nucleate ascospores ranging from 1.5 to 2.0 μ in diameter. Such ascospores, when isolated in single-spore cultures (*8*), germinate and give rise to characteristic *B. dermatitidis* colonies that form typical conidia. We have successfully infected mice with inocula from monoascospore cultures and observed the characteristic tissue forms of *B. dermatitidis*.

Since cleistothecia have been produced only after the pairing of cultures, it is assumed that this fungus is heterothallic. Five combinations of strains have produced fertile fruiting bodies. Twenty-six isolates, when paired with at least one other strain, have produced incipient cleistothecia or mature ascocarps. These results are compatible with heterothallism. Since the fungus is multinucleate, we realize that final determination will depend on the results of pairings of monoascospore cultures.

Comparison of this fungus with descriptions (*6*) of members of the Gymnoascaceae indicates that the organism belongs to this family and to a new genus (*9*). A detailed account of the origin and development of the cleistothecium, a Latin diagnosis, and of the results of pairing of monoascospore cultures is in preparation.

E. S. MCDONOUGH
ANN L. LEWIS

Biology Department,
Marquette University,
Milwaukee, Wisconsin 53233

References and Notes

1. J. W. Carmichael, *Can. J. Botany* **40**, 1137 (1962).
2. J. F. Denton, E. S. McDonough, L. Ajello, R. J. Ausherman, *Science* **113**, 1226 (1961); J. F. Denton and A. F. Di Salvo, *Amer. J. Trop. Med. Hyg.* **13**, 716.
3. E. S. McDonough, in *Recent Progress in Microbiology*, N. E. Gibbons, Ed. (Univ. of Toronto Press, Toronto, 1963), pp. 69–83; ———, R. Van Prooien, A. L. Lewis, *Amer. J. Epidemiol.* **81**, 86 (1965); J. J. Prochnow, *Amer. Rev. Respirat. Diseases* **94**, 761 (1966).
4. A. L. Lewis, thesis, Marquette Univ., 1966.
5. C. W. Emmons, *Mycologia* **36**, 188 (1944).
6. R. K. Benjamin, *El Alisio* **3**, 301 (1956); H. H. Kuehn, *Mycologia* **50**, 417 (1958); **51**, 665 (1959); A. E. Apinis, *Revision of British Gymnoascaceae* (Brit. Mycol. Inst., London, 1964), pp. 1–56.
7. C. D. Smith, *Mycopathol. Mycol. Appl.* **22**, 99 (1964). (Agar, 20 g; filter-sterilized 15-percent yeast extract, 6.0 ml; cycloheximide, 1 g in 10 ml of acetone; penicillin, 20,000 units; streptomycin, 4000 units; polymyxin B, 100,000 units; distilled water, 1000 ml.)
8. The ascospores, isolated with a De Fonbrune micromanipulator, were grown on brain-heart infusion agar containing 5 percent blood and 1 percent yeast extract by Shu-lan Cheng, Mycololgy Section, National Communicable Disease Center, Atlanta, Georgia.
9. R. K. Benjamin has examined prepared slides of the fungus; he agrees with us that it represents a new genus and is a member of the Gymnoascaceae.
10. Supported by NIH grants AI 02211-(08-09) and GM 09996 and by a grant by the Marquette University Committee on Research.

28 February 1967

Heritability of Plasma Cholinesterase Activity in Inbred Mouse Strains

Abstract. *The activity of cholinesterase was measured in the plasma of 543 mice of both sexes from 23 inbred mouse strains. Differences between strains were highly significant. The heritability of this characteristic was 0.70 in females and 0.67 in males.*

In a continuing collaborative study designed to characterize experimental animals used in certain biochemical and radiation research, plasma cholinesterase activity has been measured in mice of both sexes from 23 different inbred strains. The data are of particular interest to those working with the genetic control of enzyme activity and to those concerned with correlating enzyme activity and behavior patterns.

All mice were born, raised, and maintained in the same laboratory. At weaning they were transferred from individual breeding rooms to one room where they remained until assay. By this means, stress of shipping was eliminated, and other environmental stresses were minimized and kept uniform. With the exceptions noted, all mice were housed four to six to a cage and were given free access to water and food (Old Guilford laboratory chow). Male mice of strains AKR/J, BALB/cJ, RF/J, and SJL/J fight vigorously; therefore, it was necessary to cage some of them singly. All the mice were 3½ to 4½ months old at the time of assay. Enzyme activity was measured in each of the 543 mice; to minimize possible environmental fluctuations, all measurements were made within a 4-day period. Under light ether anesthesia, the femoral artery and vein were dissected and transected. The freely flowing blood was aspirated into heparinized pipettes. It was then placed in plastic centrifuge cones (0.4 ml) and centrifuged sufficiently to separate the plasma from the red cells. Portions (0.2 ml) of plasma were placed in cups and diluted to 0.5 ml with distilled water. The cups were then placed on automatic analysis equipment which removed a sample of 0.1 ml from each cup for analysis of cholinesterase activity. With the remainder of each sample, assay for other enzymes was performed.

