TABLE II. Mean Blood Pressure in Choline-Deprived Cockerels (mm Hg).

	Months after end of initial choline-deficient period						
	0	1	2	3	4	6	7
Controls	138 ± 5.7* (7)†	141 ± 3.8 (5)		139 ± 5.1 (5)	150 ± 7.1 (5)	168 ± 5.7 (11)	173 ± 6.4 (6)
Choline-def.	157 ± 4.6 (7)	149 ± 4.3 (5)	152 ± 3.7 (5)	150 ± 6.5 (5)	144 ± 4.8 (5)	147 ±10.5 (8)	157 ± 8.8 (7)
Repeat choline-def.				146 ± 3.8 (5)	133 ± 5.8 (5)	130 ± 5.9 (4)	

* Mean ± S.E.
† No. in parentheses refer to No. of determinations, one determination/bird each month.

phenomenon. Male chicks and capons, but not females, are said to show increases in blood pressure with age after puberty, and this increased pressure in adult males and capons(3) is reduced by suppression of pituitary gonadotrophin secretion by estrogens or drugs(3). These findings indicate that pituitary gonadotrophins rather than androgens are required for normal rise in blood pressure with age.

TABLE III. Chick Kidney and Testis Weights at 6 Months after End of Initial Choline-Deficient Period.

	Wt of testes, g/100 g body wt	Comb index, CM^2	Wt of kidneys, g/100 g body wt
Controls	.38 ± .10* (5)†	128 ± 8.3 (5)	.85 ± .07 (5)
Repeat choline-def.	.10 ± .01 (4)	84 ± 8.9 (4)	1.06 ± .08 (4)

* Mean ± S.E. † No. of birds.

Summary. It is concluded that in the present experiments lack of rise in blood pressure with age in choline-deprived chicks was due directly to suppression of pituitary gonadotrophins secretion caused by lack of choline in early weeks of life, and not to consequent lack of androgens.

The author wishes to express his thanks to Mr. Anthony Ellis for technical assistance.

1. Hartroft, W. S., Best, C. H., *Brit. Med. J.*, 1949, v1, 423.
2. Weiss, H. S., Ringer, R. K., Sturkie, P. D., *Am. J. Physiol.*, 1957, v188, 383.
3. Sturkie, P. D., Ringer, R. K., *ibid.*, 1955, v180, 53.

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Parasitic *Philornis* Flies as Possible Sources of Arbor Virus Infections (Diptera, Anthomyidae).* (24444)

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In the course of a nestling bird bleeding program conducted in Trinidad as part of a general study of arthropod-borne (ARBOR) viruses, it has been observed that many nestlings are infected with immature fly parasites. Some of the early reared material was sent to Dr. Curtis Sabrosky at U. S. National Museum in Washington, D. C., who identified the flies as belonging to genus *Philornis* Macquart in the family Anthomyidae. They are thus biologically similar to North American bird parasites of the genus *Apaulina* Hall and *Protocalliphora* Hough of the Old World, family Calliphoridae(1). Continuing observations soon made it evident that we were dealing with several species of *Philornis* and that various degrees of parasitism were in-

* Studies and observations upon which this paper is based were conducted in the Trinidad Regional Virus Lab. with the support and under auspices of Government of Trinidad and Tobago, Colonial Development and Welfare Scheme, and Rockefeller Fn.

volved (including subcutaneous and external parasitism). All early material was reared out for identification and is being studied by Dr. H. R. Dodge, who is revising the genus. Now we are also becoming interested in the potentialities of *Philornis* as possible virus carriers, and are experimenting with much of the material presently being obtained. The purpose of this note is to record certain preliminary observations made along these lines.

On 16 July, 1957, a communal nest of anis (*Crotophaga ani*) was seen at Vega de Oropouche in which 4 of the 6 nestlings were infested with several *Philornis* larvae (designated by us "species F"). These were external parasites feeding on host by rasping the skin with oral hooks and ingesting the oozing serous fluid and blood. The same day in the laboratory, the birds were inoculated intramuscularly with 0.1 ml of a 10^{-1} dilution (868 MLD_{50}) of St. Louis virus (TRVL 9404 strain, pool #3). One *Philornis* larva which was placed on bird #882 20 July (fourth postinoculation day) and allowed to feed overnight, dropped the following morning and was ground up and inoculated into a group of 6 adult mice 0.03 ml i.c. A 2/6 mortality ratio resulted, with deaths on days 6 and 7. The agent was isolated in further passage and confirmed as St. Louis virus in a specificity test using a known St. Louis immune serum. Circulating virus was demonstrated in the host bird on 21 July (fifth postinoculation day), when there was a titer of $10^{3.5}$. A specificity test confirmed its identity with St. Louis virus.

