estimated to be 4 to 8 weeks (*2*). *Pneumocystis carinii* has been transmitted from cortisone-treated rats to cortisone-treated germfree rats by close contact and airborne routes (*10, 13*). Heavier degrees of infection were found after prolonged periods (3 or more weeks) of exposure.

The nude mouse offers the potential for performing definitive epidemiologic studies with *P. carinii*. The light infection produced in many nude mice closely resembles the carrier state that exists in different animals in nature. With this model, it should be possible to determine the incubation period of *P. carinii*, to establish dose-response curves, and study the development of the infection. The nude mouse should provide an in vivo counterpart to the in vitro culture system of *P. carinii* (*14*). The potential problems with the animal model system are illustrated by the spread of *P. carinii* throughout the closed nude mouse colony (Table 2), which has interfered with interpretation of lung injection and serial passage studies performed thus far. We now perform all studies in germfree isolators to avoid problems of cross contamination.

The role of specific host immune factors in the development of *P. carinii* infection is poorly understood. Clinical studies of patients with primary immune deficiency diseases, lymphoreticular neoplasms receiving immunosuppressive agents, and the role of malnutrition suggest that defects in both B and T cells contribute (*15*). Yet, the occurrence of pneumocystis pneumonia after corticosteroids have been tapered and after bone marrow transplantation suggest that some degree of host immune reaction is important for overt disease to develop. Using thymic transplantation and other immunologic manipulations, this animal model offers a method for clarifying the complex factors in the host-parasite relationship of *P. carinii* infection.

PETER D. WALZER,* VERA SCHNELLE DONALD ARMSTRONG,† P. PETER ROSEN
Infectious Disease Service, Department of Medicine, and *Department of Pathology, Memorial Sloan-Kettering Cancer Center*, and *Cornell University Medical College, New York 10021*

References and Notes

1. B. Goodell, J. B. Jacobs, R. D. Powell, V. T. DeVita, *Ann. Intern. Med.* **72**, 337 (1970); D. Armstrong, H. Chmel, C. Singer, M. Tapper, P. P. Rosen, *Eur. J. Cancer Suppl.* **11**, 79 (1975); P. D. Walzer, D. P. Perl, D. J. Krogstad, P. G. Rawson, M. G. Schultz, *Ann. Intern. Med.* **80**, 83 (1974).
2. D. C. Gadjusek, *Pediatrics* **19**, 543 (1957).
3. K. Kucera, *Ann. Parasitol. Hum. Comp.* **42**, 465 (1967); F. G. Poelma, *Z. Parasitenkd.* **46**, 61 (1975); R. Lainson and J. J. Shaw, *Trans. R. Soc. Trop. Med. Hyg.* **69**, 505 (1975).
4. J. K. Frenkel, J. T. Good, J. A. Schultz, *Lab. Invest.* **15**, 1559 (1966).
5. W. H. Sheldon, *J. Exp. Med.* **110**, 147 (1959).
6. J. A. Minielly, S. D. Mills, K. E. Holley, *Can. Med. Assoc. J.* **100**, 846 (1969); B. R. H. Farrow, A. D. J. Watson, W. J. Hartley, C. R. R. Ituxtable, *J. Comp. Pathol.* **82**, 447 (1972).
7. H. K. Kim, W. T. Hughes, S. Feldman, *Proc. Soc. Exp. Biol. Med.* **141**, 304 (1974); S. K. Lim, W. C. Eveland, J. R. Porter, *Appl. Microbiol.* **26**, 666 (1973).
8. L. Norman and I. G. Kagan, *Infect. Immun.* **8**, 317 (1973).
9. R. H. Jacobson and N. D. Reed, *Proc. Soc. Exp. Biol. Med.* **147**, 667 (1974); I. A. Clark and A. C. Allison, *Nature (London)* **252**, 328 (1974); P. Emmerling, H. Finger, J. Bockemuhl, *Infect. Immun.* **12**, 437 (1975); D. D. Isaak, R. H. Jacobson, N. D. Reed, *ibid.*, p. 1478; J. L. Sullivan, R. E. Mayner, D. W. Barry, F. A. Ennis, *J. Infect. Dis.* **133**, 91 (1976); C. K. Hsu, S. H. Hsu, R. A. Whitney, C. T. Hanson, *Nature (London)* **262**, 397 (1976); M. J. Colston and G. R. F. Hilson, *ibid.*, p. 399.
10. J. O. Hendley and T. H. Weller, *Proc. Soc. Exp. Biol. Med.* **137**, 1401 (1971).
11. P. Rosen, N. Martini, D. Armstrong, *Am. J. Med.* **58**, 794 (1975).
12. C. Singer, D. Armstrong, P. P. Rosen, D. Schottenfeld, *Ann. Intern. Med.* **82**, 722 (1975); H. J. Meuwissen, W. J. Brzosko, A. Nowoslawski, R. A. Good, *Lancet* **1970-I**, 1124 (1970).
13. J. H. Meuwissen, *Natl. Cancer Inst. Monogr.* **43**, 133 (1976).
14. L. Pifer and W. T. Hughes, *Pediatr. Res.* **9**, 344 (abstr.) (1975).
15. P. D. Walzer, M. G. Schultz, K. A. Western, J. B. Robbins, *J. Pediatr.* **82**, 416 (1973); B. A. Burke and R. A. Good, *Medicine* **52**, 23 (1973); D. Rifkind, T. D. Faris, R. B. Hill, *Ann. Intern. Med.* **65**, 943 (1966); L. O. Gentry, J. Ruskin, J. S. Remington, *Calif. Med.* **116**, 6 (1972); P. Rosen, D. Armstrong, C. Ramos, *Am. J. Med.* **53**, 428 (1972); W. T. Hughes *et al.*, *Am. J. Dis. Child.* **128**, 44 (1974).
16. We thank Dr. James R. Tillotson of Albany Medical College for supplying a lung specimen on one of the patients used in this study. Supported by the American Cancer Society, clinical investigation grant 26, and Memorial Hospital grant 3610; P.D.W. was supported by NIH training grant CA-05110-15.
* Present address: Division of Infectious Diseases, Department of Medicine, University of Kentucky Medical Center, Veterans Administration Hospital, Lexington.
† To whom correspondence and reprint requests should be addressed.

