evaluated. For example, addition of various concentrations of the antibabesial drug 1,3-bis(*p*-amidinophenyl)triazene-bis(*N*-acetylglycinate)-diaceturate (*13*) to cultures with initial parasitemias of 0.3 to 0.5 percent enables us to titrate the drug's action, because a lethal concentration (3.5×10^{-6} g/ml) prevents culture darkening. A gradation of color change is observed between lethal and nonlethal dosages and this color change is closely correlated with parasitemias.

Extracellular babesias (merozoites) are rarely observed in cultures during the exponential phase of growth, indicating that almost all the parasites are able to rapidly infect new cells. However, when cultures are removed from a CO_2 incubator to an incubator containing ambient air (CO_2-deprived), merozoites accumulate in the medium (Fig. 1B). These merozoites can be separated from the erythrocytes by differential centrifugation (*14*) and they remain infectious for several hours.

Examination of the physiological, biochemical, and antigenic properties of *B. bovis* has been hampered by the absence of a method for cultivating it in vitro. The MASP culture system described here will facilitate investigations of this, and perhaps other related pathogens. Parasite antigens generated in vitro in the absence of host immune and metabolic elements and heretofore unobtainable from infected animals should now become available. A study of isolated infectious merozoites made possible by this culture method may provide valuable insight into the characteristics of merozoites of other hemoprotozoa, for example, *Plasmodium* sp., the cause of human malaria.

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References and Notes

1. H. O. Köwigshöefer, Ed., *PAO-WHO-OIE Animal Health Yearbook* (Food and Agriculture Organization, Rome, 1977).
2. G. R. Healy, A. Spielman, W. Gleason, *Science* **192**, 479 (1976); M. Ristic and G. R. Healy, *Babesiosis—The Parasitic Zoonosis*, vol. 1, *Protozoan Zoonoses* (CRC Press, Cleveland, in press).
3. J. F. Anderson, L. A. Magnarelli, C. S. Donner, A. Spielman, J. Piesman, *Science* **204**, 1431 (1979).
4. W. Trager and J. B. Jensen, *ibid.* **193**, 673 (1976).
5. E. Erp, S. M. Gravely, R. D. Smith, M. Ristic, M. B. Osorno, C. A. Carson, *Am. J. Trop. Med. Hyg.* **27**, 1061 (1978).
6. E. Erp, R. D. Smith, M. Ristic, M. B. Osorno, *Am. J. Vet. Res.*, in press.
7. In contrast to the spinner flask method (*6*) which will support parasite multiplication of 6.4×10^5 times over a 32-day period, increases of 1×10^{18} times are achieved by the method described here over a similar time span.
8. A Mexican isolate of *B. bovis* was used.
9. With Hanks salts (Gibco).
10. Freshly collected bovine blood obtained from mature *Bos taurus* cows is defibrinated by shaking with glass beads and then centrifuged at 1000*g* for 10 minutes at 4°C. The serum may be used immediately, stored for up to 3 days at 4°C, or frozen at −20°C for later use.
11. We have used two procedures for preparing cultures. Erythrocytes and serum may be separated as outlined (*10*) and mixed with Medium 199 in the proper ratios. Alternatively, after determining the packed cell volume of defibrinated blood and adjusting for its serum content, additional serum and Medium 199 may be added to achieve the proper proportions. *Babesia* are damaged by centrifugation (they show, for example, altered morphology and staining properties, and retarded growth for 24 to 48 hours) so the latter method is recommended for initial isolation. However, there is no difference in the ability of new culture prepared by either method to support growth of parasites.
12. An initial *p*H range of 6.9 to 7.2 is satisfactory.
13. Berenil; Farbwerke Hoechst Ag., Frankfurt, Germany.
14. Cultures deprived of CO_2 for 4 to 6 hours are decanted into 50-ml conical centrifuge tubes and sedimented at 400*g* for 5 minutes at 4°C. The top 30 ml is removed and sedimented at 750*g* for 10 minutes. This supernatant is removed and again sedimented at 750*g* for 10 minutes. Centrifugation of the final supernatant at 1000*g* for 20 minutes produces a pellet containing free merozoites and some erythrocyte stroma. These merozoites may be used to infect normal MASP cultures that have been in a CO_2 incubator for at least 1 hour.
15. When infectious merozoites were added to new cultures that had been incubated for 1 hour under CO_2, most of them invaded normal erythrocytes within 1 hour after inoculation, and infection was completed within 3 hours. Merozoites stored in new cultures at 4°C for 16.6 hours under air retained 20 percent of their initial infectivity.
16. Supported in part by research grant 1-44-44-30-377 from the Rockefeller Foundation. The expert technical assistance of G. Clabaugh is gratefully acknowledged.