On 18 March, 1958, 22 larvae of *Philornis* "species G" were expressed from under the skin of 2 week-old nestling doves (*Leptoptila* sp.) taken at Fishing Pond. In contrast to the previous fly, species "G" is a subcutaneous parasite. The same day 12 of these larvae were inoculated by glass capillary pipette, each with an estimated 0.002 ml of a 10^{-5} dilution of Ilhéus virus (TRVL 10507 strain, pool #5). This pool titered $10^{8.16}$ in a 0.03 ml intracerebral inoculation of adult mice; calculation of virus titers was made by the method of Reed and Muench (2). The calculated inoculum is 96 MLD_{50}. All larvae pupated during the next 2 days. The first adult (female) emerged 28 March after 10 days of pupation (it was also the tenth postinoculation day). This adult (specimen #9) and one pupa (#8) were ground individually in 1 ml of bovalbumin diluent fortified with antibiotics, centrifuged at 8,000 rpm for 10 minutes and inoculated into 2 groups of 6 adult mice 0.03 ml i.c. The following day 7 more adults emerged and were treated similarly. The remaining 3 pupae failed to survive.

Of the 8 adult flies and one pupa tested, 2 flies (#9 and #11) were positive for virus; a specificity test confirmed it to be Ilhéus. As indicated above, these flies incubated the virus for 10 and 11 days before being tested. Virus from fly #9 titered $10^{-5.16}$ and that from fly #11 titered $10^{-1.76}$. When these figures are adjusted for amount of diluent to give a figure for total virus content/fly, we find that the amount of virus recovered from fly #9 was about 4,750,000 $MLD_{50's}$ and from fly #11 about 1,900 $MLD_{50's}$. Since the original inoculum going into the larvae contained approximately 96 $MLD_{50's}$, evidence of multiplication of virus was obtained in both flies. The reason for the low percentage of infected flies is not known, but it may be that the capillary needle became plugged.

A third experiment was initiated 24 April, 1958, when a large number of larvae of *Philornis* "species A" were taken from the nest hole of a jacamar, *Galbula ruficauda*, at Vega de Oropouche. This appears to be a coprophilous species of *Philornis* in that the larvae feed on bird excrement. Fifty of these larvae were inoculated the same day by glass capillary pipette with a 10^{-5} dilution of St. Louis virus (TRVL 13375 strain, pool #2) which titered $10^{6.64}$ in baby mice (intracerebral inoculum of 0.02 ml). The larval inoculum of 0.002 ml is estimated to contain approximately 4 MLD_{50} of virus. All larvae pupated within the next 2 days and adults appeared 9-10 days after pupation, at which time they were stored at –60°C.

Eight adults were subsequently tested for virus. Each was ground individually in 1 ml of diluent (called 10^{-1}), centrifuged for ½ hour and inoculated into baby mice. Six of the flies proved to harbor virus which killed

all the mice in their respective groups. Subsequently a specificity test confirmed the virus to be St. Louis. Titrations were made of 5 flies, and titers of 10^{-2}, $10^{-2.2}$, $10^{-2.5}$, $10^{-3.8}$ and $<10^{-4.3}$ were obtained. This indicates virus quantities ranging from 3,300 to >650,000 MLD_{50} per fly.

Discussion. The role that *Philornis* flies may play in the epidemiology of certain arthropod-borne viruses, such as St. Louis or Ilhéus, is not known. The evidence presented indicates that flies may become infected as larvae and remain infected on reaching the adult state 10 days later. Inasmuch as the adult fly is not a bloodsucker and a single fly generation is associated with only one nest, some other means must exist for insuring virus survival. Possibly susceptible birds become infected through eating infected flies, or the virus may be transmitted through the fly's egg to its progeny. At the moment these possibilities are still unknowns. Thus far, no naturally infected *Philornis* have been encountered.

Summary. Fly parasites of the genus *Philornis* (Anthomyidae) attacking nestling birds have been shown capable of becoming infected with St. Louis and Ilhéus viruses. In one instance, a larva was found positive for St. Louis virus after feeding on a nestling ani that was circulating this virus. In a second case, 2 of 9 adult flies became infected with Ilhéus virus after they had been inoculated as larvae 10 and 11 days previously. Similarly, 6 of 8 adult flies proved positive for St. Louis virus after being inoculated 10 days previously as larvae.

We are grateful for the advice of Dr. Richard M. Taylor who suggested the inoculation of *Philornis* larvae.

1. Hall, D. G., *The Blowflies of North America,* The Thomas Say Fn., 1948, 477pp.

2. Reed, L. J., Muench, H., *Am. J. Hyg.,* 1938, v27, 493.

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Effect of Autonomic Blocking Agents on Depot Fat Mobilization in Normal and Adrenalectomized Animals.* (24445)

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Hausberger(1) showed that the functional activity of fat cells was regulated by an abundant nerve supply, not only to blood vessels, but to parenchymal cells as well. Trophic influence of the autonomic nervous system on fat tissue has since been demonstrated by several other investigators(2-4). Wool *et al.*(5) using liver fat as index of fat mobilization in normal and adrenal demedullated animals, were able to reduce degree of fat accumulation in liver by pretreatment with ergotamine tartrate. Since the level of liver lipids is a resultant of many processes and does not necessarily mirror depot fat activity, we investigated the rate of fat mobilization from a peripheral depot after treatment with various autonomic blocking agents. The fat depot used was the epididymal fat body of the rat. Our results indicate that certain sympathetic system blocking agents do alter the rat's ability to move fat rapidly from this depot during a fast. To demonstrate this, it was necessary to remove the adrenal glands prior to administration of the blocking drugs.

Methods. Male Sprague-Dawley rats weighing 90-110 g were used. In larger animals, the bilateral symmetry of the epididymal fat body disappears. Animals were used

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