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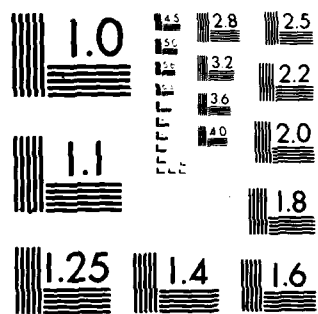
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CHEMOTHERAPY OF LEISHMANIASIS

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Final Report

by

Wallace Peters, MD, DSc.

December 1978

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US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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Liverpool School of Tropical Medicine
Pembroke Place, Liverpool L3 5QA
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Simplified screening procedures for <u>in vivo</u> evaluation in mice of potential antileishmanial compounds are described in this report. Data are provided on the antileishmanial activity of 12 WRAIR compounds and 6 other compounds against a visceral parasite, <u>L.infantum</u> (LV9), and two cutaneous parasites, <u>L.major</u> (LV39) and <u>L.m.amazonensis</u> (LV78). The taxonomic status of a number of newly isolated strains of <u>Leishmania</u> is described and an updated list of isolates maintained in the reference (see over)		

(continued)

collection is appended. Other work has involved studies on antimonial leishmanicides in liposomes, separation of amastigotes from tissue using a cellulose column, purine and pyrimidine synthesis in L.m.amazonensis promastigotes and immunological studies of visceral and cutaneous leishmaniasis.

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INTRODUCTION

An interim Progress Report was submitted on the work of this unit in April 1978. This report included the test procedures now adopted for in vivo evaluation of potential antileishmanial activity. These are repeated below for convenience. A number of compounds was supplied by WRAIR for testing on four strains of Leishmania in December 1977. Preliminary data were supplied to WRAIR by the following month together with a request for additional supplies of certain drugs. Unfortunately, because of faulty communications we have only been in a position to continue with certain in vivo tests as from November 1978. The data requested by WRAIR a year ago are therefore not yet all available and top priority is currently being given to completing this work. Additional in vitro studies to parallel the in vivo work are also in hand at the present time.

Please note that the Progress Report sent in April 1978 was not intended for general circulation. It is replaced by the present Final Report which is now being submitted in the format recommended in a letter from WRAIR SGRD-AJ dated 11 October 1978.

SCIENTIFIC ACTIVITIES

1. CHEMOTHERAPY

1.1 Techniques

We have reviewed and simplified to some degree the procedures for drug testing that were set out in our application for research support, dated February 1977. Experience showed us that the original techniques developed by Mrs. Trotter, were unnecessarily cumbersome and did not give as consistent results as we had anticipated. The following techniques have now been adopted in this Department.

We are currently investigating the possible use of Balb/C mice as hosts to replace the NMRI line that we have used so far. Information received from colleagues in other centres suggests that Balb/C's may be as satisfactory as, or better than NMRI's and they are easier to obtain in bulk from breeders in this country than the latter variety.

1.1.1 Visceral leishmaniasis

The laboratory model used for the investigation of drug activity against visceral infection in this laboratory is L. donovani sensu lato (Strain LV9 from Ethiopia). The parasite is maintained by continuous passage in Syrian hamsters (Mesocricetus auratus). (This parasite is probably a variant of L. infantum).

Infected animals are anaesthetised with ether and killed by cervical dislocation. The skin surface is swabbed with alcohol and the enlarged spleen removed aseptically into sterile Locke's solution in a sterile tissue homogeniser. After homogenisation the resultant suspension is centrifuged at 200 g for five minutes to remove tissue debris and the supernatant is then further centrifuged at 800 g for ten minutes to separate the amastigotes. These are resuspended in Locke's solution and their numbers counted in a Neubauer haemocytometer. The suspension is further diluted with Locke's solution to produce a final concentration of 50×10^6 amastigotes/ml.

20 g NMRI mice from a random bred colony are inoculated intravenously with 0.2 ml of the amastigote suspension. Parasites reach the liver and spleen rapidly by this route and may be detected one hour after infection. Infection is carried out randomly and the infected mice are subsequently divided at random into groups of six. Two untreated control groups are established and one treated group for each dose level of the compounds under test are set up. Additionally, Pentostam (sodium stibogluconate) at 400 mg base/kg is used as a positive drug control.

One control group is sacrificed on D+7 and liver impression smears are prepared, stained with Giemsa stain and examined to confirm the presence of parasites. Treatment of the dosed groups is commenced on D+8 and continued for five days. On the day following the final dose (D+13), all surviving animals are sacrificed and liver impression smears are prepared.

Assessment of the effect of treatment is made by examining liver impression smears. The smears are fixed with methanol, stained with filtered Giemsa stain and the numbers of parasites/1000 host cell nuclei are counted. The mean parasite count of each treated group is expressed as a percentage of the mean parasite count of the untreated control group and a dose response curve is plotted on log/probit graph paper to determine the ED_{50} and ED_{90} of the test compound.

The test result is also expressed, by comparison with Pentostam, as a Pentostam Index.

$$\frac{ED_{90} \text{ Pentostam}}{ED_{90} \text{ Test compound}} = \text{Pentostam Index}$$

1.1.2 Cutaneous leishmaniasis

Experimental compounds are tested against both Old World and New World forms of cutaneous leishmaniasis. The Old World type is represented by L. major = L. tropica major (Strain LV39 from USSR) and the New World cutaneous leishmaniasis by L. mexicana amazonensis (Strain LV78 from Brazil). The test system used is essentially the same for both species, differing only in the nature of the inoculum. L. major infections are produced by the intradermal inoculation of promastigotes from culture, whilst L. m. amazonensis infections are induced by the inoculation of amastigotes harvested from lesions in Syrian hamsters.

Studies are still proceeding to find a suitable rodent host for L. panamensis (Strain LIV641 = WR 128)

(i) Preparation of inocula

a) L. major

Using sterile techniques L. major amastigotes are isolated from developing nodules that have not ulcerated, by homogenisation and differential centrifugation. Rabbit blood agar slopes are seeded with a drop of the resultant amastigote suspension, 200 units/ml penicillin and 20 µg/ml streptomycin are added and the slopes are incubated at room temperature. Cultures are passed when the promastigotes are in their log phase of growth. Cultured promastigotes are concentrated by centrifugation at 800 g for ten minutes, resuspended in Locke's solution and the number of flagellates present estimated in a counting chamber. Suspensions are adjusted to contain between 5 and 25 x 10⁶ promastigotes/ml.

b) L. mexicana amazonensis

A suspension of amastigotes is obtained by grinding tissue from infected hamster feet with sterile sand in Locke's solution using a pestle and mortar, and differentially centrifuging it at 200 g for five minutes and the supernatant at 800 g for fifteen minutes. The amastigote pellet is resuspended in Locke's solution, parasite numbers are counted in a haemocytometer, and the suspension is diluted to contain 5 x 10⁷ amastigotes/ml.

(ii) Test procedure

20 g TFW mice are shaved over an area of 1.5 cm² above the tail base using animal clippers, and inoculated in random order with a volume of 0.2 ml of either L. major (or L. m. amazonensis) suspension intradermally 1 cm above the tail base in the midline. The animals are randomly caged in groups of five. The experiment is controlled with one untreated group and one positive drug control group which receives Pentostam equivalent to 400 mg antimony/kg. Four groups of mice, receiving a range of dose levels, are set up for each compound under test.

Treatment is commenced at three hours post-infection and continued daily for five days in all. Compounds are routinely administered subcutaneously in the interscapular region.

The site of infection is kept shaved throughout the experiment and the size and appearance of the lesion is assessed for each mouse weekly for up to seven weeks and a lesion score from 0-4 allocated as follows:

- | | |
|---|---|
| 0 | No thickening of skin |
| 1 | Thickening of skin |
| 2 | Small discreet nodule (up to 0.3 cm diameter) or extensive thickening |
| 3 | Discreet nodule (0.3-0.6 cm diameter) |
| 4 | Larger lesions and ulcerated lesions. |

The lesion score is assessed by two workers each examining the animals independently and a mean lesion score is derived from the sum of both sets of readings in order to minimise the human error inherent in any such subjective system.

