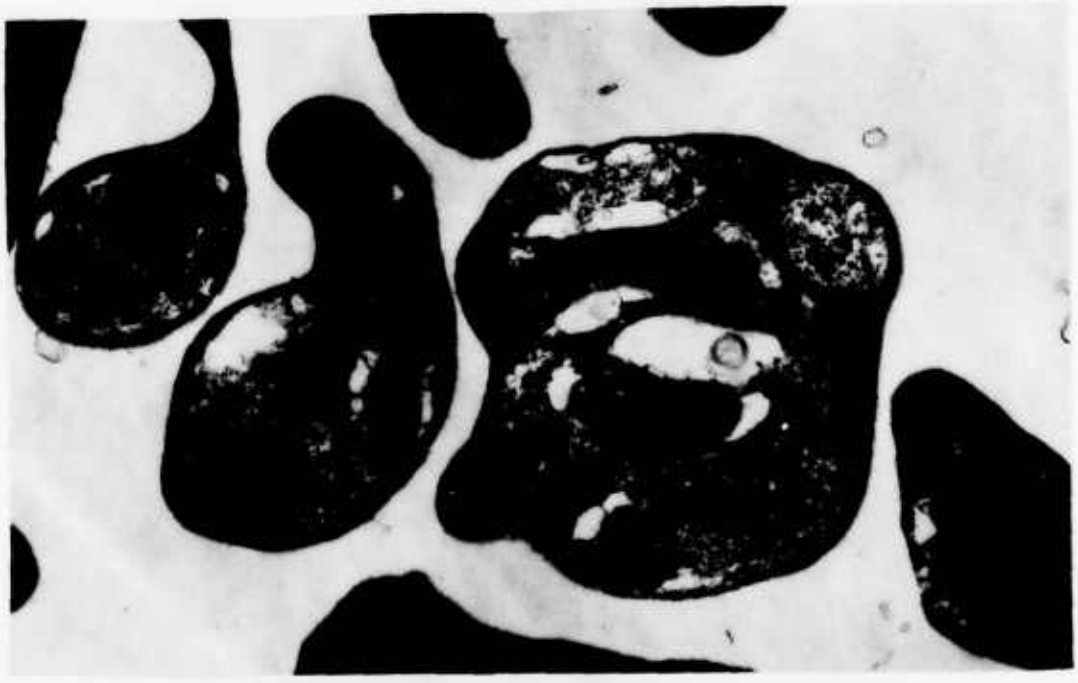

Plate 6. Plasmodium berghei N strain clone (X 13,200)

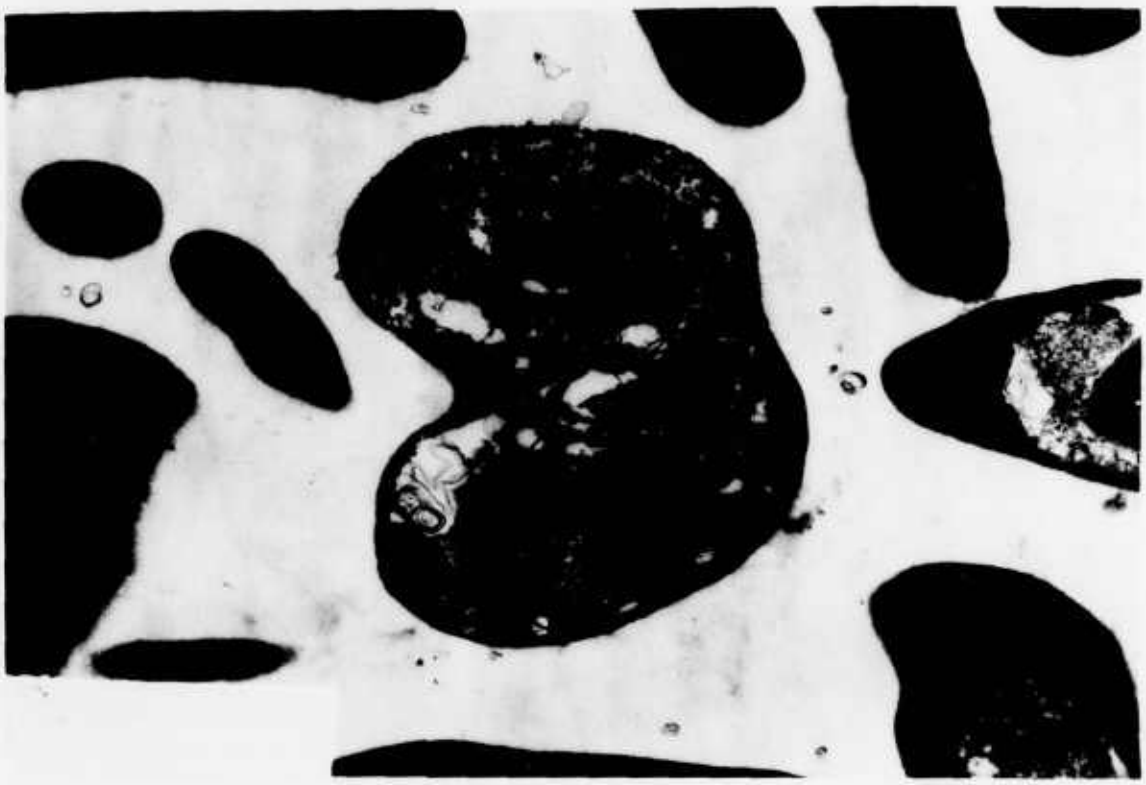

Plate 7. Plasmodium berghei N strain clone (X 16,600)

3.3.2 P.berghei RC strain.

The RC strain of P.berghei is a highly chloroquine-resistant line which was produced by gradually increasing dosing of N strain with chloroquine. The parasite is confined to immature erythrocytes and the growth curve follows the pattern of the reticulocyte response. High parasitaemias occur only when the reticulocyte response is artificially stimulated, for example by treatment with phenylhydrazine. The infection is chronic and, usually, self-resolving.

RC strain is almost unaffected by chloroquine and cross-resistance is present to amodiaquine, primaquine, quinine, cinchonine, quinidine, mefloquine, halofantrine, artemisinin, pyronaridine, mepacrine and Mannich bases (such as WR 228258). It is hypersensitive to pyrimethamine, sulfadoxine, Fansidar and

floxacrine.

Ultrastructurally, untreated RC strain parasites have a pale "foamy-looking" cytoplasm with few ribosomes. The pigment is rather fine and may be difficult to identify.

Plate 8. Untreated P.berghei RC strain trophozoite (X 20,000)

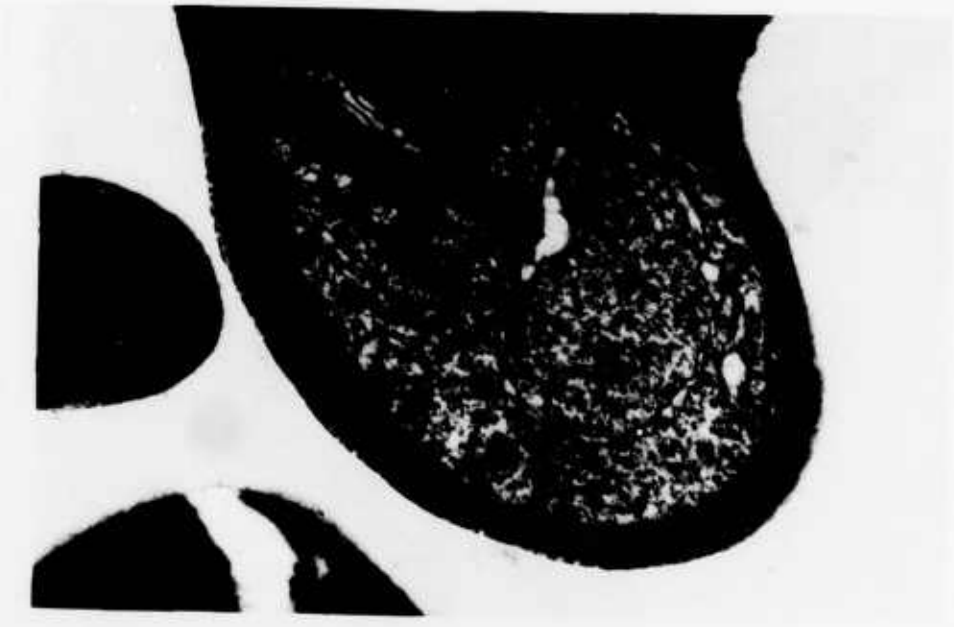

Plate 9. P.berghei RC strain. Passaged with daily dosage of 60 mg/kg chloroquine diphosphate. (X 34,800)

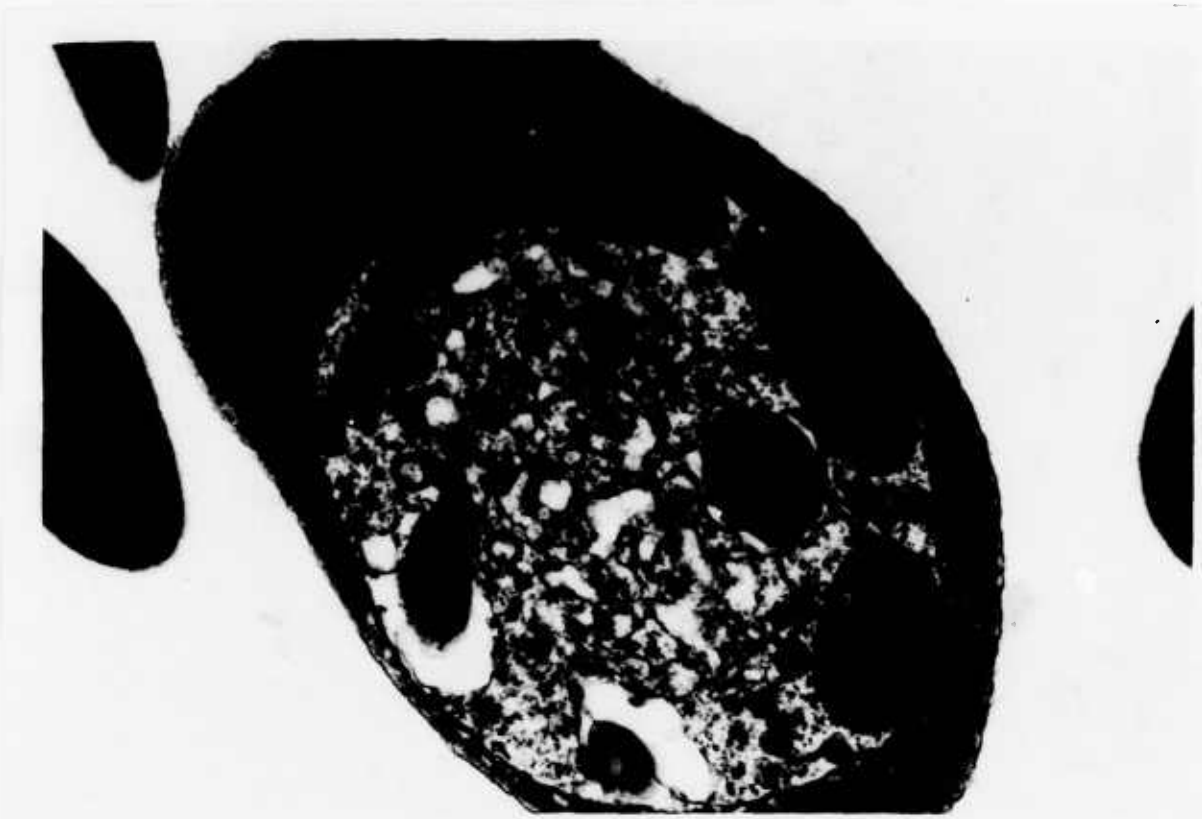

Plate 10. P.berghei RC strain. Passaged under daily chloroquine pressure (60 mg/kg). (X 32,000)

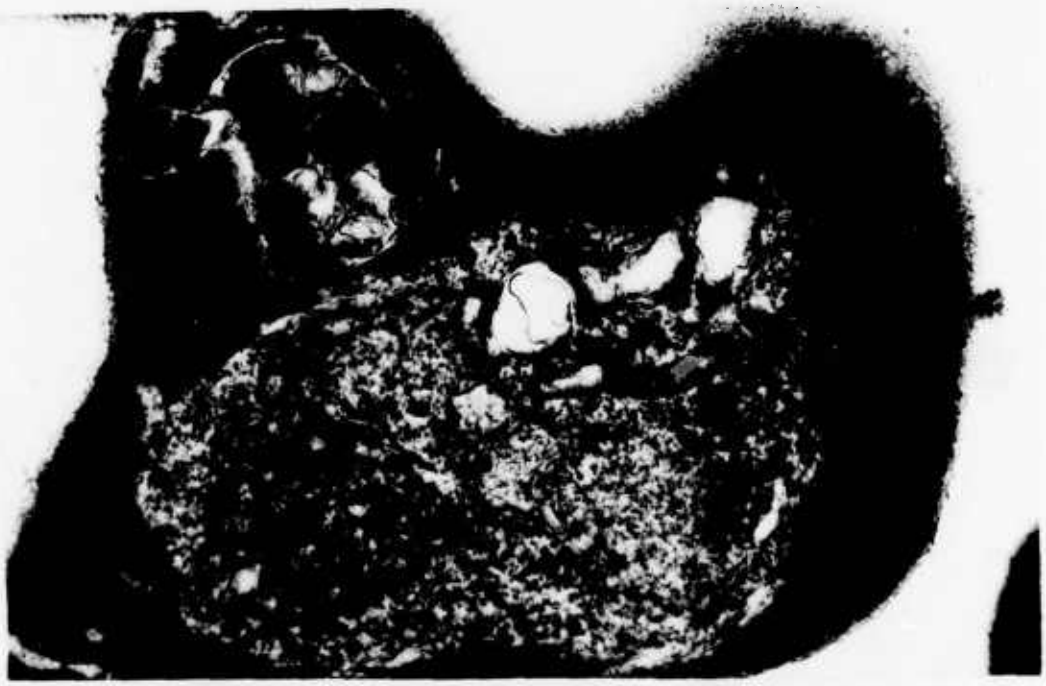

Plate 11. Untreated cloned P.berghei RC strain. Note the fine pigment coalescing in a "residual body". (X 32,000)

Plate 12. Chloroquine treated P.berghei clone. (X 32,000)

3.3.3 P.berghei P strain.

P.berghei P strain was derived from N strain in the same way as RC. It is markedly resistant to primaquine and possesses slight cross-resistance to quinidine, mefloquine, artemisinin. There is also a marked cross-resistance to cycloguanil. P strain is hypersensitive to chloroquine, cinchonine, sulfadoxine, Fansidar, floxacrine, clindamycin and WR 228258.

EM examination shows that the untreated parasites are in good condition with the nucleus and its associated membranes appearing normal. The endoplasmic reticulum is active looking and many healthy mitochondria are present. The pigment has a soft, rounded appearance. When subjected to primaquine pressure (60 mg/kg/day), some internal vacuolation is seen and there is a

certain amount of membrane disorganisation, notably in the mitochondria. All the pigment appears soft and there are occasional aggregations into single vacuoles.

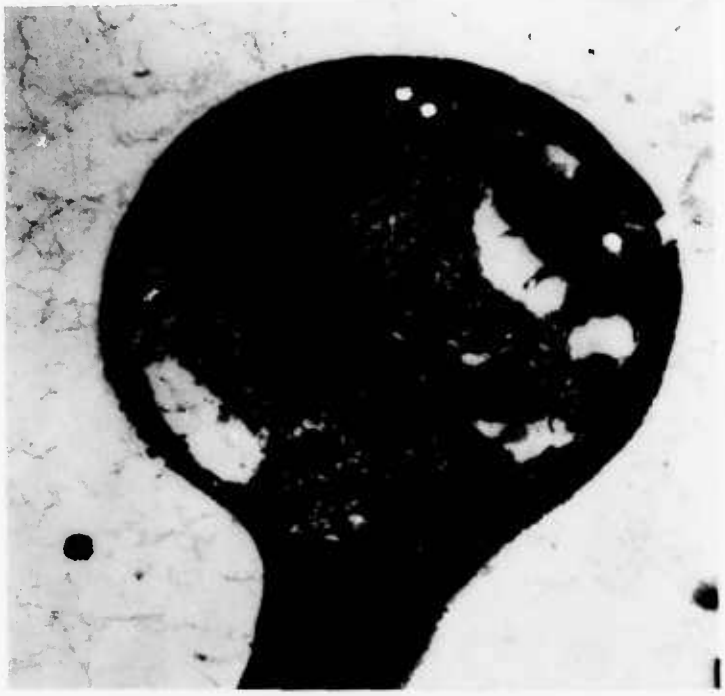

Plate 13. Untreated P.berghei P strain. (X 26,000)

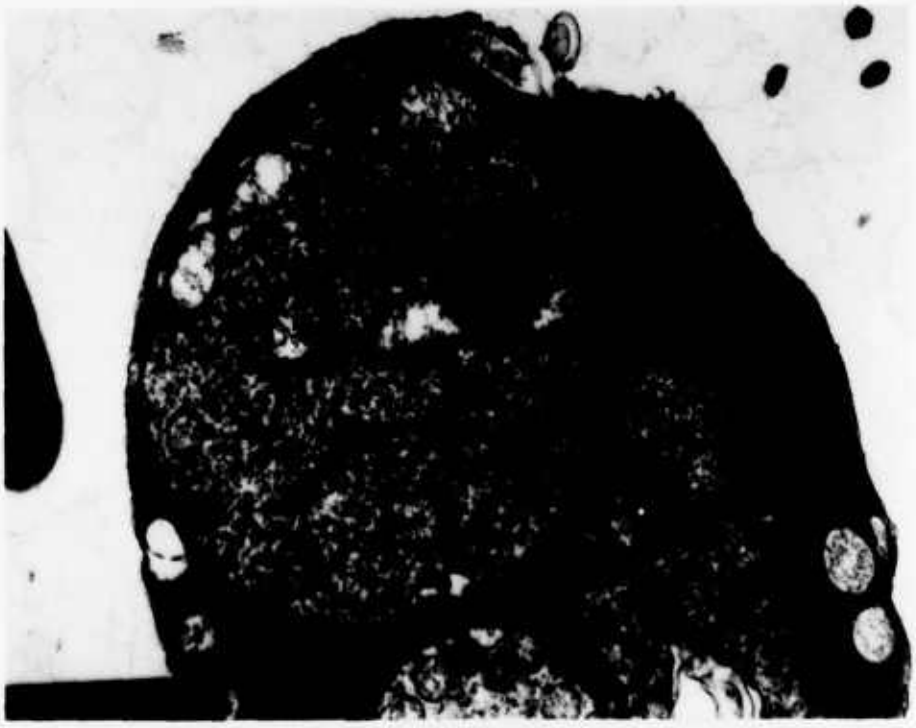

Plate 14. Untreated P.berghei P strain. Note the numerous, large mitochondria. (X 32,000)

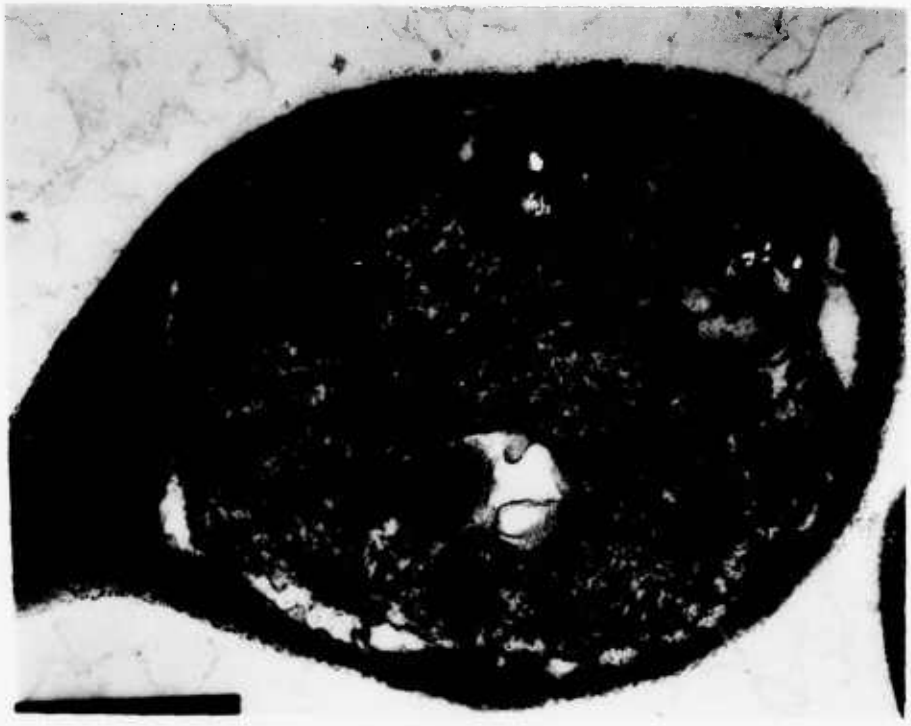

Plate 15. Primaquine treated P.berghei P strain. (X 40,000)

3.3.4 P.berghei B strain.

P.berghei B strain has a marked primary resistance to cycloguanil, which was developed by application of a gradually increasing dose of cycloguanil to successive passages. Cross-resistance is present in this strain to pyrimethamine, menoctone and doxycycline. B strain is hypersensitive to cinchonine, sulfadoxine, floxacrine and WR 228258.

Ultrastructurally, both treated and untreated control parasites of B strain look almost the same. The main difference is seen in the appearance of the plentiful pigment, which in the control is soft and arranged in very long thin rods. In cycloguanil treated parasites the pigment rods are shorter and paler in appearance.

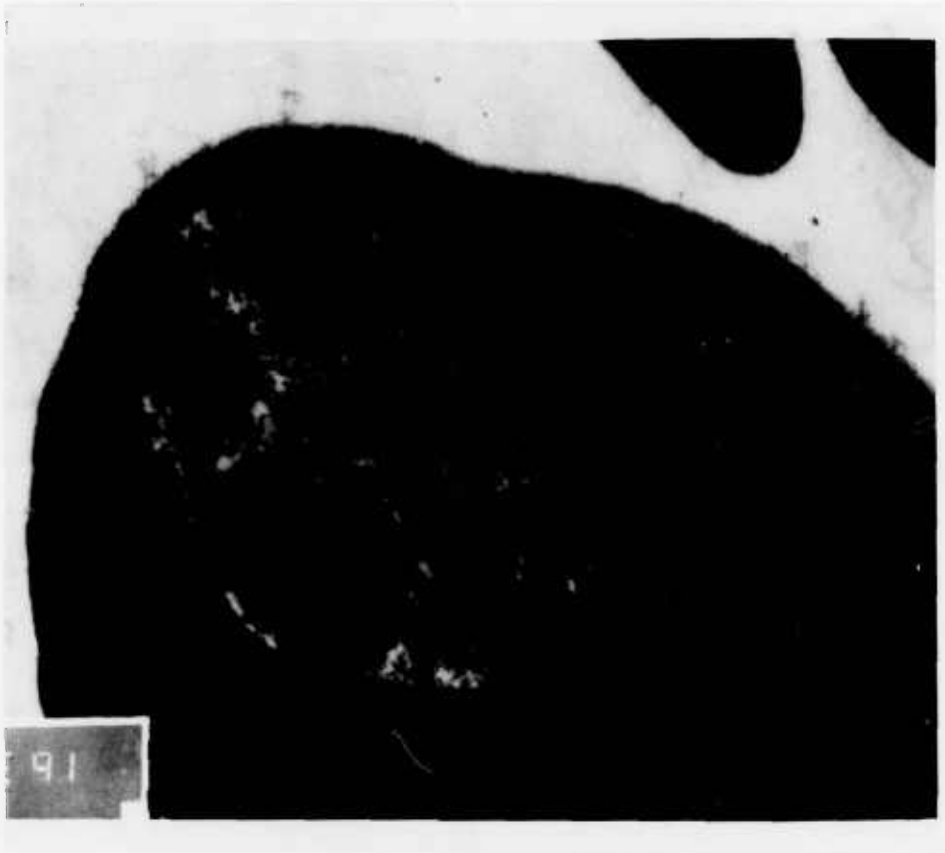

Plate 16. Untreated P.berghei B strain. (X 32,000)

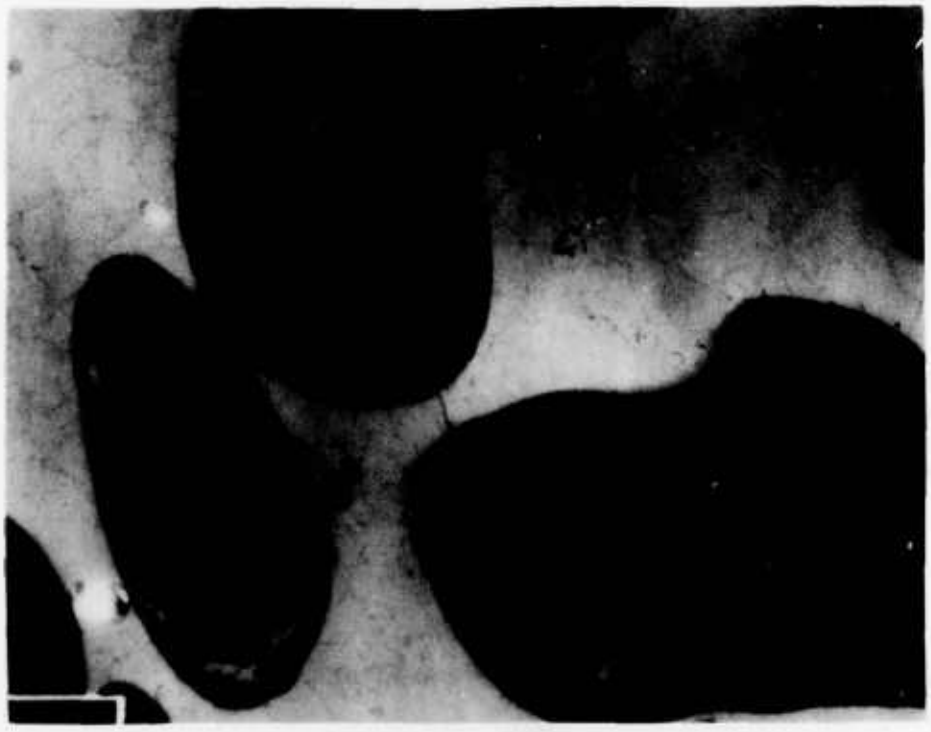

Plate 17. Untreated P.berghei B strain. (X 60,000)

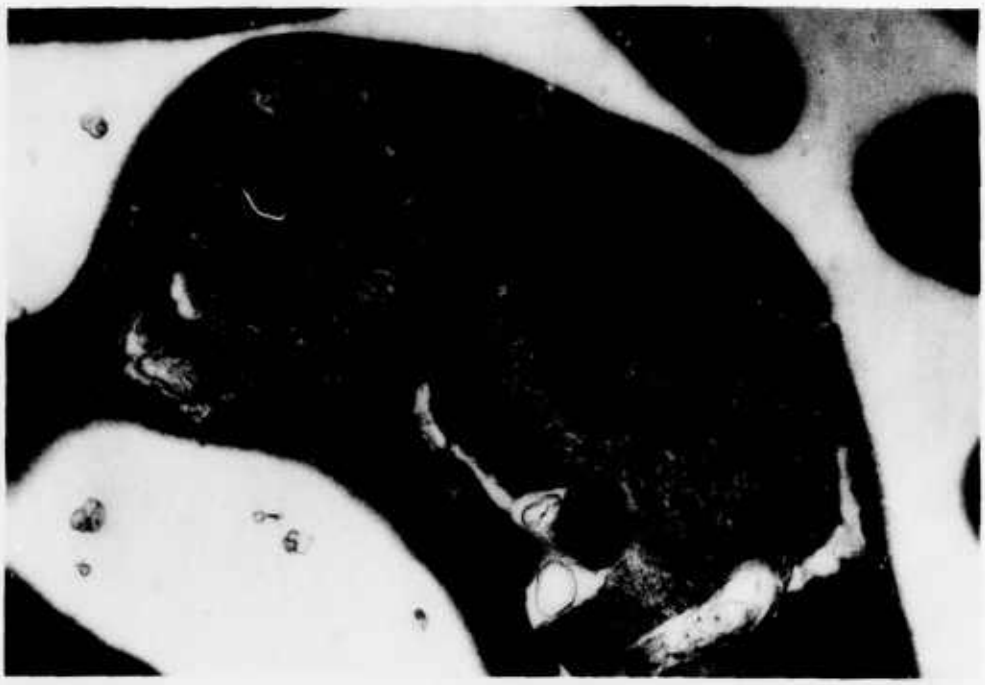

Plate 18. Cycloguanil treated P.berghei B strain. (X 26,000)

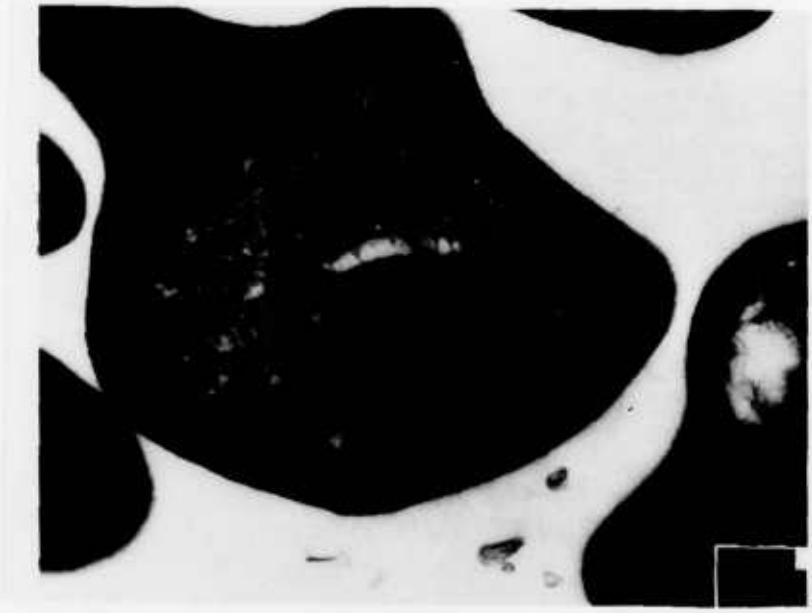

Plate 19. Cycloguanil treated P.berghei B strain.

3.3.5 P.berghei PYR strain.

Unlike the preceding strains, the PYR strain was developed from P.berghei NY strain. A high level of resistance to pyrimethamine was produced as a result of a single exposure to this compound and has remained as a stable feature. Cross-resistance to primaquine, quinidine, cycloguanil, menoctone and doxycycline are present and PYR strain is hypersensitive to cinchonine, sulfadoxine, floxacrine, clindamycin, mepacrine and WR 228258.

Electron microscopy reveals that PYR parasites display a normal, healthy appearance. The mitochondria have dark matrices and there is plenty of soft, pale pigment present. Apart from the presence of some cytoplasmic spaces, particularly around the nucleus, pyrimethamine treated parasites are morphologically identical to the untreated controls.

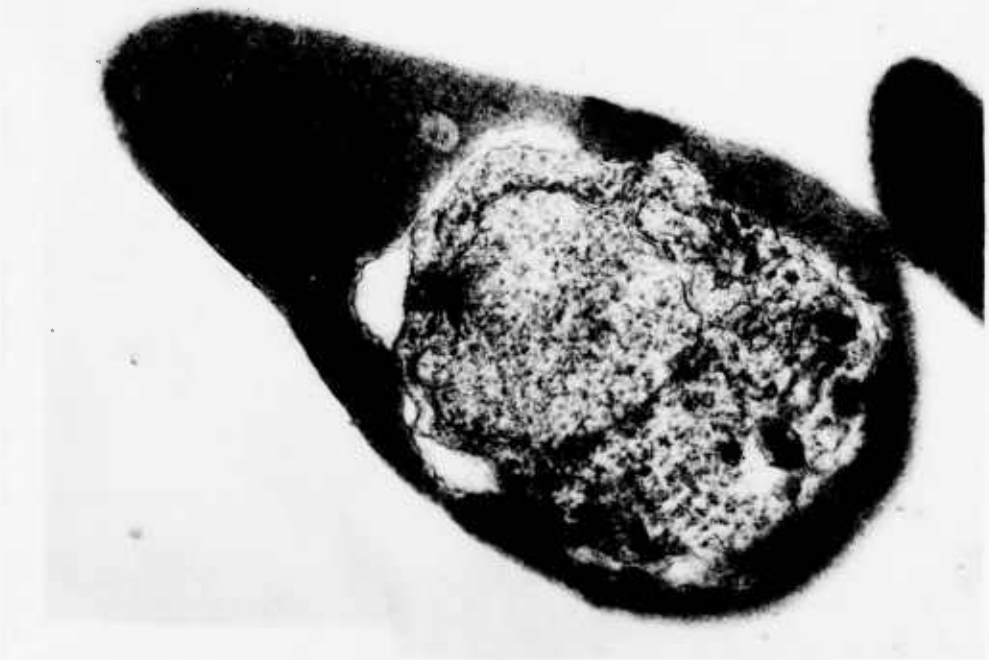

Plate 20. Untreated P.berghei PYR strain. (X 32,000)

Plate 21. Pyrimethamine treated P.berghei PYR strain. (X 32,000)

3.3.6 P.berghei ORA strain.

The ORA strain was developed by the gradually increasing dose technique from P.berghei NY using sulphaphenazole (Orisulf). A very high level of resistance developed, not only to this drug but also to all other sulphonamides. In addition, cross-resistance is present to pyrimethamine, cycloguanil and doxycycline. This strain is hypersensitive to primaquine, cinchonine, floxacrine, clindamycin and WR 228258.

Ultrastructural studies of the ORA strain shows healthy looking parasites, which in the untreated controls are unusually full of ribosomes and contain large pigment crystals in vacuoles. Treated parasites are much vacuolated, with some aggregation of ribosomes and an increased number of mitochondria. The pigment is conspicuous and has a similar appearance to that of the controls.