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Mebendazole Therapy of Parenteral Trichinellosis

Abstract. *Mebendazole was highly effective against the helminth parasite* Trichinella spiralis *in mice subjected to a 3-day course of treatment during the invasive and encystment phases of experimental trichinellosis. When treatment began either 2 or 4 weeks after the mice were inoculated with parasites, the number of larvae developing in the host musculature was greatly reduced by twice-daily oral administration of 3.125, 6.25, or 12.5 milligrams of mebendazole per kilogram of body weight.*

Recent reports have demonstrated the chemotherapeutic effectiveness of a benzimidazole anthelmintic, mebendazole, in the treatment of *Trichinella spiralis* infection in rats and mice (*1*–*7*). Because mebendazole (methyl 5-benzoyl-2-benzimidazolecarbamate) is used for the treatment of other helminthoses in man, and because it is highly active against enteral and parenteral forms of *Trichinella* in laboratory animals, this compound is one of great potential utility in the treatment of human trichinellosis. However, there have been comparatively few studies of the susceptibility of *T. spiralis* to mebendazole treatment during the invasive phase of experimental trichinellosis in mice (that is, when the second generation larvae, shed by adults in the gut, have penetrated the muscle cells and when they are in various early stages of encapsulation within muscle fibers) or of the efficacy of dosage regimens similar to those used in man.

Table 1. Number of *T. spiralis* larvae recovered from the musculature of control mice and mice treated by stomach tube with mebendazole twice daily for three consecutive days (days 14 to 16) during the invasive phase of infection.

Larvae per mouse at mebendazole dose (mg/kg)			
0.0	3.125	6.25	12.5
Individual counts			
3,200	*	0	*
11,000	1,200	200	0
11,142	1,320	800	660
11,600	1,400	1,400	1,000
12,100	1,760	2,280	1,200
12,540	3,520	3,200	1,400
13,250	5,850	3,500	1,600
23,000	6,400	6,250	2,600
Mean			
12,229	3,064†	2,204†	1,209†
Efficacy (%)	75	82	90

*Mouse dead before scheduled necropsy. †$P < .05$.

Evidence for a lethal effect of mebendazole on *T. spiralis* during the invasive phase in mice was obtained recently (*7*) by administering mebendazole (50 mg/kg) by stomach tube once daily for five consecutive days (days 14 to 18) of infection. This dosage regimen significantly reduced by 96 percent the number of larvae subsequently recovered from the host musculature on day 45 after inoculation. Although this dosage was extremely high relative to other anthelmintic applications of the drug, the results clearly indicated that the first larval stage of *T. spiralis* was vulnerable to mebendazole treatment. It was then reasoned that a comparable level of drug activity against *Trichinella* might be obtained with a much lower unit dosage of mebendazole if the anthelmintic were administered in a series of divided daily oral doses during the invasive phase (and presumably the encystment phase as well) of infection. In this way, very small quantities of the drug would continuously be available to the parasite via the host bloodstream and tissue fluids and the total dosage required to achieve efficacy

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Table 2. Number of *T. spiralis* larvae recovered from the musculature of control mice and mice treated by stomach tube with mebendazole twice daily for three consecutive days (days 14 to 16) during the invasive phase of infection.

Larvae per mouse at mebendazole dose (mg/kg)		
0.0	0.3125	1.0
Individual counts		
19,700	15,100	*
22,700	16,300	14,600
24,200	16,600	16,800
24,500	27,000	20,800
29,300	28,800	23,200
36,500	28,900	31,000
36,600	31,300	40,100
49,900	41,400	43,800
Mean		
30,425	25,675†	27,186†
Efficacy (%)	0	0

*Mouse dead before scheduled necropsy. †No significant difference compared to controls that received no mebendazole.

would be greatly reduced. It was therefore decided to use a treatment regimen that is now recommended for the control of ascariasis, trichuriasis, and ancylostomiasis and necatoriasis in man. Accordingly, the drug was administered in a series of twice-daily oral doses on three successive days during the invasive and encystment phases of a *Trichinella* infection.