(iii) Determination of drug activity

a) Percentage suppression during the first four weeks

The sum of the weekly mean lesion scores for the first four weeks of the experiment in the treated groups is expressed as a percentage of the untreated control group score for the same period. These values are plotted on log/probit graph paper and the percentage suppression is determined.

b) Suppressive dose

The sum of the weekly mean lesion scores of the treated groups over the course of the whole experiment is expressed as a percentage of the untreated control values for the total period. The results obtained are plotted on log/probit graph paper and the SD_{50} and SD_{90} are determined. (SD_n = that dose which causes n% suppression of lesion development compared to the untreated control as reflected by the sum of the mean lesion scores.)

c) Pentostam Index

$$\frac{SD_{90} \text{ Pentostam}}{SD_{90} \text{ Test compound}} = \text{Pentostam Index}$$

1.2 Data on WRAIR and other compounds tested

Table 1 summarises data on 12 compounds provided by WRAIR and 6 other compounds. Details of the activity of these compounds as assessed so far against L. infantum (LV9), L. major (LV39) and L. mexicana amazonensis (LV78) in mice are provided in Tables 2 through 30. The outstanding points that arise from these data are the following:

- (i) Glucantime sc is marginally less active than sodium stibogluconate (Pentostam) sc
- (ii) Only 2 8-aminoquinolines showed significant activity against skin-dwelling parasites (namely WR 226292 and WR 226257). (Pentostam itself is only effective at high doses against these organisms.)
- (iii) WR 226292 (the 5,6 methoxy analogue of WR 6026) is highly active against L. infantum and L. major when given po, (Pentostam Index 155) but little better than Pentostam given sc. WR 226292 is indeed the most active compound with the broadest spectrum of activity that we have encountered (other than Pentostam in liposomes versus L. infantum).
- (iv) The lepidines WR 212579, and 226257 are less active than WR 6026 but WR 211666 is about as effective as WR 6026. All these compounds have a similar level of activity sc and po except WR 211666 which, like 226292 is more active po.

- (v) WR 181023 (4-methyl primaquine) is about as active as WR 212579 against L. infantum but is ineffective at tolerated doses against the cutaneous species.
- (vi) Clindamycin shows a modest activity against L. infantum.
- (vii) Amphotericin B has poor activity against L. infantum but is superior to Pentostam against L. major.
- (viii) An old batch of WR 6026 (AG 75499) showed poor activity compared to newer material. This may indicate chemical instability.

It is interesting to compare these data obtained in mice with data published recently by Kinnamon et al. (Am. J. trop. Med. Hyg., 1978, 27, 751) based on studies with the Khartoum strain of L. donovani (= L. infantum) in hamsters.

1.3 Studies on antimonial leishmanicides in liposomes

In association with the Department of Biochemistry in this University (Dr. New) we have been investigating the effects of incorporating Pentostam and other antimonials into liposomes, using L. infantum and L. major in NMRI mice as our target organisms. We were unaware that two other groups were working independently on this question, namely that of Black at the Nuffield Institute in London, and Steck at WRAIR. We have arrived at similar conclusions to both these groups, namely that the activity of organic antimonials is greatly enhanced against visceral leishmaniasis when the compounds are incorporated into liposomes. The activity against cutaneous infection is still being investigated, and information will be provided later. Other forms of liposome and other antileishmanial drugs will also be investigated in an attempt to enhance their therapeutic index and effectiveness. A report on our liposome studies is attached as Appendix I.

2. PARASITE BIOCHEMISTRY AND MODE OF ACTION

A new technique has now been described by Mr. Brazil for separating pure amastigotes from tissue using a cellulose column. (Appendix II). Preliminary data on the purine and pyrimidine synthetic activities of L. m. amazonensis promastigotes are summarised in Appendix III, details of which are being prepared for publication. This preliminary work shows a clear distinction between the metabolism of this parasite and others studied by Marr and Berens.

3. BIOCHEMICAL IDENTIFICATION OF LEISHMANIAL ISOLATES

This Department has now been officially designated as the "WHO International Collaborating Centre for the Biochemical Characterisation of Leishmania".

We have received several interesting new isolates including several from cases of Uta (from Dr. Herrer, Peru) and isolates from patients with kala-azar in the current Indian epidemic in Bihar. We have also received further material from Senegal isolated from man, rodents and P. duboscqui. This material is currently being grown and identified. The Indian material, all from man, is particularly interesting as it differs from strains of L. donovani that we received several years ago from Professor P. C. Sen Gupta of Calcutta.

A strain of Leishmania isolated by Mutinga and Ngoka from a dog in Kenya (Trans. R. Soc. trop. Med. Hyg., 1977, 71, 447) has proved to be identical to strains causing visceral disease in man in that area. This is the first time that the dog has been implicated as a reservoir of kala-azar in Kenya where the reservoir previously has been suspected to be rodents of various species.

We are currently examining all available isolates from kala-azar from India, the Middle East, Africa and South America in an attempt to resolve several enigmas that have arisen regarding the taxonomic status of the causative organisms. We still hope to receive more cultures of Honduras isolates from WRAIR to include in this survey.

The organisms responsible for cutaneous and mucocutaneous disease in the New World are currently receiving attention, and it is already becoming clear that mucocutaneous disease may result from infection with more than one species or subspecies of Leishmania.

A paper reviewing the biochemical taxonomy of isolates from the Aethiopian zoogeographical region has now been published (Appendix IV).

Further isolates have been received from WRAIR for identification and work on these is proceeding. The data available so far are as follows:

LV	<u>Original designation</u>			
639	117 Herrera	Man	Honduras	visceral
640	142	Animal	Honduras	visceral (trypanosome)
641	158 Murray	Man	Panama	cutaneous
642	182 Herrera	Man	Honduras	visceral
643	195	Animal	Honduras	visceral
644	220 Hendricks	Man		cutaneous
645	219	Man	India	visceral
646	234	Dog	Spain	cutaneous?
647				
648	LN158 Murray	Man	Panama	cutaneous
649	WR168 Khartoum	Man	Sudan	visceral
650	WR271	Man	Kenya	visceral
651	WR272 McGillivray	Man	Kenya	cutaneous
652	WR275 Orviss	Man		cutaneous

Preliminary characterisation of LV639, LV642 and LV644 indicate that the GPI and 6PGDH of all three are identical. These enzymes are quite different from other isolates from patients with visceral leishmaniasis of the Old World. Although the material has not yet been compared with markers for South American L. mexicana, the enzymes looked at so far appear to be very close to these organisms. Work is continuing on the identification of the whole series.

In Appendix V are listed isolates received in our WHO Collaborating Centre ✓ in addition to those listed as Annex 1 of our Final Technical Report of March 1977. The total number of isolates in our collection is now 677.

4. IMMUNOLOGICAL STUDIES

The ELISA test designed for the diagnosis of visceral leishmaniasis in our Department (Hommel et al., Ann. trop. Med. Parasit., 1978, 72, 213-218). has proved invaluable in identifying cases of kala-azar in India and helping to differentiate those probably suffering from malaria but misdiagnosed. This work is still continuing in collaboration with the staff of the National Institute of Communicable Diseases, Delhi, and will be reported in due course. Filter paper blood samples from man and a variety of domestic animals are being examined in Liverpool by Mrs. Sells, and those from man are being examined by the FAT by Dr. Lanotte of Montpellier University.

In vitro studies of T-cell function in cutaneous leishmaniasis and kala-azar are being conducted by Mrs. Sells. The leucocyte migration test is proving negative in patients known to have had Oriental Sore, but further investigations are being carried out including the use of modified techniques. A relationship between the type of cutaneous cellular response in infection with different Leishmania species is suspected to determine whether ELISA-detectable antibodies are produced in a given patient or not. This is being followed up in patients seen at clinics in England and overseas.

5. PUBLICATIONS

Papers published since last Final Report (March 1977)

Ashford, R. W. (1977). The comparative ecology of Leishmania aethiopica. Colloques Internationaux du CNRS, No. 239, 225-232.

Brazil, R. P. (1977). On the identity of Brazilian Leishmania strains and the use of the name L. braziliensis. Trans. R. Soc. trop. Med. Hyg., 71, 360-361 (correspondence).

Brazil, R. P. (1978). Electrophoretic variation of the enzyme phosphoglucosomutase in different strains of Leishmania. Ann. trop. Med. Parasit., 72, 289-291.

Brazil, R. P. (1978). Isolation of the intracellular stages of Leishmania mexicana amazonensis using cellulose column. Ann. trop. Med. Parasit., 72, 579-580.

Chance, M. L., Gardener, P. J. and Peters, W. (1977). The biochemical taxonomy of Leishmania as an ecological tool. Colloques Internationaux du CNRS, No. 239, 53-61.

Chance, M. L., Schnur, L. F., Thomas, S. C. and Peters, W. (1978). The biochemical and serological taxonomy of Leishmania from the Aethiopian zoogeographical region of Africa. Ann. trop. Med. Parasit., 72, 533-542.

Croft, S. L. (1977). Ultrastructural aspects of the nucleus of Leishmania hertigi. J. Protozool., 24, p. 42A (Abstract No. 14).

Croft, S. L., Schnur, L. F. and Chance, M. L. (1978). The morphological, biochemical and serological characterisation of strains of Leishmania hertigi from Panama and Brazil and their differentiation. Ann. trop. Med. Parasit., 72, 93-94.

Gardener, P. J. (1977). Taxonomy of the genus Leishmania. Tropical Diseases Bulletin, 74, 1069-1088.

Hommel, M. (1978). The genus Leishmania: biology of the parasites and clinical aspects. Bulletin de l'Institut Pasteur, 75, 5-102.

