fish solubles sometimes failed to give an additional response in diets containing whey. One supplement alone sometimes failed to elicit a response, as in the experiments reported here.

These results are not interpreted as contradicting reports from other laboratories that fish solubles and whey contain different unknown factors. They seem to indicate that the dietary requirements for the different unknowns vary from time to time for obscure reasons.

Other species have been observed previously to respond favorably to dietary purines and purine derivatives under certain conditions. Frost and Elvehjem(8) noted a growth response when adenine nucleotides were fed to rats on a low niacin diet. Huff and Bosshardt(9) found that mice which were fed 2% succinylsulfathiazole in a low fat diet gave a growth response to purines and purine derivatives.

Summary. In 5 experiments growth of chicks was increased by feeding adenosine at 150 or 300 mg kg of purified diet. Fish solubles at the rate of 3% of the diet gave a more pronounced response than did adenosine. Adenosine did not consistently elicit a response in the presence of either fish solubles or dried whey.

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Received May 7, 1956. P.S.E.B.M., 1956, v92.

Influence of Season on EEE Infection in English Sparrows. (22483)

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Clinical eastern equine encephalomyelitis (EEE) is not observed in man or horses in the United States during the winter months. Antibody surveys and attempts to isolate virus from wild birds and mosquitoes indicate that the virus is not active or is at a very low level of activity during the winter in the southern United States(1). Cessation of mosquito activity may explain this pattern in more northern areas but some species are active in considerable numbers throughout the winter in lower southern areas. It has been shown that lower temperatures such as occur in southern areas during the winter do not adversely influence the ability of mosquitoes to transmit the virus(2). Neither can the lack of virus activity be correlated with the absence of a susceptible bird population since susceptible birds migrate into the southern areas during the winter months. It has been shown that changes in day length and an internal reproductive rhythm influence the physiology, reproduction, and migratory behavior of birds(3,4). It is possible, therefore, that the cessation of virus activity may be due to changes in response to the virus in birds brought about by seasonal changes.

The present study was conducted to determine whether the response of birds to EEE infection was altered by seasonal influences.

Materials and methods. English sparrows (*Passer domesticus domesticus*) were trapped

near Montgomery, Alabama. The reproductive status of the sparrows was determined by bill color in the males(4), sexual display, and nesting activity. The breeding season in this locality extends from about February 15 to September 15. All captured birds were held from 3 days to a week in 2′ x 3′ x 3′ wire cages to allow adjustment to captivity before inoculation, with the exception of a few birds held for a month in a roofed 8′ x 8′ x 8′ wire cage. EEE virus used was strain AR167, which was isolated from *Culiseta melanura*(5) and had received 2 mouse intracerebral and one chick embryo passage. Birds were inoculated subcutaneously over the breast with 0.03 ml of varying virus dilutions. Amounts of virus inoculated are expressed as mouse intracerebral LD_{50} units of virus. Birds were bled from the jugular vein. At each bleeding 0.1 ml of blood was withdrawn and diluted immediately with 0.9 ml of 10% horse serum in buffered water. Virus titrations and neutralization tests were performed by the intracerebral inoculation of 3-week-old CFW mice. Tests for neutralizing antibody were made by mixing the 1:10 diluted blood samples with 10-fold dilutions of virus. LD_{50} endpoints were calculated by the method of Reed and Muench(6). When deaths occurred, birds were autopsied and suspensions of brain and liver were inoculated into mice for the isolation of virus.

Results. Eight groups of birds were inoculated between July 6, 1953 and April 27, 1954. Each group was divided into 3 subgroups; one of the subgroups received an inoculum expected to infect none of the birds; a second received an inoculum expected to infect half; and a third received an inoculum expected to infect all. The dates of inoculation, amounts of virus inoculated, maximum viremia titers, duration of viremia, and outcome in representative groups are shown in Table I.

The smallest dose of virus which infected any bird (3/10) was 0.13 LD_{50} unit. Doses of 1.3 LD_{50} or greater always infected at least half of the birds. Doses of 3.2 LD_{50} or more infected all birds inoculated. No variation in susceptibility was apparent at different seasons of the year.

TABLE I. EEE Infection in English Sparrows.

Date of inoc.	LD_{50} inoc.	Sex	Max blood virus titer	Duration of viremia (days)	Died or survived
11/19/53	.4	♂			S
		♂	5.8	4	S
		♀	3.3	1	D
		♀			S
	.13	♂			S
		♂			S
		♀			S
		♀	7.3	2	D
	.04	♂			S
		♂			S
		♀			S
		♀			S
2/ 2/54	1.0	♂	5.5	4	D
		♂	5.5	3	S
		♀	5.5	3	D
		♀	5.5	5	S
	.32	♂			S
		♂	6.5	3	D
		♀	7.0	3	D
		♀			S
	.1	♂			S
		♂			S
		♀			S
		♂			S
4/27/54	1.3	♀			S
		♀	6.5	4	D
		♂	7.5	2	D
		♂			S
	.4	♀			S
		♀			S
		♂	7.0	1	D
	.13	♀			S
		♀			S
		♂			S

Five of 40 birds which became infected survived. The amounts of virus which survivors had received ranged from 0.4 to 5.6 LD_{50} units. Infected individuals survived in July, September, November, and February. Amounts of virus in excess of 7.6 LD_{50} units caused the death of all of 10 birds thus exposed.

Deaths considered due to infection occurred between the second and ninth days after inoculation, the majority occurring between the second and fifth days. The death rate was highest in those groups which received the largest dosage of virus.

Virus was present in the blood of 31 of 40 infected birds within 17 hours after inocula-

tion; in 35 of 40, maximum titers were reached on the second day. In those cases where death did not intervene, blood virus titers remained above $10^{4.0}$ LD_{50} for 2 days in 8 of 22 individual birds and for 3 days in 14 of 22. After the second or third day, blood virus titers gradually declined and the virus disappeared by the fifth or sixth day. In general, viremias were similar in titer and duration regardless of the size of the inoculum, or the month of the year in which the birds were infected.

To check for prolonged incubation periods or recurrence of viremia, the surviving birds of the group inoculated October 27, 1953 were bled daily for 2 weeks. No virus was detected in any sample obtained after the fourth day following inoculation.

Preinoculation and final blood samples from the surviving birds were tested for neutralizing antibody. None of the birds showed evidence of immunity before inoculation. Only those birds in which viremia was evident developed antibody. Neutralizing antibody was detected as early as 6 days after inoculation, reached maximum levels between 21 and 84 days, and was still present 109 days after inoculation.

Discussion. The high susceptibility and blood virus titers observed in these studies support previous evidence that the English sparrow has a high potential as a source of EEE virus for infecting mosquitoes(7). However, this potential may be somewhat decreased by the high mortality rate early in the course of the viremia. The finding that only birds which developed detectable viremia subsequently developed measurable antibody is in contrast to the picture in horses in which neutralizing antibody may develop in the absence of detectable viremia(8). These studies did not indicate that susceptibility, viremia levels attained, or mortality were influenced by the age, sex, or reproductive status of the bird or by the season of the year.

Summary. 1. Eight groups of wild caught English sparrows totaling 78 individuals were inoculated with eastern equine encephalomyelitis virus from July 1953 to April 1954. 2. No significant differences in response to infection were observed which could be related to age, sex, reproductive status or season. 3. Information on infective dosage, blood virus levels developed, mortality, and development of neutralizing antibody is presented.

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Received May 15, 1956. P.S.E.B.M., 1956, v92.