Plate 22. Untreated P.berghei ORA strain. (X 26,000)

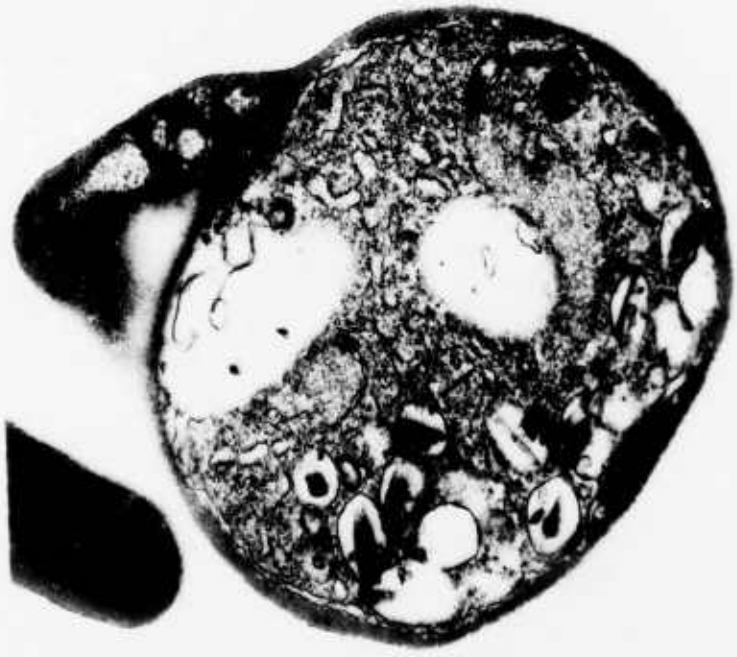

Plate 23. Untreated P.berghei ORA strain. (X 26,000)

Plate 24. Orisulf treated P.berghei ORA strain. (X 50,000)

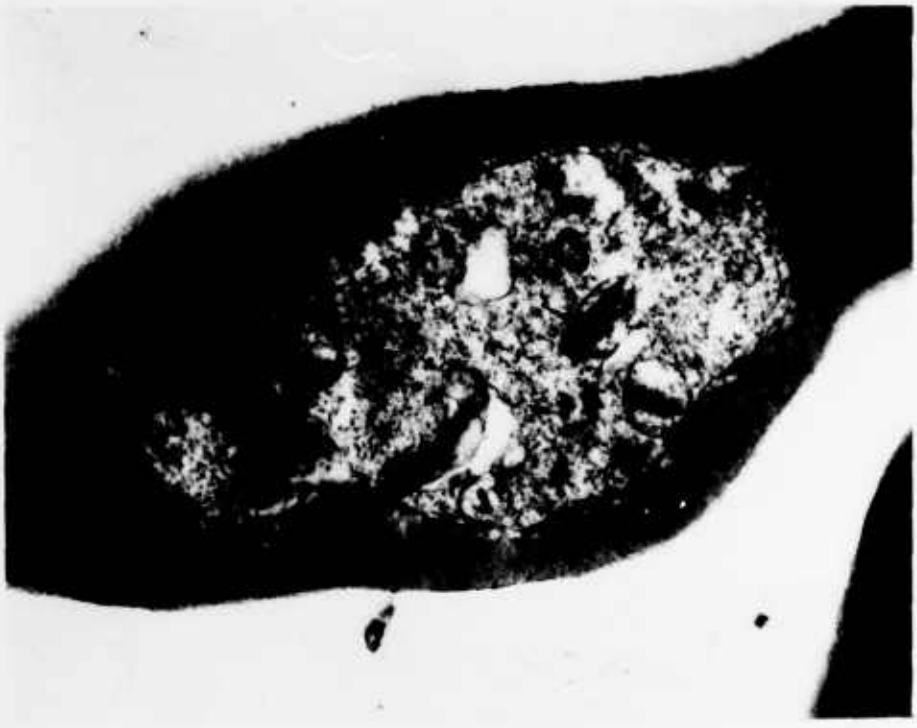

Plate 25. Orisulf treated P.berghei ORA strain. (X 40,000)

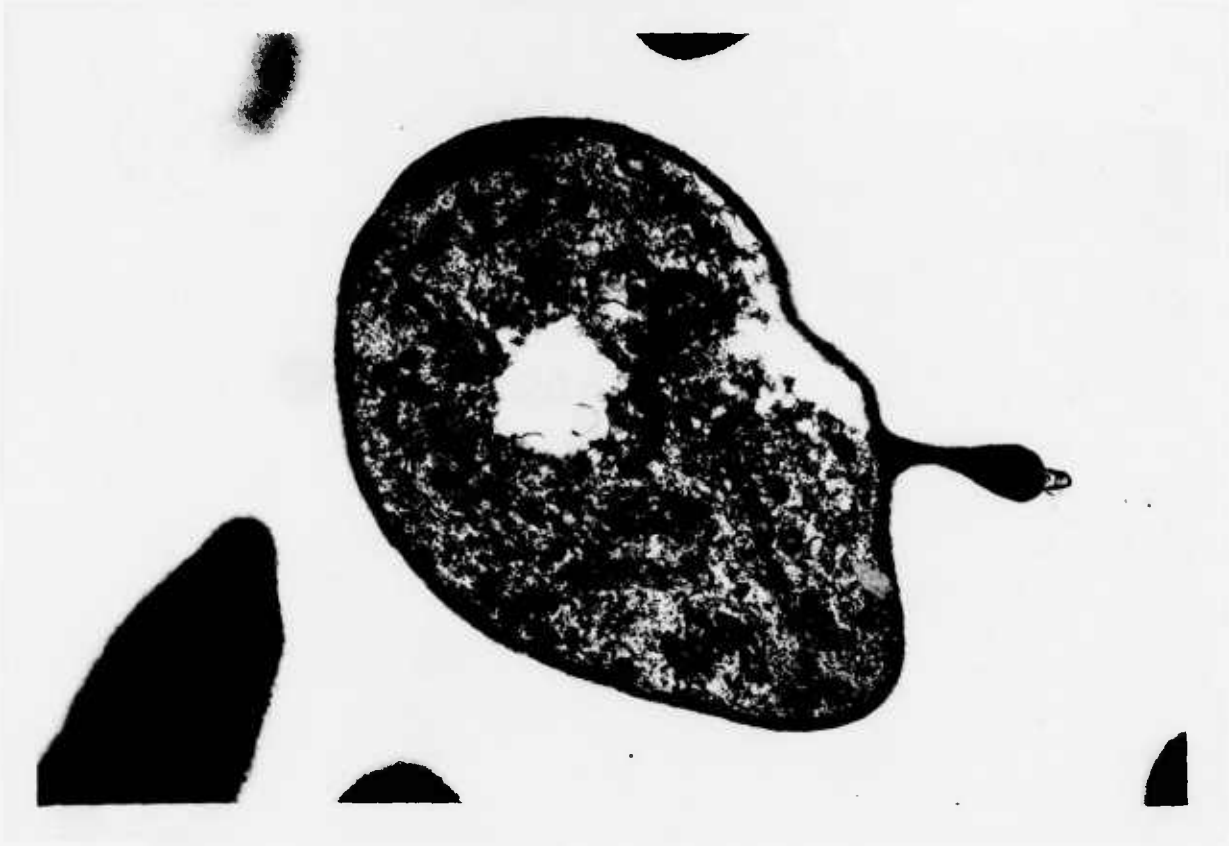

Plate 26. Orisulf treated P.berghei ORA strain, possibly a macrogametocyte. (X 20,000)

3.3.7 P.berghei MEN strain

The highly menoctone-resistant MEN strain, which is derived from P.berghei N strain, shows only slight cross-resistance to pyrimethamine, doxycycline and LON 1765. This strain is hypersensitive to primaquine, quinine, cinchonine, mefloquine, halofantrine, sulfadoxine, Fansidar, clindamycin and WR 228258.

The ultrastructural appearance of the treated and untreated parasites differs quite substantially. The untreated controls have very active looking endoplasmic reticulum, unusually large mitochondria and large pigment granules. Much of the pigment is located in central vacuoles rather than as usual at the periphery of the parasite. Parasites which have been exposed to menoctone show extensive peripheral vacuolation and the endoplasmic

reticulum and its membranes appear disordered. In contrast to the control, the pigment is somewhat softened. Some mitochondria show thickening of the membranes.

Plate 27. Untreated P.berghei MEN strain. (X32,000)

Plate 28. Untreated P.berghei MEN strain. (X 32,000)

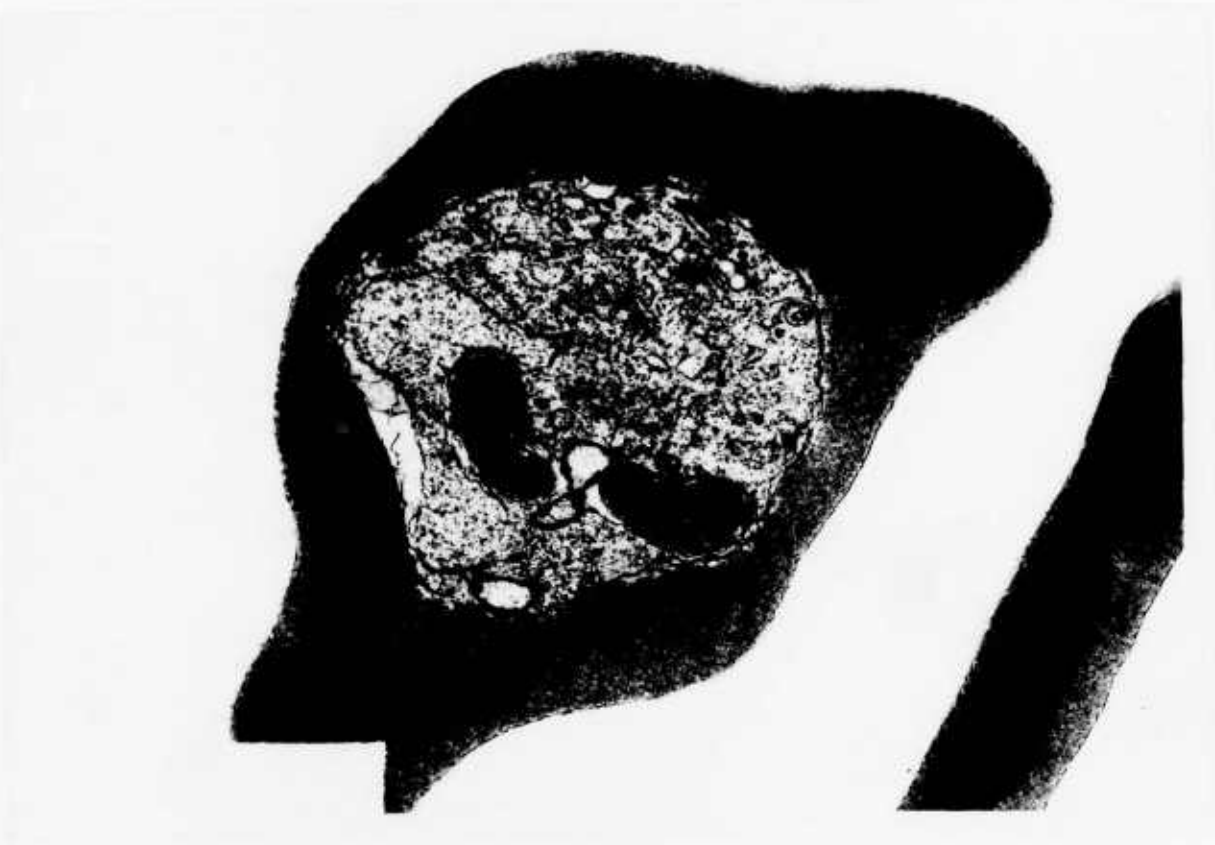

Plate 29. Untreated P.berghei MEN strain. (X 26,000)

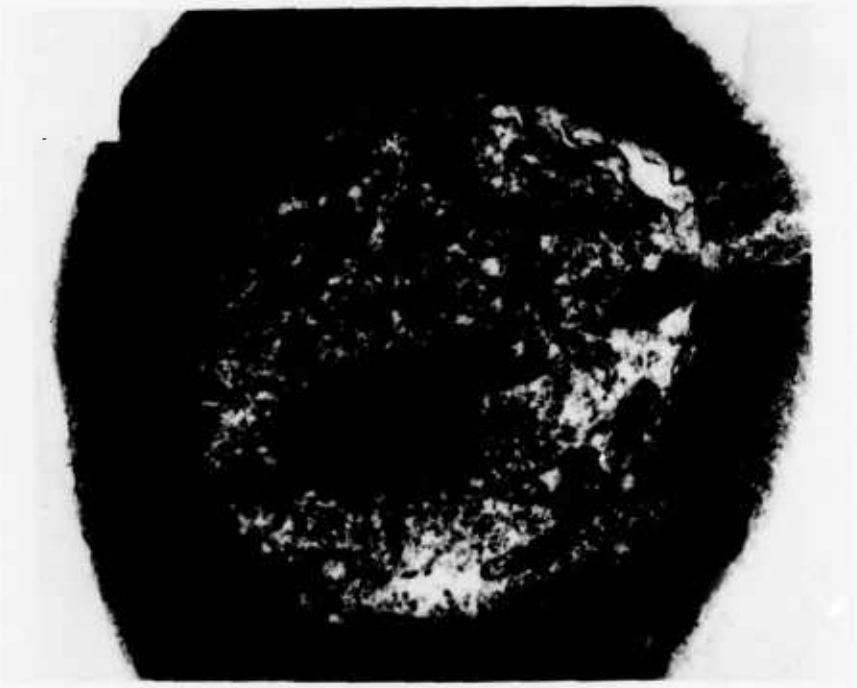

Plate 30. Untreated P.berghei MEN strain. (X 52,000)

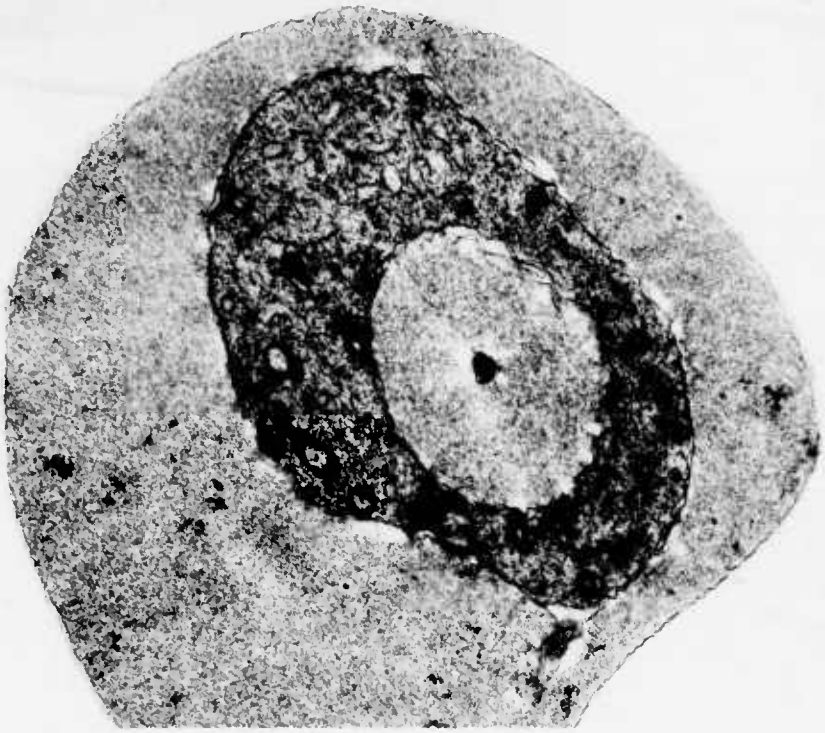

Plate 31. Untreated P.berghei MEN strain. (X 26,000)

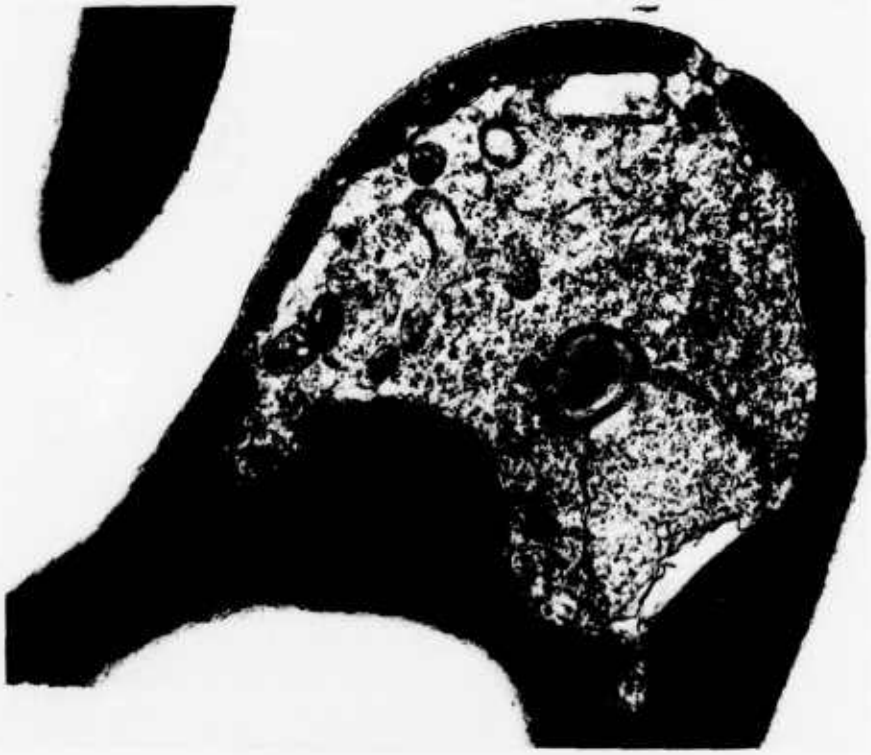

Plate 32. Menoctone treated P.berghei MEN strain. (X 40,000)

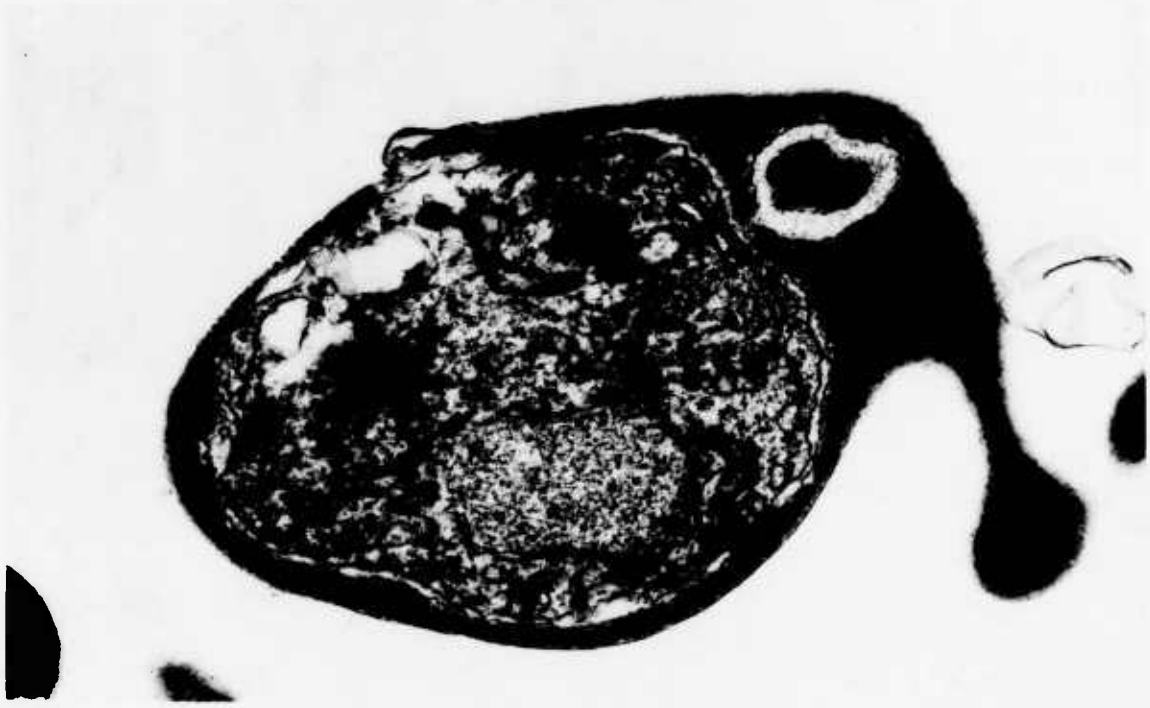

Plate 33. Menoctone treated P.berghei MEN strain. (X 40,000)

3.3.8 P.berghei N/1100 strain.

The mefloquine-resistant N/1100 strain was produced from P.berghei N strain by the relapse technique. In addition to being highly resistant to mefloquine, N/1100 is also cross-resistant to amodiaquine, quinine, cinchonine, quinidine, halofantrine, artemisinin, doxycycline, mepacrine, WR 228258 and LON 1765. Hypersensitivity is present to pyrimethamine, sulfadoxine, Fansidar, floxacrine and clindamycin.

When examined with the electron microscope, untreated N/1100 strain parasites are seen to have rather prominent mitochondria and small pigment granuoles in single vacuoles. Exposure to mefloquine causes some mitochondrial swelling. The pigment tends to aggregate into small vacuoles (3 or 4 pieces in each vacuole) and becomes rather "soft" looking.

Plate 34. Untreated P.berghei N/1100 strain. (X 32,000)

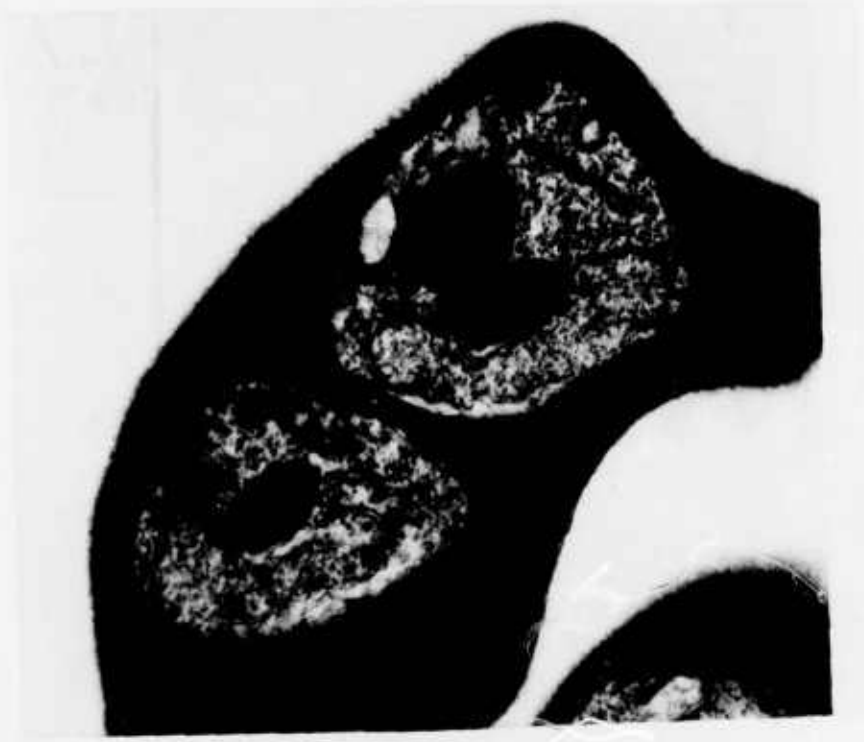

Plate 35. Untreated P.berghei N/1100 strain. (X 32,000)

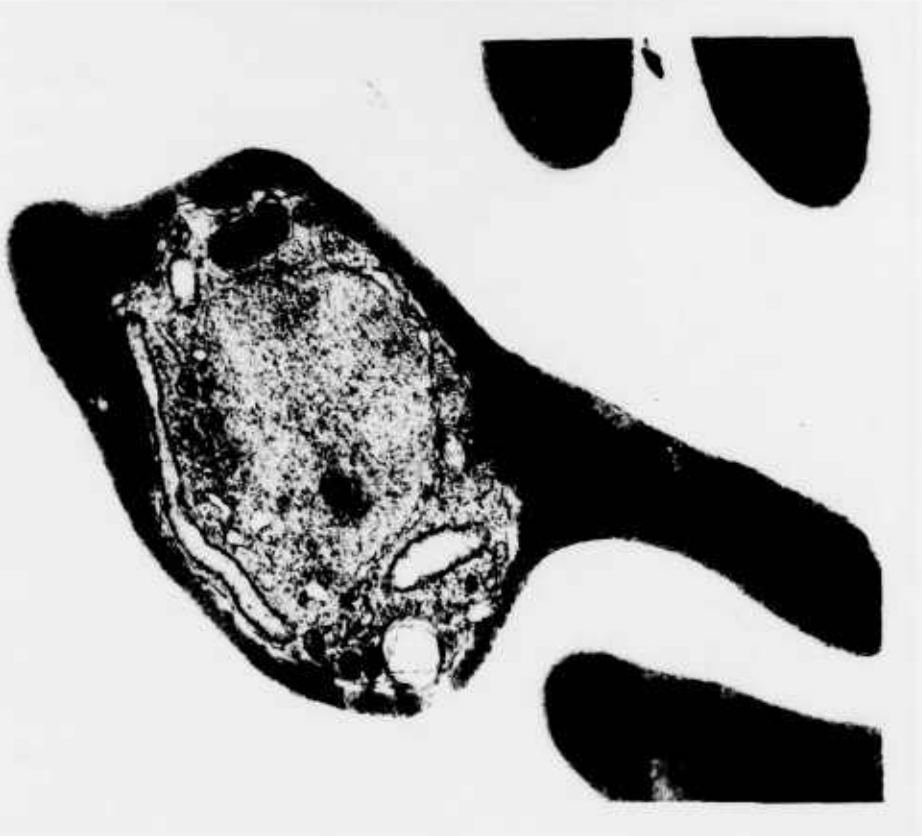

Plate 36. Untreated P.berghei N/1100 strain. Early schizogony.(X 32,000)

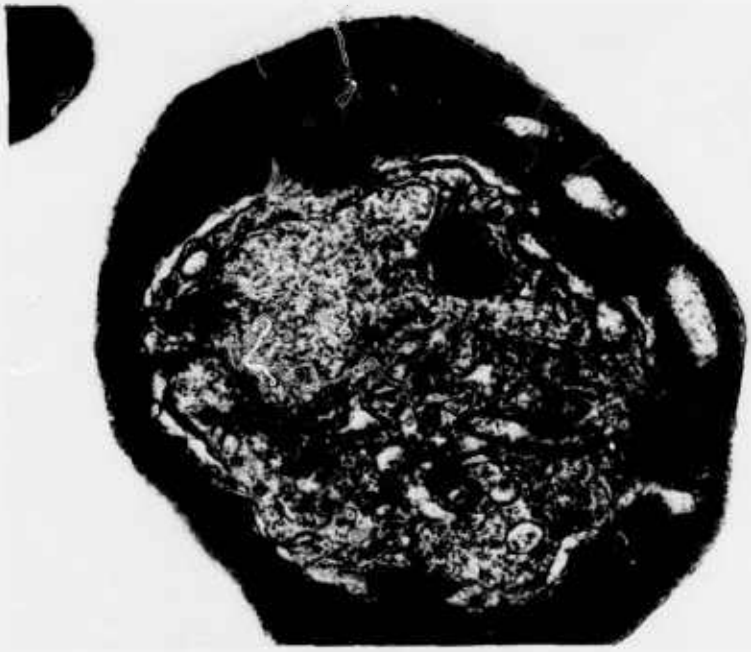

Plate 37. Untreated P.berghei N/1100 strain. Early schizogony.(X 40,000)

Plate 38. Mefloquine treated P.berghei N/1100 strain. (X 50,000)

Plate 39. Mefloquine treated P.berghei N/1100 strain. (X 40,000)

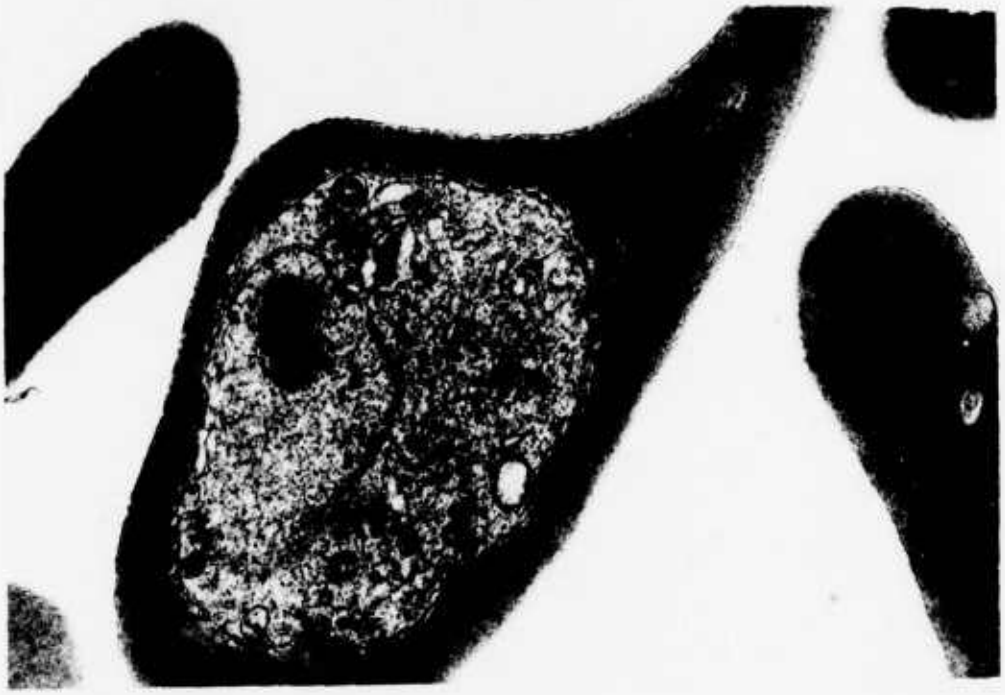

Plate 40. Mefloquine treated P.berghei N/1100 strain. (X 32,000)

3.3.9 P.berghei N/1708 strain.

The N/1708 strain was produced by inducing resistance to the Mannich base WR 228258 in P.berghei N strain by the relapse technique. N/1708 is cross-resistant to chloroquine, quinidine, doxycycline, mepacrine, and LON 1765. Hypersensitivity was noted to cinchonine, sulfadoxine, Fansidar and floxacrine.

Ultrastructurally, it can be seen that untreated N/1708 has very extensive endoplasmic reticulum, fairly small mitochondria and very large pigment vacuoles containing small, crystalline pigment granules. When exposed to WR 228258, the mitochondria become swollen and the pigment, which is no longer crystalline, aggregates into clumps.