Hommel, M., Peters, W., Ranque, J., Quilici, M. and Lanotte, G. (1978). The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. Ann. trop. Med. Parasit., 72, 213-218.

Killick-Kendrick, R., Molyneux, D. H., Hommel, M., Leaney, A. and Robertson, E. (1977). Leishmania in phlebotomid sandflies, V. The nature and significance of infections of the pylorus and ileum of the sandfly by leishmaniae of the braziliensis group. Proc. R. Soc. Lond., B., 198, 191-199.

Molyneux, D. H., Lewis, D. H. and Killick-Kendrick, R. (1977). Aspects of the microecology of Leishmania. Colloques Internationaux du CNRS, No. 239, 31-40.

New, R. R. C., Chance, M. L., Thomas, S. C. and Peters, W. (1978). The antileishmanial activity of antimonials entrapped in liposomes. Nature, 272, 55-56.

Peters, W., Chance, M. L., Mutinga, M. J., Ngoka, J. M. and Schnur, L. F. (1977). The identification of human and animal isolates of Leishmania from Kenya. Ann. trop. Med. Parasit., 71, 501-502.

Schnur, L. F. (1977). Insect flagellate excreted factors from leptomonad, crithidial and herpetomonad strains. J. Protozool., 24, p. 5A (Abstract No. 74).

Papers submitted for publication

Brazil, R. P. (1978). In vitro susceptibility of mouse peritoneal macrophages to Leishmania spp. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

Brazil, R. P. and McCarthy, J. D. (1979). Purine and pyrimidine synthesis in promastigotes of Leishmania mexicana amazonensis. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

Chance, M. L., New, R. R. C., Thomas, S. C. and Heath, S. (1979). The treatment of visceral leishmaniasis with liposomes. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

Croft, S. L. (1979). Ultrastructural study of the nucleus of Leishmania hertigi. Protistologica.

- Croft, S. L. and Molyneux, D. H. (1979). Further studies of the virus-like particles of Leishmania hertigi. J. Protozool.
- Croft, S. L. and Molyneux, D. H. (1979). Studies on the ultrastructure, virus-like particles and infectivity of Leishmania hertigi. Ann. trop. Med. Parasit.
- Croft, S. L. and Schnur, L. F. (1979). The Noguchi-Adler phenomenon: an ultrastructural study of the effects of homologous antiserum on the growth of promastigotes of Leishmania braziliensis braziliensis and L. h. hertigi. Ann. trop. Med. Parasit.
- Dedet, J.-P., Derouin, F., Hubert, B., Schnur, L. F. and Chance, M. L. (1979). Isolation of Leishmania major from Mastomys erythroleucus and Tatera gambiana in Senegal (West Africa). Ann. trop. Med. Parasit.
- Rassam, M. B., Al-Mudhaffar, S. A. and Chance, M. L. (1978). Isoenzyme characterisation of Leishmania species from Iraq. Ann. trop. Med. Parasit.

RESEARCH PLANS

These were submitted to WRAIR in April 1978 with an application for renewal of WRAIR support. We have had no written information concerning the fate of this application but understand from a verbal communication that our application has been rejected. This is the subject of a letter to Dr. McCormick dated 20th November, 1978.

APPENDICES

Tables 1-30

- Appendix I Antileishmanial activity of antimonials entrapped in liposomes.
New, R.R.C., Chance, M.L., Thomas, S.C. and Peters, W. (1978), Nature, 272, 55-56.
- Appendix II Isolation of the intracellular stages of Leishmania mexicana amazonensis using cellulose column.
Brazil, R.P. (1978), Ann.Trop.Med.Parasitol., 72, 579-580.
- Appendix III Purine and pyrimidine synthesis in promastigotes of Leishmania mexicana amazonensis.
Brazil, R.P. and McCarthy, J.D. (1979), Trans.R.Soc.Trop.Med.Hyg. (in press)
- Appendix IV The biochemical and serological taxonomy of Leishmania from the Aethiopian zoogeographical region of Africa.
Chance, M.L., Schnur, L.F., Thomas, S.C. and Peters, W. (1978), Ann.Trop.Med.Parasitol., 72, 533-542.
- Appendix V Updated list of isolates in reference collection.

Table 1A

SUMMARY OF DATA FROM ANTILEISHMANIAL DRUG TESTS ON WRAR AND OTHER COMPOUNDS.

WR	BN	LIV	L. infantum (=L. donovani) (LV9)		L. major (LV39)		L. m. amazonensis (LV78)		Comments
			ED ₉₀	PI	SD ₉₀	PI	SD ₉₀	PI	
			155	1.0	2750	1.0	860	1.0	PENTOSTAM
6026 AD	BG 14463	1425	2.8	55.4	MTD		MTD		MTD = 30
6026 AD	BG 14463	1425	2.5 p.o.	62.0					
6026 A1	AG 75499	1181	30	5.2	MTD		MTD		MTD = 30 ?Compound unstable (old batch)
226292 AA	BG 44541	1426	62	2.5	MTD				MTD = 30
226292 AA	BG 44541	1426	1.0 p.o.	155	1.0 p.o.	155	10 p.o.	15.5	
211666 AB	BG 11417	1427	5.0	31.0	MTD		MTD		MTD = 30
211666 AB	BG 11417	1427	2.7 p.o.	57.4					
211666 AB	BG 11417	1595	13.0	11.9					
212579 AB	BG 48969	1428	11.5	13.5	MTD				MTD = 30
212579 AB	BG 48969	1428	13.0 p.o.	11.9					
226257 AA	BG 44425	1429	14.5	10.7	10	15.5			
226257 AA	BG 44425	1429	11.5 p.o.	13.5					

ED₉₀/SD₉₀ = mg/kg x 5

PI = Pentostam Index

NA = not active

MTD = maximum tolerated dose

(Note: all doses s.c. unless otherwise stated)

Table 1B

SUMMARY OF DATA FROM ANTILEISHMANIAL DRUG TESTS ON WRAIR AND OTHER COMPOUNDS.

WR	BN	LIV	L. infantum (LV9) (=L. donovani)		L. major (LV39)		L. m. amazonensis (LV78)		Comments
			ED ₉₀	PI	SD ₉₀	PI	SD ₉₀	PI	
29577 AC	AW 41662	1430	145	1.1	100		100		
214975 AB	BE 45137	1593	200	0.8					GLUCANTIME
13254 AB	BB 48455	1187					100		
2975 E 19	AG 64745	1370	16.5	9.4	MTD		MTD		PRIMAQUINE PHOSPHATE MTD = 60
81023 AG	BE 50003	1373	9.8	15.8	60		100		4-METHYL PRIMAQUINE
81023	ZN 39806	1594	12.2	12.7					" "
82234	BE 17580	1596	270	0.6					2-METHYL PRIMAQUINE
203659 AC	BE 10429	1335	100	1.6					CLINDAMYCIN
		1349	30	5.2					U24729A
			950	0.2	1000	2.8			AMPHOTERICIN B
		1092	MTD		MTD				RIFAMPICIN MTD = 100
1544 BM	AR 20613	1488	MTD						CHLOROQUINE PHOSPHATE MTD = 100
1543	AR 78360	1495	MTD						MEPACRINE HYDROCHLORIDE MTD = 60

ED₉₀/SD₉₀ = mg/kg x 5

PI = Pentostam Index

NA = not active

MTD = maximum tolerated dose

(Note: all doses s.c. unless otherwise stated)

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1

Compound: Pentostam

Route of administration: s.c.

L. donovani (Strain LV9)		Experiment No.: L23	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	4	1810	
100.0 (as Sb) (30)	6	447	24.7 ± 7.1
200.0 (60)	6	132	7.3 ± 3.6
400.0 (120)	6	2	0.1 ± 0.1
600.0 (180)	6	0	0

ED₅₀ 75 (59-104)

ED₉₀ 155 (123-215)

Pentostam Index 1.0

as Sb 22.5 (17.7-31.2)

as Sb 46.5 (36.9-64.5)

L. major (Strain LV39)		Experiment No.:							Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	0.2	0.2	0.5	0.5	0.8	1.2	1.4				
600 (Sb) 180	0.1	0.2	0.4	0.6	0.8	0.8	0.6	92.9	7.1	72.9	
1000 (300)	0.2	0.3	0.4	0.4	0.7	0.6	0.8	92.9	7.1	70.8	
1500 (450)	0.2	0.2	0.2	0.2	0.2	0	0.4	57.2	42.8	25.0	
2000 (600)	0	0	0.2	0.2	0.2	0.2	0.2	28.6	71.4	20.8	

SD₅₀ 1050
as Sb 315

SD₉₀ 2750
as Sb 825

Pentostam Index 1.0.