The strain of *T. spiralis* used was originally obtained from G. Stewart, University of Texas at Arlington, and has been maintained in my laboratory by passage through mice since 1975. The mice were ICR male Swiss albinos (Harlan Industries) that were 8 to 10 weeks old and weighed 25 to 35 g at the time of exposure to infection. They were housed and maintained in accord with principles of the Committee on Laboratory Animal Care, Indiana University, and had free access to commercial feed and water.

The parasitological procedures used for isolation, preparation of inocula, and administration of infective larvae were basically those of Campbell (*8*). Larvae were recovered from the skeletal musculature of skinned and eviscerated mice with infections of at least 5 weeks' duration by digestion at 37°C for 3 to 4 hours in Krebs-Ringer saline containing commercial pepsin (1 percent, weight to volume) and concentrated HCl (1 percent by volume). Mice were routinely infected by oral inoculation with 200 to 250 larvae suspended in 0.30 to 0.50 ml of 5 percent gelatin.

The efficacy of mebendazole against *T. spiralis* during the invasive phase of infection was determined by administering the drug by stomach tube twice daily for three consecutive days beginning on day 14 after the mice were inoculated with *T. spiralis*. Prepared as an aqueous suspension of the granular commercial formulation containing, per gram, 167 mg of active drug (Telmin, Pitman-Moore) the mebendazole was given to the mice at approximately 9 a.m. and 4 p.m. on each treatment date. The volume was adjusted so that each test animal received 0.1 ml of the final suspension per 10 g of body weight. Control mice were dosed orally with comparable volumes of water. The number of larvae in the musculature of treated and untreated mice was determined by digesting each carcass separately in a solution of 1 percent pepsin, 1 percent HCl, and estimating the number of larvae in equal portions. Data were subjected to the nonparametric Kruskal-Wallis one-way analysis of variance by ranks; Ryan's method of adjusted significance levels was used for the separation of treatment means (*9*). By this method of analysis it was possible to make statistical comparisons between the mean worm burdens of unmedicated and treated groups as well as among the mean worm burdens of the treated groups. A probability greater than 0.05 was not considered significant.

In experiment 1, mebendazole was given as oral doses of 3.125, 6.25, or 12.5 mg/kg, twice daily, on days 14 to 16 of infection. At necropsy on day 63 the treated mice harbored an average of, respectively, 75, 82, and 90 percent fewer muscle larvae than did the control mice (Table 1). The mean worm burden in the treated groups was highly significantly different ($P \leq .001$) from the controls. There was no significant difference between the reduction in worm burden obtained with a dosage of 3.125 mg/kg and that obtained with the 6.25 or 12.5 mg/kg treatments.

In experiment 2, the mice were treated as before, being given mebendazole twice a day for three consecutive days starting 2 weeks after they were infected with *T. spiralis*. On each occasion, each group received water or mebendazole (0.3125 or 1 mg/kg). At necropsy on day 42 of infection, the carcasses yielded the numbers of larvae shown in Table 2. Mebendazole had no effect on *T. spiralis* during the invasive phase at these two low dosages; there was no significant difference (χ^2, 0.85; $0.50 < P < .70$, Kruskal-Wallis test) in numbers of larvae recovered from controls and the experimental mice.

If the larvae of *Trichinella* are encysted in the muscles it is still possible to kill them by mebendazole therapy, with the same dosages as are effective during the invasive phase. In experiment 3, mice received mebendazole (3.125, 6.25, or 12.5 mg/kg) by stomach tube twice daily on three consecutive days beginning on day 28 after inoculation of *T. spiralis*. At 14 days after the last treatment the mebendazole-treated mice harbored an average of, respectively, 86, 92, and 84 percent fewer muscle larvae than controls (Table 3). The mean worm burden in the treated groups was highly significantly different ($P = .005$) from that of the controls; there was no significant difference between the reduction in worm burden obtained with a dosage of 3.125 mg/kg and that obtained with the 6.25 or 12.5 mg/kg treatments. Moreover, larvae recovered from each of the mebendazole-treated mice were less than normal size, and all were loosely coiled or arc-like, and motionless. No infectivity tests were made on these patently dead larvae.

Table 3. Number of *T. spiralis* larvae recovered from the musculature of control mice and mice treated by stomach tube with mebendazole twice daily for three consecutive days (days 28 to 30) during the encystment phase of infection.