Plate 41. Untreated P.berghei N/1708 strain. (X 26,000)

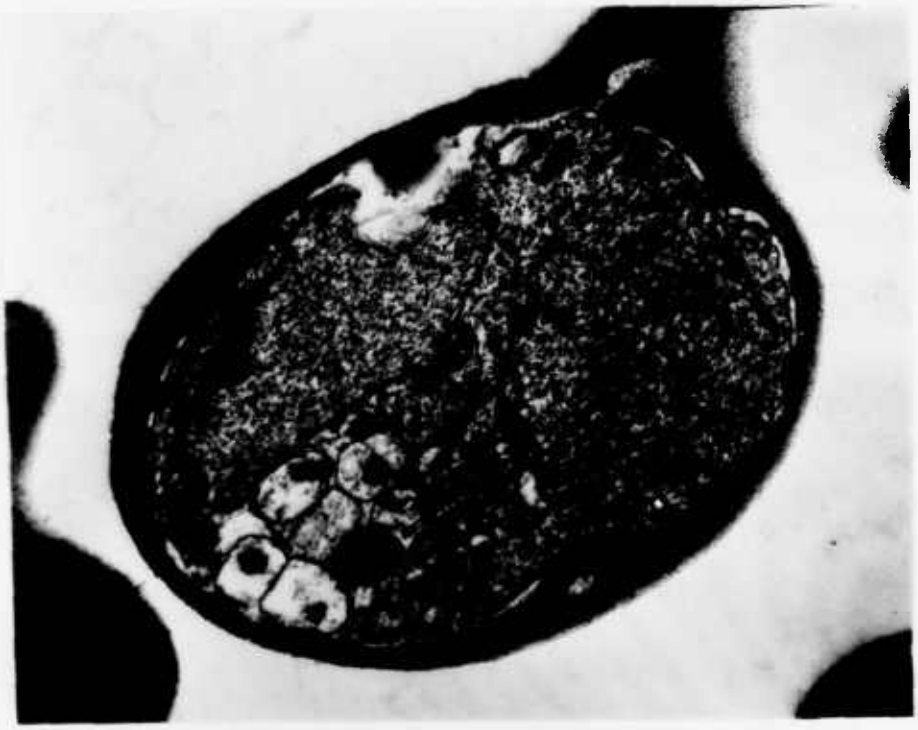

Plate 42. Untreated P.berghei N/1708 strain. (X 32,000)

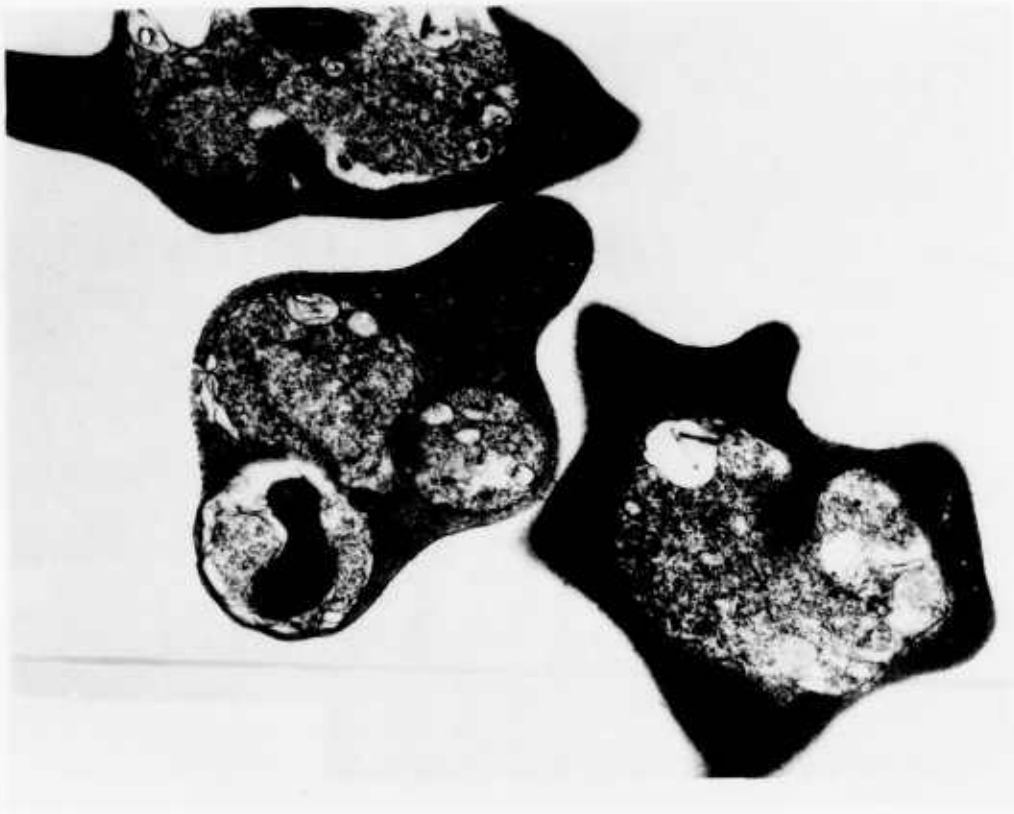

Plate 43. Untreated P.berghei N/1708 strain. (X 16,600)

Plate 44. Untreated P.berghei N/1708 strain. (X 20,000)

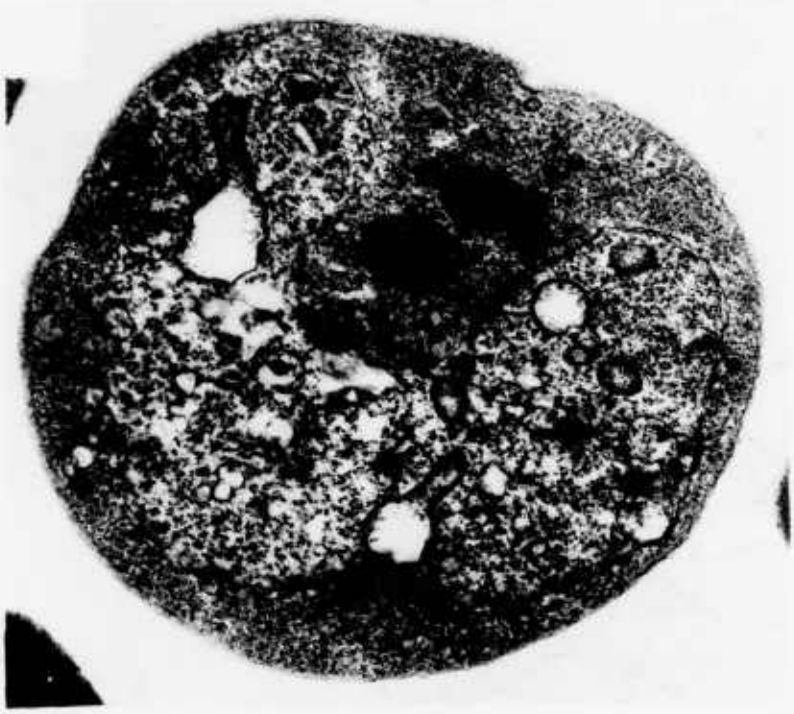

Plate 45. WR 228258 treated P.berghei N/1708 strain. Pigmented parasite in reticulocyte. (X 32,000)

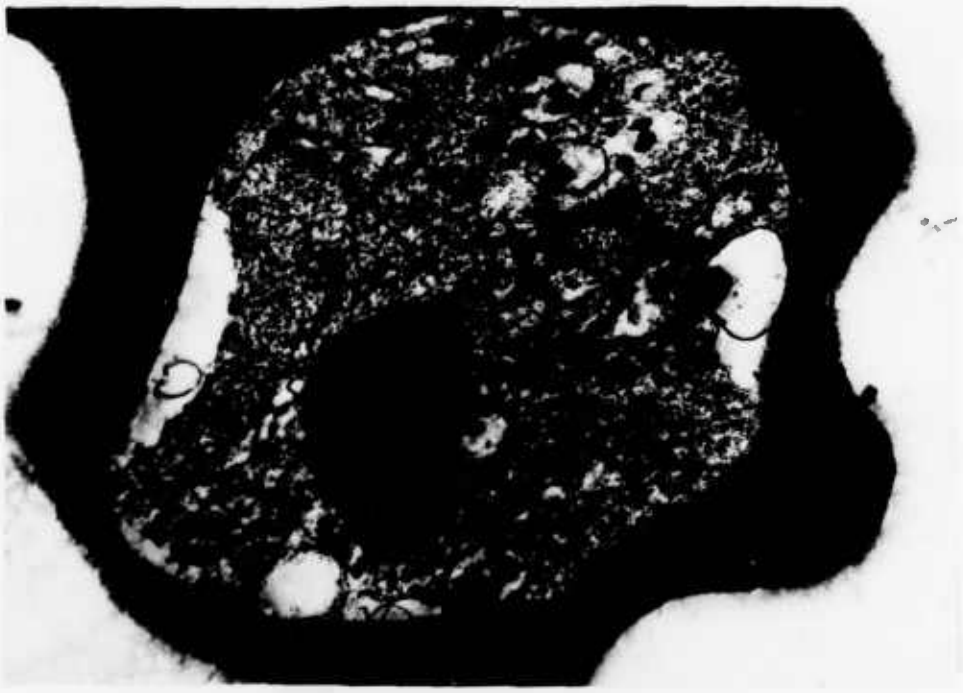

Plate 46. WR 228258 treated P.berghei N/1708 strain. (X 50,000)

3.3.10 P.berghei NH strain.

The halofantrine-resistant NH strain of P.berghei was developed by the relapse technique from N strain. The high sensitivity of N strain to halofantrine limited the maximum dose which could be used to 5 mg/kg and as a result the NH strain has only a low level of resistance to halofantrine (I_{90} = 3.3). Cross-resistance is present to quinidine, mepacrine and artemisinin of approximately the same degree, but marked cross-resistance to doxycycline, LON 1765 and WR 228258 is apparent. Slight hypersensitivity to sulfadoxine, Fansidar and floxacrine is present.

Electron microscopy reveals that the untreated parasites contain numerous, rather irregularly distributed ribosomes, large mitochondria and discreet, "pale" crystalline pigment granules. Treatment with halofantrine produces some vacuolation, nuclear blebbing and some reduction in the number of ribosomes seen. Pigment changes are variable, some appearing "fuzzy" (e.g. Plate 51).

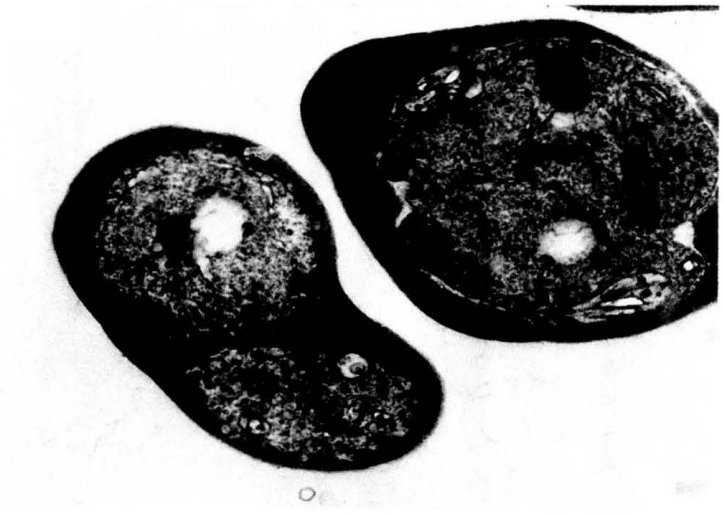

Plate 47. Untreated P.berghei NH strain. (X 16,600)

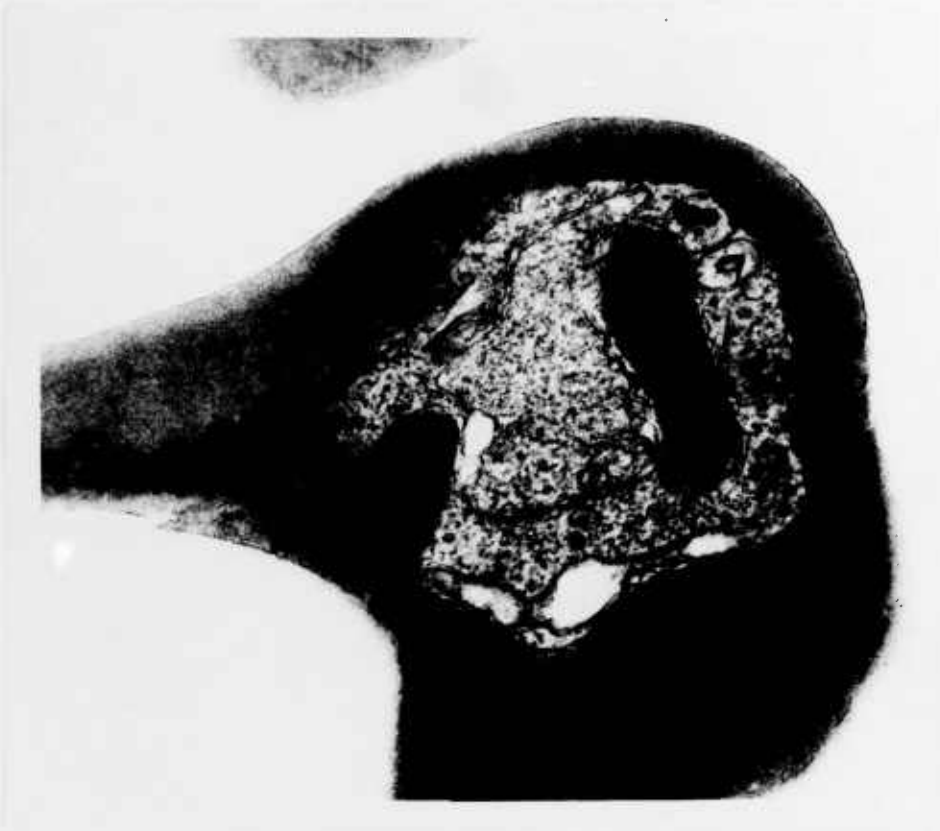

Plate 48. Untreated P.berghei NH strain. (X 32,000)

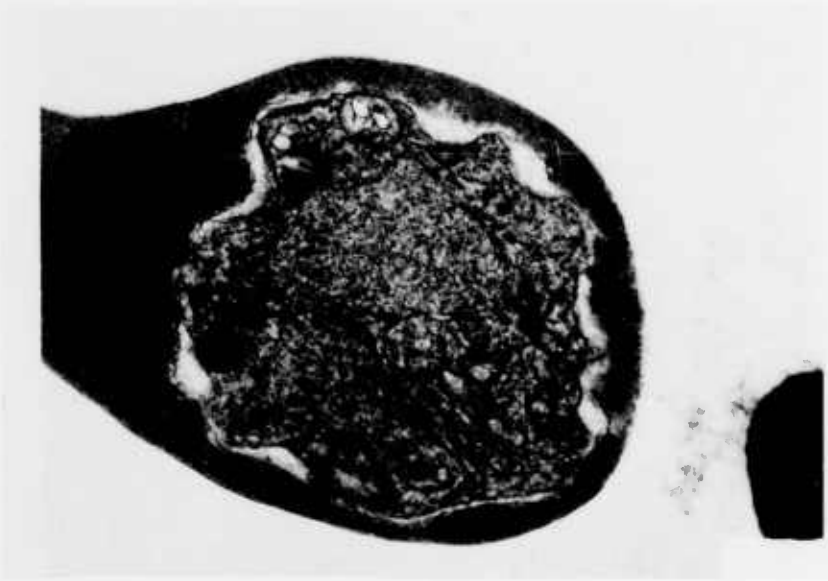

Plate 49. Untreated P.berghei NH strain. Early schizont.(X 26,000)

Plate 50. Halofantrine treated P.berghei NH strain. (X 16,600)

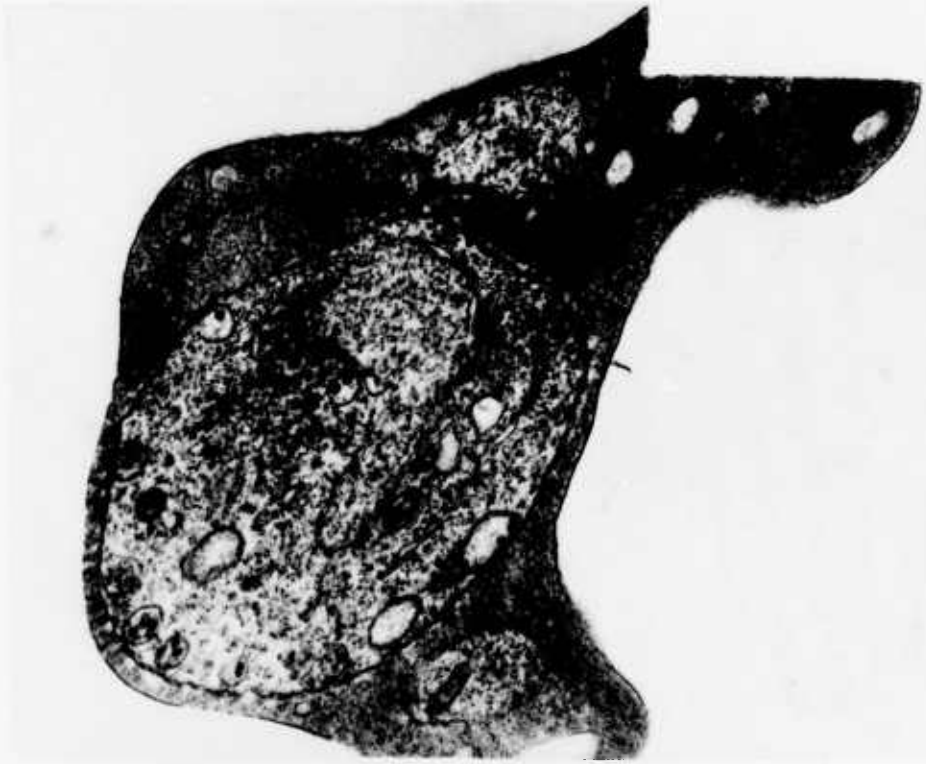

Plate 51. Halofantrine treated P.berghei NH strain. (X 32,000)

Plate 52. Halofantrine treated P.berghei NH strain. (X40,000)

3.3.11 P.berghei Q strain.

P.berghei Q strain, which was developed from N strain by the relapse technique, has a high level of resistance to quinine. This strain also shows marked cross-resistance to chloroquine, amodiaquine, primaquine, cinchonine, quinidine, mefloquine, halofantrine, artemisinin, pyronaridine, mepacrine, doxycycline, LON 1765 and WR 228258.It is hypersensitive to pyrimethamine, sulfadoxine, Fansidar, floxacrine and clindamycin.

At the ultrastructural level, the untreated parasites appear normal but contain only small amounts of non-crystalline, "fuzzy" pigment. After exposure to quinine, cytoplasmic vacuolation and nuclear blebbing are apparent and there is a marked reduction in the numbers of ribosomes and mitochondria seen. The pigment in

these treated parasites is seen as very small, dark, round granules.

Plate 53. Untreated P.berghei Q strain. (X 26,000)

Plate 54. Untreated P.berghei Q strain. (X 32,000)

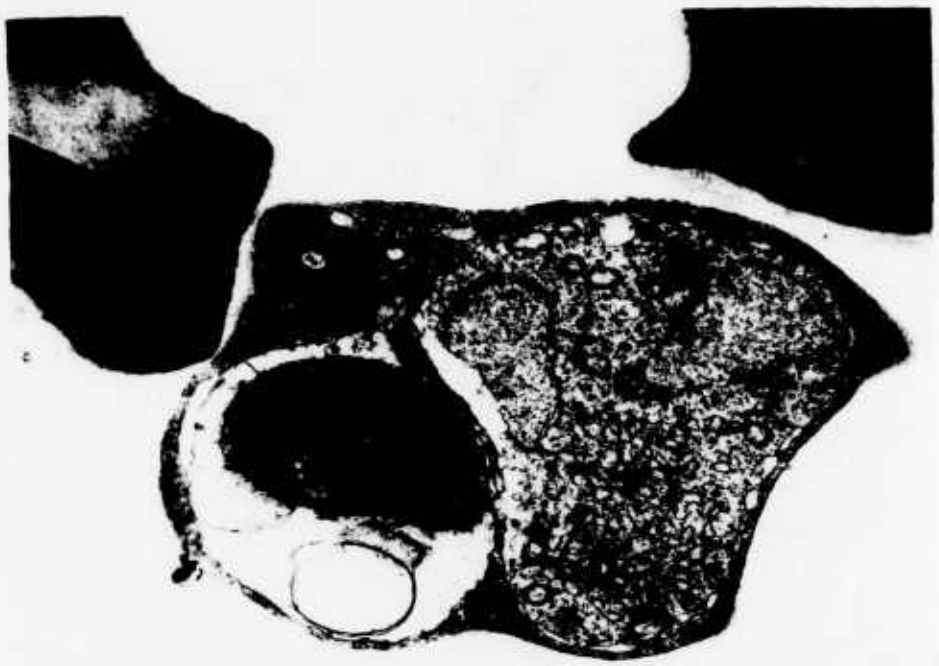

Plate 55. Quinine treated P.berghei Q strain. (32,000)

Plate 56. Quinine treated P.berghei Q strain. (X 40,000)

3.3.12 P.berghei NPN strain.

Resistance to pyronaridine was produced by the relapse technique from P.berghei N strain. The NPN strain is cross-resistant to chloroquine, amodiaquine, quinine, cinchonine, quinidine, halofantrine, artemisinin, cycloguanil, doxycycline, mepacrine, LON 1765 and WR 228258. There is hypersensitivity to sulfadoxine, Fansidar, floxacrine and clindamycin.

Untreated parasites show some cytoplasmic vacuolation and nuclear blebbing when examined with the electron microscope. Such small amount of pigment as was seen appeared crystalline. Pyronaridine treated parasites showed a number of distinct changes. There was duplication of some nuclear membranes, fewer ribosomes than the controls, the mitochondria were swollen with thickened membranes and the pigment was non-crystalline with occasional clumping.

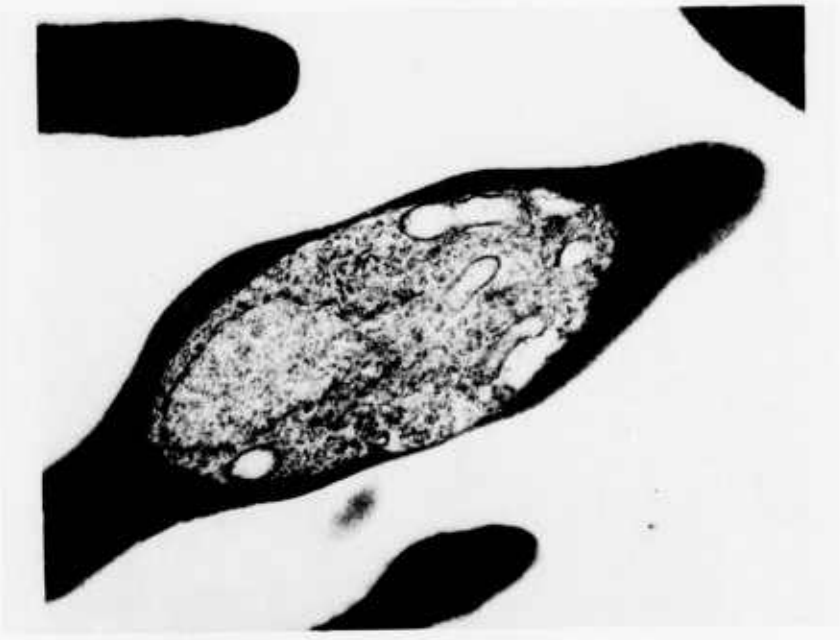

Plate 57. Untreated P.berghei NPN strain. (X 20,000)

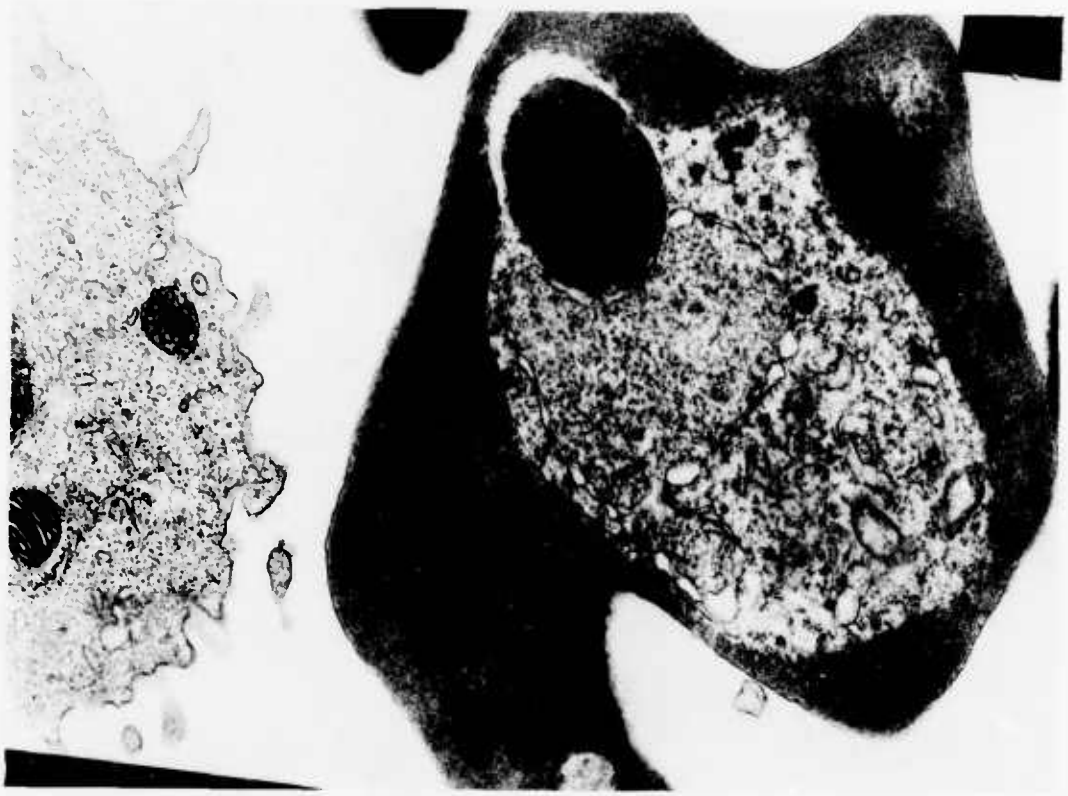

Plate 58. Untreated P.berghei NPN strain. (X 32,000)

Plate 59. Pyronaridine treated P.berghei NPN strain. (X 26,000)

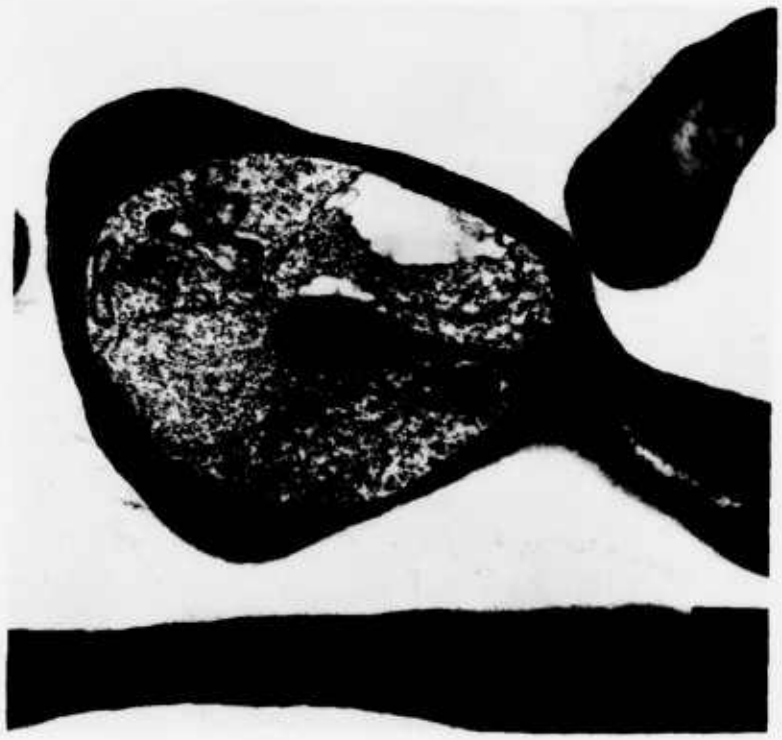

Plate 60. Pyronaridine treated P.berghei NPN strain. (X 26,000)

3.3.13 P.berghei NAM strain.

The NAM strain was derived from P.berghei N strain by the relapse technique using amodiaquine pressure. Cross-resistance studies are not yet completed on this strain, but so far cross-resistance has been shown against quinidine, halofantrine, artemisinin, doxycycline, mepacrine and WR 228258. The NAM strain is hypersensitive to floxacrine.

Ultrastructural studies show that the cytoplasm of the untreated trophozoite has a rather vacuolated, "foamy" appearance and the pigment is fuzzy. Mitochondria are increased in number. Treated NAM has a very pale and "foamy" cytoplasm with many spaces (not vacuoles). There are extensive mitochondria, some of which are swollen, and the pigment is rather amorphous.

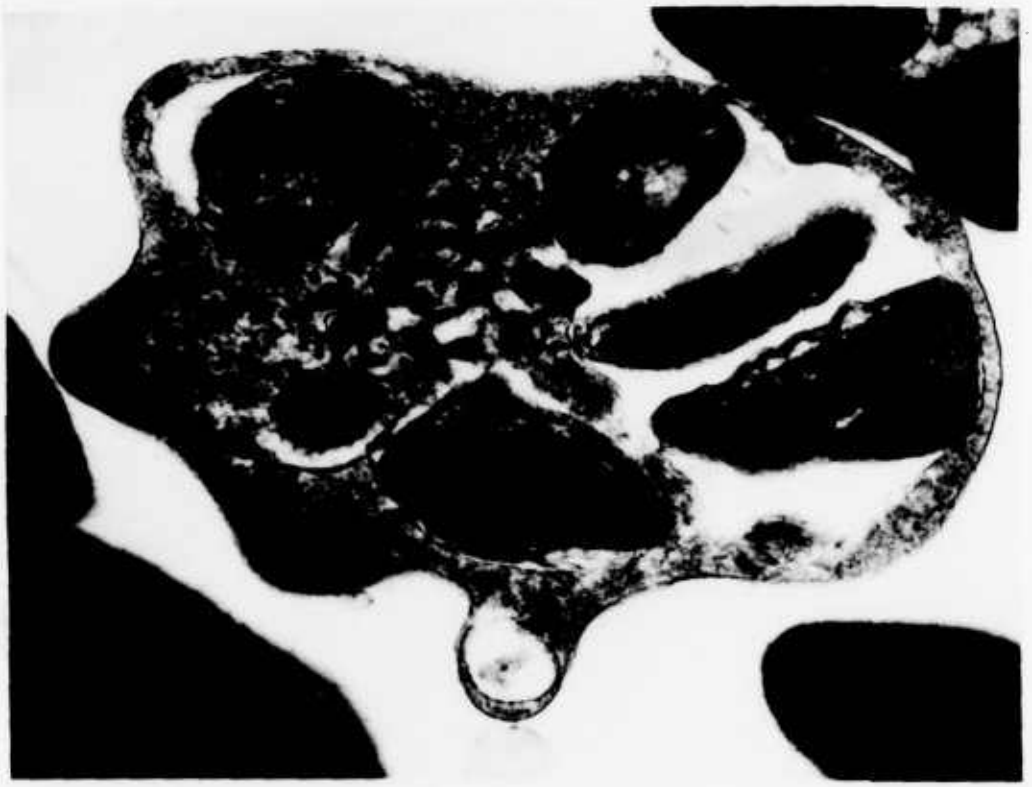

Plate 61. Untreated P.berghei NAM strain schizont. (X 26,000)

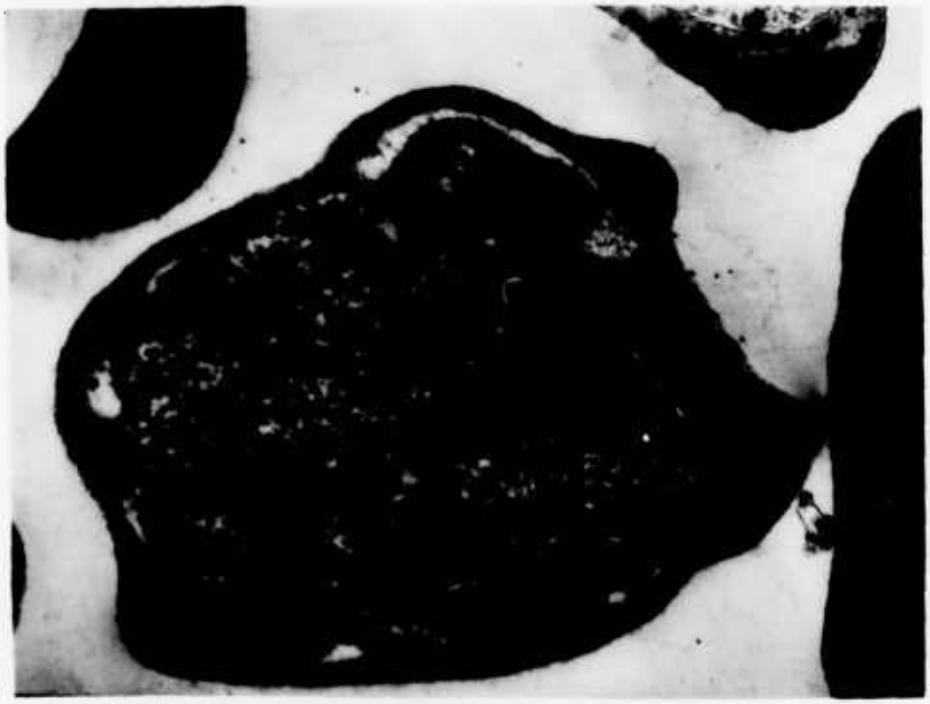

Plate 62. Untreated P.berghei NAM strain. (X 26,000)

Plate 63. Amodiaquine treated P.berghei NAM strain. (X 26,000)

3.3.14 P.berghei PFMA strain.

The PFMA strain was developed as an attempt to induce resistance in P.berghei N strain to a triple combination of mefloquine, pyrimethamine and sulfadoxine. Complete cross-resistance data from this strain are not yet available, but preliminary results show that cross-resistance occurs to quinidine, artemisinin, doxycycline and mepacrine. PFMA is hypersensitive to pyrimethamine, floxacrine and WR 228258.

Ultrastructural studies of the untreated controls show generally normal looking parasites. There are however mitochondrial differences from N strain as the lumen of some contain structures slightly resembling cristae. There is plenty of pigment but none is hard enough to drop out. Parasites which

have been exposed to a single dose of the triple drug combination show some general vacuolation with enlargement of the ribosomes and hyperactive mitochondria. In addition, multiple membranes are to be found in various organelles. Some of the pigment is hard and dropping out of the section, although most is not.

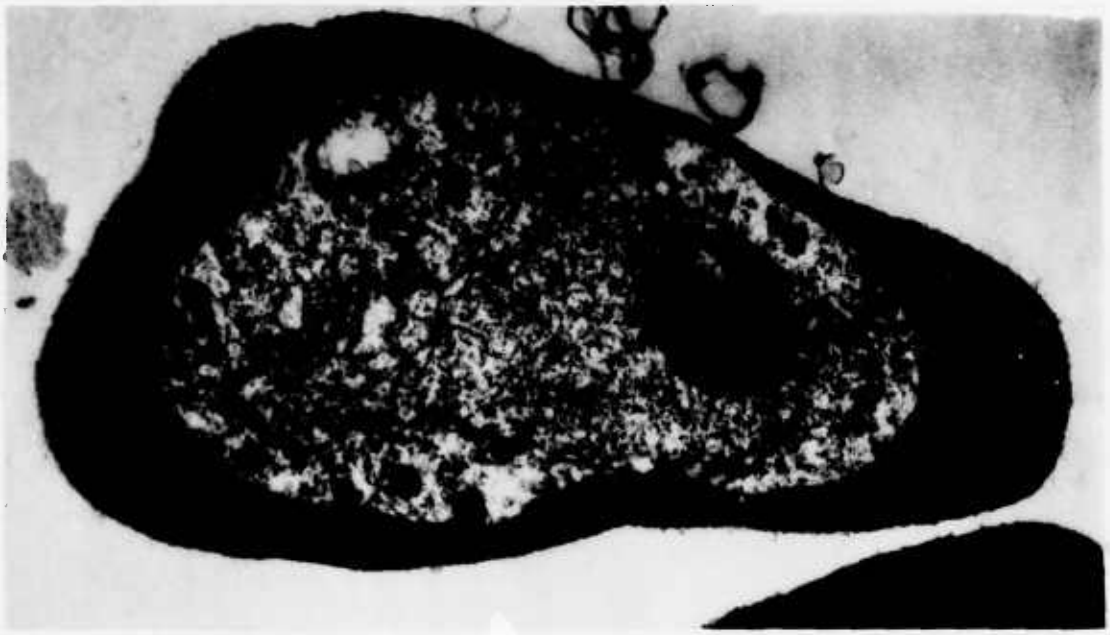

Plate 64. Untreated P.berghei PFMA strain. (X 32,000)

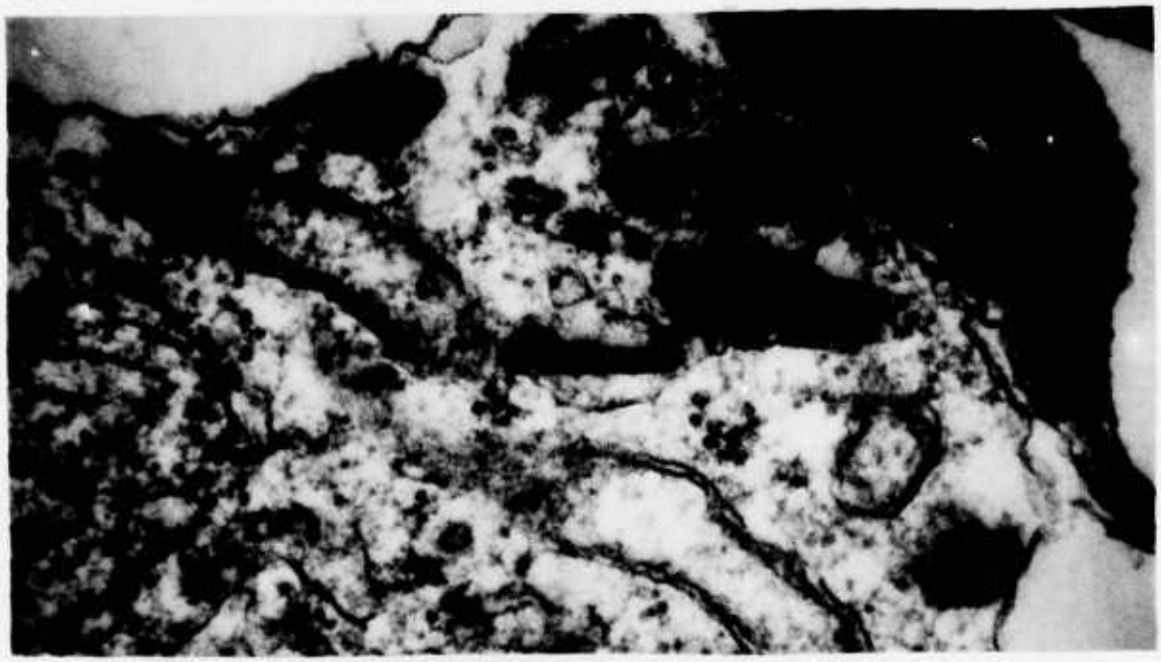

Plate 65. Untreated P.berghei PFMA strain. (X 100,000)

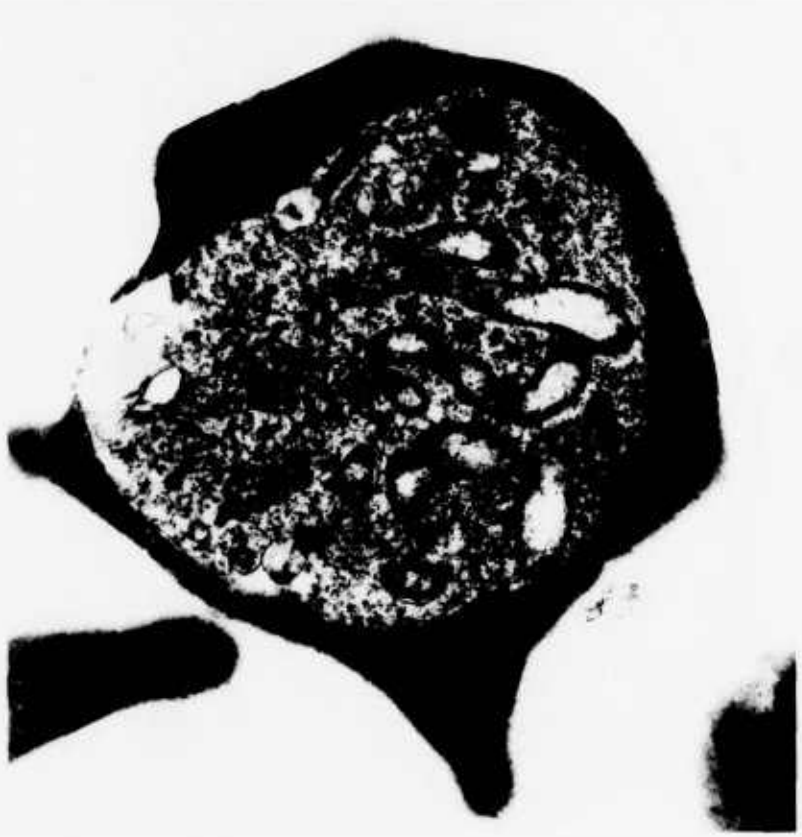

Plate 66. P.berghei PFMA strain following exposure to the triple combination of mefloquine, pyrimethamine and sulfadoxine. (X 20,000)

3.3.15 P.berghei KFY strain.

The KFY strain was developed as a model for Fansidar resistance by inducing resistance in N strain to a 1:3 combination of pyrimethamine and sulfadoxine. KFY has a high degree of resistance to pyrimethamine but results for sulfadoxine are not yet available. The strain is hypersensitive to quinidine and WR 228258.