L. m. amazonensis (Strain LV78)		Experiment No.:							Date:		
Dose (mg/kg)		Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
		1	2	3	4	5	6	7			
Control		0.6	1.0	1.3	1.3	1.4	1.4	2.3			
(as Sb)											
100	(30)	0.9	1.1	1.4	1.2	1.3	1.3	1.2	100	0	90.3
200	(60)	0.9	0.4	0.4	0.3	0.6	0.8	0.4	47.6	52.4	40.9
400	(120)	0.5	0.3	0.3	0.6	0.5	0.6	0.6	40.5	59.5	36.6
600	(180)	0.3	0.2	0.1	0.1	0.6	0.3	0.4	- 16.7	83.3	21.5

SD₅₀ 290
(as Sb) 87

SD₉₀ 860
as Sb 258

Pentostam Index 1.0

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1425

WR 6026AD
BG 14463

Route of administration: SC

<u>L. donovani</u> (Strain LV9)				Experiment No.:			Date:
Dose (mg/kg)	No. of animals			Mean amastigotes/1000 host cell nuclei			% Control
	L6	L12	L41	L6	L12	L41	
Control	3	4	3	5460	4459	4050	
1.0	5			1360			33.6 ± 5.2
3.0	3			1357			24.9
10.0	3			103			1.9
30.0	6			0			0

ED₅₀ 1.2 (0.6-1.9)

ED₉₀ 2.8 (1.5-4.6)

Pentostam Index 55.4

<u>L. major</u> (Strain LV39)								Experiment No.: L56		Date:
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.2	0.2	0.6	0.9	1.6	2.0	1.7			
3.0	0	0.3	0.4	1.4	1.6	2.0	2.4	100	0	100
10.0	0.5	0.8	1.9	1.0	2.0	2.8	2.9	100	0	100
30.0	0	0.1	0.4	1.0	2.0	2.2	2.7	32	68	100
60.0 (~ LD80)	0	0	1.0	1.5	2.0	3.0	2.0	100	0	100

SD₅₀ > MTD

SD₉₀ > MTD

Pentostam Index -

<u>L. m. amazonensis</u> (Strain LV78)								Experiment No.: L52		Date:
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.2	0.2	0.6	0.9	1.6	2.0	2.4			
3.0	0.6	0.5	0.7	1.3	1.2	1.8	2.7	100	0	100
10.0	0.2	0.3	0.7	0.4	0.5	1.6	1.6	94	6	87
30.0	0	0.4	0.4	1.0	0.9	2.0	2.0	53	47	100
60.0 (~ LD80)	0	0	1.0	1.0	2.0	2.0	2.0	100	0	100

SD₅₀ > MTD

SD₉₀ > MTD

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1445

WR 6026AD
BG 14463

Route of administration: PO

L. donovani (Strain LV9)		Experiment No.: 124	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
1.0	5	514	22.6 ± 4.7
3.0	5	232	10.2 ± 4.2
10.0	5	28	1.2 ± 0.7

ED₅₀ 0.4 (0.2-0.6)

ED₉₀ 2.5 (1.7-4.4)

Pentostam Index 62.0

L. major (Strain LV39)				Experiment No.:					Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)							Experiment No.:		Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 3

Compound: LIV 1111

WR 6016A-1
AG 75499

Route of administration: SC

<i>L. donovani</i> (Strain LV9)		Experiment No.: L43	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	4600	
3.0	5	1490	32.4 $\pm$ 3.3
10.0	5	730	15.9 $\pm$ 3.1
30.0	5	560	12.2 $\pm$ 4.2
60.0 (MTD)	5	125	2.7 $\pm$ 0.8

ED₅₀ 2.2(1.0-3.9)ED₉₀ 30(14-63)

Pentostam Index 5.2

L. major (Strain LV39)				Experiment No.: L47				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.6	0.6	1.3	1.9	2.4			
3.0	0.1	0.2	0.4	0.3	1.0	0.8	1.9	58.8	41.2	64.4
10.0	0.1	0.4	0.2	0.1	0.6	0.6	0.9	47.1	52.9	39.7
30.0	0.1	0.4	0.2	0.4	0.5	0.4	1.0	64.7	35.3	41.1

SD₅₀ 7.0SD₉₀ > MTD

Pentostam Index -

L. m. amazonensis (Strain LV78)		Experiment No.: L48							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.4	0.5	0.8	0.9	1.2	0.7	1.0			
3.0	0.2	0.4	0.5	0.4	0.4	0.1	0.1	58	42	38
10.0	0.4	0.3	0.2	0.6	0.5	0.6	1.1	58	42	67
30.0	0.4	0.2	0.4	0.6	0.8	0.8	1.2	62	38	80
60.0 (LD80)	1.0	1.5	0.5	0.5	0.5	1.0	2.0	- 100	0	100

SD₅₀SD₉₀ > MTD

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1

Compound: LIV/1426

WR 126292AA

Route of administration: SC

BG 44541

<u>L. donovani</u> (Strain LV9)		Experiment No.: L4 & L12		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei		% Control
Control	L4 3	L12 4	L4 4525	L12 4459
30.0	3		4550	100
60.0	3		723	16.0
100.0	6		LD ₁₀₀	-

ED₅₀ ~ 50ED₉₀ ~ 62

Pentostam Index ~ 2.5

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
30.0	0	0	0	0.5	2.0	3.5	3.0	11.4	88.6	63.8
100.0	-	-	-	-	-	-	-	LD ₁₀₀		

SD₅₀ ~ 30SD₉₀ > MTD

Pentostam Index -

L. m. amazonensis (Strain LV78)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 7

Compound: LIV1456

WR 226292AA
BG 44541

Route of administration: PO

L. donovani (Strain LV9)		Experiment No.: L64	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
1.0	5	28	1.2 ± 0.7
3.0	5	0	0
10.0	5	0	0

ED₅₀ < 1.0ED₉₀ < 1.0

Pentostam Index > 135

L. major (Strain LV39)		Experiment No.: L61							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.8	1.2	2.4	3.0	3.3	2.8	3.0			
1.0	0.4	0.2	0.1	0.2	0.2	0.2	0.6	12	88	12
3.0	0.4	0.4	0.2	0.4	0	0	0	19	81	8
10.0	0.4	0	0	0.4	0	0	0	11	89	5

SD₅₀ < 1.0SD₉₀ ~ 1.0

Pentostam Index ~ 155

L. m. amazonensis (Strain LV78)								Experiment No.: L53		Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	1.2	2.8	3.0	3.0	2.6	2.3	2.3				
1.0	0.6	0	0.2	0.4	0.8	0.7	0.8	12	88	20	
3.0	0.2	0.2	0.8	0.6	0.7	1.0	0.9	18	82	26	
10.0	0.4	0.2	0.4	0.6	0.5	1.0	1.2	16	84	25	

SD₅₀ < 1.0SD₉₀ > 10.0

Pentostam Index < 15.5

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1417

WR 211666AB
BG 11417

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: L12 & L41		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei		% Control
	L12 L41	L12 L41		
Control	4 3	4459 4050		
1.0	5	2260		55.8 ± 11.6
3.0	5	1680		41.5 ± 5.9
10.0	6	198		4.5 ± 1.7
30.0	6	0		0

ED₅₀ 1.9 (0.8-3.2)

ED₉₀ 5.0 (2.5-7.5)

Pentostam Index 31.0

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
10.0	0	0	0.5	2.6	2.8	3.3	3.1	70.5	29.5	87.2
30.0	0	0	0	0.7	2.0	2.4	2.4	15.9	84.1	53.2

SD₅₀ ~ 33

SD₉₀ > MTD

Pentostam Index -

L. m. amazonensis (Strain LV78)								Experiment No.:		Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	0.1	0.4	0.5	0.8	1.2	1.2	1.8				
10.0	0.1	0.5	0.8	1.2	1.4	2.2	2.1	100	0	100	
30.0	0	0.2	0.7	1.0	1.0	1.8	2.0	100	0	100	

SD₅₀ > MTD

SD₉₀ > MTD

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 14.7

WR11166AB
BG11417

Route of administration: PO

L. donovani (Strain LV9)		Experiment No.: L24	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
1.0	5	1022	45.0 ± 8.4
3.0	5	394	17.3 ± 3.4
10.0	5	2	0.2 ± 0.1

ED₅₀ 1.0 (0.8-1.7)

ED₉₀ 2.7 (1.9-4.2)

Pentostam Index 57.4

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 11417

WB 21166AB
BG 11417

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: 100	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	3110	
3.0	5	1470	47.2 ± 5.5
10.0	5	330	10.6 ± 1.2
30.0	5	130	4.2 ± 0.6

ED₅₀ 2.4(1.6-3.5)

ED₉₀ 13(9.5-21)

Pentostam Index 11.9

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 11417 WR 11140 AB
BG 11417

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: 20	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	1030	
100	5(5 died)	-	
200	5(5 died)	-	
300	5(5 died)	-	