Cholinesterase activity was assayed by the method of Garry and Routh (*1*) as modified by Levine, Scheidt, and Nelson (*2*). This method entails the hydrolysis of the substrate acetylthiocholine by interaction with plasma; the resultant thiocholine is then treated with 5,5′-dithiobis-2-nitrobenzoic acid, a specific thiol indicator. The resultant color is determined colorimetrically at 420 mμ. The released sulfhydryl is determined by comparison to standard solutions of reduced glutathione. The unit of cholinesterase activity is defined as the amount of enzyme per milliliter of plasma that liberates 1 μmole of sulfhydryl groups in 3 minutes of incubation.

Table 1. Plasma cholinesterase activity in 23 inbred mouse strains.

Strain	Activity*			
	Females		Males	
	No.	Mean* ± S.E.	No.	Mean* ± S.E.
A/J	10	37.2 ± 1.20	13	28.7 ± 1.08
AKR/J	16	29.8 ± 0.57	18	16.1 ± 0.71
AU/SsJ	6	67.7 ± 2.97	5	47.2 ± 2.92
BALB/cJ	10	33.1 ± 1.08	12	26.5 ± 0.93
CBA/J	12	41.8 ± 0.87	16	28.1 ± .82
CE/J	9	37.1 ± .94	17	27.9 ± 1.45
C57BL/6J	11	34.5 ± 1.06	10	30.3 ± 0.78
C57BR/cdJ	10	31.9 ± 0.82	18	26.2 ± .49
C57L/J	11	33.8 ± .42	15	26.6 ± .49
C58/J	9	37.8 ± 1.13	16	29.5 ± .65
C3HeB/FeJ	15	33.3 ± 0.67	14	26.1 ± .50
DBA/1J	9	52.9 ± 1.71		
DBA/2J	10	44.7 ± 0.84	10	29.8 ± .29
LP/J			7	20.7 ± 1.41
MA/J	23	45.6 ± .78	21	23.3 ± 0.25
RF/J	12	31.0 ± .44	17	17.8 ± .30
RIII/J	8	42.8 ± 3.07	11	32.7 ± 1.18
PL/J	15	46.0 ± 1.20	16	30.9 ± 0.82
SEC/1ReJ	10	42.6 ± 1.07	10	36.0 ± 1.03
SJL/J	12	34.0 ± 0.60	10	19.4 ± 0.88
SM/J	11	35.5 ± .55	13	25.2 ± .58
ST/bJ	11	35.6 ± .93	13	26.8 ± .48
129/J	11	39.1 ± .83	10	35.8 ± .81

* Enzyme activity is expressed as micromoles of sulfhydryl per milliliter of plasma.

Table 2. Analysis of variance of plasma cholinesterase activity. *df*, Degrees of freedom; *MS*, mean square; *F*, *F* test.

Source of variation	Variance			
	Females		Males	
	df	*MS*	*df*	*MS*
Between strains	21	651.66	21	445.50
Within strains	229	12.76	270	9.05
Significance:				
	$F = 51.05$		$F = 49.22$	
	$P < .001$		$P < .001$	

In females the lowest mean activity was found in strain AKR/J and the highest in strain AU/SsJ, the means differing by a factor of nearly 2 (Table 1). The activity was consistently lower in the males; the lowest and highest mean values were found in the same above-mentioned strains, and differed by a factor of nearly 3. Analysis of variance (Table 2) has shown that these strain differences are highly significant.

Genetic determination or heritability in the broad sense (h^2) was calculated by methods described by Falconer (*3*). The values obtained were: $h^2 = 0.70$ for females, and $h^2 = 0.67$ for males. These values, which are quite high, are in excellent agreement with the value of 0.78 obtained by Roderick (*4*) for realized heritability in two outbred stocks of rats selected for brain cholinesterase activity.

No attempt has yet been made to investigate further the genetics of cholinesterase activity, nor has any attempt been made to correlate enzyme activities with other traits. However, our data provide the necessary background information for a rational choice of experimental animals for such studies.

