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.

Received September 4, 1958. P.S.E.B.M., 1958, v99.

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

ALAN C. LEVY† AND ESTELLE R. RAMEY (Introduced by J. C. Rose)
Dept of Physiology, Georgetown University School of Medicine, Washington, D. C.

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

* Supported in part by Grant from NIAMD, NIH, Bethesda.

† In partial fulfillment of requirements for degree of Doctor of Philosophy, Graduate School, Georgetown University. Predoctoral fellow of Nat. Inst. of Neurological Diseases, U. S. P. H. S.

Present address: Dept. of Physiology, Howard University, Washington, D.C.

TABLE I. Effect of Autonomic Blocking Agents on Depot Fat Mobilization (12 Hr Fast).

Procedure	No. of animals	Depot wt, mg		Mobilization	
		Initial	Final	mg	mg/100 mg initial depot wt
Intact					
Saline	17	89.8 ± 5.6†	79.0 ± 5.5	+10.8 ± 2.2	+13.0 ± 2.4
Dibenzyline (.2 mg)*	10	98.3 ± 5.3	87.7 ± 5.8	+10.6 ± 1.7	+11.2 ± 2.0
Hexamethonium chloride (1.25 mg ion)*	11	82.5 ± 5.0	74.9 ± 5.1	+ 7.6 ± 1.4	+ 9.5 ± 1.6
Ergotamine tartrate (.5 mg)*	15	97.5 ± 2.1	87.0 ± 2.4	+10.5 ± 2.4	+11.3 ± 2.3
Idem (.25 mg) + Dibenzyline (.1 mg)*	10	94.3 ± 8.1	83.2 ± 7.9	+11.1 ± 2.0	+14.9 ± 2.8
Sham adrenalectomy					
Saline	16	71.0 ± 4.5	69.9 ± 5.1	+ 1.1 ± 1.6	+ 2.5 ± 2.8
Dibenzyline	8	64.5 ± 2.6	66.9 ± 4.3	— 2.4 ± 2.6	— 3.1 ± 4.0
Hexamethonium chloride	9	76.9 ± 5.6	77.4 ± 5.7	— .5 ± 2.9	— .9 ± 3.6
Ergotamine tartrate	9	61.3 ± 6.7	60.4 ± 6.5	+ .9 ± 3.1	+ 1.5 ± 4.6
Idem + Dibenzyline	7	57.6 ± 4.5	53.7 ± 4.5	+ 3.9 ± 1.6	+ 7.0 ± 2.8
Adrenalectomy					
Saline	30	39.5 ± 2.1	29.3 ± 1.9	+10.2 ± .9	+26.3 ± 2.2
Dibenzyline	10	61.9 ± 7.0	46.2 ± 5.8	+15.7 ± 2.0	+25.8 ± 2.0
Hexamethonium chloride	14	45.3 ± 4.7	34.3 ± 3.2	+11.0 ± 3.7	+22.6 ± 3.7
Ergotamine tartrate	14	51.7 ± 4.4	52.6 ± 4.5	— .9 ± 2.3	— 1.9 ± 4.2
Idem + Dibenzyline	8	53.4 ± 4.8	49.2 ± 5.1	+ 4.2 ± .8	+ 8.5 ± 2.0

* Per 100 g body wt. † ± S.E. + = Mobilization of fat. — = Deposition of fat.

as their own controls. The first epididymal fat body was removed at onset of fasting and the second at end of this period. The fat pad was removed under ether anesthesia by orchiectomy through a lateral scrotal incision. A fat-free area of the spermatic cord was identified and ligated. The testis with its fat pad was excised. Fat was then dissected from its attachment to the epididymis and testis. Total lipids were extracted and weighed according to Bloor's method(6). The difference between amount of extractable lipid in each depot before and after a 12-hour fast, was used as index of peripheral fat mobilization. Bilateral adrenalectomies were performed under ether anesthesia and these animals were maintained on Purina Rat Chow and 0.9% saline *ad lib.* Adrenalectomized animals were studied on seventh post-operative day. Sham operations were performed in a similar fashion. Ergotamine tartrate was administered in a dose of 0.5 mg/100 g body weight at start of 12-hour fast. Dibenzyline, 0.2 mg/100 g body weight was injected at beginning of the fasting period and again after 6 hours of fast. Hexamethonium chloride was given at a dose of 1.25 mg ion/100 g body weight at start of fasting period. 0.25 mg ergotamine tartrate plus 0.1 mg Dibenzyline/100 g body weight was injected at start of 12-hour fast. All doses were given subcutaneously.