ED₅₀

ED₉₀

Pentostam Index

L. major (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1428

WR 212573AB
BG 48969

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: L12 & L13		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei		% Control
	L12 4	L13 4	L12 4459 L13 2515	
Control				
1.0	6		1920	76.4 ± 4.6
3.0	6		1632	64.9 ± 4.5
10.0	6	1890		42.4 ± 4.5
30.0 (MTD)	6	0		0

ED₅₀ 7.0(3.0-10)ED₉₀ 11.5(5.4-17)

Pentostam Index 13.5

L. major (Strain LV39)		Experiment No.: L40 & L22							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control L40	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
3.0	0.5	2.0	2.0	2.4	2.4	2.8	3.0	100	0	86.8
Control L22	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
10.0	0.3	0.7	0.8	1.3	2.2	3.3	2.9	70.5	29.5	81.6
30.0 (MTD)	0.2	1.0	1.0	1.5	1.0	2.5	3.0	84.1	15.9	72.3

SD₅₀ > MTDSD₉₀ > MTD

Pentostam Index -

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 41426

WR 210573AB
BG 48969

Route of administration: PO

L. donovani (Strain LV9)		Experiment No.: 124	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
3.0	5	1330	58.5 ± 4.2
10.0	5	668	29.4 ± 7.3
30.0	5	18	0.8 ± 0.5

ED₅₀ 4.3 (2.6-7.5)

ED₉₀ 13 (8.0-23)

Pentostam Index 11.9

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 14

Compound: LT 1429

WR 226257AA

Route of administration: SC

BG 44425

<u>L. donovani</u> (Strain LV9)		Experiment No.: L13 & L18		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei		% Control
Control	L13 4	L18 4	L13 4020	L18 2515
1.0	6		1828	72.7 ± 8.1
3.0	6		1513	60.2 ± 5.0
10.0	6		1067	42.4 ± 2.9
30.0	6		95	2.4 ± 0.8
100.0	6		0	0

ED₅₀ 5.3 (3.2-9.5)ED₉₀ 14.5 (9.0-26)

Pentostam Index 10.7

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
1.0	0.5	2.0	2.2	2.5	2.7	3.0	3.6	100	0	94.8
3.0	0.2	1.4	2.0	2.6	2.4	3.3	4.0	100	0	91.4
10.0	0.3	1.4	1.6	2.4	2.5	2.6	3.0	91.9	9.1	79.3

SD₅₀ > 10.0SD₉₀ > 10.0

Pentostam Index -

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1

Compound: LIV 1429

WR 206257AA
BO 14425

Route of administration: PO

L. donovani (Strain LV9)		Experiment No.: 142	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	4050	
1.0	5	2570	63.5 ± 9.5
3.0	5	1760	43.5 ± 10.0
10.0	5	1590	39.3 ± 6.2

ED₅₀ 3.6(1.0-8.0)

ED₉₀ 11.5(3.5-24)

Pentostam Index 13.5

L. major (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1a

Compound: LIV 4130

WR 129577AC
AW 41861

Route of administration: SC

<i>L. donovani</i> (Strain LV9)		Experiment No.: L45	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	5	2070	
3.0	5	1710	82.6 $\pm$ 7.2
10.0	5	1390	67.2 $\pm$ 10.9
30.0	5	1100	53.1 $\pm$ 9.3
60.0	5	740	35.8 $\pm$ 3.3
100.0	5	363	17.5 $\pm$ 3.5

ED₅₀ 20 (9.5-44)ED₉₀ 145 (67-300)

Pentostam Index 1.1

L. major (Strain LV39)		Experiment No.: L50							Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	0	0.1	0	0.8	0.8	1.4	1.6				
10.0	0	0	0	0.4	1.2	2.1	2.6	44	56	100	
30.0	0	0.1	0	0.4	1.8	2.4	2.8	56	44	100	
60.0	0	0.1	0	0.5	1.6	2.0	2.7	67	33	100	
100.0	0	0	0	0.6	2.0	2.2	2.5	67	33	100	

SD₅₀ > 100SD₉₀ > 100

Pentostam Index -

<i>L. m. amazonensis</i> (Strain LV78)								Experiment No.: L49		Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	0.1	0	0.4	0.5	0.3	0.2	0.7				
10.0	0.1	0	0.4	0.2	0.7	1.0	1.3	70	30	100	
30.0	0	0.1	0.4	0.3	0.5	0.4	0.5	80	20	95	
60.0	0	0	0.4	0.2	0.3	0.5	1.5	60	40	100	
100.0	0	0.4	0.4	0.4	0.3	0.7	1.2	100	0	100	

SD₅₀ > 100SD₉₀ > 100

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1593 WR 214075AB
GLUCANTIME BE 48137

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: L65	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	3120	
3.0	5	1950	62.6 $\pm$ 8.6
10.0	5	1910	61.3 $\pm$ 8.9
30.0	5	1630	52.3 $\pm$ 12.9

ED₅₀ > 30

ED₉₀ > 30

Pentostam Index -

L. major (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 15

Compound: LIV/1593
GLUCANTIMEWR 214975AB
BE 45137

Route of administration: SC

<u>L. donovani</u> (Strain LV9)		Experiment No.: L66	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	1030	
100	5	420	40.8 $\pm$ 19.2
200	5	170	16.5 $\pm$ 12.1
300	5	60	5.8 $\pm$ 1.9

ED₅₀ 35 (12-90)ED₉₀ 200 (70-500)

Pentostam Index 0.8

<u>L. major</u> (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)					Experiment No.:				Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀Pentostam Index NOT TESTED.Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 12

Compound: LIV/1187

WR 113254AB
BB 48455

Route of administration: SC

<i>L. donovani</i> (Strain LV9)		Experiment No.:	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control			

ED₅₀ED₉₀Pentostam Index NOT TESTED.

L. major (Strain LV39)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.8	1.0	1.1	1.6	2.4			
3.0	0.1	0.6	1.3	0.5	1.1	1.6	1.2	100	0	86.5
10.0	0.1	0.4	0.6	0.6	1.6	2.1	2.2	73.9	26.1	100
30.0	0	0.1	0.4	0.5	0.9	1.1	1.6	43.5	56.5	62.6
100.0	0	0.4	0.4	0.5	1.1	1.6	1.2	56.5	43.5	70.3

SD₅₀ > 100SD₉₀ > 100

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 10

Compound: LIV 1470 WR 2475E-19 AG 64745

Route of administration: SC

PRIMAGWINE DIPHOSPHATE

L. donovani (Strain LV9)		Experiment No.: L9 & L41		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei		% Control
	L9 L41	L9 L41		
Control	3 3	5153 4050		
1.0	5	3040		75.1 ± 12.1
3.0	5	2390		59.0 ± 12.8
10.0	5	1000		24.7 ± 5.0
30.0	5	487		9.5 ± 1.6
60.0 (MTD)	5	10		0.2 ± 0.04

ED₅₀ 4.6(2.7-9.0)

ED₉₀ 16.5(9.5-33)

Pentostam Index 9.4

L. major (Strain LV39)		Experiment No.: L40 & L22							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control L40	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
1.0	0.5	2.4	2.4	3.8	4.0	3.6	4.0	100	0	100
3.0	0.1	0.6	1.4	2.1	2.3	2.0	2.8	67.7	32.3	64.9
10.0	0.1	0.8	1.4	2.5	2.4	2.8	3.2	77.4	22.6	75.9
Control L22	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
30.0	0	0	0.2	1.0	1.2	2.4	2.7	40.9	59.1	53.2
60.0 (MTD)	0	0	0	1.5	2.2	2.7	3.0	34.1	65.9	66.7

SD₅₀

SD₉₀ > MTD

Pentostam Index -

L. m. amazonensis (Strain LV78)				Experiment No.: L42				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.5	0.8	1.2	1.2	1.8			
30.0	0	0.3	0.6	0.6	0.8	1.7	1.9	83.3	16.7	98.3
60.0 (MTD)	0	0	0.4	1.2	1.4	1.9	2.0	88.9	11.1	100

SD₅₀

SD₉₀ > MTD

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1873
4-METHYL PRIMAQUINE

WR 181023AG
BE 50003

Route of administration: SC

<i>L. donovani</i> (Strain LV9)		Experiment No.: L43	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	4600	
3.0	5	1804	39.2 $\pm$ 9.3
10.0	5	450	9.8 $\pm$ 4.2
30.0	5	60	1.3 $\pm$ 0.6
60.0	5	10	0.2 $\pm$ 0.2
100.0	5	0	0

ED₅₀ 2.7 (1.6-4.5)

ED₉₀ 9.8 (4.8-15.5)

Pentostam Index 15.8

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.6	0.6	1.3	1.9	2.4			
3.0	0.1	0.2	0.4	0.7	0.5	0.4	1.2	82.4	17.6	50.7
10.0	0.1	0.2	0.6	0.5	0.7	0.8	1.0	82.4	17.6	53.4
30.0	0	0.2	0.4	0.4	0.8	0.8	1.2	58.8	41.2	52.1
60.0	0	0	0.3	0.5	0.7	1.3	1.3	47.1	52.9	56.2