CHARLES R. ANGEL, DORSEY T. MAHIN
RICHARD D. FARRIS
KENT T. WOODWARD
Walter Reed Army Institute of Research, Washington, D.C.
JOHN M. YUHAS, JOHN B. STORER
The Jackson Laboratory, Bar Harbor, Maine

References and Notes

1. P. J. Garry and J. I. Routh, *Clin. Chem.* **11**, 91 (1965).
2. J. B. Levine, R. A. Scheidt, V. A. Nelson, in *Technicon Symposium, Automation in Analytical Chemistry* (Mediad, Inc., New York, 1965), p. 582.
3. D. S. Falconer, *Introduction to Quantitative Genetics* (Ronald Press, New York, 1960).
4. T. H. Roderick, *Genetics* **45**, 1123 (1960).
5. Supported in part by U.S. Army contract DA-49-193-MD-2289.

17 February 1967

Bluetongue Virus Infection: Pathologic Responses of Nervous Systems in Sheep and Mice

Abstract. *Lesions caused by bluetongue virus infection of the central nervous systems of sheep and mice vary with age of the host. The character of the lesions appears to be influenced by the stage of immunological maturity of the infected animals.*

During the fall of 1954, field cases of congenital anomalies were noted in lambs born to ewes that had been vaccinated against bluetongue with a modified live-virus vaccine (*1*). The affected lambs were chiefly from ewes vaccinated between the 4th and 8th weeks of gestation. Similar lesions were produced experimentally in fetuses of 6 out of 29 ewes vaccinated on the 40th day of gestation but not in those of ewes vaccinated on the 19th to the 29th days (*2*). The congenital anomalies in the nervous system ranged from hydranencephaly to subcortical cerebral cysts in the less severely affected animals; in some specimens obtained during the gestation period an acute necrotizing meningoencephalitis was found (*2, 3*).

The interesting nature of these lesions prompted an investigation of the responses of mature and developing nervous systems to bluetongue virus infection. Since this virus has been adapted to the nervous system of mice (*4*), lesions were examined in both sheep and mice.

Chicken-embryo-adapted bluetongue virus, Californian strain 11, was used to inoculate sheep. Laparotomies were performed on ewes, marked by the ram at mating, and fetuses were inoculated through the uterine wall. Lambs and sheep were infected intracerebrally. Mice received either low-passage (passage 15) or high-passage (passage 62) mouse-adapted bluetongue virus. They were inoculated intracerebrally when 1, 10, or 40 days old, so that in each age group 10 received high-passage and 10 low-passage virus.

Table 1. Lesions in the central nervous system of sheep inoculated with bluetongue virus at various ages.

Age (days)	No. inoculated	Lesions
		Fetuses
36–39	3	Resorbed, 2; necrotic, 1
57–59	3	Hydranencephaly, 1
74-89	4	Small cerebral cysts, 1; nonsuppurative meningoencephalitis, 1
		Lambs and sheep
	11	Mild nonsuppurative meningoencephalitis, 9

Observations on the sheep are summarized in Table 1. Fetuses in the group 36 to 39 days old died, which was discovered when two of the ewes returned to estrus. The third ewe was killed 17 days after inoculation, and a small necrotic fetus was recovered. Of the three fetuses in the group 57 to 59 days old, one inoculated at 57 days and collected 24 days later had hydranencephaly typical of the bluetongue encephalopathy. The other two fetuses were twins and did not have gross lesions. In a fetus, infected on the 76th day of gestation and left for birth, small cysts were found in the cerebral hemispheres; and another, infected at 89 days and collected 18 days later, had a moderately severe nonsuppurative meningoencephalitis and focal loosening of the neuropil of the cerebral subcortical tissues. Lambs and sheep reacted with a mild nonsuppurative meningoencephalitis.

Lesions in mice varied with age of the animals and passage level of the virus. High-passage virus in 1-day-old mice produced a focal necrotizing encephalitis with very few inflammatory cells in the lesions; these mice died rapidly within 4 days of inoculation. Ten-day-old mice reacted with more inflammatory cells and perivascular cuffing as well as with focal necrosis, and in 40-day-old mice a mild nonsuppurative meningoencephalitis with some foci of necrosis was found. The difference in responses of the age groups was seen more distinctly in mice infected with low-passage virus. In 1-day-old mice there was a severe necrotizing encephalitis with little inflammatory reaction affecting especially, but not only, the cerebral hemispheres; these mice usually died about the 7th day after inoculation. Ten-day-old mice did not die and in them the low-passage virus produced a mild nonsuppurative meningoencephalitis with slight necrosis. Lesions in the 40-day-old mice consisted only of a very mild nonsuppurative meningitis.

These findings, supported by the previous observations (*1, 2*), indicate that young fetal sheep respond to bluetongue virus infection by developing cerebral anomalies, whereas older animals respond with nonsuppurative meningoencephalitis. The time during which the response of the nervous system changes from one type to the other