Larvae per mouse at mebendazole dose (mg/kg)			
0.0	3.125	6.25	12.5
Individual counts			
*	500	600	500
*	2,500	1,700	2,300
20,833	4,300	1,800	3,150
34,750	5,000	2,500	4,700
40,000	5,300	3,800	4,900
52,700	6,917	4,000	14,000
Mean			
30,071	4,086†	2,400†	4,925†
Efficacy (%)	86	92	84

*Mouse dead before scheduled necropsy. †$P < .05$.

The greatest interest in the field of trichinellosis chemotherapy lies in efficacy directed against the parasite outside the gastrointestinal tract (*10*). In man, invasion of muscles has usually occurred by the time a diagnosis has been made, so that lack of specific drugs effective against parenteral forms of *Trichinella* at well-tolerated dosages has been a major handicap to the clinician. Considered in conjunction with the other reports cited here (*1-7*), this investigation illustrates the degree to which efficacy may be influenced by the frequency of drug administration and division of the daily oral dose. The optimum dosage regimen for the treatment of human trichinellosis has yet to be established. The dosage schedule usually recommended for control of the common gastrointestinal helminths

in man applies to both children and adults; that is, one 100-mg tablet of mebendazole, administered orally, morning and evening, on three consecutive days, regardless of body weight. Thus, for a standard 65-kg male the dose would be 1.5 mg/kg. The findings of the present study with mice, if they are applicable to man, suggest that children would get an effective dosage, but an average adult would receive only a partially effective dosage; in adults weighing 82 kg or more the dosage would be ineffective. It is possible that with timely diagnosis of the disease and a revised dosage regimen, mebendazole might give a consistent therapeutic response. Trial of the drug in severe trichinellosis in man should be considered.

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References and Notes

1. S. de Nollin and H. Van den Bossche, *J. Parasitol.* **59**, 970 (1973).
2. S. de Nollin, M. Borgers, O. Vanparijs, H. Van den Bossche, *Parasitology* **69**, 55 (1974).
3. D. Thienpont, O. Vanparijs, R. Vandesteeve, in *Trichinellosis*, C. W. Kim, Ed. (Intext Educational Publishers, New York, 1974), pp. 515–527.
4. R. Spaldonova, O. Tomasovicova, J. Corba, *Proceedings of the Third International Congress of Parasitology* (Munich, Germany, 25 to 31 August 1974), pp. 675–676.
5. R. Spaldonova and J. Corba, *Proceedings of the Fourth International Congress of Parasitology* (Warsaw, Poland, 19 to 26 August 1978), p. 146.
6. S. S. Fernando and D. A. Denham, *J. Parasitol.* **62**, 874 (1976).
7. R. O. McCracken, *ibid.* **64**, 214 (1978).
8. W. C. Campbell, *ibid.* **53**, 395 (1967).
9. R. E. Kirk, *Experimental Design: Procedures for the Behavioral Sciences* (Brooks/Cole, Belmont, Calif., 1968), pp. 493–497.
10. W. C. Campbell and L. S. Blair, *Exp. Parasitol.* **35**, 304 (1974).
11. We thank W. C. Campbell of the Merck Institute for Therapeutic Research and M. J. Ulmer, Iowa State University, for helpful suggestions and critical review of the manuscript. This work was supported in part by PHS grant S07 RR 7031 to Indiana University under the Biomedical Research Support Grant Program and a summer faculty fellowship to R.O.McC. from Purdue University.

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Properties of a Normal Mouse Cell DNA Sequence (sarc) Homologous to the src Sequence of Moloney Sarcoma Virus

Abstract. *A 15.0-kilobase (kb)* Eco *RI DNA fragment from normal mouse Balb/c genomic DNA that contains sequences (sarc) homologous to the acquired cell sequences (src) of Moloney sarcoma virus (MSV) has been cloned in phage* λ. *The sarc region (1.2 to 1.3 kb) of the 15.0-kb cell fragment is indistinguishable from the src region of two isolates of MSV as judged by heteroduplex and restriction endonuclease analyses. The cellular sequences flanking sarc show no homology to other MSV sequences. Whereas cloned subgenomic portions of MSV that contain src transformed NIH-3T3 cells in vitro, the cloned sarc fragment is inactive.*

Sarcoma retroviruses provide a system for studying the contribution of normal cell DNA sequences to malignancy. The genomes of these viruses contain sequences, presumably acquired from the host cell, which are a prerequisite for their tumorigenic potential and their ability to morphologically transform cells in vitro (*1–4*). By isolating these sequences from normal cells with recombinant DNA cloning techniques, the specific molecular elements responsible for their oncogenic potential can be defined, and the contribution of other viral sequences to the transforming process can be determined. By convention, we use the term src for the acquired cell sequences found in sarcoma viruses and the term sarc for the normal cell DNA sequences homologous to them (*4*).