SD₅₀ > 60

SD₉₀ > 60

Pentostam Index -

L. m. amazonensis (Strain LV78)								Experiment No.:	L48	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)	
	1	2	3	4	5	6	7				
Control	0.4	0.5	0.8	0.9	1.2	0.7	1.0				
10.0	1.0	1.0	0.5	0.6	0.6	0.7	1.1	100	0	100	
30.0	0.4	0.5	0.6	0.6	1.0	0.7	1.0	73	27	87	
60.0	0.4	0.6	0.7	0.4	1.1	0.7	1.3	81	19	95	
100.0	0.6	0.6	0.6	0.6	0.6	0.8	1.3	- 92	8	93	

SD₅₀ > 100

SD₉₀ > 100

Pentostam Index -

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1

Compound: LIV 1594
4-METHYL PRIMAQUINE

WR 131023
ZN 13604

Route of administration: SC

<i>L. donovani</i> (Strain LV9)		Experiment No.:	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	3120	
3.0	5	780	25.0 ± 6.2
10.0	5	460	14.8 ± 1.9
30.0	5	280	9.0 ± 1.2
100.0	5	0	0

ED₅₀ 4.0(1.4-10.5)

ED₉₀ 12.2(4.3-32)

Pentostam Index 12.7

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Reference

Compound: LIV/1104
2-METHYL PRIMAQUINEWR 161104
BENTEC

Route of administration: i.p.

<u>L. donovani</u> (Strain LV9)		Experiment No.: 100	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	5	3120	
3.0	5	2510	80.4 ± 1.1
10.0	5	2950	94.5 ± 0.7
30.0	5	940	30.1 ± 0.4

ED₅₀ED₉₀

Pentostam Index

L. major (Strain LV37)				Experiment No.:					Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)							Experiment No.:		Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: 100-100
1-METHYL-2-PYRIDINE

WR 18114
BF 17747

Route of administration: i.p.

<u>L. donovani</u> (Strain LV9)		Experiment No.: 100	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	-	1040	
100	5	250	24.1 ± 4.1
200	5 (2 died)	213	20.6 ± 7.1
300	5 (5 died)	-	-

ED₅₀ 34 (12-80)

ED₉₀ 270 (90- MTD)

Pentostam Index 0.6

<u>L. major</u> (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

<u>L. m. amazonensis</u> (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: *L. donovani* (Strain LV9)
CLIN AMFON BR 10419

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: L	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	5460	
1.0	3	4710	86.3
3.0	3	3647	66.8
10.0	3	2090	38.3
30.0	3	1930	35.4
100.0	3	1633	29.9

ED₅₀ 10

ED₉₀ > 100

Pentostam Index < 1.6

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1149

U24729A

Route of administration: SC

<u>L. donovani</u> (Strain LV9)		Experiment No.: 26	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	5460	
30.0	3	2420	44.3

ED₅₀ < 30

ED₉₀ > 30

Pentostam Index < 5.2

<u>L. major</u> (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		Sum Week 1-7 (as % control)
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

<u>L. m. amazonensis</u> (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		Sum Week 1-7 (as % control)
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: AMPHOTERICIN B

Route of administration: i.p.

<i>L. donovani</i> (Strain LV9)		Experiment No.: 11	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	6	2377	
10.0	6	1817	76.9 ± 3.9
30.0	6	898	34.9 ± 2.2
100.0	6	600	23.3 ± 3.4
300.0	6	527	20.5 ± 4.8

ED₅₀ 11 (4.5-32)

ED₉₀ 950 (450- 1000)

Pentostam Index 0.2

L. major (Strain LV39)		Experiment No.:							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0	0.9	1.5	2.1	2.9	3.2	3.2			
10.0	0	0.3	1.6	2.8	2.4	2.4	2.0	100	0	87
30.0	0	1.0	1.4	1.8	1.6	1.8	1.6	93	7	67
100.0	0	0	0.6	1.8	2.8	2.8	2.4	53	47	75
300.0	0	0.2	0.4	0.2	0.4	0.8	0.8	18	82	20

SD₅₀ ~ 1.35

SD₉₀ ~ 1000

Pentostam Index

L. m. amazonensis (Strain LV78)								Experiment No.:	Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1047
RIFAMPICINRoute of administration: PO

<u>L. donovani</u> (Strain LV9)		Experiment No.: <u>L18</u>	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	4	2573	
10.0	6	870	33.8 ± 6.3
30.0	6	809	31.4 ± 7.1
100.0	6	702	27.3 ± 6.2
300.0 (LD ₅₀)	6	350	13.6
600.0 (LD ₁₀₀)	6	--	-

ED₅₀ 8.5 (2.5-12.5)ED₉₀ > MTD

Pentostam Index -

L. major (Strain LV39)		Experiment No.: L35							Date:	
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0	0.2	0.4	1.2	1.0	1.7	2.0			
60.0	0	0.2	0.4	1.0	1.2	1.2	1.4	89	11	83
100.0	0	0	0.4	1.0	1.3	1.3	2.5	78	22	100
300.0	0	0	0.2	0.8	1.6	1.6	1.6	56	44	89
600.0	0	0	0.2	0.6	0.7	1.0	1.2	50	50	100

SD₅₀SD₉₀ > MTD

Pentostam Index

L. m. amazonensis (Strain LV78)				Experiment No.:				Date:		
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										

SD₅₀SD₉₀Pentostam Index NOT TESTED.Department of Parasitology
Liverpool School of Tropical MedicineSigned:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1044a WR 1844BM
CHLOROQUINE PHOSPHATE AP 10413

Route of administration: s.c.

L. donovani (Strain LV9)		Experiment No.: 100	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	4	1810	
60.0	6	1137	62.8 ± 15.6
100.0 (MTD)	6	890	49.2 ± 3.6

ED₅₀ ~ 100

ED₉₀ > MTD

Pentostam Index -

L. major (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: MIV 1145 WR 1843
METHAMIN HYDROCHLORIDE AR 7840

Route of administration: s.c.

L. donovani (Strain LV9)		Experiment No.: 523	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	4	1810	
30.0	6	1480	81.8 ± 10.7
60.0	6	827	45.7 ± 12.3

ED₅₀ ~ 60

ED₉₀ > 60

Pentostam Index -

L. major (Strain LV39)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)		Experiment No.:	Date:
Dose (mg/kg)	Weekly mean lesion score	Sum Week 1-4 (as % control)	% suppression
	1 2 3 4 5 6 7		
Control			

SD₅₀

SD₉₀

Pentostam Index NOT TESTED.

Department of Parasitology
Liverpool School of Tropical Medicine

Signed:
Date:

Principal Investigator: Professor W. Peters

Antileishmanial activity of antimonials entrapped in liposomes

THE use of liposomes in chemotherapy as a method of ensuring that drugs reach the diseased or defective tissues suffers from the drawback that, on intravenous injection, the bulk of the material is rapidly cleared by the phagocytic cells of the liver¹, and does not reach the desired target. We report here a system in which advantage is taken of this behaviour to treat visceral leishmaniasis in mice, in which the amastigote parasites reside unharmed in vacuoles inside the Kupffer cells of the liver².

Liposomes were prepared from lecithin (purified from egg yolk by the method of Pangborn³) by ultrasonication as described by Bangham and Horne⁴. Lecithin (200 mg) was dispersed in 10 ml fluid, and sonicated in the cold for 5 min. For liposomes containing drug, 2 g either of antimony potassium tartrate or sodium antimony gluconate were included in the dispersion. Otherwise, the material was dispersed in distilled water alone. After sonication, the suspension was passed through a glass fibre filter pad, dialysed for 3 d against 2 l of normal saline (with three changes of medium) and then concentrated 10-fold using an Amicon XM100 filter. The quantity of antimonial drug retained in the concentrate was measured by atomic absorption spectroscopy. The filter held back up to 90% of the antimony contained in the dialysate. This material was found to sediment within 30 min at 100,000g. The above procedure gave liposomes containing typically a total of 5 mg drug. After storing for 1 week at room temperature, at least 50% of the antimony was retained within sedimentable liposomes, the remainder having escaped into solution. A fresh preparation was used for each experiment described. Figure 1 shows an example of an antimony-containing liposome which displays the characteristic multilamellar structure. Preliminary observation by X-ray microanalysis has provided additional evidence for the presence of antimony in these vehicles.



Fig. 1 Electron micrograph of section of mouse liver showing liposome containing potassium antimony tartrate 1 h after intravenous injection. Tissue fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer post-fixed in 1% osmium tetroxide and embedded in Araldite. ($\times 200,000$).