13 December 1976; revised 25 February 1977

Pineal Vasotocin: Release into Cat Cerebrospinal Fluid by Melanocyte-Stimulating Hormone Release-Inhibiting Factor

Abstract. *The intracarotid injection of both synthetic melanocyte-stimulating hormone release-inhibiting factor (MIF) and purified MIF prepared from bovine hypothalami induces arginine vasotocin release into cerebrospinal fluid of cats and significantly decreases the pineal arginine vasotocin content at 5 minutes after the injection. The present results demonstrate an extrapituitary endocrine effect of synthetic and purified bovine MIF.*

We demonstrated previously that the mammalian pineal gland contains (*1, 2*) and synthesizes (*3*) the nonapeptide arginine vasotocin (AVT), and that very low concentrations of AVT (0.0001 pg) injected into the third ventricle significantly decrease the pituitary melanocyte-stimulating hormone (MSH) content in normal mice, and completely reverse the pituitary MSH increase in pinealectomized mice (*4*). Since at the same time pinealectomy significantly increases the content of a MSH release-inhibiting factor (MIF) in mice hypothalami, and AVT reverses this increase (*5*), it is reasonable to assume that AVT induces alterations in the pituitary MSH content by interfering with the synthesis or release of MIF.

Although several peptides have been proposed as MIF (*6*), there is no agreement about the nature of the physiological MIF. One of the most potent and most investigated MIF's is the tripeptide Pro-Leu-Gly-NH_2 (*7*) also designated MIF-I (*6*). The preferential uptake of radioactivity by the pineal after the intravenous injection of ^{3}H-labeled MIF-I in both mice and rats (*8*), as well as the concentration of radioactivity in the ependymal cells bordering the ventricles after the intracarotid injection of ^{3}H-labeled MIF-I in rats (*9*), prompted us to investigate the possible effects of synthetic MIF-I, and of a purified MIF from bovine hypothalami, on the AVT content of the pineal and cerebrospinal fluid (CSF) of cats.