The electron microscope shows the untreated parasite to have a normal appearance apart from some peripheral vacuolation which may be artefactual in origin. The pigment is normal, hard and dropping out. Treated parasites show cristae-like structures resembling those seen in the PFMA strain (cf Plates 69 and 64).

Plate 67. Untreated P.berghei KFY strain showing nuclear spindle. (X 32,000)

Plate 68. Untreated P.berghei KFY strain. (X 40,000)

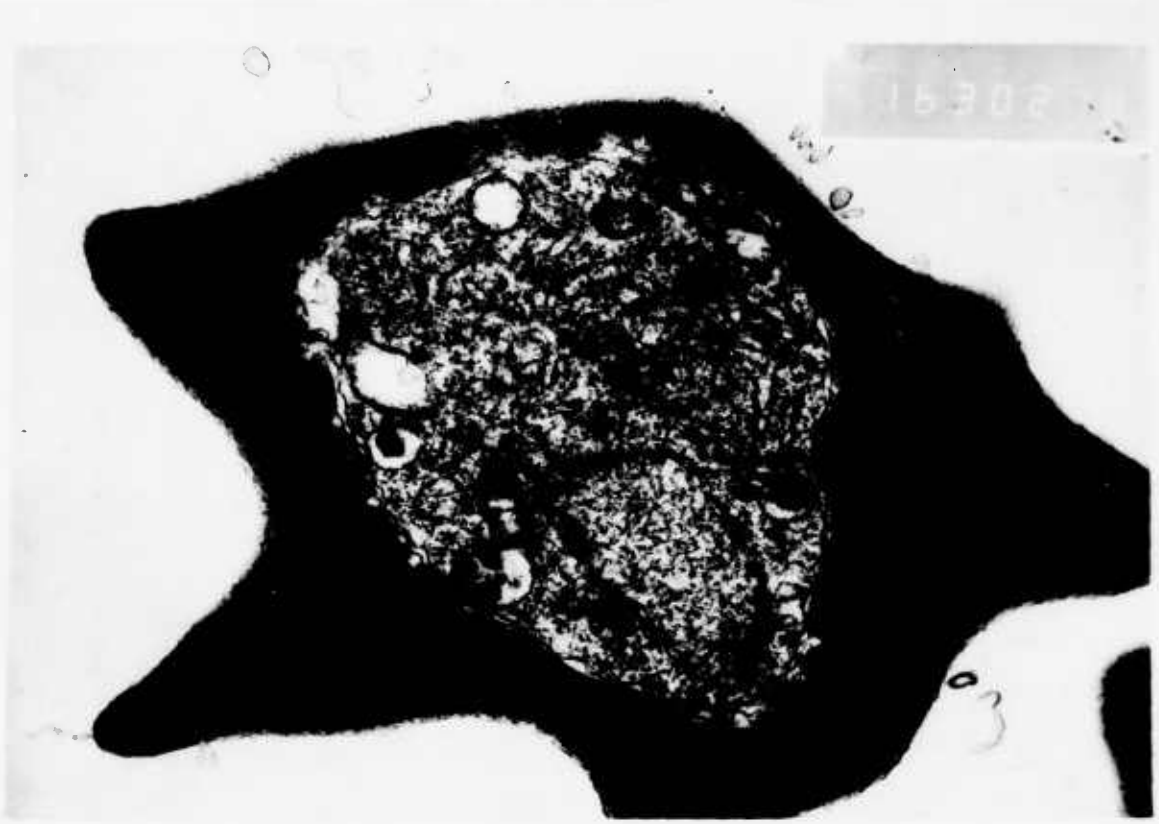

Plate 69. Fansidar treated P.berghei KFY strain. (X 32,000)

3.3.16 P.berghei MFY strain.

The MFY strain was produced by putting P.berghei KFY strain under drug pressure from the triple combination of mefloquine, pyrimethamine and sulfadoxine. Cross-resistance studies are still in progress but first results show a marked enhancement of pyrimethamine resistance with some cross-resistance to quinidine, doxycycline and mepacrine being apparent. There is marked hypersensitivity to WR 228258.

The ultrastructural appearance of the untreated parasites is generally normal although the pigment appears paler than usual. Exposure to the drug combination produces little change other than grouping or clumping of the pigment and some duplication of membranes.

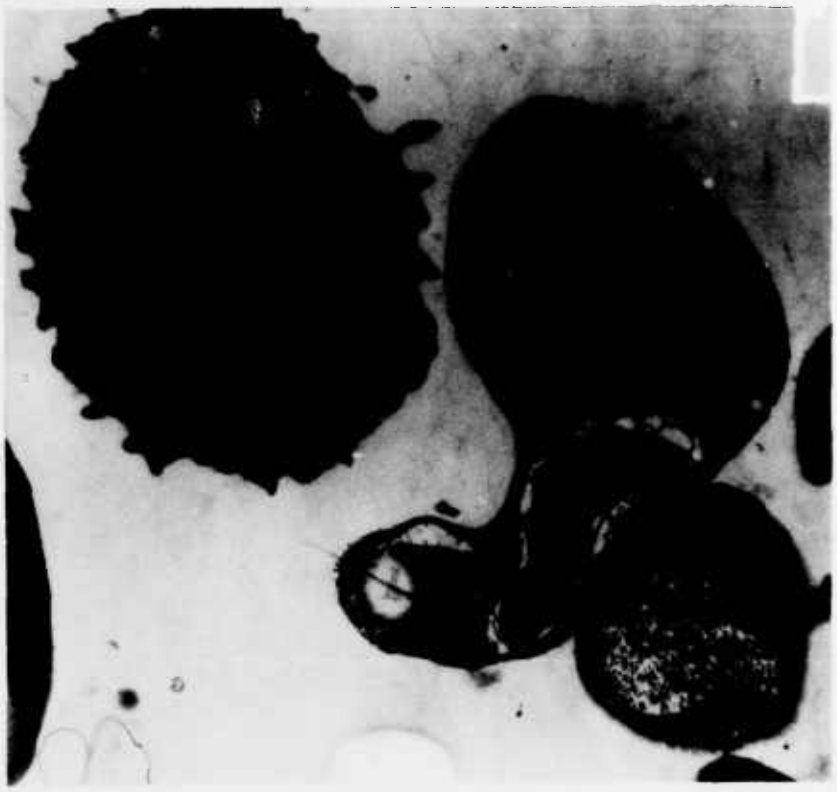

Plate 70. Untreated P.berghei MFY strain. (X 13,200)

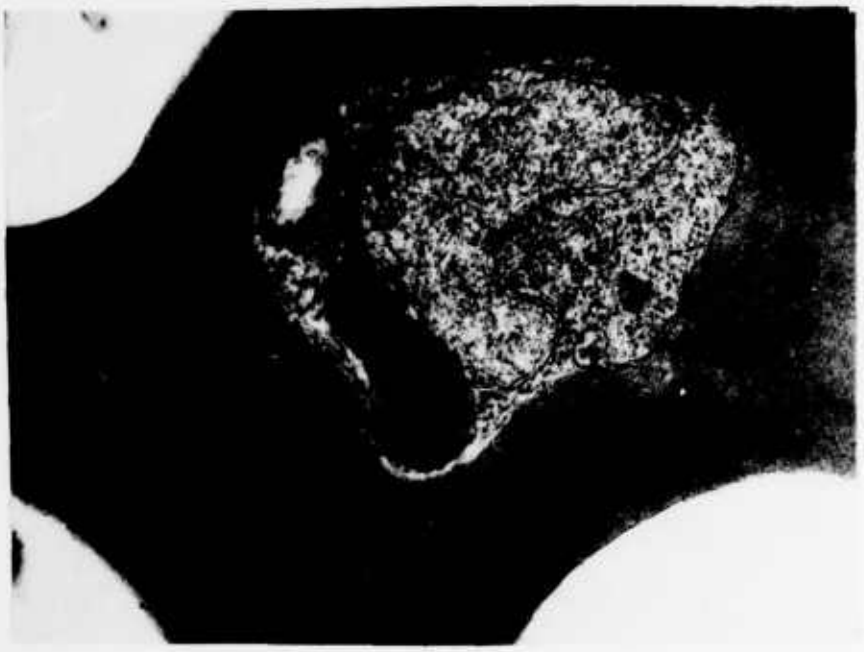

Plate 71. Untreated P.berghei MFY strain. (X 40,000)

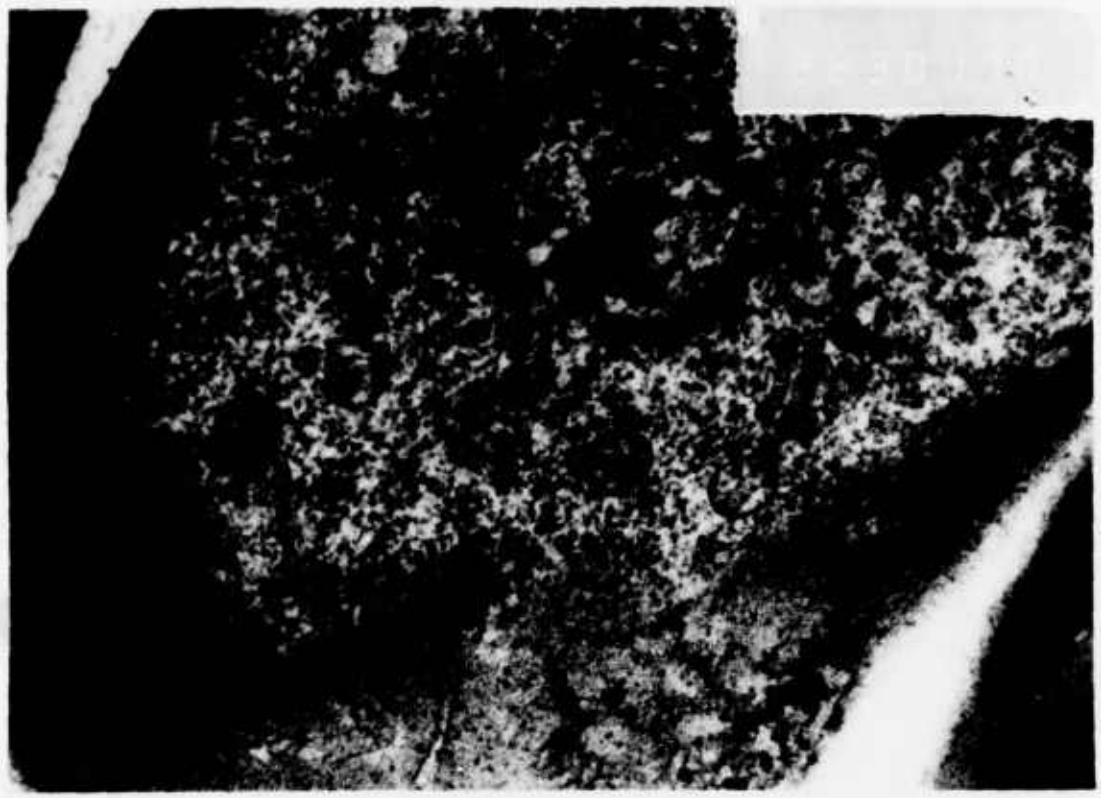

Plate 72. Untreated P.berghei MFY strain. (X 52,000)

Plate 73. P.berghei MFY strain after exposure to a combination of mefloquine and Fansidar. (X 13,200)

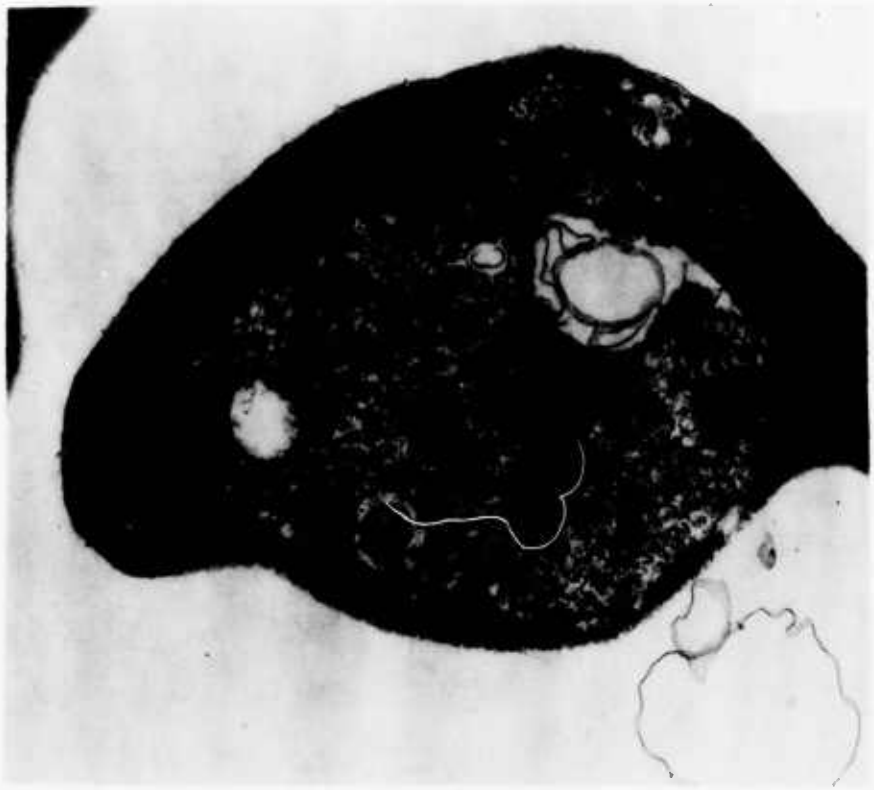

Plate 74. P.berghei MFY strain after exposure to a combination of mefloquine and Fansidar. (X 26,000)

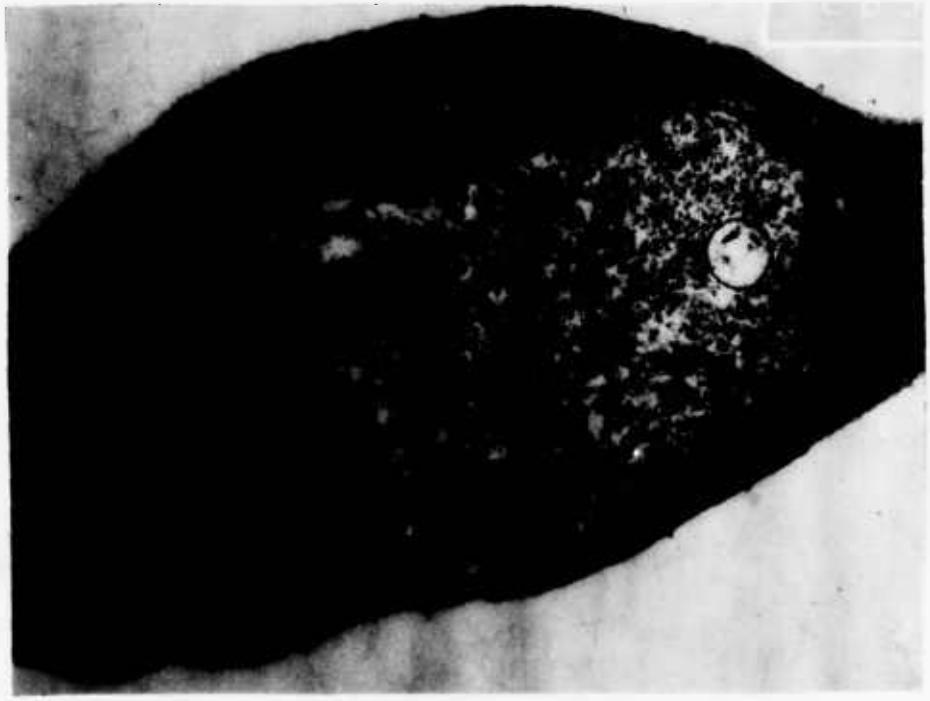

Plate 75. P.berghei MFY strain after exposure to a combination of mefloquine and Fansidar. (X 40,000)

3.3.17 P.berghei QM strain

The QM strain was produced by subjecting P.berghei N strain to pressure with a combination of artemisinin and mefloquine using the relapse technique. The resulting resistant strain was not only resistant to artemisinin and mefloquine but there was also cross-resistance to chloroquine, amodiaquine, primaquine, quinine, cinchonine, quinidine, halofantrine, doxycycline, mepacrine and WR 228258. Hypersensitivity was seen to pyrimethamine, sulfadoxine, Fansidar and floxacrine. Pyronaridine, menoctone and clindamycin have yet to be tested against the QM strain.

The electron microscope showed untreated QM strain parasites to be normal but possibly with cristae in some mitochondria and normal, sometimes pale pigment. Parasites which had been exposed to a single 30 mg/kg dose of a 2:3 mixture of artemisinin and mefloquine showed marked peripheral vacuolation and nuclear blebbing. No pigment at all was seen, but empty vacuoles that would probably have contained pigment were present.

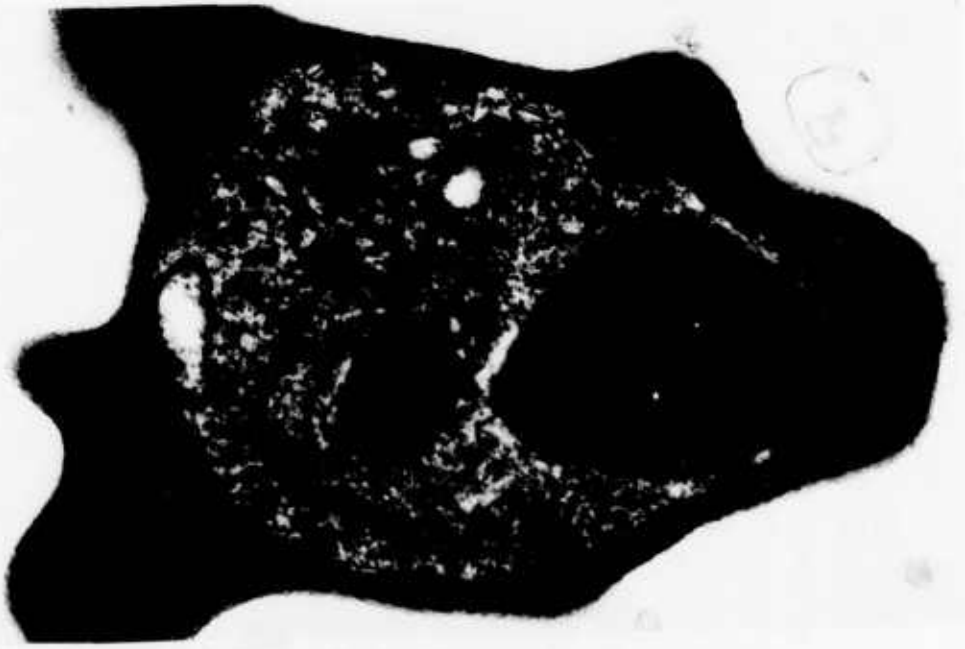

Plate 76. Untreated P.berghei QM strain. (X 26,000)

Plate 77. P.berghei QM strain after exposure to 2:3 artemisinin and mefloquine. (X 26,000)

3.3.18 P.berghei N/1765 strain

Resistance to LON 1765, an experimental indolo(3,2-c) quinoline-N-oxide, was developed in P.berghei N strain by the relapse technique. Slight cross-resistance was noted to quinine, quinidine, artemisinin and doxycyline whilst there was appreciable cross-resistance to mepacrine and WR 228258. The N/1765 strain is hypersensitive to sulfadoxine, floxacrine and clindamycin.

Other than the presence of many small, hard pigment granules (many of which were in very large vacuoles), there were no noteworthy features seen in untreated parasites. However, when exposed to drug pressure the endoplasmic reticulum was swollen and the contents of the mitochondria were dark staining. The

pigment vacuoles became enormously enlarged and contained soft round pigment.

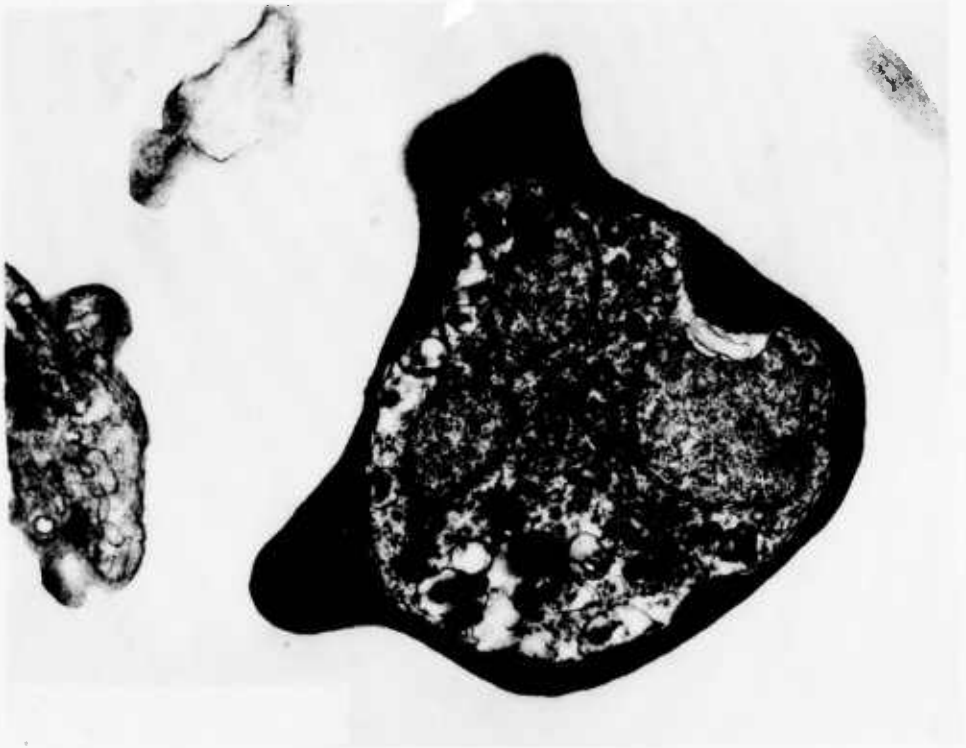

Plate 78. Untreated P.berghei N/1765 strain. (X 20,000)

Plate 79. LON 1765 treated P.berghei N/1765 strain. (X 32,000)

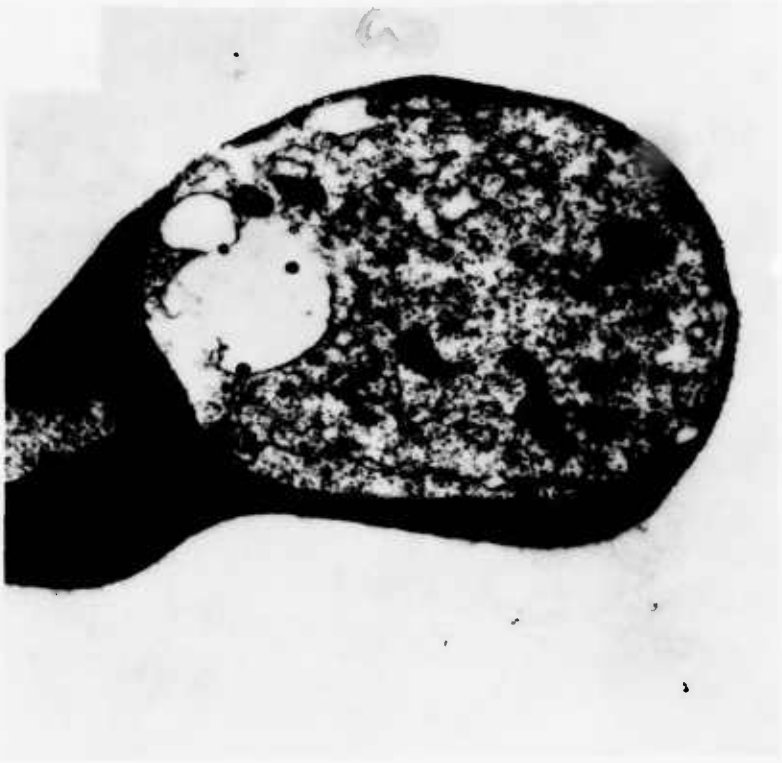

Plate 80. LON 1765 treated P.berghei N/1765 strain. (X 26,000)

3.3.19 P.yoelii ssp NS strain.

The moderately chloroquine-resistant NS strain was isolated from the mixture of P.berghei and a subspecies of P.yoelii which comprised the original Keyberg 173 isolate. Separation of the NS was achieved by passage through Anopheles stephensi after submitting the mixture of parasites to chloroquine pressure. Apart from its probably inherent resistance to chloroquine, which we have enhanced, the NS strain is also resistant to amodiaquine, quinine, quinidine, doxycycline, mepacrine, LON 1765 and menoctone. Hypersensitivity is noticeable to sulfadoxine, Fansidar, floxacrine and WR 228258.

Examination of the ultrastructure of the untreated parasite reveals dark staining mitochondrial membranes on prominent

mitochondria and large crystalline pigment. When exposed to chloroquine, vacuolation of both nucleus and cytoplasm can be seen and the ribosomes are "clumped". The mitochondria appear normal but are sparse and the pigment is "fuzzy" but no massive clumping occurs.

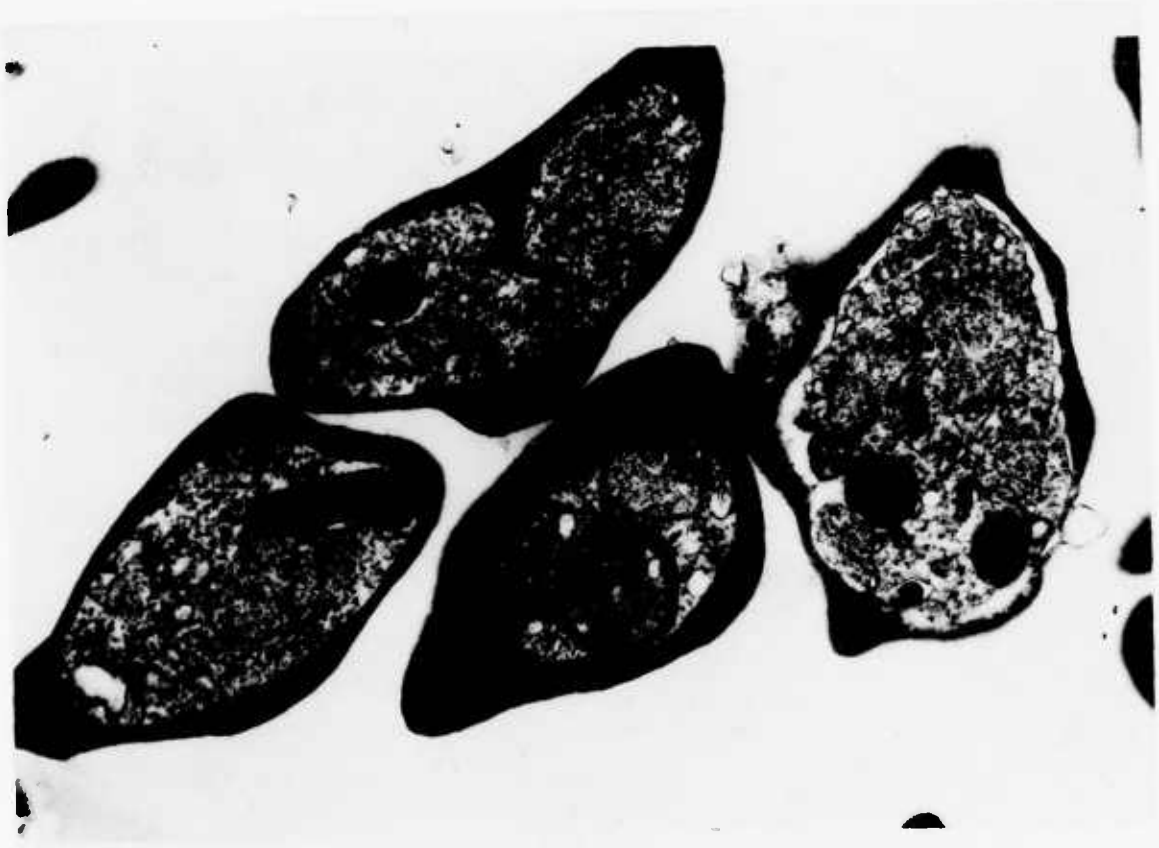

Plate 81. Untreated P.yoelii ssp. NS strain. (X 12,600)

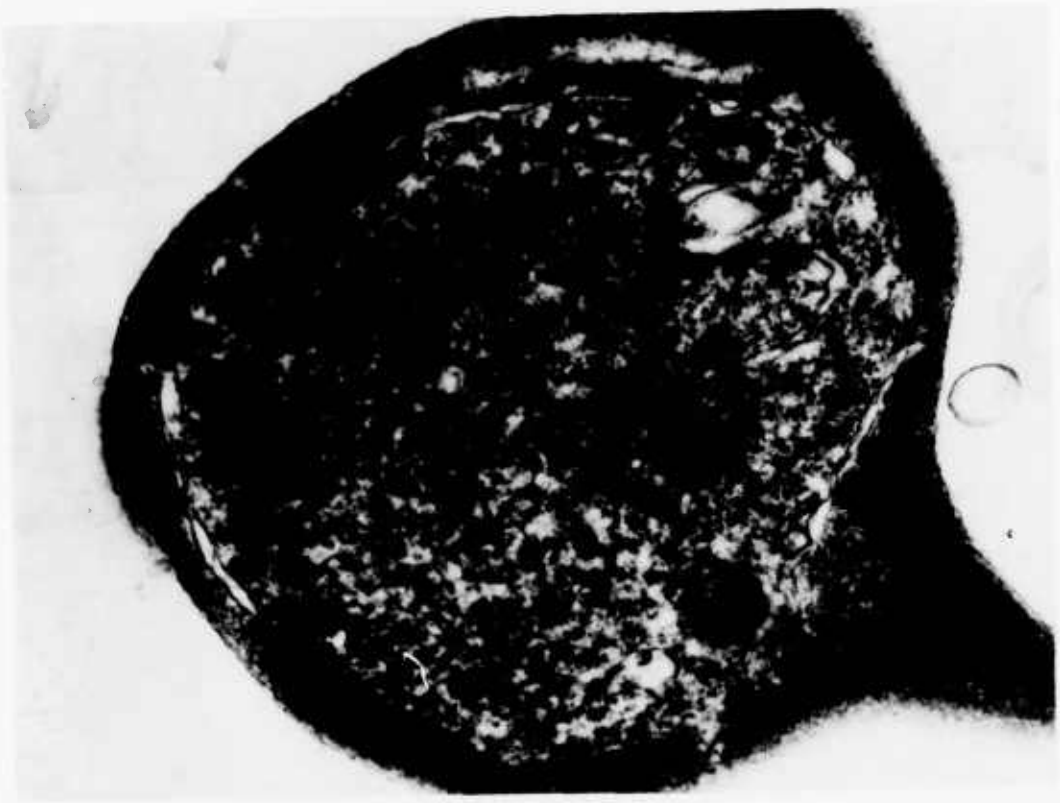

Plate 82. Untreated P.yoelii ssp. NS strain. (X 50,000)