Table 1 Survival of amastigotes in the liver after treatment with drug-loaded liposomes

Expt	Drug	Treatment	Dose administered (mg per kg body weight)	Parasites per 200 cell nuclei $\pm$ s.e.
1	Potassium antimony tartrate	None	None	240 $\pm$ 50
		Drug alone	20	260 $\pm$ 17
		*Drug within liposomes	20	0
2	Potassium antimony tartrate	None	None	849 $\pm$ 86
		Liposomes alone	None	863 $\pm$ 81
		Drug alone	20	760 $\pm$ 63
		+Drug - liposomes	20	304 $\pm$ 66
		*Drug within liposomes	20	0
3	Potassium antimony tartrate	None	None	297 $\pm$ 16
		*Drug within liposomes	0.125	247 $\pm$ 29
			0.25	225 $\pm$ 17
			0.5	199 $\pm$ 15
			1.0	113 $\pm$ 30
			2.0	101 $\pm$ 18
			None	508 $\pm$ 86
4	Potassium antimony tartrate	Drug alone	10	513 $\pm$ 34
		*Drug within liposomes	10	46 $\pm$ 19
			5	86 $\pm$ 21
	Sodium antimony gluconate	Drug alone	10	638 $\pm$ 77
		*Drug within liposomes	10	63 $\pm$ 15
			5	172 $\pm$ 29

Groups of six NMRI mice were inoculated with amastigotes of *L. donovani* on day 0, and received their first dose of drug on day 10. In Expt 1, the drug was administered over 3 d (days 10, 11, 12) and the animals were killed on day 14. In Expts 2, 3 and 4, the drug was given as a single dose, and the livers taken for assay on day 12.

*Drug incorporated into liposomes during sonication. 1 mg of drug is contained in approximately 40 mg phospholipid.

+Drug mixed with a pure liposome suspension 10 min before injection.

The effect of drugs on a leishmanial infection was assayed by a modification of the method of Stauber *et al.*⁵ using the mouse model of Bradley⁶. The concentration of drug was adjusted to 1 mg ml⁻¹, and injected intravenously into NMRI mice (approximate weight 20 g) which had been inoculated intravenously 10 d previously with amastigotes of an Ethiopian isolate of *Leishmania donovani* s.l. (isolate no. HU3, Liverpool designation LV9). This parasite is maintained routinely by passage through hamsters and transferred to mice in the form of a spleen cell suspension. The mice were killed 2 d after injection of the drug, and the extent of the infection assayed microscopically by counting the number of amastigotes per 200 liver cell nuclei in an impression smear.

Table 1 shows that drug incorporated into liposomes had a much greater effect in reducing the parasite count in the liver than equivalent quantities of either liposomes or drug injected alone. Tartrate, 0.4 mg per animal (20 mg per kg body weight), given over a period of 3 d, was sufficient to abolish the infection completely as judged by light microscopy (Expt 1). An identical result was obtained when the same quantity was administered in a single dose (Expt 2). A significant reduction in the number of parasites was also observed after administration of a liposome suspension in which the tartrate had been dissolved at a concentration of 0.8 mg per ml just before injection. This result suggests that there may be some uptake of the drug by lecithin liposomes from the aqueous medium. Over the range of doses tested, there is a linear response of probit activity against log dose (Fig. 2). In addition, Table 1 shows that the enhanced activity exhibited by tartrate in the liposomes was also given by the drug

sodium antimony gluconate, which is formulated for clinical use under the trade name Pentostam (Burroughs Wellcome).

The ideal drug carrier has three important properties. First, it must protect the drug from host metabolic processes and, vice versa, protect the non-target host tissues during the time taken for the drug to reach the target. Second, it must transport the drug directly to the desired tissue, and, finally, it may enhance the uptake of the drug by the cells of the target tissue, and perhaps increase its efficacy within the defective cell.

In the experimental model described here, antimony-filled liposomes seem to satisfy most of these requirements. Antimony potassium tartrate was one of the first and most potent agents used for curing leishmaniasis, but effective treatment necessitated prolonged exposure to highly toxic levels of drug. In our system, levels close to the lethal dose of 50 mg per kg were without effect; when entrapped in liposomes, however, a single dose of 20 mg per kg (Expt 2) was completely effective in eliminating the infection. Measurement of the

concentration of antimony in plasma samples after injection of tartrate within liposomes showed that the material was cleared from the blood with a half-life of approximately 1 h.

At this time, antimony-containing liposomes were found in the liver (Fig. 1). In contrast, the free drug was cleared rapidly from the plasma, and was not detectable by our method 15 min after intravenous administration. Lippincott *et al.* have shown that free tartrate is taken up by erythrocytes during this time. Also, other workers have shown that liposomes are taken up by all organs encompassing the mononuclear phagocyte system (MPS), and that uptake of therapeutic drugs into lysosomes of phagocytic cells can be enhanced *in vitro* and *in vivo* by incorporation of the drug into liposomes.

In view of the fact that liposomes and leishmanial parasites share a propensity for cells of the MPS, it is reasonable to hope that targetting of antimonial drugs at present in use, by entrapment within liposomes, may be feasible in a range of clinical manifestations of the disease, and especially where amastigotes reside within macrophages of the liver, spleen, bone marrow and lymphatic system.

We thank Mr G. Moore for technical assistance, and Dr C. Green for helpful discussion. Financial support was provided by the Wellcome Trust, WHO and the US Army Research and Development Command (Contract DA-ERO-124-74-G0049).

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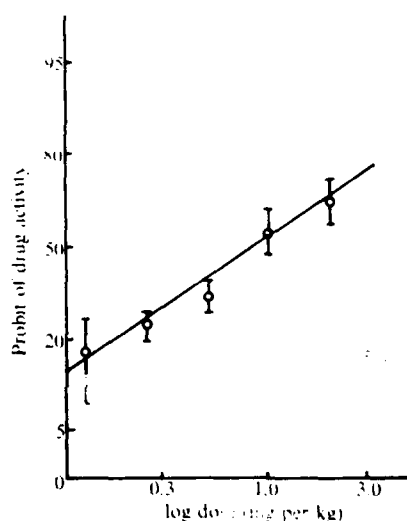


Fig. 2 Relation between probit of activity and dose of potassium antimony tartrate, using data from Table 1 (Expt 3).

Laboratory Demonstration presented at the Royal Society of Tropical Medicine and Hygiene Laboratory Meeting held in Liverpool, 16th November, 1978.

Trans.R.Soc.Trop.Med.Hyg. 73(4)

PURINE AND PYRIMIDINE SYNTHESIS IN PROMASTIGOTES OF LEISHMANIA MEXICANA AMAZONENSIS.

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The occurrence of salvage pathway for purines is generally assumed in Leishmania. This assumption is made from the study of growth requirements of the organisms in defined medium (Steiger & Steiger, 1977). There is little information on the uptake of radiolabelled nucleic acid precursors in Leishmania spp. (Croft, 1977; Marr & Berens, 1978) and only recently have some of the enzymes of purine metabolism been examined (Konigk & Rasoul, 1978).

In the present communication we investigated purines and pyrimidines metabolism in promastigotes of L.m.amazonensis using radiolabelled precursors. The incorporation of 2-³H adenosine, 6-³H thymidine, 5-³H uridine, U-¹⁴C glycine and 6-¹⁴C orotic acid into nucleic acids was measured by scintillation counting methods (Neame et al., 1974). Radioactive adenosine, thymidine, uridine and glycine were incorporated into the nucleic acids of the parasites, but there was no incorporation of orotic acid. These results show that promastigotes of L.m.amazonensis are capable of both salvage and de novo synthesis of purine, but they rely on the salvage pathway for pyrimidine synthesis.

The inability of L.donovani and L.braziliensis sensu lato to synthesize purines de novo (Marr & Berens 1978) could account for the different reaction of Leishmania spp. to different antileishmanial agents.