Bovine MIF, obtained from stalk median eminence tissues, was purified by gel filtration on Sephadex columns as recommended by Schally and Kastin (*10*). The intravenous administration of 0.5 μg of this purified material produces an increase of the pituitary MSH levels in mice equivalent to that of 0.2 μg of Pro-Leu-Gly-NH_2 (Hoechst). The assay methods used have been described (*1*) and included rat antidiuretic, rat uterine, and frog bladder (*Rana temporaria*) assays. Synthetic AVT having a potency of 110 ± 18 rat antidiuretic and 18,000 ± 2000 hydroosmotic units per milligram was used as standard in the biological assays. Highly purified crystalline trypsin (twice crystallized, chymotrypsin-free, Serva) was used for the tryptic digestion of crude pineal extracts and CSF samples. The pineals were extracted in 0.25 percent acetic acid as described (*11*). Seventeen cats (3 to 4 kg) anesthetized with urethane were injected in the left carotid either with 1.5 μg of MIF-I (Hoechst) or with 3.5 μg (the equivalent

Table 1. Hydroosmotic and antidiuretic activities of cat cerebrospinal fluid after the intracarotid injection of 1.5 μg of MIF-I or of 3.5 μg of purified bovine MIF. The values are expressed as microunits of synthetic AVT per milliliter (± standard error).

Time after injection (minutes)	Activity			
	Hydroosmotic		Antidiuretic	
	MIF-I	Purified bovine MIF	MIF-I	Purified bovine MIF
−5	< 50	< 50	< 5	< 5
5	4350 ± 713	5216 ± 918	27 ± 3	32 ± 5
15	4050 ± 825	4695 ± 642	25 ± 3	30 ± 4
30	1280 ± 250	1699 ± 150	9 ± 2	11 ± 2
60	< 50	< 50	< 5	< 5

Table 2. Hydroosmotic and antidiuretic activities of cat pineal glands after the intracarotid injection of 1.5 μg of MIF-I or of 3.5 μg of purified bovine MIF. The values are expressed as microunits of synthetic AVT per gland (± standard error).

Time after injection (minutes)	Activity			
	Hydroosmotic		Antidiuretic	
	MIF-I	Purified bovine MIF	MIF-I	Purified bovine MIF
Control	8415 ± 1077		55 ± 5	
5	4550 ± 810	4285 ± 734	30 ± 5	28 ± 3
30	5230 ± 912	5415 ± 699	95 ± 15	100 ± 17
60	6697 ± 823	6372 ± 931	181 ± 23	177 ± 21

of one-half hypothalamus) of purified bovine MIF diluted in a volume of 0.5 ml of 0.9 percent NaCl and 0.01*M* acetic acid. Controls received the diluent alone. Additional controls were injected with 1 μg of synthetic oxytocin (Syntocinon, Sandoz) or with 1 μg of 8-leucine vasopressin ("oxypressin"). The CSF samples obtained by cisternal puncture, as well as the crude pineal extracts, were assayed immediately after the experiment.

The hydroosmotic and antidiuretic activities of cat CSF and pineals after the intracarotid injection of MIF-I and purified bovine MIF are shown in Tables 1 and 2, respectively. No rat uterine activities could be detected in the CSF samples (that is, less than 25 μunit/ml). In cats injected with oxytocin, oxypressin, or with the diluent alone, no hydroosmotic activities could be detected in the CSF samples at 15 minutes after the injection (< 50 μunit/ml). Both hydroosmotic and antidiuretic activities of cat CSF and pineals were completely destroyed after 3 hours of incubation with 25 μg of trypsin per milliliter at *p*H 8 and 38°C (that is, they were reduced in hydroosmotic activity to < 50 μunit/ml and in antidiuretic activity to < 5 μunit/ml). The ratio of hydroosmotic to antidiuretic activity of CSF samples, as well as that of control pineals and of pineals assayed at 5 minutes after the injection of MIF-I or purified bovine MIF, did not differ significantly from the value of 159 that we found for synthetic AVT by the same assays. This ratio is extremely specific for AVT (*12*). Therefore, the biological activities of cat CSF and pineals, in terms of the ratios of their hydroosmotic to antidiuretic activities as well as their susceptibility to tryptic digestion, demonstrate that both MIF-I and purified bovine MIF induce the release into the CSF of a basic peptide indistinguishable from synthetic AVT (*12*).