The src sequences of sarcoma viruses were acquired during putative recombination between parental leukemia viruses and host cell information (*4*). The Moloney murine sarcoma virus (MSV) arose spontaneously during passage of Moloney murine leukemia virus (MuLV) in Balb/c mice (*5*) and contains both MuLV and normal Balb/c mouse cell sequences (*2*). The mechanism by which the viral src sequence participates in cell transformation is unknown. Likewise, the physiological function of the normal cell sarc sequence is unknown. Sarc sequences are represented in the normal mouse genome from one to, at most, a few copies per cell, and their rate of evolutionary divergence parallels that of mouse globin DNA sequences (*6*). As a normal cell sequence, the tumorigenic potential of sarc is obviously of great interest. We have cloned the normal Balb/c sarc sequence in phage λ and present a comparative analysis of the physical and biological properties of sarc and src.

We previously cloned the integrated proviruses of two MSV isolates, m1 and HT1, into phage λ (*7*), and identified in each provirus the src portion as well as the sequences derived from MuLV (*8*). The src regions of both m1 and HT1 MSV are identical by heteroduplex analysis and restriction endonuclease mapping (*8*). To detect the cellular sarc sequence, we hybridized a src specific restriction fragment (4.5-kb *Kpn* I to 5.2-kb *Hind* III of λHT1) (Fig. 2) labeled by nick translation (*10*), to a Southern (*9*) trans-

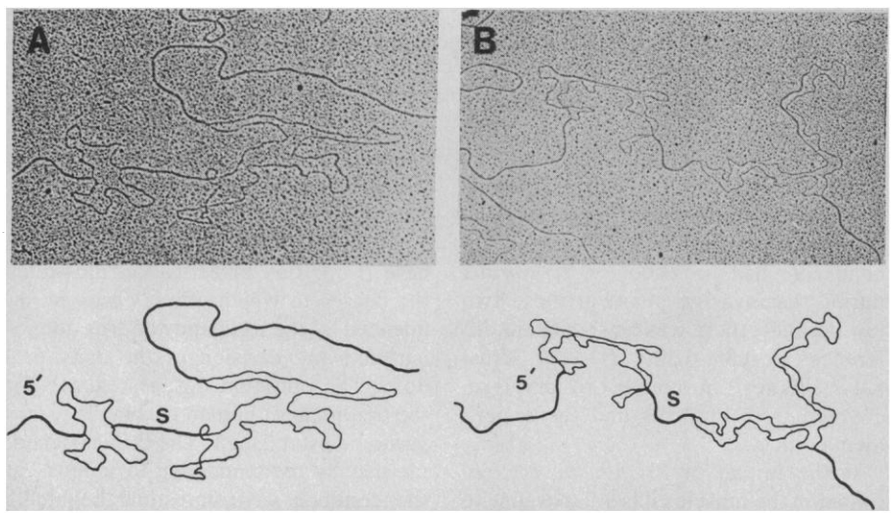

Fig. 1. Heteroduplex analyses of λMsarc with λm1 and λHT1. Heteroduplex analyses were performed on DNA extracted from λMsarc and λm1 (A) or λHT1 (B) as described (*11*). Restriction endonuclease mapping showed that the sarc sequence in λMsarc was in the same 5′ to 3′ orientation as the src in λHT1 and λm1 (*8*). The 5′ ends of the inserted fragments are adjacent to the left vector fragment of λgtWES (21.9 kb). Twenty-seven molecules were measured for (A) and 25 for (B). The measurements from the 5′ end to the 3′ end for the single-stranded, double-stranded, and single-stranded regions of the inserted fragments are, respectively, in (A) for λMsarc, 2.70 ± 0.34, 1.17 ± 0.18, and 10.84 ± 0.76 kb, and for λm1, 3.44 ± 0.34, 1.17 ± 0.18, and 2.19 ± 0.25 kb; in (B) for λMsarc, 3.0 ± 0.17, 1.30 ± 0.17, and 11.2 ± 0.5 kb; and for λHT1, 7.24 ± 0.42, 1.30 ± 0.17, and 3.34 ± 0.26 kb.

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