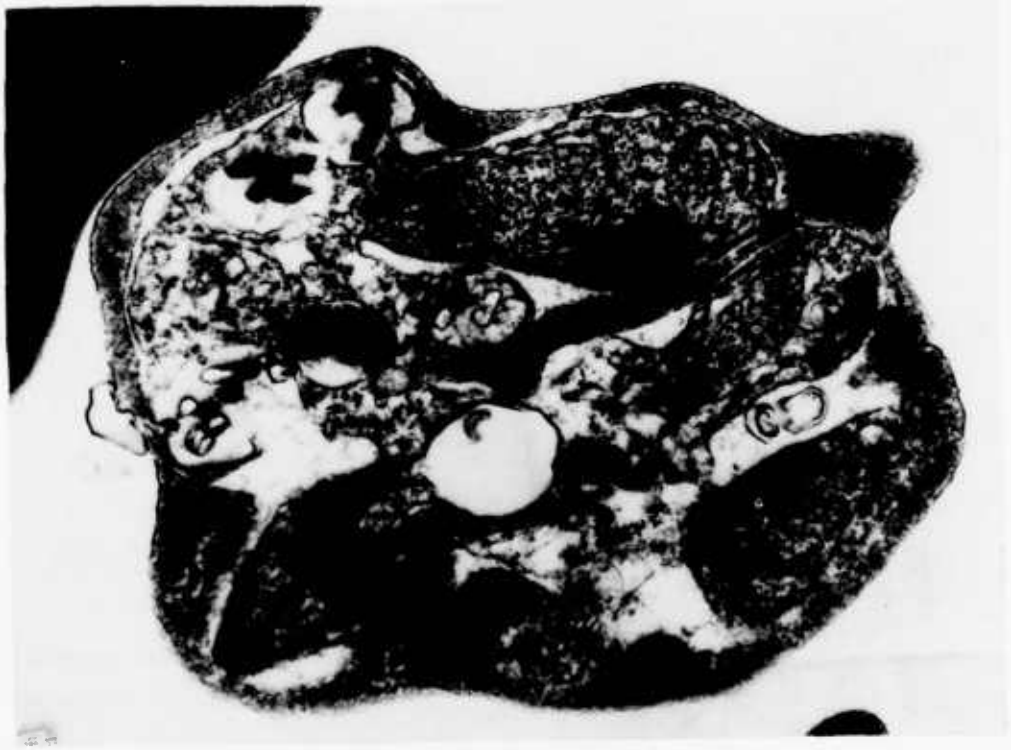

Plate 83. Untreated P.yoelii ssp. NS strain schizont. (X 32,000)

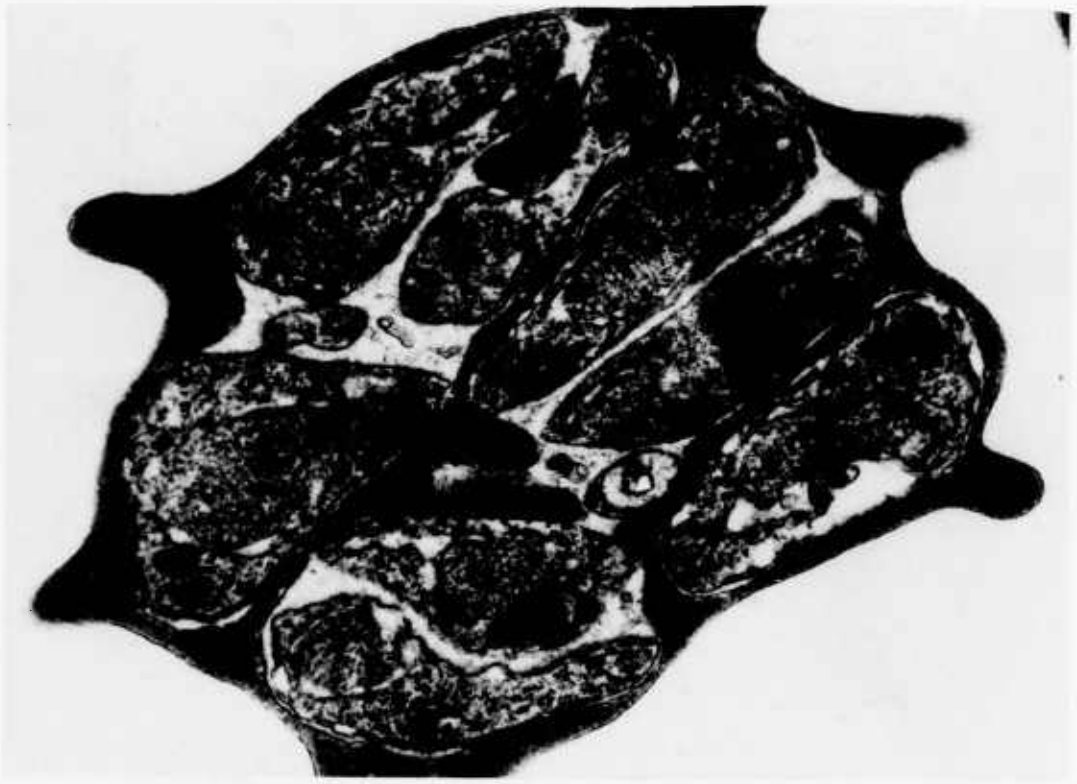

Plate 84. Untreated P.yoelii ssp NS strain schizont. (X 32,000)

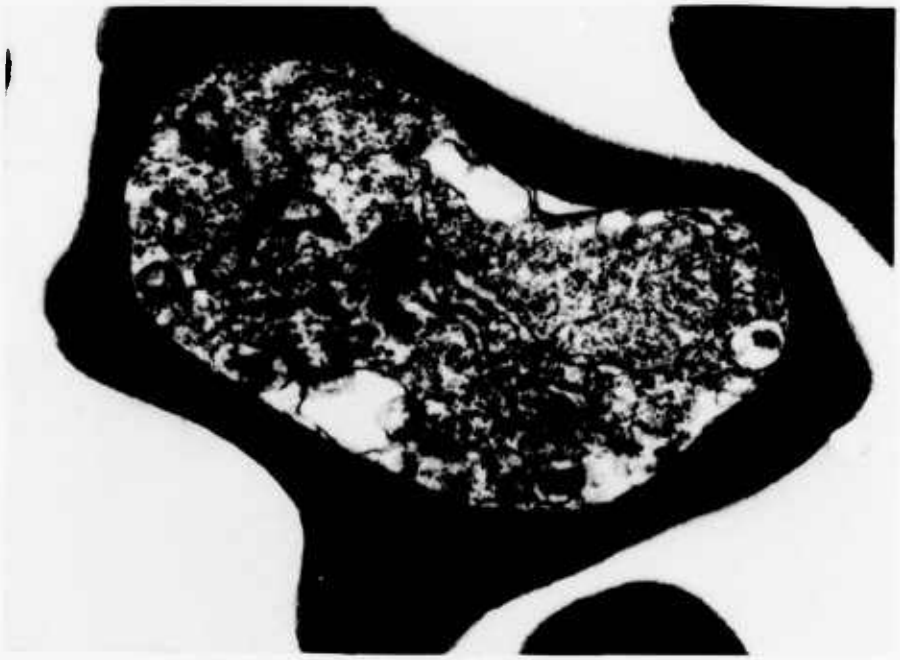

Plate 85. Chloroquine treated P.yoelii ssp. NS strain. (X 32,000)

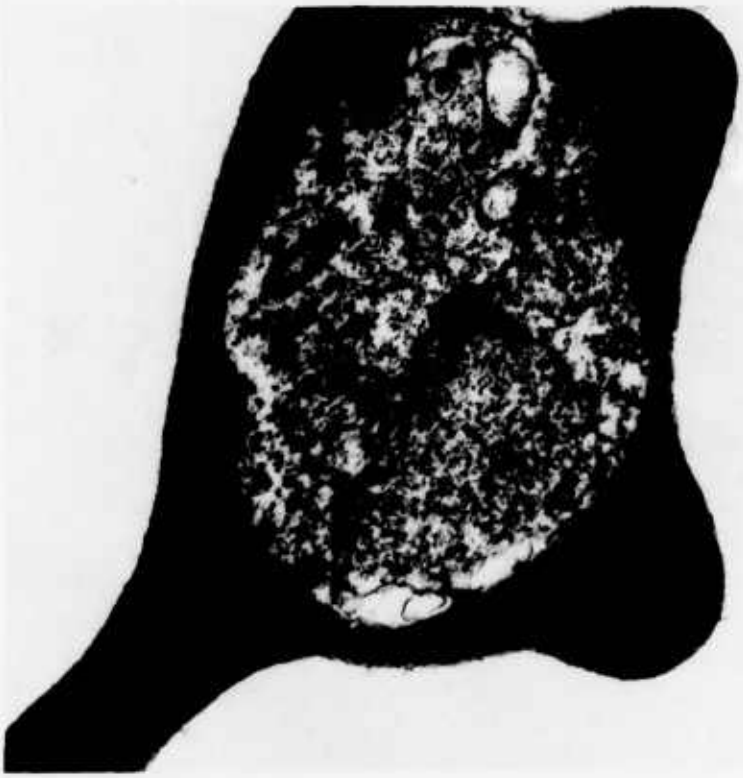

Plate 86. Chloroquine treated P.yoelii ssp. NS strain. (X 32,000)

3.3.20 P.yoelii ssp. ART strain.

The highly artemisinin resistant ART strain was developed by subjecting P.yoelii ssp NS strain to artemisinin pressure in the relapse technique. Cross-resistance occurs to chloroquine, amodiaquine, quinine, cinchonine, quinidine, mefloquine, halofantrine, pyronaridine, doxycycline, mepacrine, LON 1765 and WR 228258. Hypersensitivity to pyrimethamine, sulfadoxine, Fansidar, floxacrine and clindamycin is present in this strain.

The untreated ART parasite is abnormal in appearance with a large number of mitochondria, some with extra membranes, and dark, hard pigment. When treated with artemisinin, the appearance of the parasites is significantly changed. The nuclear membranes are irregularly darkened and the nucleoplasm shows patchy

clumping. Some mitochondrial membranes are thickened. The pigment is relatively unchanged.

Plate 87. Untreated P.yoelii ssp. ART strain. (X 26,000)

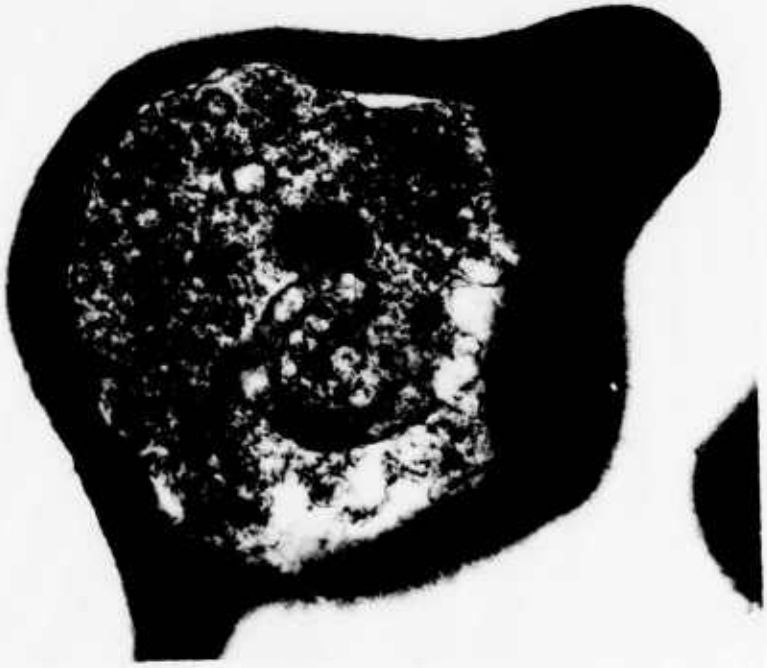

Plate 88. Untreated P.yoelii ssp. ART strain. (X 32,000)

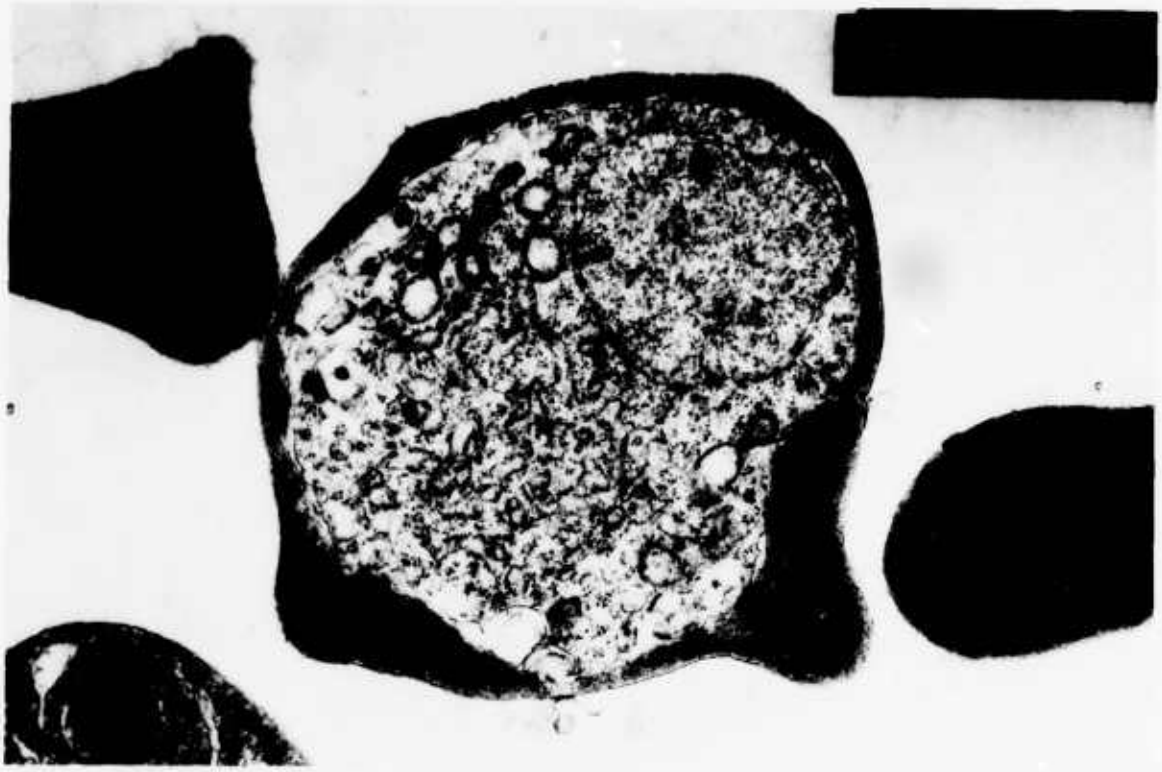

Plate 89. Artemisinin treated P.yoelii ssp. ART strain. (X 32,000)

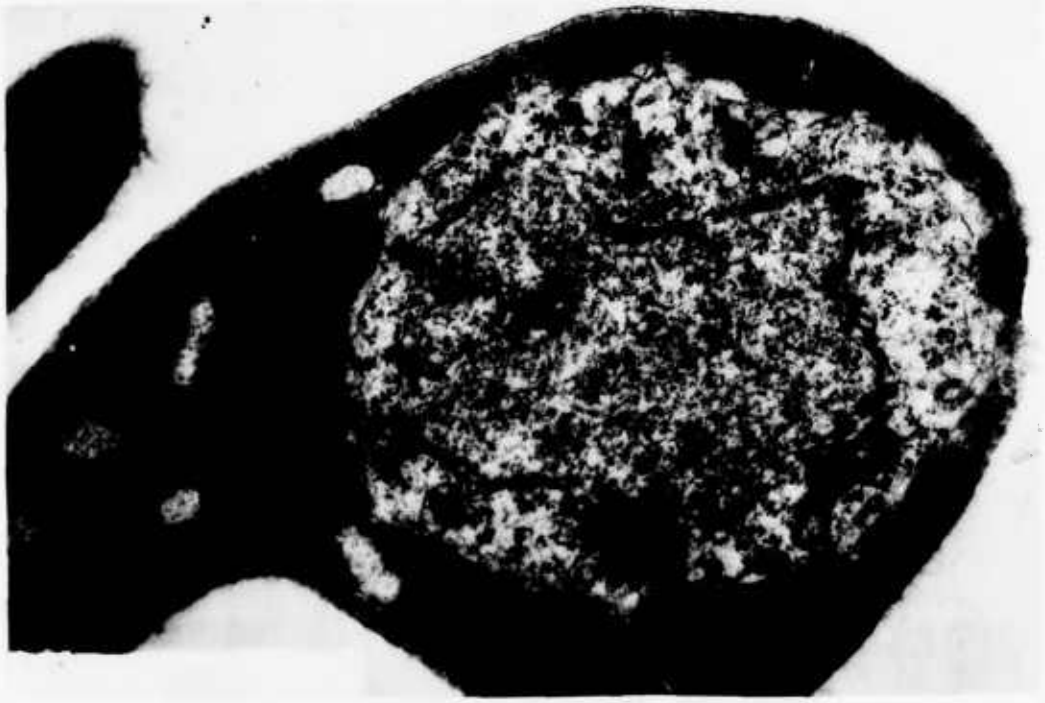

Plate 90. Artemisinin treated P.yoelii ssp. ART strain. (X 32,000)

3.3.21 P.yoelii ssp. SH strain.

The SH strain was developed in parallel with the NH strain of P.berghei as a halofantrine resistant line of P.yoelii ssp. (NS). As well as being highly resistant to halofantrine, marked cross resistance is present to chloroquine, amodiaquine, cinchonine, quinidine, mefloquine, artemisinin, pyronaridine, doxycycline, mepacrine and LON 1765. Slight cross resistance to menoctone also occurred. The strain is hypersensitive to sulfadoxine, Fansidar, floxacrine, clindamycin and WR 228258.

Electron microscopy shows that untreated parasites of this strain possess increased mitochondria with thickened or double membranes. The pigment appears normal. After treatment with halofantrine, peripheral membrane and nuclear blebbing occur and the ribosomes are depleted. Pigment is reduced in quantity, rounded and "fuzzy".

Plate 91. Untreated P.yoelii ssp. SH strain. (X 5,200)

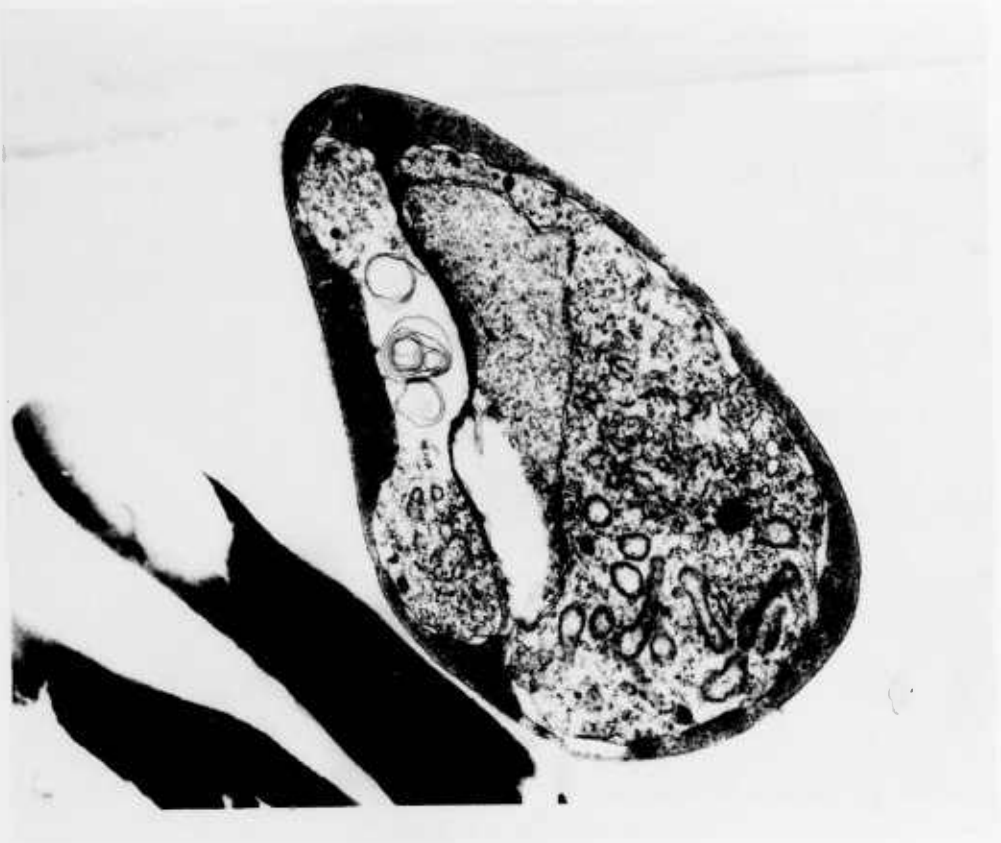

Plate 92. Untreated P.yoelii ssp. SH strain. (X 20,000)

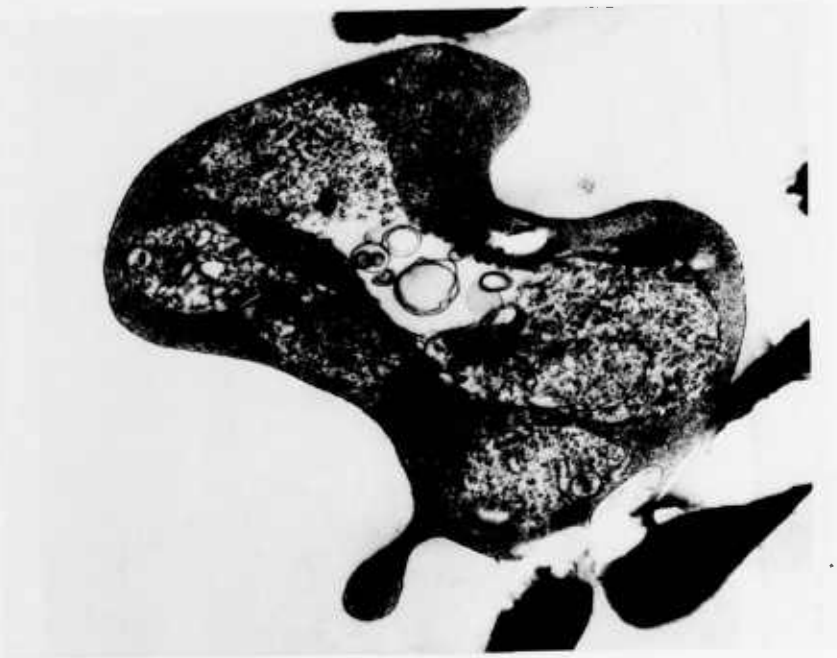

Plate 93. Untreated P.yoelii ssp. SH strain. (X 16,600)

Plate 94. Halofantrine treated P.yoelii ssp. SH strain. (X 20,000)

Plate 95. Halofantrine treated P.yoelii ssp. SH strain. (X 20,000)

Plate 96. Halofantrine treated P.yoelii ssp. SH strain. (X 26,000)

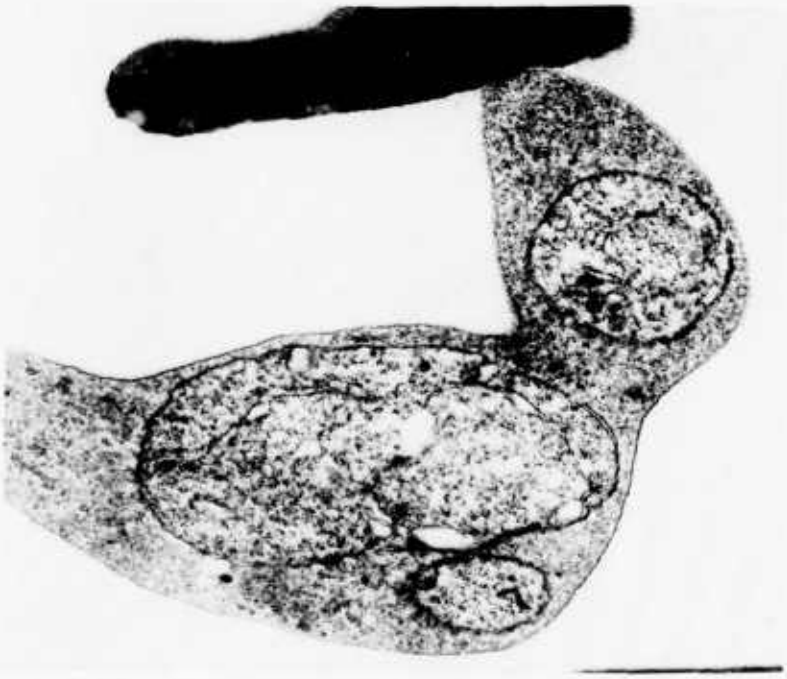

Plate 97. Halofantrine treated P.yoelii ssp. SH strain. (X 30,000)

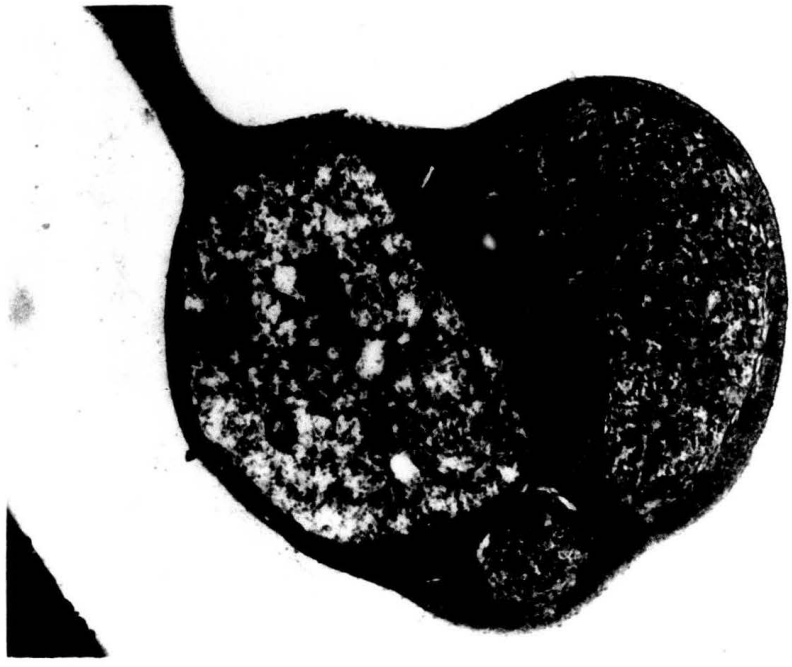

Plate 98. Halofantrine treated P.yoelii ssp. SH strain. (X 40,000)

3.3.22 P.yoelii ssp. NS/1100.

Derived from the NS strain by the relapse technique, using mefloquine pressure, the NS/1100 is highly resistant to mefloquine and cross-resistant to chloroquine, primaquine, quinine, quinidine, halofantrine, artemisinin, doxycycline, mepacrine, LON 1765 and WR 228258. Sensitivity is enhanced to cinchonine, pyimethamine, sulfadoxine, Fansidar, floxacrine and clindamycin.

Very little of note is apparent in electron micrographs of the untreated parasites. After exposure to mefloquine, the endoplasmic reticulum is swollen and there is thickening of the mitochondrial membranes. Very little pigment is present but there is an increase in the number of food vacuoles.

Plate 99. Untreated P.yoelii ssp. NS/1100. (X 32,000)

Plate 100. Untreated P.yoelii ssp. NS/1100. (X 20,000)

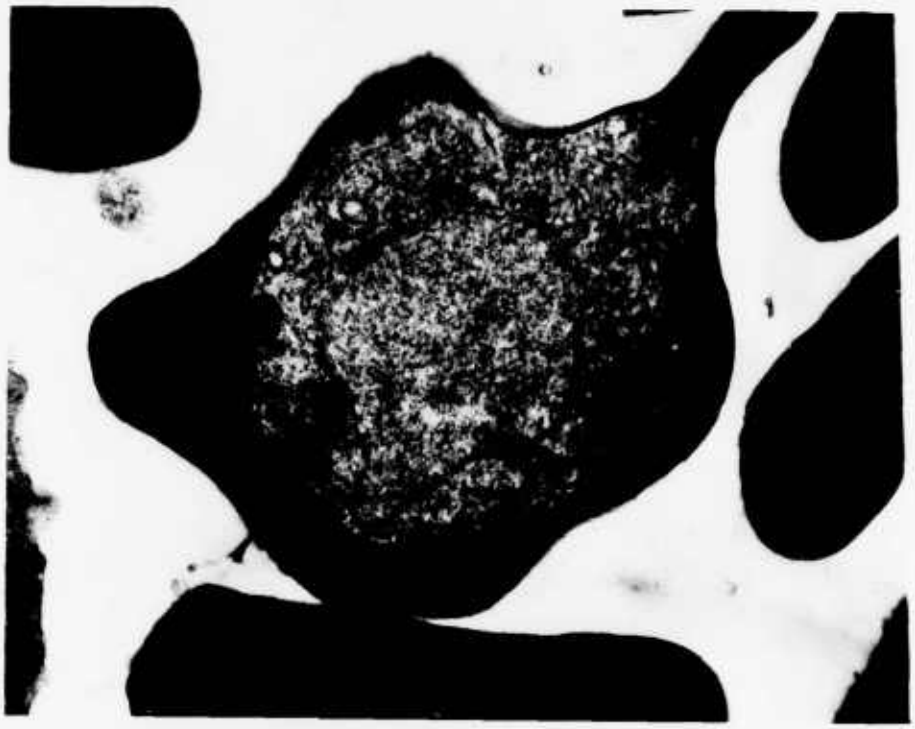

Plate 101. Mefloquine treated P.yoelii ssp. NS/1100. (X 32,000)

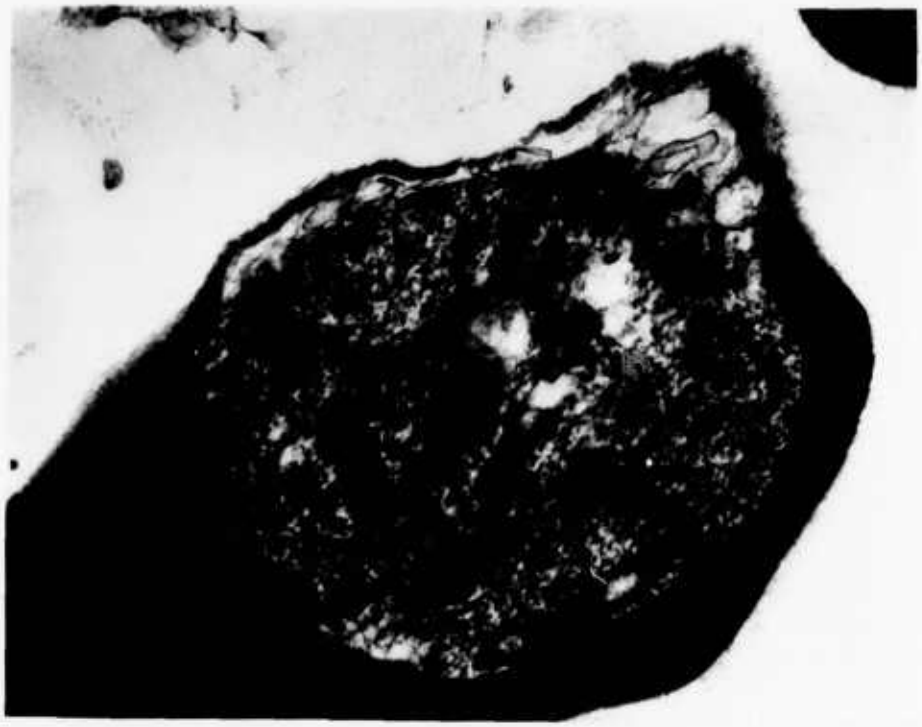

Plate 102. Mefloquine treated P.yoelii ssp. NS/1100. (X 40,000)

3.3.23 P.yoelii ssp SAM strain.

This amodiaquine resistant strain, derived from NS, is still being tested to determine the cross-resistances which it possesses. To date we have identified cross-resistance to quinidine, halofantrine, artemisinin, pyronaridine, doxycycline, mepacrine, LON 1765 and WR 228258.

Ultrastructurally, it can be seen that in untreated parasites the ribosomes are irregularly dispersed, and there are prominent mitochondria with thick membranes. After exposure to amodiaquine the cytoplasm becomes foamy and full of vacuoles containing "fuzzy" pigment. This is accompanied by some apparent pigment clumping.

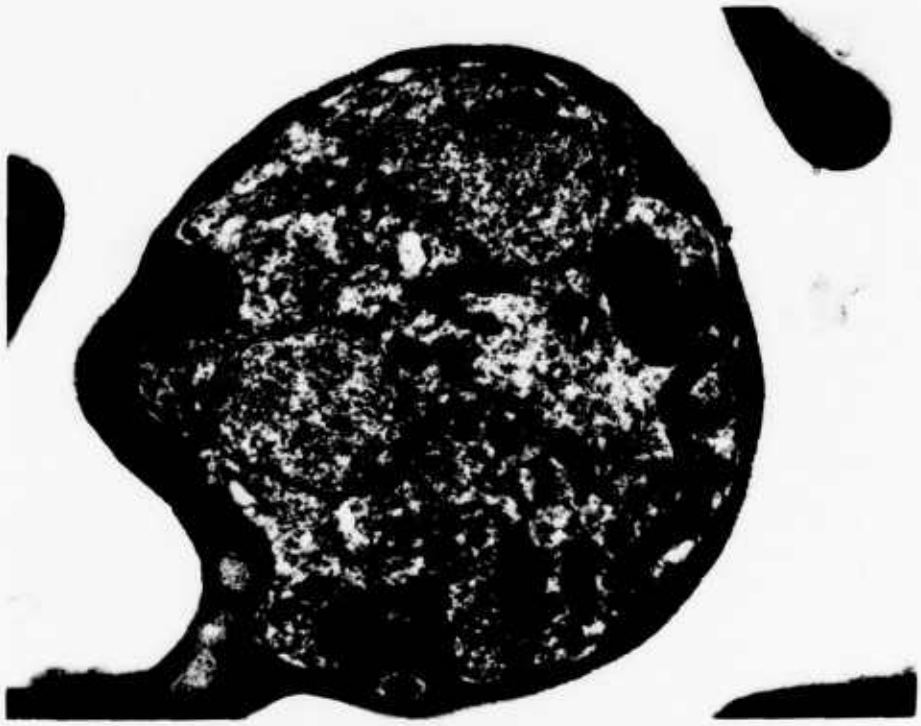

Plate 103. Untreated P.yoelii ssp. SAM strain. (X 26,000)

Plate 104. Amodiaquine treated P.yoelii ssp. SAM strain. (X 32,000)

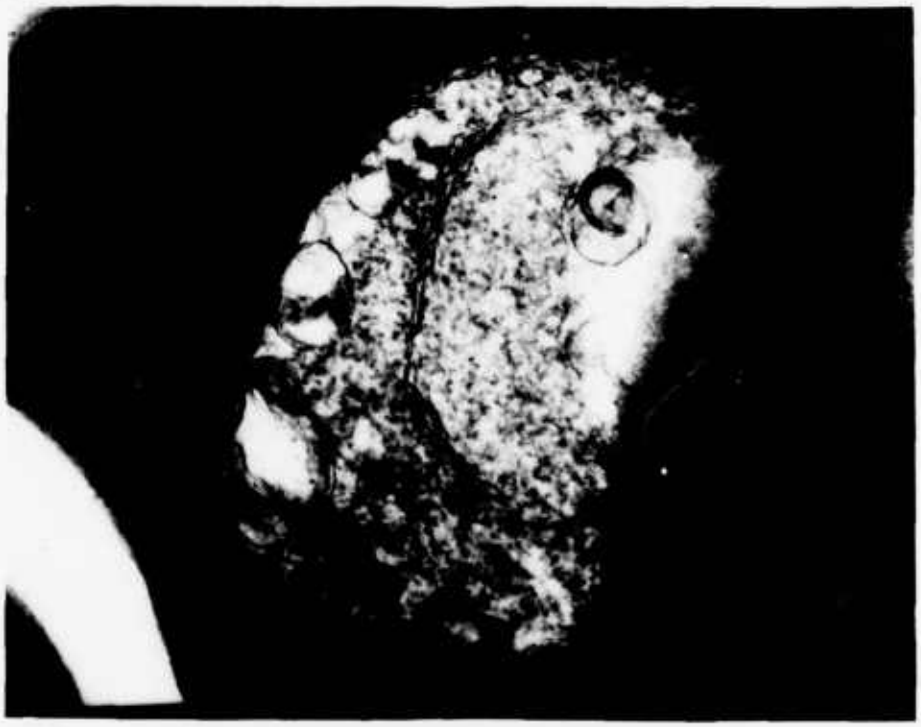

Plate 105. Amodiaquine treated P.yoelii ssp. SAM strain. (X 52,000)

3.3.24 P.yoelii ssp. QMS strain.

Derived from P.yoelii ssp. NS strain, the QMS was developed for primary resistance to a 2:3 combination of artemisinin and mefloquine. Resistance studies are in still in progress but cross-resistance has already been identified to quinidine, halofantrine, artemisinin, pyronaridine, doxycycline, mepacrine, LON 1765 and WR 228258.