The specific biological activities of AVT appeared in the CSF 5 minutes after the injection and disappeared within 30 minutes (Table 1). However, at 60 minutes after the injection of both MIF-I and purified bovine MIF, while the hydroosmotic activity of pineals was not restored, the antidiuretic activity exceeded the control values by more than three times (Table 2). This increase of the pineal antidiuretic activity became apparent at 30 minutes and declined after 60 minutes. The ratios of hydroosmotic to antidiuretic activities of 37 and 36 that were found in the pineals 60 minutes after the injection of MIF-I and purified bovine MIF, respectively, differed significantly from the same ratio of 159 found for synthetic AVT, demonstrating the presence of an additional antidiuretic activity unrelated to AVT. Indeed, we recently demonstrated (*13*) that during the night the rat pineal releases AVT and stores arginine vasopressin (AVP). In hypophysectomized rats, while the nocturnal release of AVT from the pineal was not affected, the storage of AVP by the pineal was completely abolished indicating its neurohypophyseal origin (*13*). In hypophysectomized rats (24 hours after the operation), MIF-I failed to increase the antidiuretic activity of the pineal 60 minutes after the intravenous injection, although it significantly decreased the hydroosmotic activity (*14*). The high uptake of radioactivity by the neurohypophysis after the intravenous injection of [^{3}H]MIF-I (*9*) also supports the above results, suggesting that both MIF-I and purified bovine MIF stimulate the release of AVT from the pineal and that of AVP from the neurohypophysis. The parallelism between the effects of MIF-I and purified bovine MIF suggests that Pro-Leu-Gly-NH_2 is a true MIF or that the true MIF has a similar structure. On the other hand, the fact that both MIF-I and purified bovine MIF are able to mimic the effects of a naturally occurring biological rhythm (*13*) suggests the possible physiological involvement of MIF in the nocturnal release of AVT and AVP.

Whatever might be the significance of the present results, it is evident that MIF has an extrapituitary endocrine effect and that feedback may occur between the hypothalamic hormone and pineal AVT.

S. Pavel
R. Goldstein
Carmen Gheorghiu, Maria Calb
Institute of Endocrinology, Bucharest, Rumania

References and Notes

1. S. Pavel, *Endocrinology* **77**, 812 (1965).
2. ——— and S. Petrescu, *Nature (London)* **212**, 1054 (1966).
3. S. Pavel, M. Dorcescu, R. Petrescu-Holban, E. Ghinea, *Science* **181**, 1252 (1973); S. Pavel, R. Goldstein, E. Ghinea, M. Calb, *Endocrinology* **100**, 205 (1977).
4. S. Pavel, C. Gheorghiu, M. Calb, M. Petrescu, *Endocrinology* **97**, 674 (1975).
5. S. Pavel, M. Calb, C. Gheorghiu, in preparation.
6. A. J. Kastin, N. P. Plotnikoff, A. V. Schally, C. A. Sandman, in *Reviews of Neuroscience*, S. Ehrenpreis and I. J. Kopin, Eds. (Raven, New York, 1976), p. 111.
7. R. M. G. Nair, A. J. Kastin, A. V. Schally, *Biochem. Biophys. Res. Commun.* **43**, 1376 (1971).
8. A. Dupont, F. Labrie, G. Pelletier, R. Puviani, D. H. Coy, A. V. Schally, A. J. Kastin, *J. Endocrinol.* **64**, 243 (1975).
9. G. Pelletier, F. Labrie, A. J. Kastin, D. H. Koy, A. V. Schally, *Pharmacol. Biochem. Behav.* **3**, 675 (1975).
10. A. V. Schally and A. J. Kastin, *Endocrinology* **79**, 768 (1966).
11. S. Pavel, R. Goldstein, M. Calb, *J. Endocrinol.* **66**, 283 (1975).
12. W. H. Sawyer, *Pharmacol. Rev.* **13**, 225 (1961).
13. M. Calb, R. Goldstein, S. Pavel, *Acta Endocrinol. (Copenhagen)* **84**, 523 (1977).
14. S. Pavel, unpublished results.
15. We thank M. Bodanszky, Case Western Reserve University, Cleveland, Ohio, for the synthetic AVT and M. von der Ohe, Hoechst Aktiengesellschaft, Frankfurt, for the synthetic MIF-I. Oxypressin was supplied by the late J. Rudinger, Institute of Molecular Biology and Biophysics, Zurich, Switzerland.

13 December 1976