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APPENDIX V

Updated list
of isolates maintained in the reference collection

Key to numbers

- 1 Dr. A. A. Herrero, Balboa Heights, Panama Canal Zone
- 2 Drs. R. Lainson and J. J. Shaw, Belém, Brazil
- 3 Professor W. H. R. Lumsden, London School of Hygiene and Tropical Medicine
- 4 Professor A. Zuckerman, WHO International Reference Centre for Leishmaniasis,
Jerusalem, Israel
- 5 Hoffman La Roche, Basle, Switzerland
- 6 Professor F. Pifano, Caracas, Venezuela
- 7 Professor J. W. Torrealba, Valencia, Venezuela
- 8 Dr. J. Convit, Caracas, Venezuela
- 9 Professor D. Bradley, Ross Institute, London School of Hygiene and Tropical Medicine
- 10 Professor W. Barbosa, Goiania, Brazil
- 12 Colonel B. C. Walton, Balboa Heights, Panama Canal Zone
- 13 Dr. R. Neal, Beckenham, England
- 14 Professor J. A. Rioux, Montpellier, France
- 15 Professor W. Mayrink, Belo Horizonte, Brazil
- 16 Liverpool School of Tropical Medicine
- 17 Professor J. Ranque, Marseille, France
- 18 Dr. R. Zeledon, San Jose, Costa Rica
- 19 Professor J. J. Laarman, Instituut voor Tropische Hygiene, Amsterdam
- 20 Professor R. Bray, Fajara, The Gambia
- 21 Professor V. Saf'janova, Gameleya Institute, Moscow
- 22 Dr. M. J. Mutinga, Nairobi, Kenya
- 23 Professor M. La Placa, Università di Bologna, Italy
- 24 Dr. R. P. Brazil, Rio de Janeiro, Brazil
- 25 Dr. R. E. Abdalla, Khartoum, Sudan
- 26 Dr. M. Hommel, Liverpool School of Tropical Medicine
- 27 Dr. R. W. Ashford, Liverpool School of Tropical Medicine
- 28 Dr. Radim, University of Teheran, Iran
- 29 Dr. M. Sharma, National Institute of Communicable Diseases, New Delhi, India
- 30 Professor H. Muhlþfordt, Bernhard-Nochi Institut, Hamburg, Germany
- 31 Dr. N. Mansour, NAMRU-3, Cairo, Egypt
- 32 Dr. E. Tikasingh, Caribbean Epidemiology Centre, Trinidad
- 33 Dr. Grové, South African Institute of Medical Research, Johannesburg
- 34 Dr. A. Lemma, Addis Ababa, Ethiopia
- 35 Professor G. Piekarski, Institute of Medical Parasitology, Bonn, W. Germany
- 36 Dr. J. Baker, Moltano Institute, Cambridge
- 37 Professor P. C. C. Garnham, Imperial College Field Station, Ascot, England
- 38 Dr. P. Rees, University of Nairobi, Kenya
- 39 Captain L. Quigg, Brooke Army Medical Center, Texas, USA
- 40 Dr. J. Dedet, Institut Pasteur d'Algerie, Algeria.
- 41 Dr. A. Bryceson, Hospital for Tropical Diseases, London
- 42 Dr. D. Weinman, Yale University, New Haven, USA
- 43 Dr. D. H. Molyneux, Liverpool School of Tropical Medicine
- 44 Miss N. Lupton, Liverpool School of Tropical Medicine
- 45 Dr. A. Ebrahimzadeh, Jondi Shapur University, Iran
- 46 Dr. J. Golenser, Institute of Medical Parasitology, University of Nijmegen
- 47 Dr. F. Sukkar, Ministry of Health, Baghdad
- 48 Dr. F. Neva, National Institutes of Health, Bethesda, USA.

Key to donors continued

- 49 Dr. L. F. Schnur, Hebrew University-Hadassah Medical School, Jerusalem, Israel
- 50 Dr. D. Le Ray, Prince Leopold Institut de Medecine Tropicale, Antwerp, Belgium
- 51 Professor A. B. Chowdhury, Calcutta School of Tropical Medicine, India
- 52 Dr. J. P. Farrell, Rutgers State University, New Jersey, USA
- 53 Dr. L. Hendricks, Walter Reed Army Institute of Research, Washington DC, USA
- 54 Dr. R. Behin, WHO Immunology Centre, Lausanne, Switzerland
- 55 Dr. Sergio Bettini Istituto Superiore di Sanita, Rome, Italy
- 56 Dr. P. Desjeux, Institut Pasteur, Dakar, Senegal
- 57 Dr. R. Custodio, Tegucigalpa, Honduras
- 58 Dr. M. Reguer, Institut Pasteur, Cayenne, French Guyana

Key to abbreviations used in notes

A	amastigotes available
CL	cutaneous lesion
DCL	disseminated cutaneous leishmaniasis
KA	kala-azar
LR	leishmaniasis recidivans
LRC	leishmaniasis reference collection (Jerusalem)
MCL	multiple cutaneous lesions
MUC	mucocutaneous
PKDL	post kala-azar dermal leishmanoid
SCL	single cutaneous lesion
V	visceral
NS	normal skin
lump	London University Medical Parasitology
T	trypanosome

Appendix V. Updated list of isolates maintained in the reference collection.

LIV626	<u>L. major</u>	DK81	Dedet (40)	Senegal	Man	CL
LIV627	<u>L. major</u>	DK83	Dedet (40)	Senegal	Man	CL
LIV628	<u>L. major</u>	DK84	Dedet (40)	Senegal	Man	CL
LIV629	<u>L. major</u>	DK85	Dedet (40)	Senegal	Man	CL
LIV630	<u>L. major</u>	DK86	Dedet (40)	Senegal	Man	CL
LIV631	<u>L. major</u>	DK87	Dedet (40)	Senegal	Man	CL
LIV632	<u>L. donovani</u>	D1	Mutinga (22)	Kenya	Dog	
LIV633	<u>L. donovani</u>	D2	Mutinga (22)	Kenya	Dog	
LIV634	<u>T. roussetti</u>	-	Hommel (26)	Gabon	<u>Russetus aegypticus</u>	
LIV635	<u>L. donovani</u>	I	Chowdhury (51)	India	Man	KA
LIV636	<u>L. donovani</u>	II	Chowdhury (51)	India	Man	KA
LIV637	<u>L. donovani</u>	VI	Chowdhury (51)	India	Man	KA
LIV638	<u>L. donovani</u>	VIII	Chowdhury (51)	India	Man	KA
LIV639	<u>L. sp.</u>	Herrera 117	Hendricks (53)	Honduras	Man	V
LIV640	<u>L. sp.</u>	142	Hendricks (53)	Honduras	Animal	
LIV641	<u>L. sp.</u>	Murray 158	Hendricks (53)	Panama	Man	CL
LIV642	<u>L. sp.</u>	Herrera 182	Hendricks (53)	Honduras	Man	KA
LIV643	<u>L. sp.</u>	195	Hendricks (53)	Honduras	Animal	V
LIV644	<u>L. sp.</u>	Hendricks 220	Hendricks (53)	India	Man	CL
LIV645	<u>L. donovani</u>	219	Hendricks (53)	India	Man	KA
LIV646	<u>L. sp.</u>	234	Hendricks (53)	Spain	Dog	skin
LIV647	<u>L. sp.</u>					
LIV648	<u>L. b. panamensis</u>	Murray LN158	Hendricks (53)	Parama	Man	CL
LIV649	<u>L. donovani</u>	Khartoum WR168	Hendricks (53)	Sudan	Man	KA
LIV650	<u>L. donovani</u>	WR271	Hendricks (53)	Kenya	Man	KA
LIV651	<u>L. sp.</u>	McGillivray WR272	Hendricks (53)	Kenya Nakuru	Man	CL
LIV652	<u>L. sp.</u>	DeViss WR275	Hendricks (53)	Kenya Nakuru	Man	CL
LIV653	<u>L. donovani</u>	SP/SKC		India	Man	KA
LIV654	<u>L. braziliensis</u>		Reguer (58)	French Guyana	Man	CL
LIV655						
LIV656						
LIV657	<u>L. donovani</u>	RB3	Behin (54)	Sudan	Man	KA
LIV658	<u>L. tropica</u>	L/6	Abdalla (25)	Sudan	Man	CL
LIV659	<u>L. mexicana</u>		Brazil (24)	Brazil Maranhao	Man	DCL
LIV660						

Appendix V. Updated list of isolates maintained in the reference collection. (continued)

LIV661	<u>L. sp.</u>	Vulpes V61	Bettini (55)	Italy, Grossetto	Fox	
LIV662	<u>L. donovani</u>	Lana	Bettini (55)	Italy, Orbitello	Dog	
LIV663	<u>L. donovani</u>	Dora	Bettini (55)	Italy, Grossetto	Dog	
LIV664	<u>L. donovani</u>	Care	Bettini (55)	Italy, Puglie	Dog	
LIV665	<u>L. sp.</u>	DK10	Desjeux (56)	Senegal	<u>Mastomys sp.</u>	V
LIV666	<u>L. sp.</u>	DK115	Desjeux (56)	Senegal		
LIV667	<u>L. sp.</u>	L69	Rioux (14)	France	Man	KA
LIV668	<u>L. sp.</u>	L70	Rioux (14)	France	Man	KA
LIV669	<u>L. sp.</u>	L71	Rioux (14)	France	Man	KA
LIV670	<u>L. sp.</u>	L72	Rioux (14)	France	Dog	V
LIV671	<u>L. sp.</u>	L73	Rioux (14)	France	Man	KA
LIV672	<u>L. sp.</u>	L76	Rioux (14)	France	Man	KA
LIV673	<u>L. sp.</u>	L77	Rioux (14)	Tunisia	Dog	V
LIV674	<u>L. sp.</u>	L78	Rioux (14)	Tunisia	Dog	V
LIV675	<u>L. donovani</u>	B14	Rioux (14)	Senegal	Dog	V
LIV676	<u>L. hertigi</u>	CM170	Zeledon (18)	Costa Rica	<u>Coendou mexicana</u>	
LIV677	<u>L. sp.</u>	Salem	Custodio (57)	Honduras	Man	CL

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