The untreated QMS trophozoites have many and abnormal mitochondria with thickened membranes. The pigment is rounded and "fuzzy". These appearances are exaggerated by exposure to the drug combination.

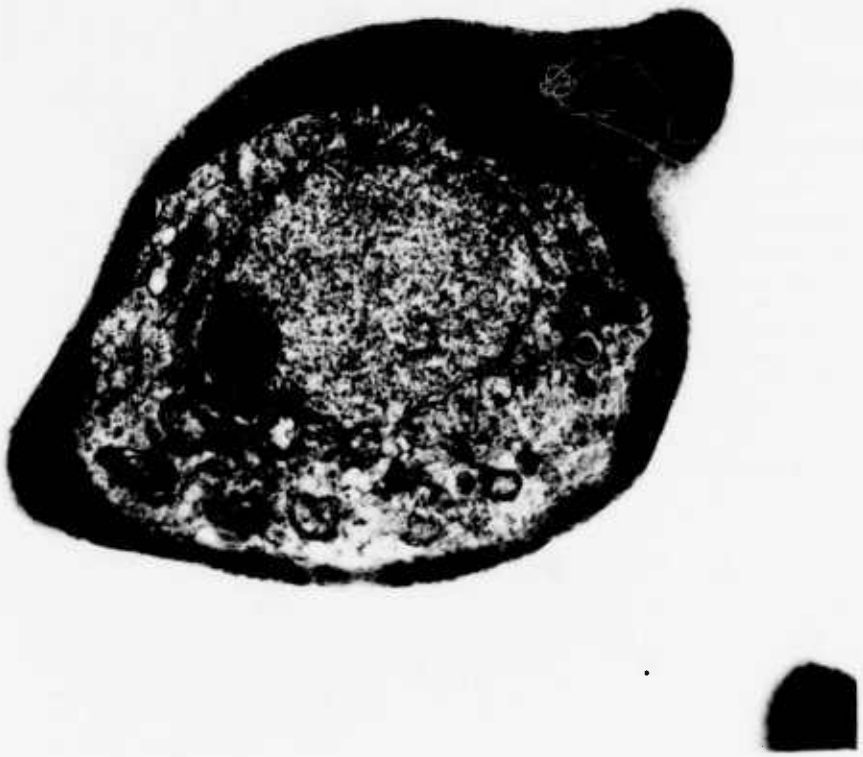

Plate 106. Untreated P.yoelii ssp. QMS strain. (X 26,000)

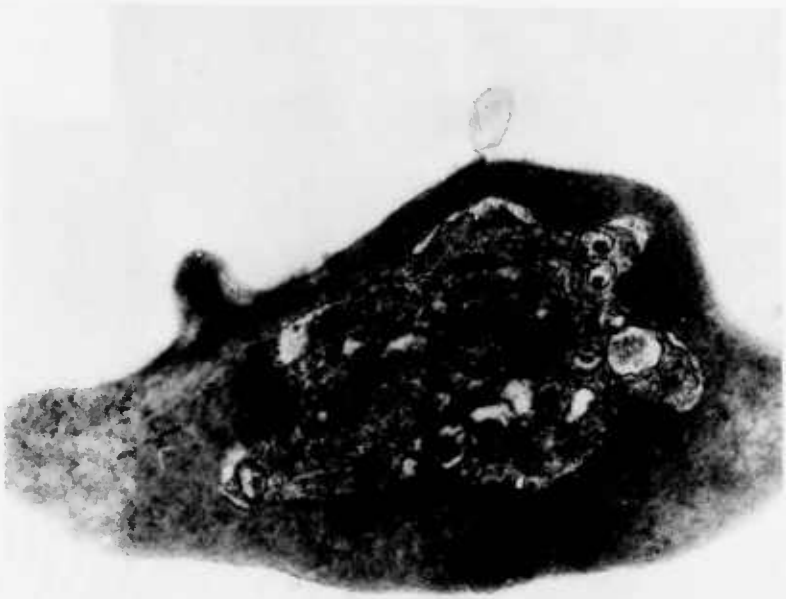

Plate 107. P.yoelii ssp. QMS strain after treatment with combined artemisinin and mefloquine. (X 26,000)

3.3.25 P.yoelii ssp. MPS strain.

The MPS strain was developed during studies into the ability of the triple combination of mefloquine, pyrimethamine and sulfadoxine to delay the emergence of resistance to the components of the mixture. Studies on the cross-resistance patterns of this strain are still continuing. Resistance has been confirmed to date against quinidine, halofantrine, artemisinin, pyronaridine, doxycycline, mepacrine, LON 1765 and WR 228258.

Further studies of the ultrastructure of this strain are required as there were insufficient parasites present in the treated group samples to draw any reliable conclusions. The untreated controls showed no abnormal features other than an apparent increase in the number of mitochondria.

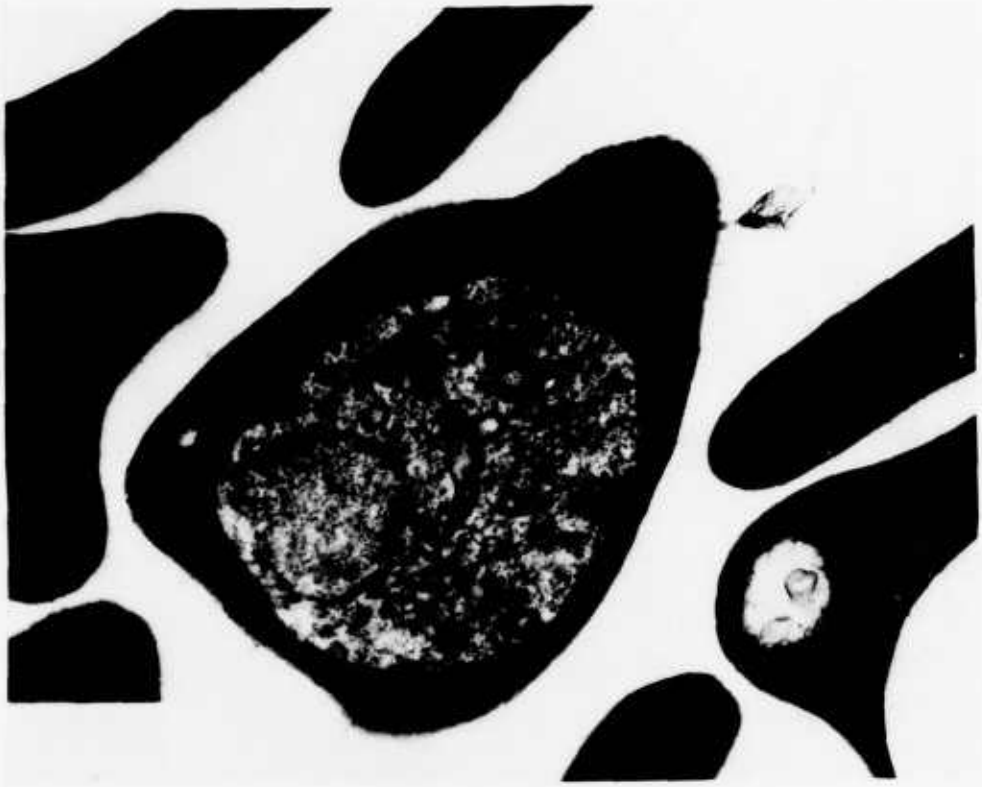

Plate 108. Untreated P.yoelii ssp. MPS strain. (X 20,000)

Plate 109. Untreated P.yoelii ssp. MPS strain. (X 26,000)

3.3.26. P.yoelii ssp. NS 1765 strain.

This strain was developed by the relapse technique from P.yoelii ssp. NS strain to have primary resistance to the indolo-(3,2-c)quinoline-N-oxide LON 1765. Cross-resistance is present in this strain to chloroquine, amodiaquine, quinidine, halofantrine, pyronaridine, doxycycline, mepacrine and WR 228258. The NS 1765 is hypersensitive to sulfadoxine, Fansidar, cycloguanil and clindamycin.

Examination of the ultrastructure of the untreated NS 1765 parasites showed that the mitochondria were normal and that many of the pigment granules were very long. The pigment vacuoles were frequently extremely large and contained short crystalline granules. Treated parasites generally presented a similar picture to the controls but with gross distension of the vacuoles containing pigment and an increase in the number of food vacuoles.

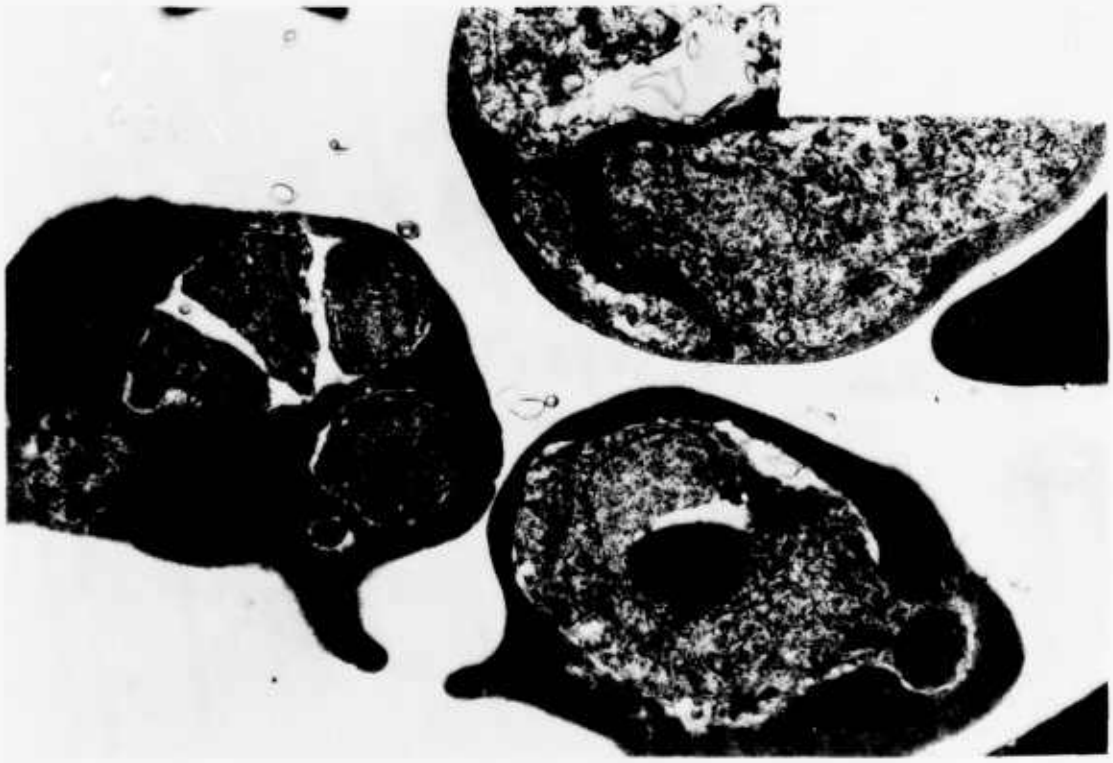

Plate 110. Untreated P.yoelii ssp. NS 1765 strain. (X 16,600)

Plate 111. Untreated P.yoelii ssp. NS 1765 strain. (X 50,000)

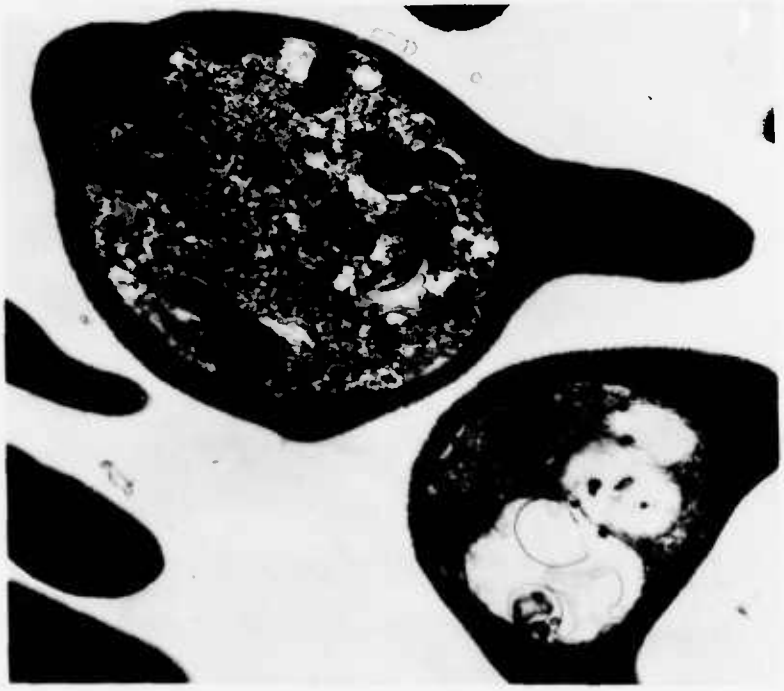

Plate 112. P.yoelii ssp. NS 1765 strain following treatment with LON 1765. (X 16,660)

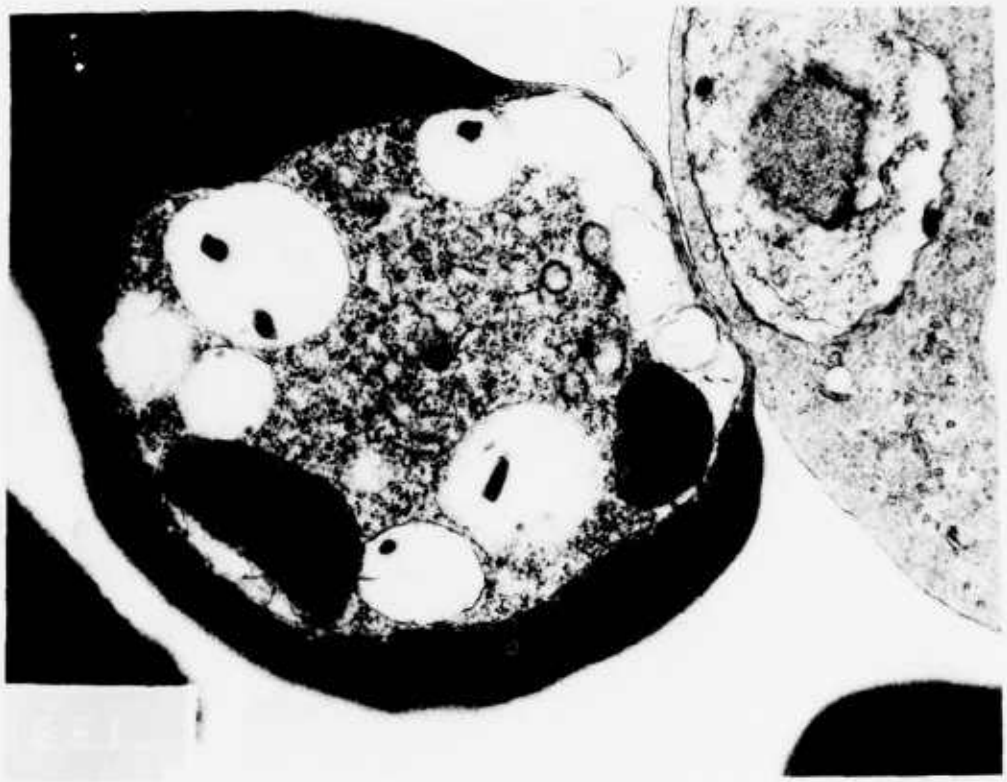

Plate 113. P.yoelii ssp. NS 1765 strain following treatment with LON 1765. (X 26,000)

3.3.27 P.yoelii ssp. NS 1708 strain.

Primary resistance was developed in P.yoelii ssp. NS strain to the Mannich base WR 228258. Cross-resistance was found to exist to chloroquine, amodiaquine, menoctone (slight), doxycycline, mepacrine, and LON 1765. The NS 1708 strain is hypersensitive to sulfadoxine, Fansidar, floxacrine and clindamycin.

Ultrastructural studies show the untreated parasites to be abnormal looking with plentiful mitochondria and normal crystalline pigment. An increased number of food vacuoles were present. Although a very dramatic clumping and reduction of pigment to a dense, black stained mass occurred, exposure to WR 228258 produced little change to other parts of the parasite.

Plate 114. Untreated P.yoelii ssp. NS 1708 strain. (X 26,000)

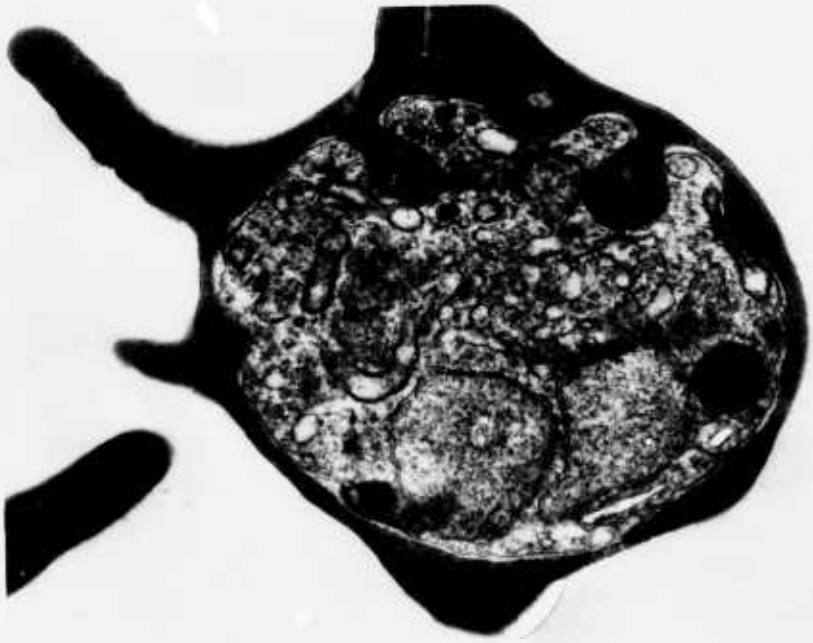

Plate 115. Untreated P.yoelii ssp. NS 1708 strain. (X 26,000)

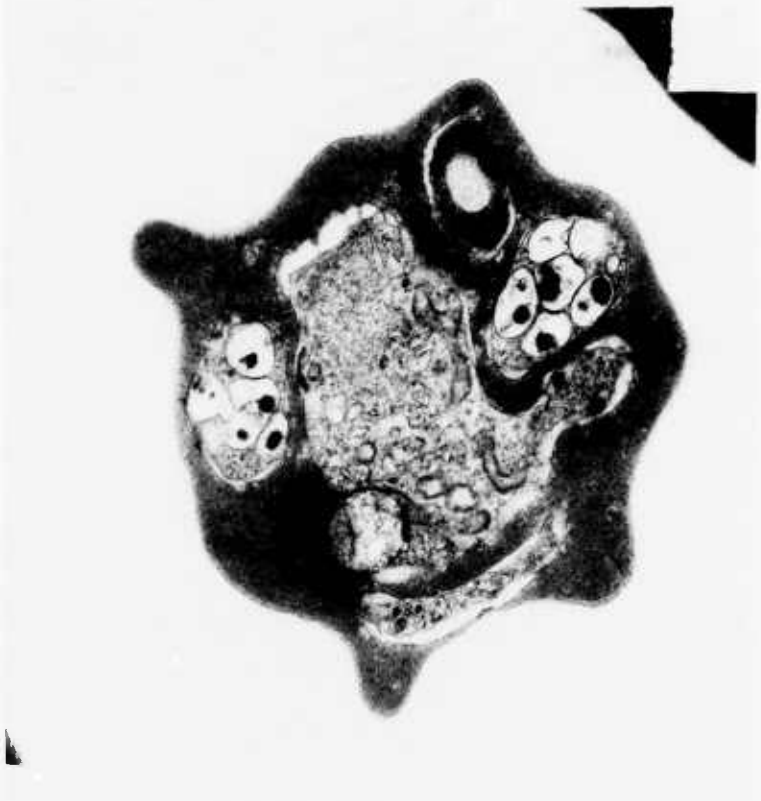

Plate 116. P.yoelii ssp. NS 1708 strain after exposure to WR 228258. (X 20,000)

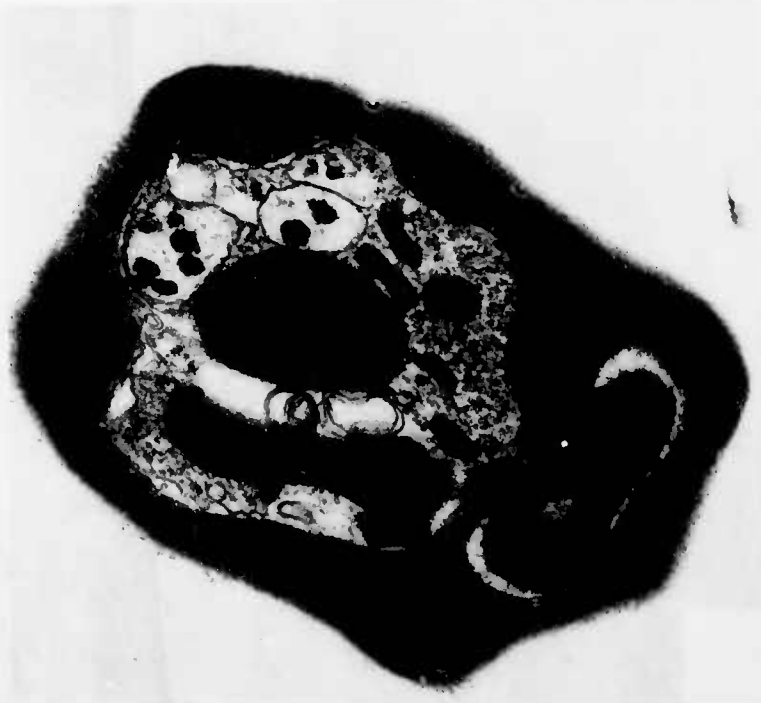

Plate 117. P.yoelii ssp. NS 1708 after exposure to WR 228258. (X 26,000)

3.3.28 P.yoelii ssp. SPN strain.

This pyronaridine resistant strain is derived from P.yoelii ssp. NS and was developed in parallel with the NPN line. Apart from its primary resistance to pyronaridine, it is also resistant to chloroquine, amodiaquine, primaquine, quinine, cinchonine, quinidine, mefloquine, halofantrine, artemisinin, cycloguanil, menoctone, doxycycline, mepacrine, LON 1765 and WR 228258. Hypersensitivity exists to sulfadoxine, Fansidar, floxacrine and clindamycin.

Electron microscope examination reveals that the untreated controls possess very large polyribosomes which are apparently very active and there are possibly extra mitochondrial membranes. large "fuzzy" pigment granules are present in enlarged vacuoles. Parasites which have been exposed to pyronaridine are vacuolated with decreased ribosomes present in the endoplasmic reticulum and the mitochondria exhibit some membrane changes. Marked peripheral and nuclear membrane blebbing are seen.Multiple invasion of the erythrocytes is a common feature of this strain.

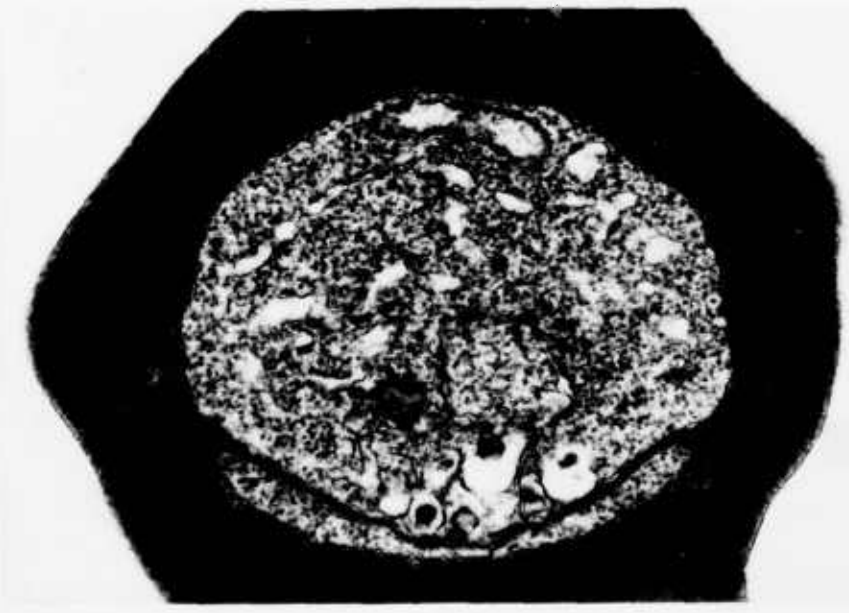

Plate 118. Untreated P.yoelii ssp. SPN strain. (X 26,000)

Plate 119. Untreated P.yoelii ssp. SPN strain. (X 52,000)

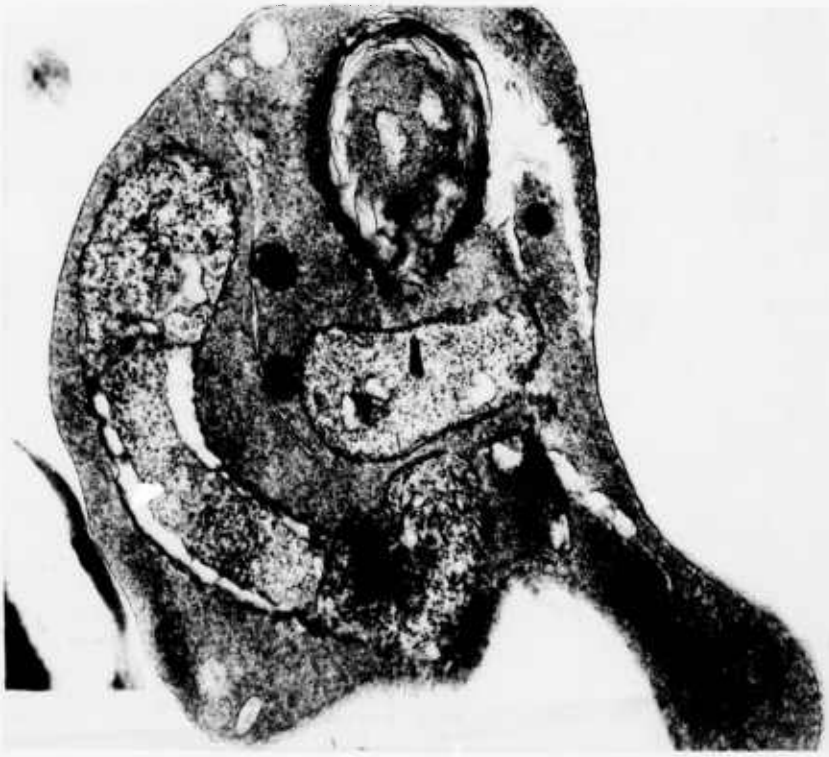

Plate 120. P.yoelii ssp. SPN strain after exposure to pyronaridine. (X 26,000)

Plate 121. P.yoelii ssp. SPN strain after exposure to pyronaridine. (X 26,000)

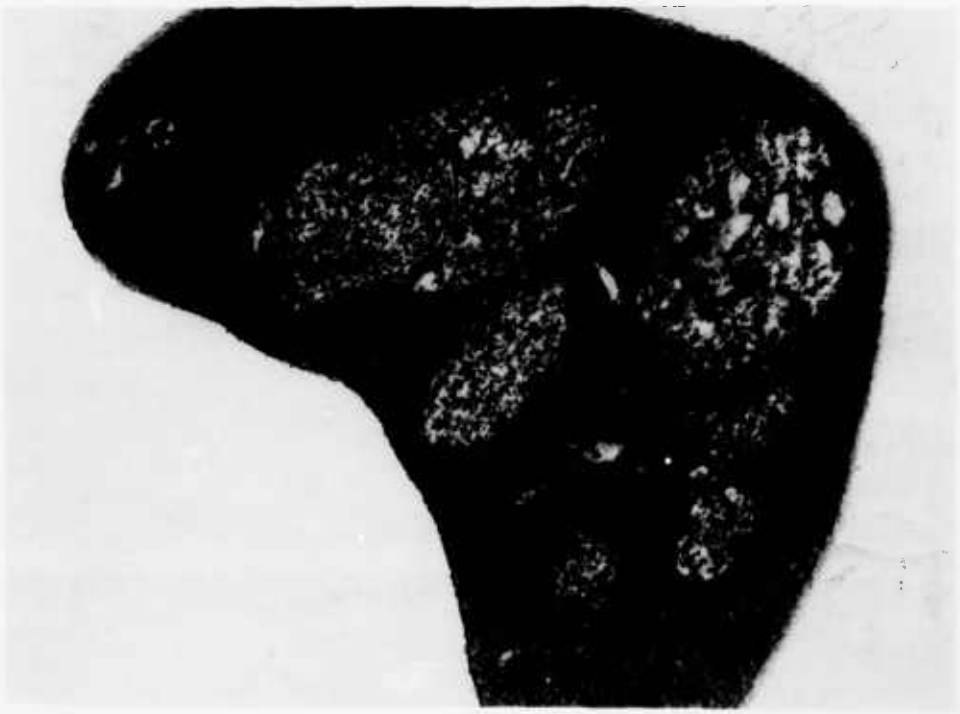

Plate 122. P.yoelii ssp. SPN strain after exposure to pyronaridine. (X 32,000)

3.3.29 P.yoelii nigeriensis NIG strain.

P.y.nigeriensis NIG strain, which is used by us for most of our vector oriented studies, has never been exposed to drug pressure of any nature. It is interesting to note, therefore, that certain inherent resistance occurs to some of the antimalarials examined. Whilst this lack of sensitivity is not of an inordinately high level, it does suggest that marked resistance would be rapidly gained to a fairly wide range of drugs. The NIG strain is slightly resistant naturally to primaquine, quinidine, artemisinin, cycloguanil, doxycycline, mepacrine and LON 1765. Enhanced sensitivity occurs to sulfadoxine, Fansidar and floxacrine.

Ultrastructurally, there is little of note to be seen. The parasites are normal and healthy in appearance with less prominent mitochondria than seen in P.berghei N strain (3.3.1). The pigment granules are long but otherwise normal.

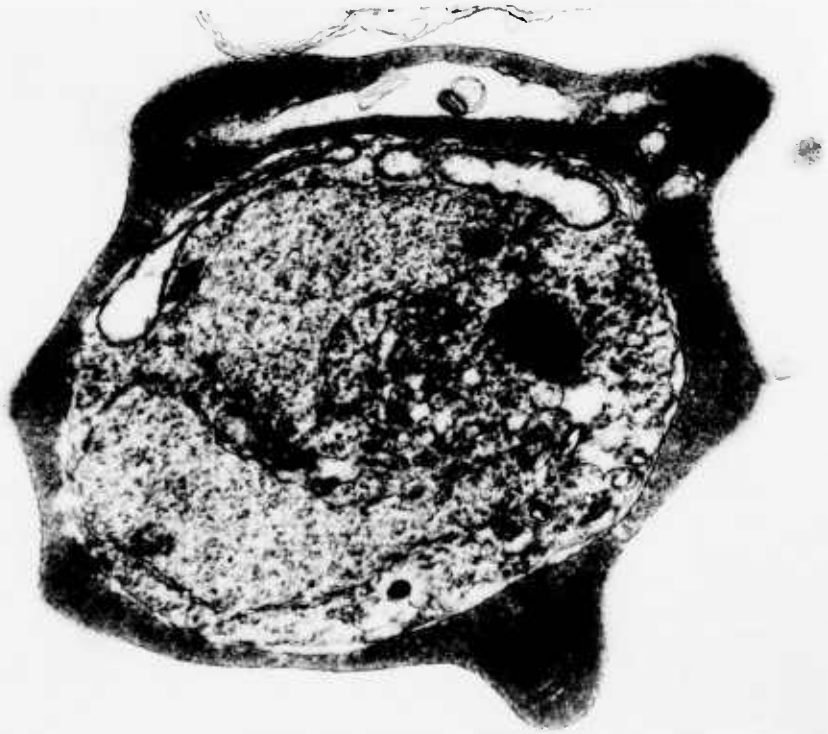

Plate 123. P.y.nigeriensis NIG strain. (X 32,000)

Plate 124. P.y.nigeriensis NIG strain. (X 50,000)

3.3.30 P.yoelii yoelii 17X strain.

No resistance data are available for this strain, but experience in vitro with the exo-erythrocytic stages in hepatocyte culture, suggests that sensitivity to primaquine is reduced when compared to P.berghei N strain. It would seem to be quite possible that this lack of sensitivity will extend to other compounds, in the same way that we have demonstrated with P.yoelii ssp. NS strain and P.y.nigeriensis, and caution should be exercised when using this strain for drug testing.

