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.

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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

is probably between the 70th and 90th days of gestation. This is an especially interesting period in the development of the fetal lamb since it is during this period that it is developing immunological responsiveness. Ovine fetuses are able to reject skin homografts after the 77th day of gestation, but not before; it should be remembered, however, that the time of onset of the ability of the lamb to respond immunologically varies with the antigenic stimulus (*5*).

Similarly, the observations on mice, and especially those obtained when low-passage virus was used, indicate that during the interval from 1 to 10 days after birth the response of the nervous system changes from one that is mainly a necrotizing lesion to one that is mainly a nonsuppurative meningoencephalitis. During this period of 1 to 10 days after birth, there is considerable development of the ability of mice to respond immunologically (*6*).

The time at which bluetongue virus is first able to provoke an immunological response in sheep and mice is not known. However, it is an attractive hypothesis that the nervous systems of immunologically immature sheep and mice respond to bluetongue virus by developing congenital anomalies or a very necrotizing lesion that may lead to such defects, whereas the nervous system of animals that have considerable immunological maturity responds with an inflammatory lesion—a nonsuppurative meningoencephalitis. It has been suggested recently that viral infections of the nervous system can produce lesions ranging from those that are largely neuronal damage to those that are largely inflammatory and that the latter seems to be associated with partial immunity (*7*). To some extent our observations support these suggestions, not in terms of acquired antibodies, but in terms of the development of the immune responsive state.

While of uncertain significance, it is interesting that the cerebral anomalies or acute necrotizing encephalitis occurred before myelination, which begins in the forebrain of the fetal lamb at 96 days (*8*) and in mice at 10 to 15 days after birth (*9*).

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Natural Free-Running Period in Vertebrate Animal Populations

Abstract. *Regression analysis (analysis of covariance) is contrasted with the conventional "mean period length" for estimating the length of period of the spontaneous activity frequency (free-running period) in population samples of Gila monsters* (Heloderma suspectum) *and kangaroo rats* (Dipodomys merriami) *in the Sonoran Desert. The mean period length in each population does not differ significantly from 24:00 hours* ($P > .05$) *and it does not differ significantly* ($P > .05$) *between the species studied; the probability that the free-running period (in constant dark) in natural populations of Gila monsters and kangaroo rats is different from 24:00.0 is less than 1 in 1000* ($P < .001$). *The so-called "mean period length" is of little or no use for precise determination of the period and phase relationships in circadian rhythms; moreover, it is entirely without value for statistical testing of differences either within or between populations.*

It is assumed that the data on circadian rhythms represent evolutionary adaptations through genetic mechanisms involving natural selection operating in natural populations, regardless of whether ultimate control of the clock is endogenous, exogenous, or both. It is surprising, therefore, that relatively little attention has been given to sampling the nature of circadian rhythms in *natural* populations of vertebrate animals, and that sophisticated statistical treatment of time series in the circadian activity cycles of samples of animals in general is virtually wanting.

During the Cold Spring Harbor symposium on biological clocks in 1960, Sollberger (*1*) suggested that appropriate statistical methods might be developed for analyzing "such typically evolutive time series as biological rhythms." Of course, powerful statistical methods have been available for analyzing some aspects of circadian rhythms, especially for the data reduction and analysis of measurements of the spontaneous free-running period of animals that lend themselves well to accurate recording of precise cyclic events. But, unfortunately, here as elsewhere in the study of biological rhythms these often necessary methods have remained essentially unused.

Perhaps the hesitance of some authors to use sophisticated statistical measures to describe and test group characteristics of activity rhythms is a continuing reaction to early misuse of statistical procedures by some workers who subjected relatively raw data to excessive manipulation in attempts to demonstrate the mere existence of certain rhythms. This flagrant misuse prompted one worker to describe a mythical rhythm in the unicorn, based on data taken from a table of random numbers (*2*). While we are aware of the dangers of overmanipulation so dramatically pointed out by Cole's article, these dangers should not be permitted to frighten workers away from using the highly legitimate and powerful statistical procedures described in this paper. We refer to regression analysis and to the concepts and techniques of the analysis of variance applied to problems in linear regression, namely, the analysis of covariance (*3–5*).

Regression methods for calculating and comparing free-running periods should replace the inaccurate "mean period length" (*6*) that is currently conventional for analysis in investigation of circadian rhythms (*7*). The value for "mean period length" is dependent only on the first and last points of a series. To illustrate, if t_1, t_2, t_3, $\cdots$ t_n are the time of initiation of activity on successive days, then the "mean period length" is usually calculated by taking

$$t = \frac{(t_2 - t_1) + (t_3 - t_2) + \cdots (t_n - t_{n-1})}{n - 1}$$

which of course reduces simply to $(t_n - t_1)/(n - 1)$. Thus the intermediate points (t_2 through t_{n-1}), that is, the majority of days, are not even included much less evaluated in the analysis. While simple in application, this method gives values that are erroneous to a