Ultrastructurally the parasites look normal. The cytoplasm is pale and contains fewer ribosomes than are found in P.berghei, the mitochondria are large and plentiful and the very prominent pigment vacuoles contain large granules of pigment.

Plate 125. P.y.yoelii 17X strain. (X 35,000)

Plate 126. P.y.yoelii 17X strain. (X 50,000)

3.4 Cytoplasmic polyhedrosis virus in Anopheles stephensi.

Cytoplasmic polyhedrosis viruses (CPV) are identified by the production of polyhedral inclusion bodies, usually in the cytoplasm of infected cells, containing isometric virus particles.

Infected mosquito larvae show little gross pathology and their behaviour and development are not noticeably altered from that of healthy larvae. Portions of the stomach of infected larvae become enlarged and have yellowish white patches which are due to large aggregates of white occlusion bodies in the cytoplasm of infected cells. Most infected larvae pupate and emerge as adults normally.

Ultrastructurally, the most marked effect on the cell is the disruption of the rough endoplasmic reticulum and Golgi apparatus. Lysis of the cell membrane eventually occurs and polyhedra are releasedinto the lumen of the midgut and excreted. Regenerative cells within the gut epithelium replace the damaged ones to some extent, which probably accounts for the chronic rather than lethal infections produced by CPV.

In mosquitoes infected with P.y.nigeriensis, inclusion bodies and virus particles have been found within oocysts and total destruction of the oocyst eventually results from the CPV infection.

The typical appearance of A.stephensi when infected with CPV and the effects of the virus on oocysts in. P.y.nigeriensis infected mosquitoes are shown in Plates 127 through 138 in the following pages.

Plate 127. Early stage of CPV infection in midgut of adult A.stephensi. (X 52,000)

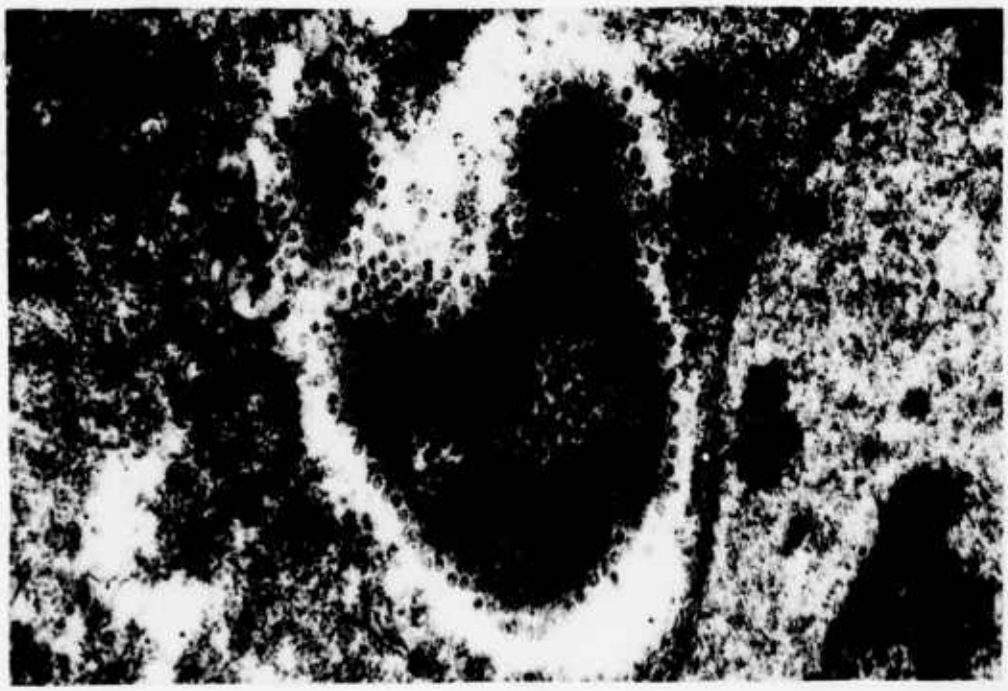

Plate 128. Early stage of CPV infection in midgut of adult A.stephensi showing the presence of both complete and incomplete virus. (X 40,000)

Plate 129. Early stage of CPV infection showing complete and incomplete virus. (X 80,000)

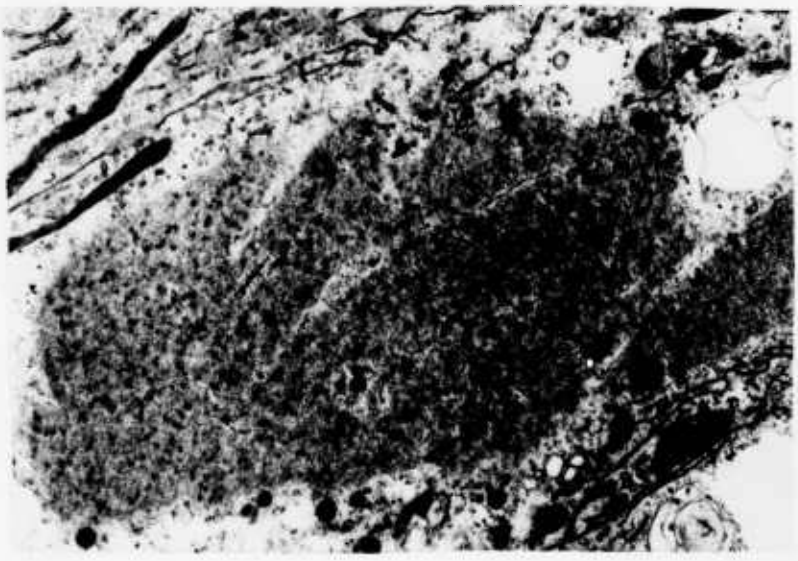

Plate 130. Membrane changes in PCV infected A.stephensi. (X 10,000)

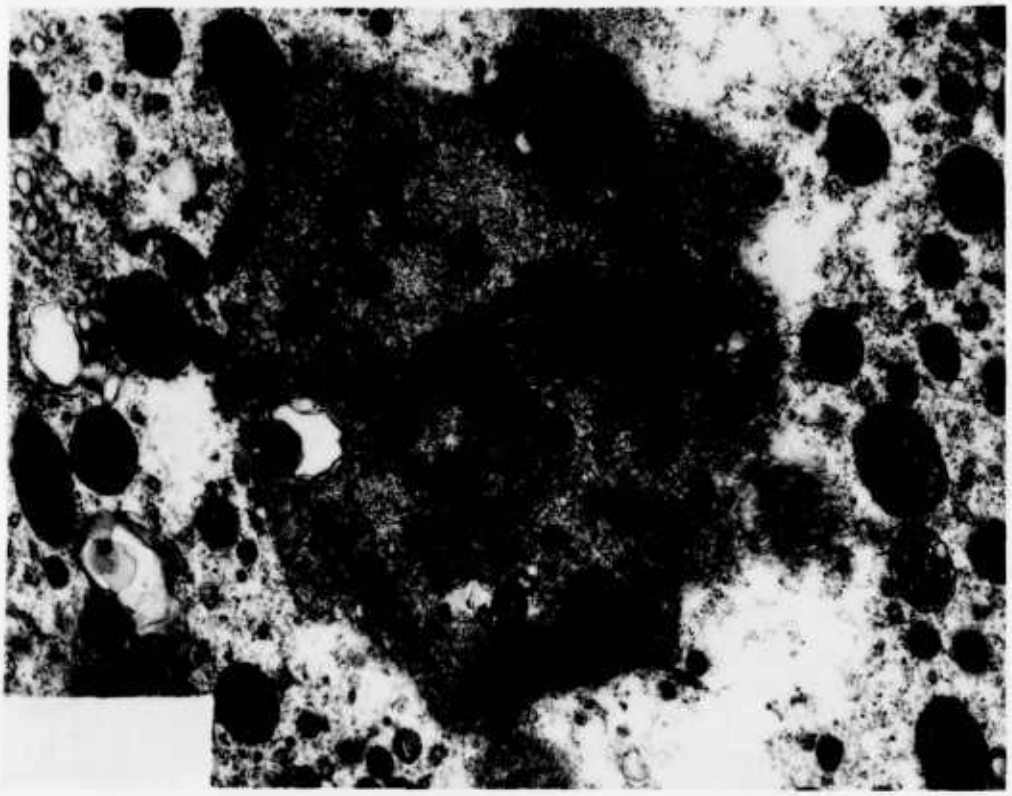

Plate 131. Further membrane changes and virus in infected midgut. (X 26,000)

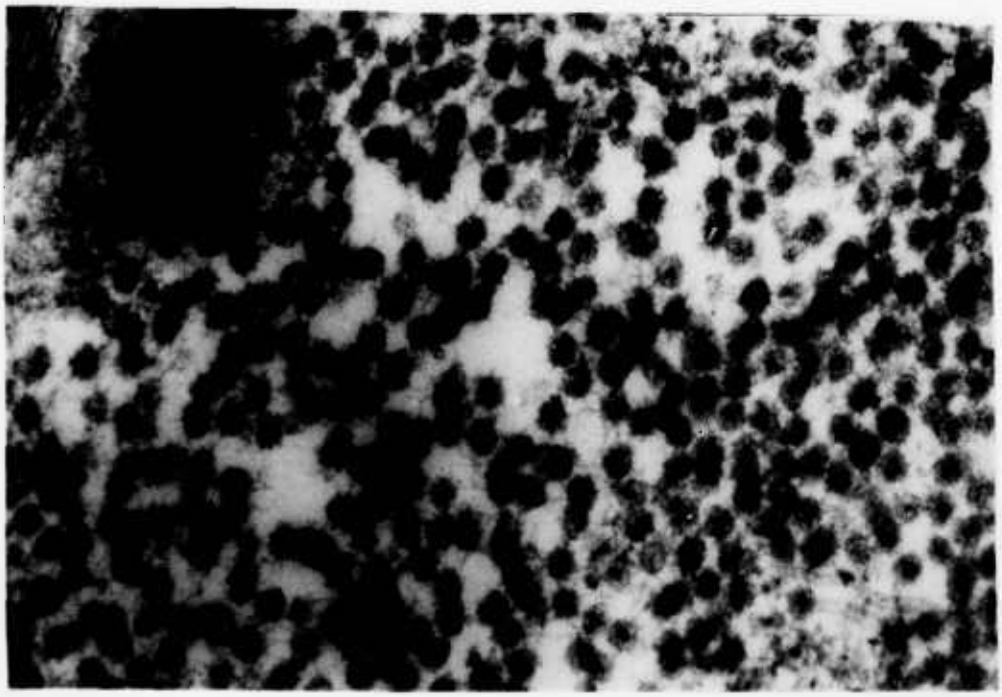

Plate 132. High magnification of CPV in A.stephensi midgut. (X 100,000)

Plate 133. CPV infected midgut showing the presence of polyhedra. (X 64,000)

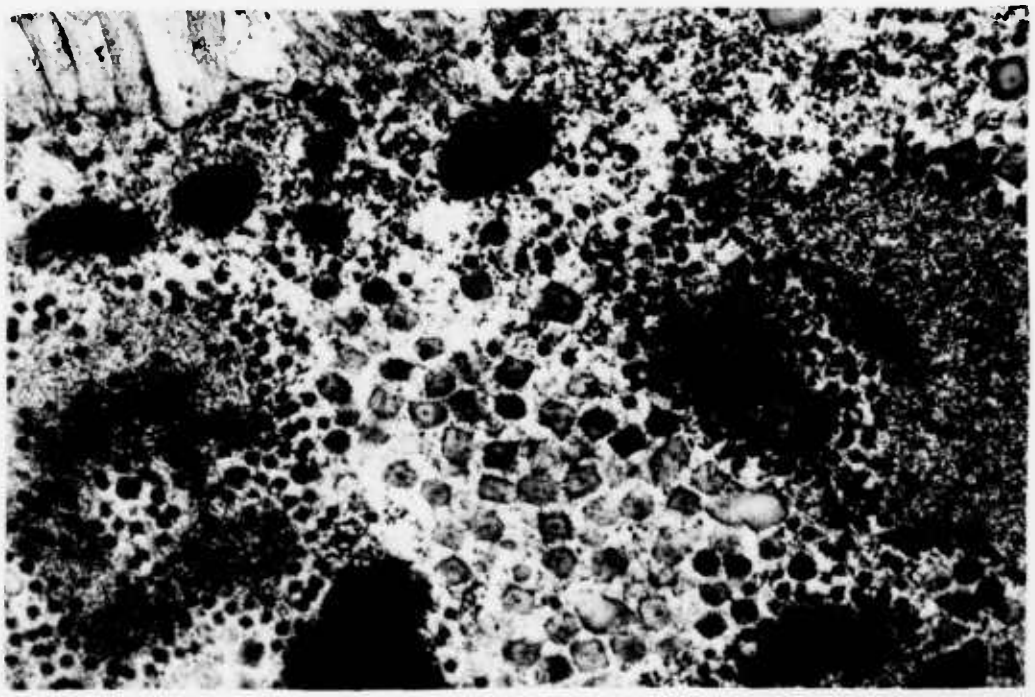

Plate 134. Infected midgut showing virus within polyhedra. (X 40,000)

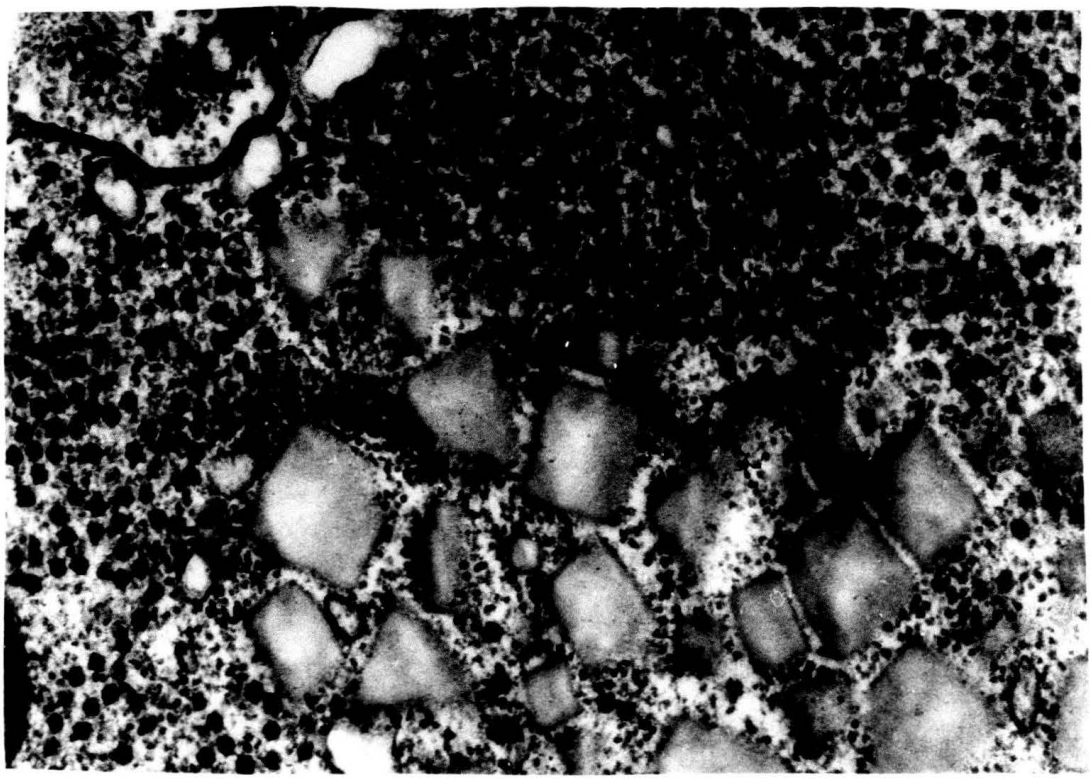

Plate 135. Infected midgut with many complete viruses and polyhedra. (X 80,000)

Plate 136. Large virus array in midgut of A.stephensi. (X 20,000)

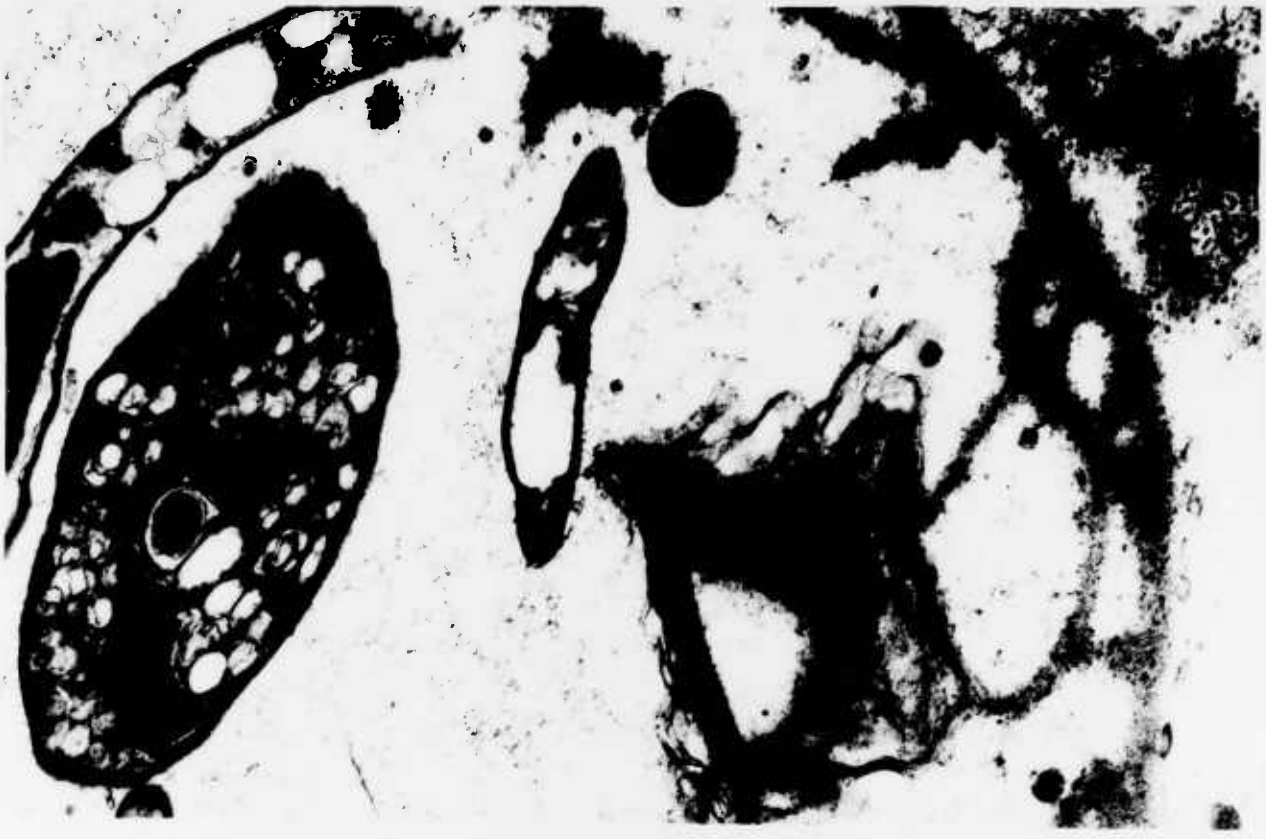

Plate 137. Infected oocyst of P.y.nigeriensis. Note the vacuolation of the sporozoite and the virus in the oocyst wall. (X 52,000)

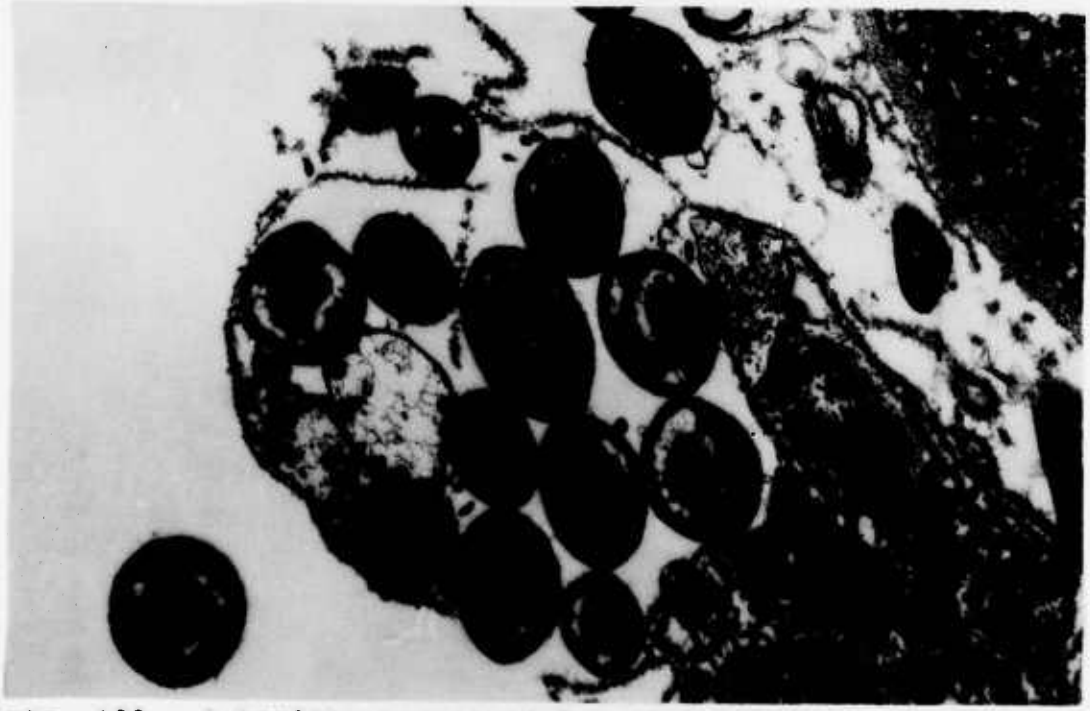

Plate 138. A healthy oocyst of P.y.nigeriensis in a virus-free A.stephensi. (X 26,000)

During the course of routine EM screening of our colony of A.stephensi for the presence of pathogenic micro-organisms such as microsporidia or viruses, the presence of CPV was detected. Since this virus interferes radically with the transmission of rodent malaria, it was considered advisable to determine the way in which PCV was being transmitted through our colony as a prerequisite to any attempt to eliminate the virus. CPV has long been known to occur in laboratory colonies of A.stephensi, but the source of this infection has not been clearly identified.

EM studies were made of all stages in the life cycle of the virus infected mosquitoes and examination of these has shown that CPV can be identified at all stages and in many locations within the host. The accompanying electron micrographs (Plates 139 - 145) trace the virus from the nurse cells of the gravid uterus and the unlaid egg through the larval stages to the adult mosquito of the next generation in a typical vertical transmission cycle.

Plate 139. CPV in the nurse cells of the gravid uterus of A.stephensi. (X 1000)

Plate 140. CPV in the nurse cells of the gravid uterus. (X10,000)

Plate 141. CPV in the nurse cells of the gravid uterus. (X 80,000)

Plate 142. Small numbers of CPV inside the ovum of A.stephensi. (X 50,000)

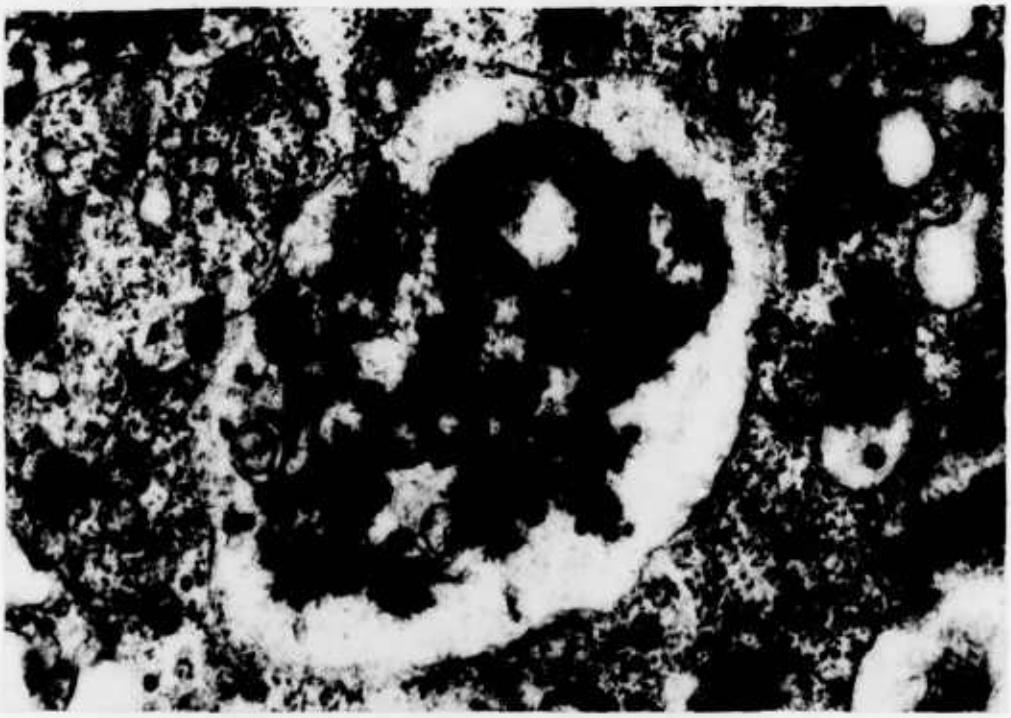

Plate 143. Low density infection with CPV in the first stage larva. (X 50,000)

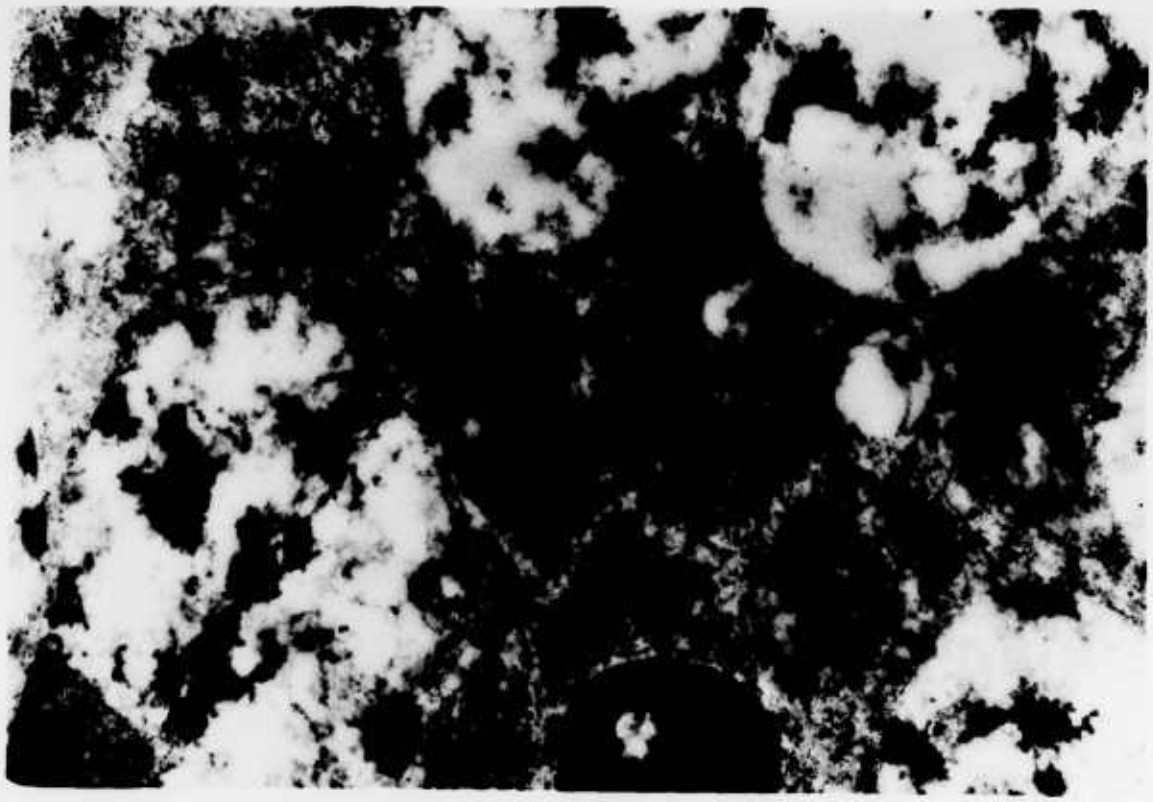

Plate 144. CPV in third stage larva of A.stephensi. (X 45,500)

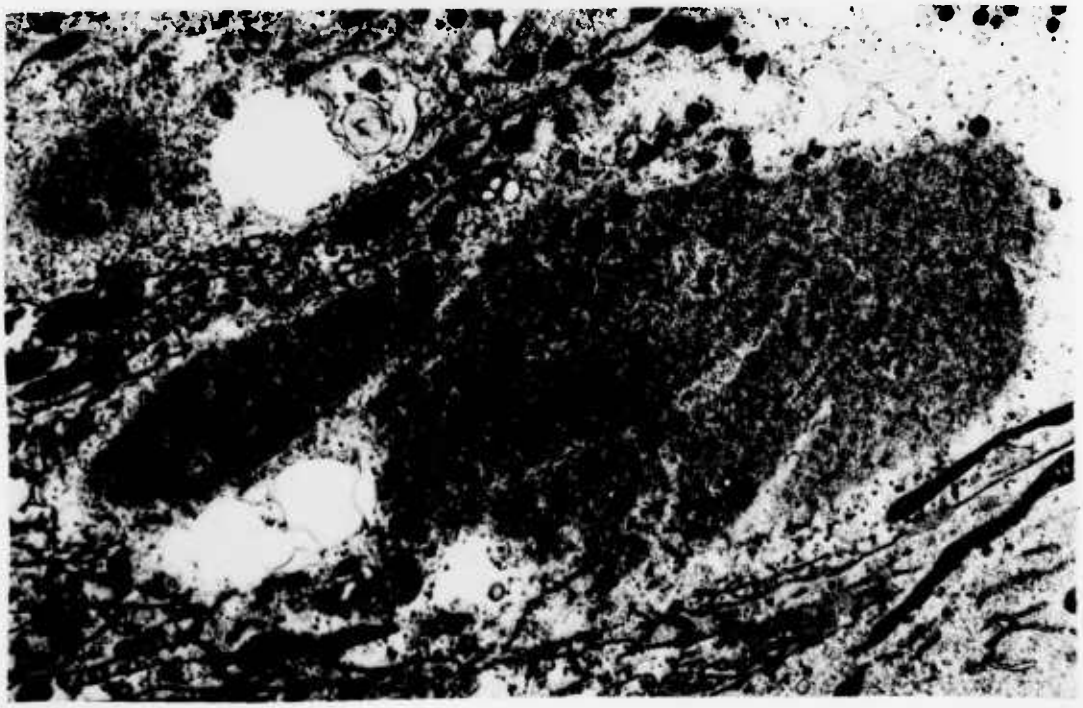

Plate 145. Heavy infection with CPV in the midgut of a female A.stephensi. (X 20,000)

The only practical course of action when encountering CPV infections in A.stephensi colonies has been to destroy the colony and establish a new one from a nucleus of clean uninfected mosquitoes. The main drawbacks to this approach are that it can take several months to breed a colony large enough to use and it is also becoming increasingly difficult to obtain clean breeding stock.

A study to determine the feasibility of treating mosquitoes with antiviral agents has identified amantadine hydrochloride as having a direct action on CPV in vivo sufficient to reduce virus numbers to a level which does not interfere with malaria transmission. Continuing studies show that a long term effect lasting through several generations can be achieved, although it is possible that the virus will remain in the colony in a latent form and re-emerge as a problem at a later date. However, it is hoped that a fully effective dose can be identified which will give a permanent solution to this problem.

In the present study, amantadine has been administered either at a 0.05% concentration in 5% sucrose solution to the adults for seven days, or added to the water in the larval trays at a concentration of 0.005%. With either of these regimes good control of the CPV infection was achieved and only very small numbers of virus could be detected in the succeeding five generations. Transmission of rodent malaria by the original treated groups and their descendants has been found to have returned to normal.

Further experiments to determine the optimal dosing regime and to assess the long term effectiveness of amantadine are continuing.

Plate 146. CPV infection in untreated control A.stephensi larva. (X 3,200)

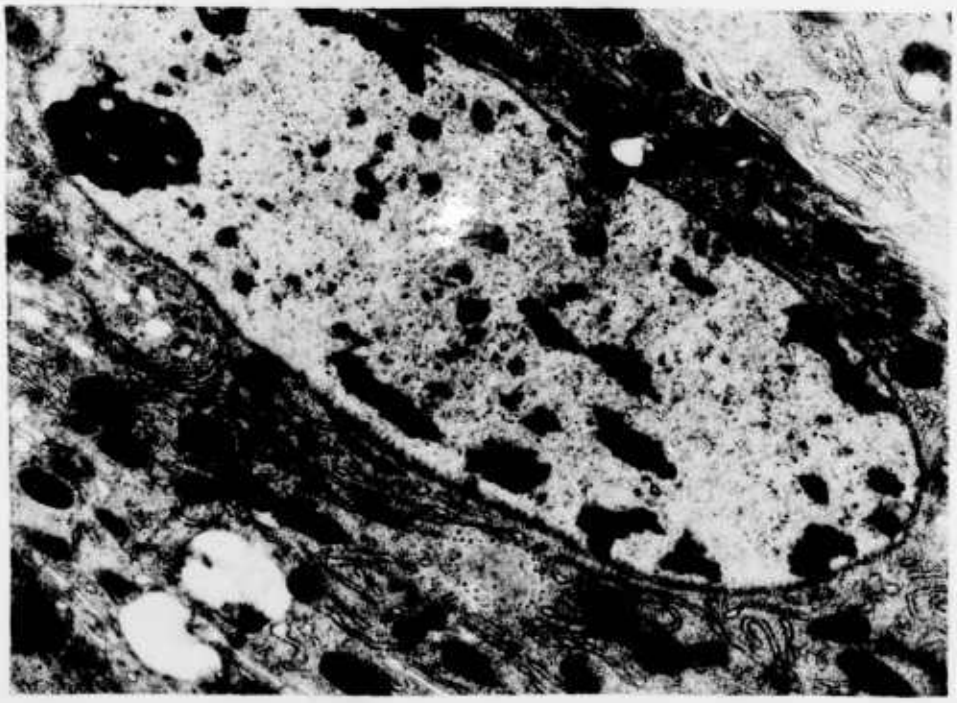

Plate 147. CPV infection in untreated control A.stephensi larva. (X 16,600)

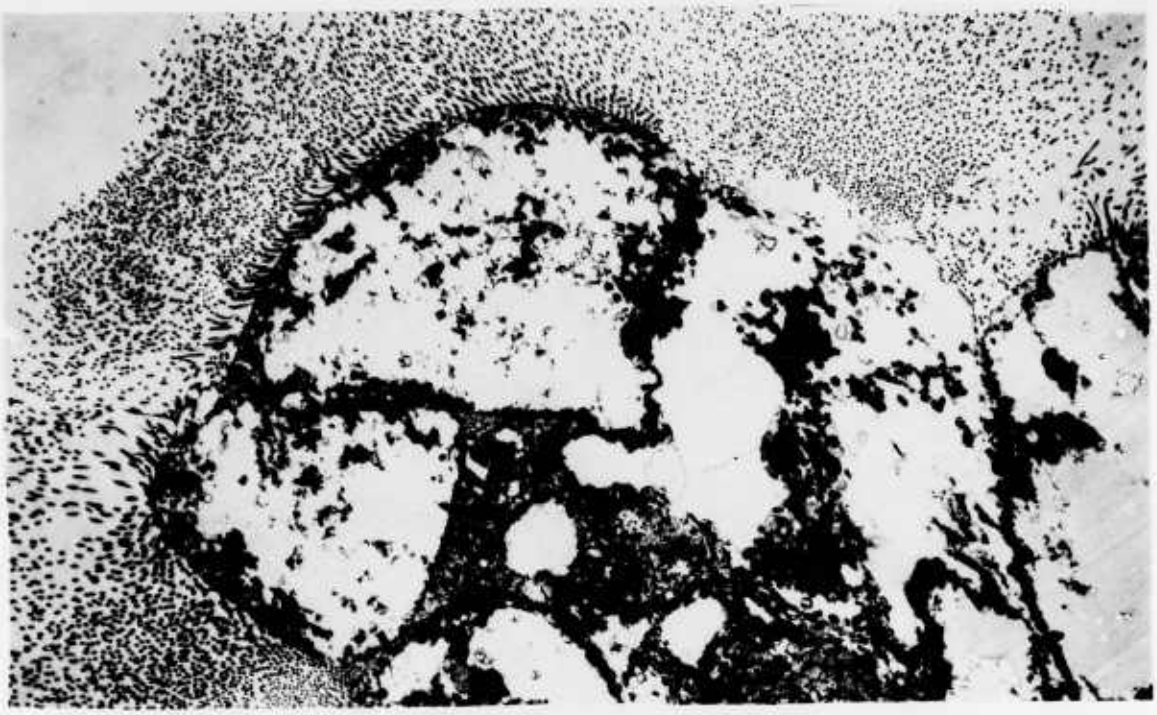

Plate 148. Midgut of untreated adult control A.stephensi infected with CPV. (X 1,300)

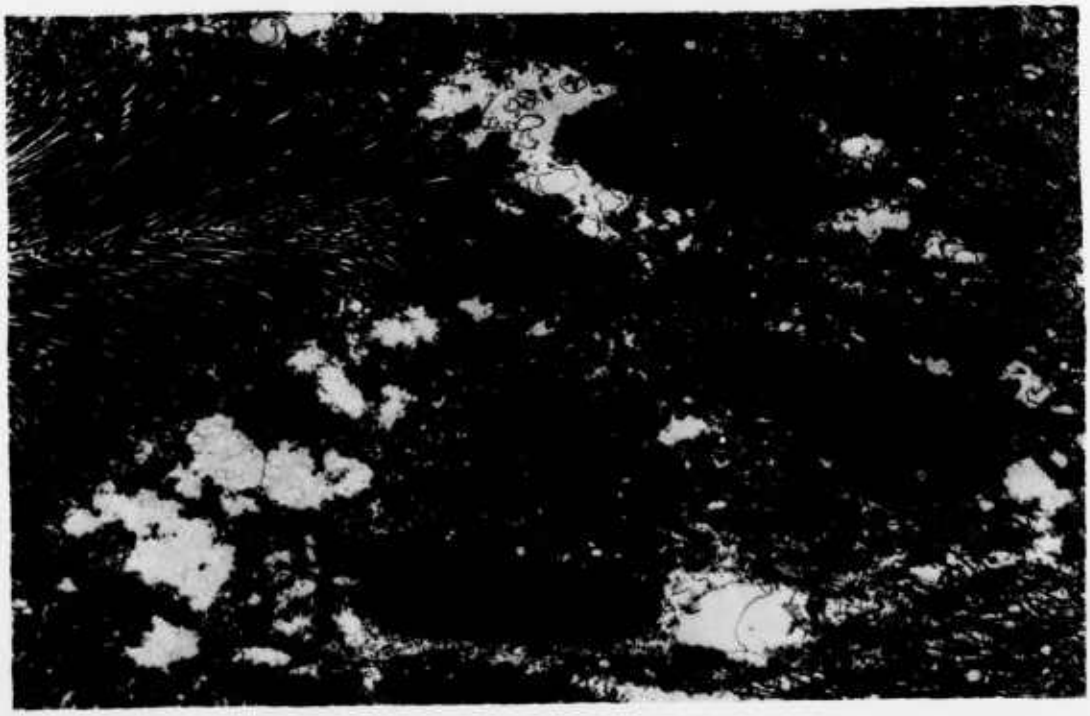

Plate 149. Midgut of untreated adult control A.stephensi infected with CPV. (X 2,600)

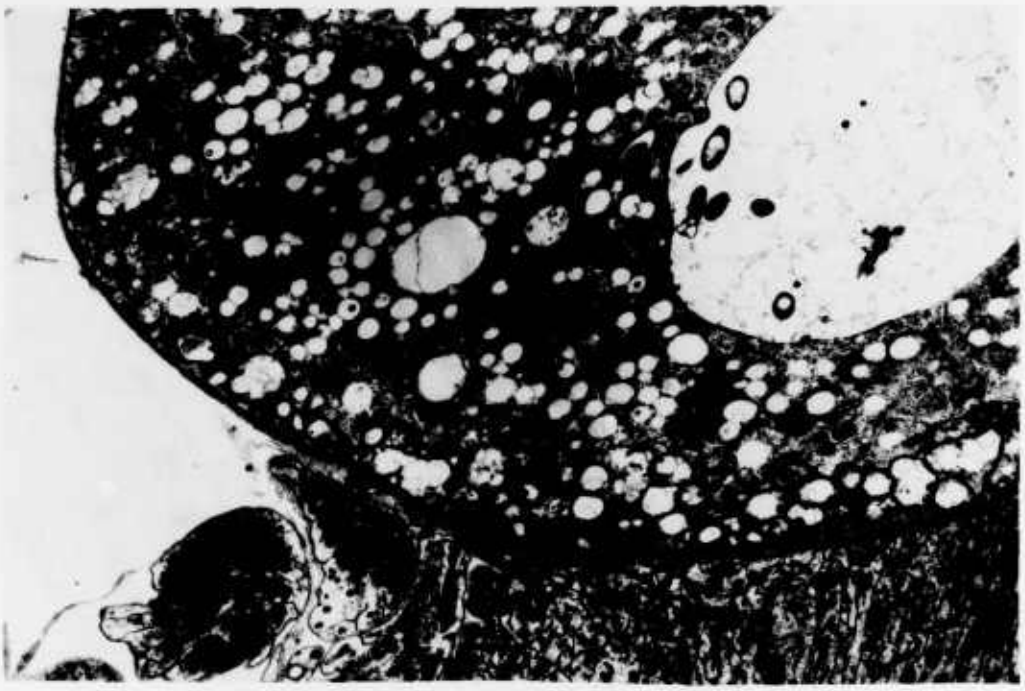

Plate 150. The effect of CPV infection on the development of P.y.nigeriensis in A.stephensi. (X 2,600)

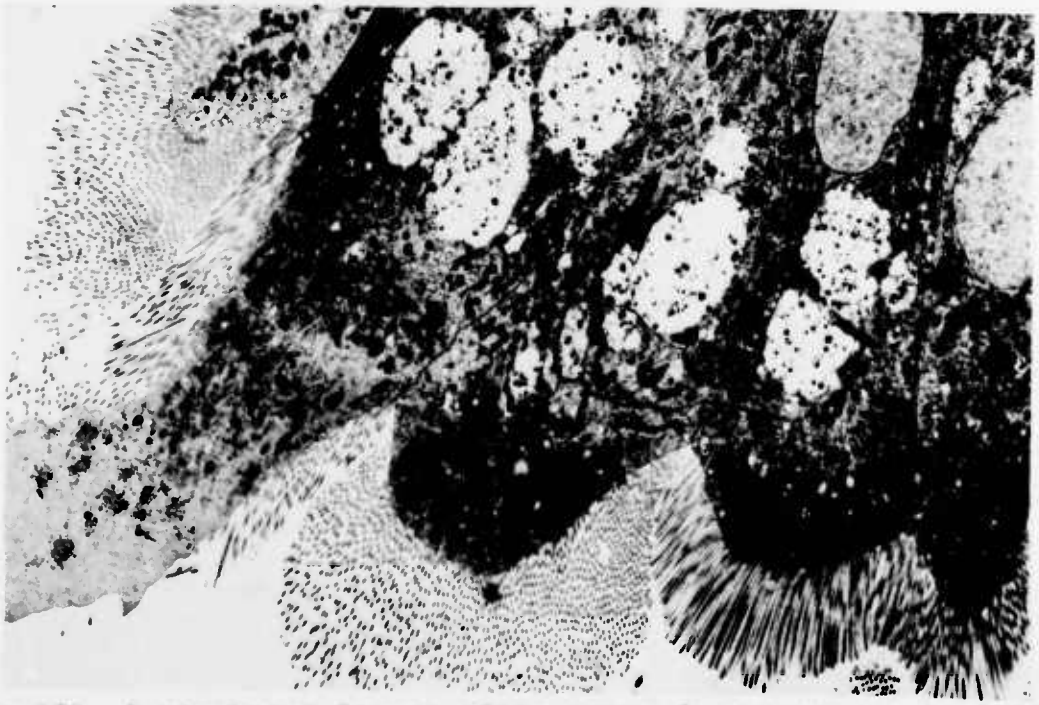

Plate 151. A.stephensi larvae after seven days exposure to 0.005% amantadine hydrochloride in the water of the larval tray. (X 1,300)

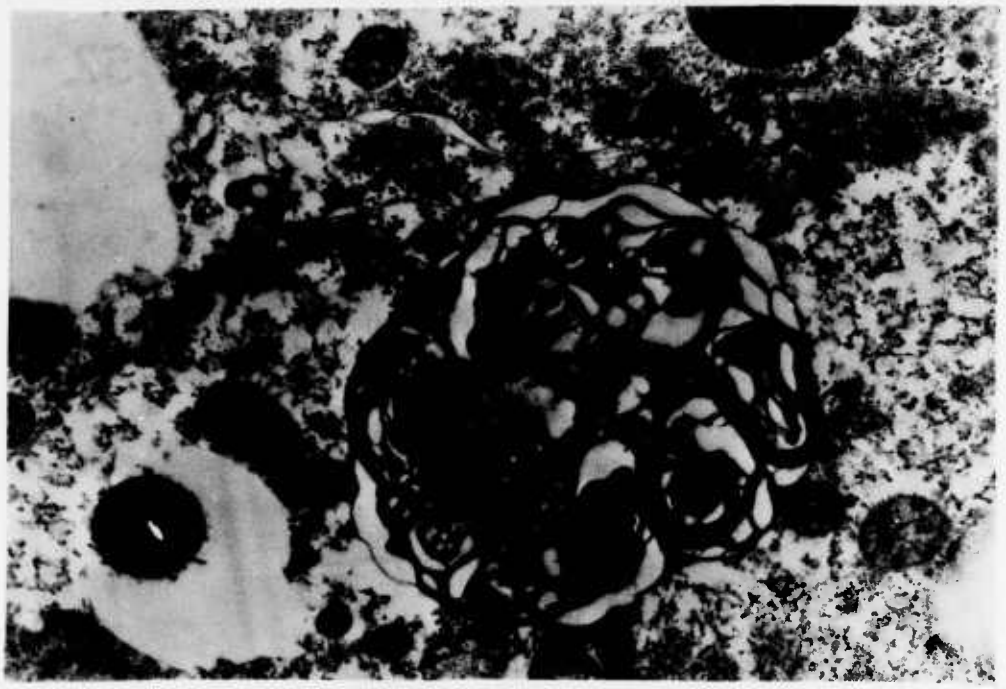

Plate 152. A.stephensi larva after seven days exposure to 0.005% amantadine hydrochloride in the water of the larval tray. (X 6,600)

Plate 153. Midgut of A.stephensi treated for seven days with 0.05% amantadine hydrochloride in the sucrose feed. (X 1,300)

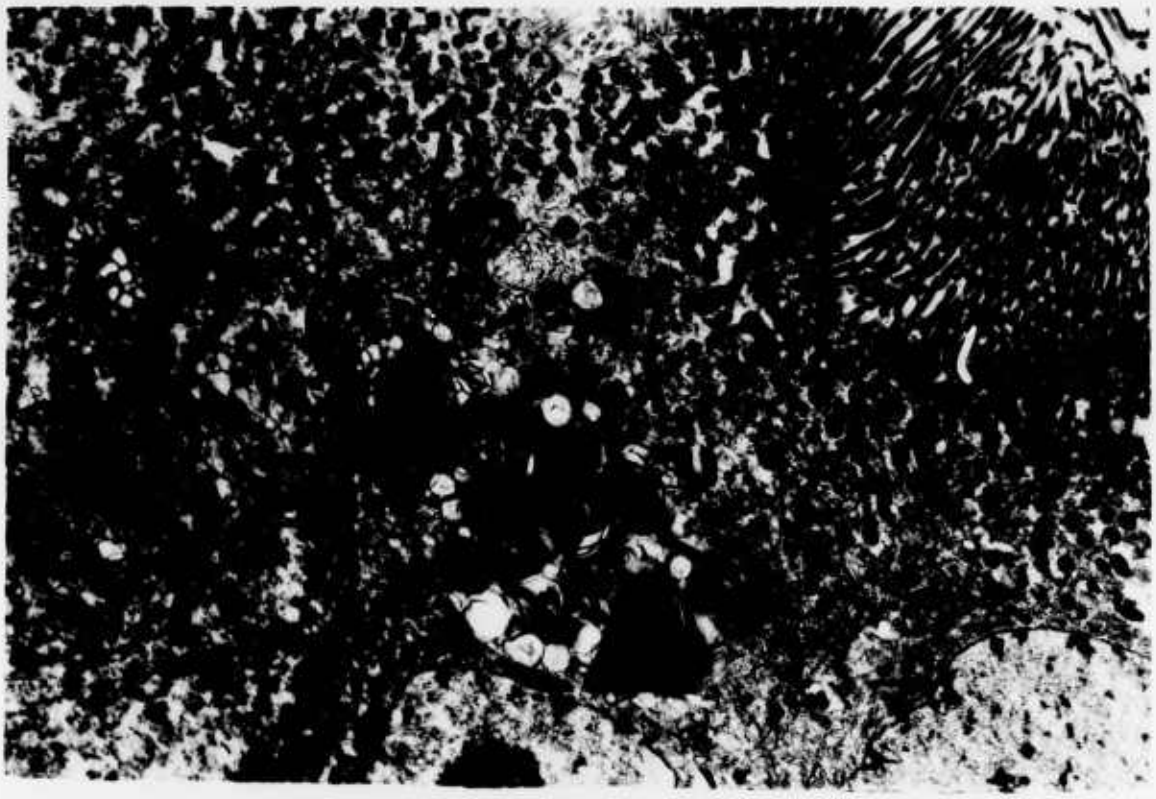

Plate 154. Midgut of A.stephensi treated for seven days with 0.05% amantadine hydrochloride in the sucrose feed. (X 4,000)

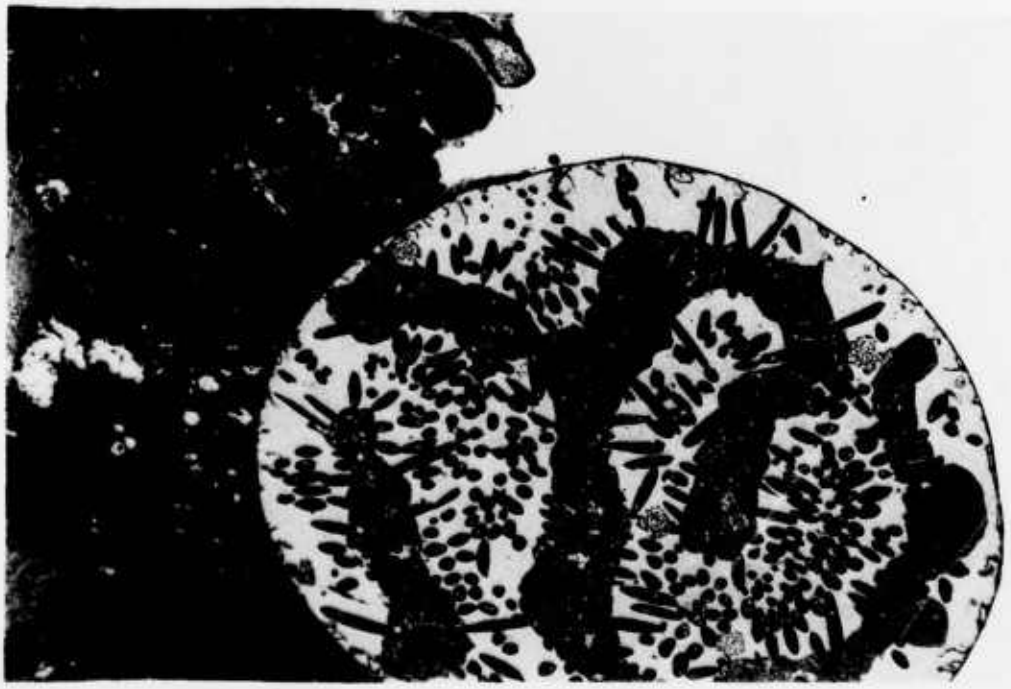

Plate 155. Development of P.y.nigeriensis oocyst in amantadine treated A.stephensi. (X 1,000)

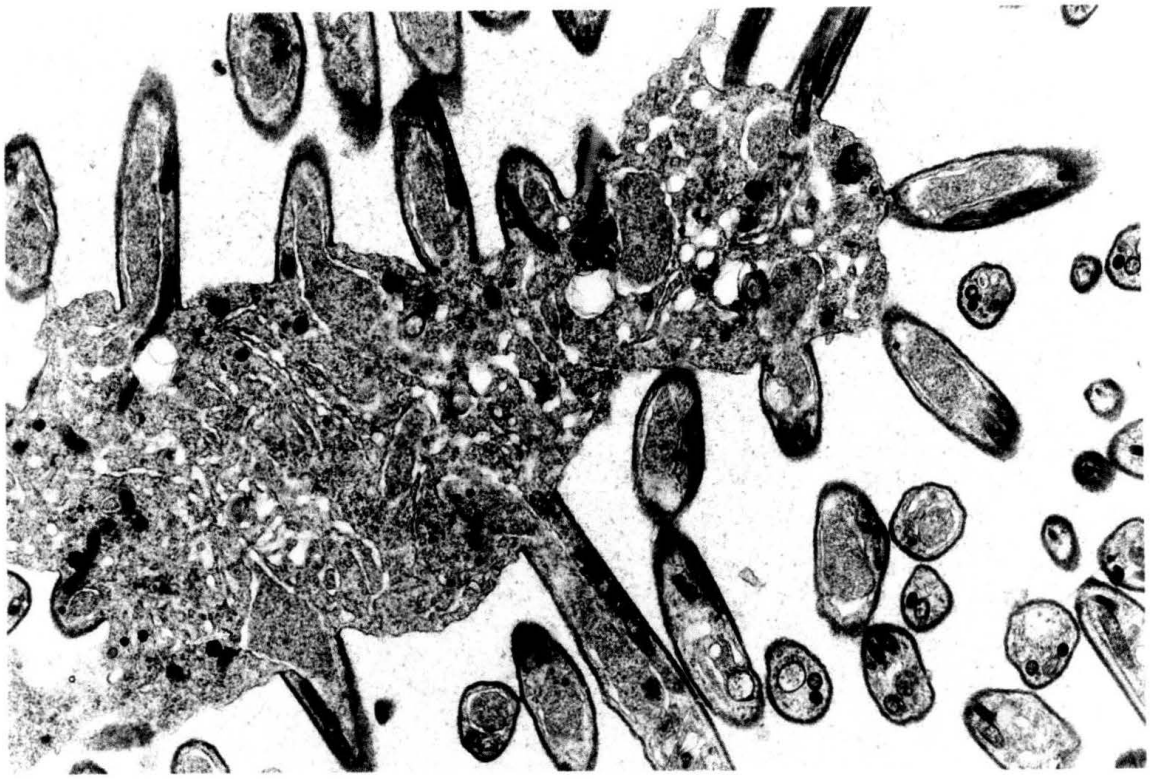

Plate 156. Development of P.y.nigeriensis oocyst in amantadine treated A.stephensi. (X 6,300)

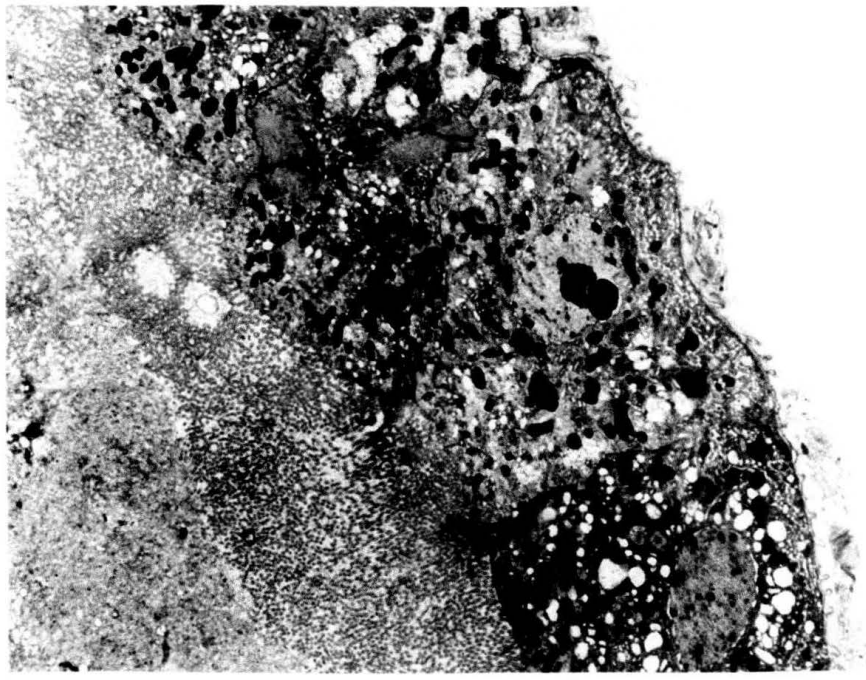

Plate 157. Midgut of adult A.stephensi treated inthe larval stage with 0.005% amantadine hydrochloride. (X 2,000)

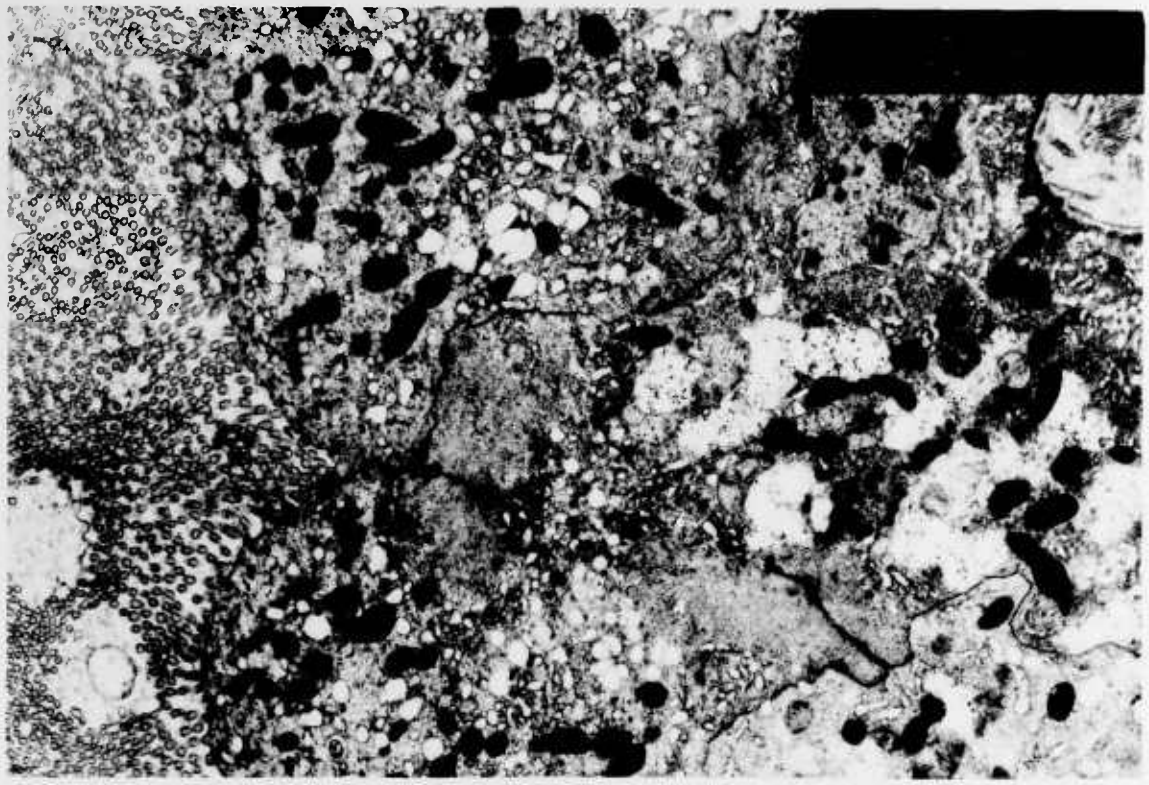

Plate 158 Midgut of adult A.stephensi treated in the larval stage with 0.005% amantadine hydrochloride.

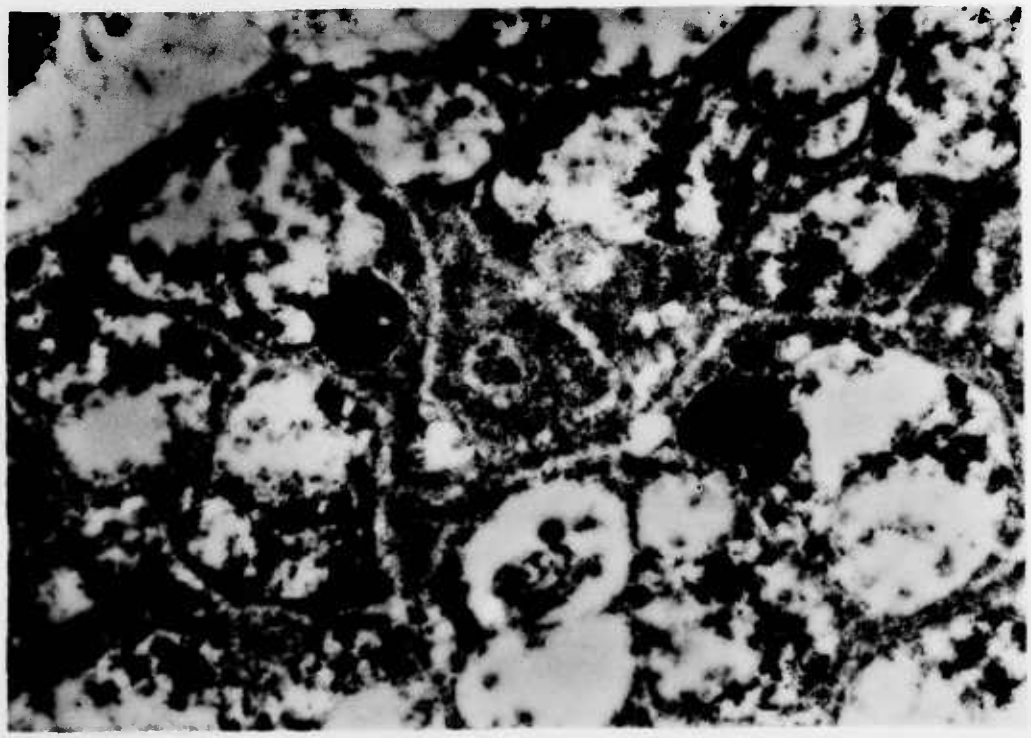

Plate 159. Second generation larva of A.stephensi descended from amantadine treated adults. (X 12,600)

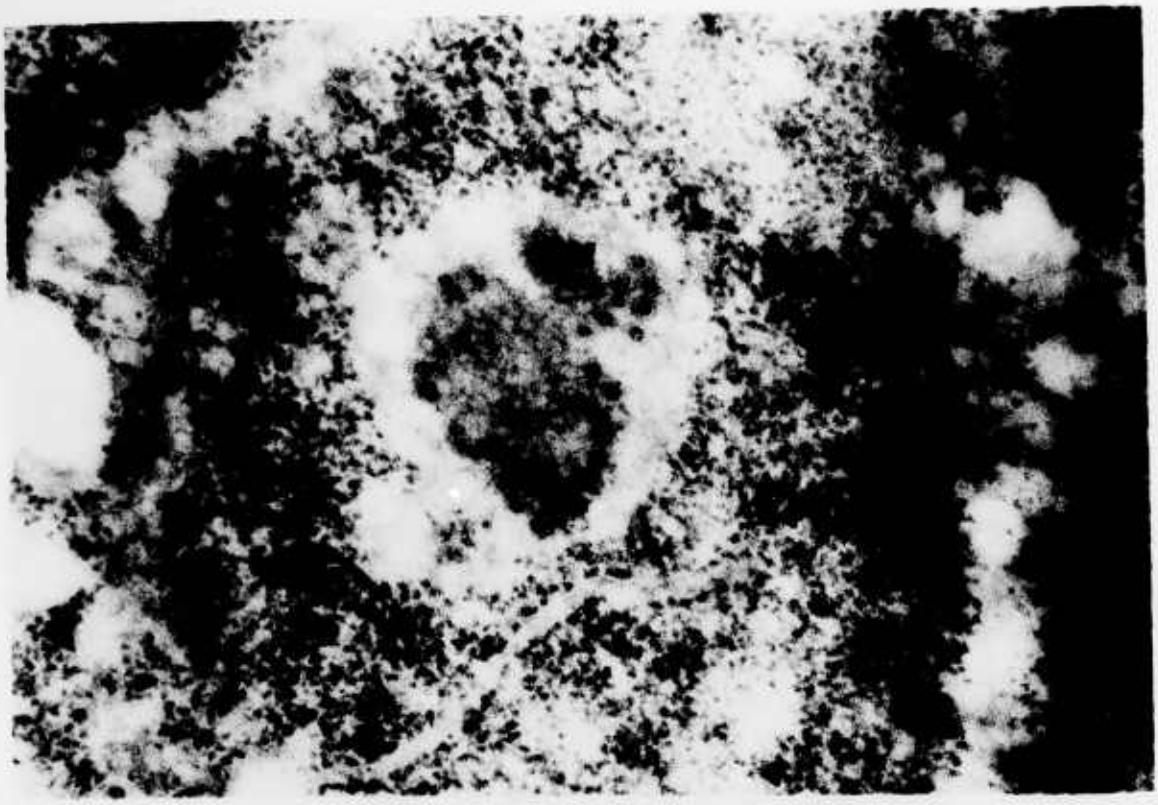

Plate 160. Second generation larva of A.stephensi descended from amantadine treated adults.

Plate 161. Midgut of second generation adult A.stephensi descended from amantadine treated adults.

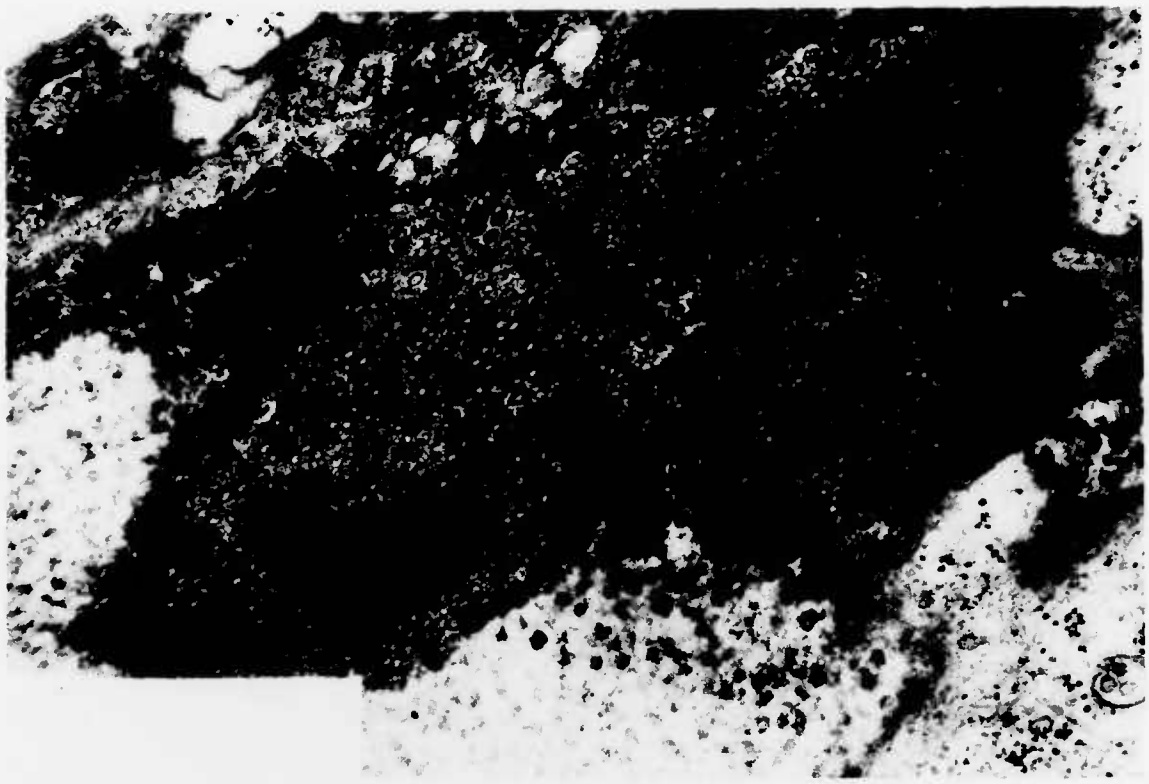

Plate 162. Midgut of second generation adult A.stephensi descended from amantadine treated adults. (X 26,000)

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