Results. In intact animals, the autonomic blocking agents used were ineffective in altering normal rate of fat movement out of the epididymal depot during a 12-hour fast (Table I).

Sham adrenalectomized animals, with no previous blocking drug treatment, showed an almost complete inhibition of fat movement out of the epididymal depot during the short 12-hour fast. This difference from intact controls may have been due to release of adrenal steroids resulting from operative procedure. The autonomic blocking agents had no further effect on this phenomenon (Table I).

Adrenalectomized animals had been shown previously to mobilize relatively more fat from the epididymal depot during a fast than do normal animals(7). Administration of ergotamine tartrate produced a marked inhibition of fat movement out of this depot (Table I). The other blocking agents listed above had no significant effect on this process. When half the dose of ergotamine was used together with half the dose of the inactive Dibenzyline, only partial inhibition of fat mobilization was observed (Table I).

Discussion. Regulation of fat mobilization

during a fast requires that some signal reaches the fat cell and produces conditions for either a diminution of fat deposition or an augmentation of fat movement out of the cell. This signal could be a blood borne humoral factor such as glucose, or certain fat moieties themselves together with hormones from various sources. There is evidence to support such an hypothesis but this does not seem to be the entire controlling mechanism that matches food intake to depot fat turnover. There are many suggestions in the literature that the nervous system and the epinephrines play a role in this phenomenon(1-4,8). There is further evidence that the adrenal steroids play a "permissive" role for certain functions of the sympathetic nervous system and its neurohumors(9). In experiments described above, a variety of autonomic blocking agents was used in an attempt to interfere with the possible neural patterns governing fat cell behavior.

There are many gaps in our knowledge of the mode and site of action of these drugs. Of those used, ergotamine tartrate is known to be the most potent inhibitor of the glycogenolytic effects of epinephrine. Dibenzyline and hexamethonium chloride apparently have little metabolic activity in this regard. It is of interest, therefore, that only ergotamine tartrate was effective in blocking the movement of fat out of the epididymal depot during a fast. This effect, however, could only be demonstrated in the adrenalectomized animal. A possible explanation for this may lie in the relationship between adrenal steroids and epinephrines. In the absence of steroids, small amounts of epinephrines do not exhibit the same potency as regards several physiologic responses(9). At the highest dose level of ergotamine tartrate used in these experiments, the presence of relatively large amounts of steroids might have overridden the blocking effect. When half the dose of ergotamine tartrate was used, a lesser degree of inhibition occurred even in the adrenalectomized animals.

Accumulation of fat in the liver due to the insult of ethionine administration appears to respond somewhat differently from peripheral depot fat metabolism with regard to blockade with ergotamine(5). Even in animals with intact adrenals, this blocking drug inhibited development of a fatty liver. It is at the liver site also that ergotamine had its most marked effect on glycogenolysis due to epinephrine. Our data suggest that the extra-hepatic effects of ergotamine on fat metabolism are masked by the presence of cortical steroids.

Summary. Ergotamine tartrate significantly inhibited movement of fat out of the epididymal fat body in adrenalectomized animals during a 12 hour fast. This effect was not observed in intact animals under similar circumstances. Neither hexamethonium chloride nor Dibenzyline affected fat mobilization in either intact or adrenalectomized animals. It is suggested that the adrenal steroids and the epinephrines may act together to regulate fat cell metabolism.

1. Hausberger, F. X., *Z. mikroskop. anat. Forsch.,* 1934, 36, 231.

2. Kure, K., Thoshin, F., Okniaka, S., *Klin. Wochschr.,* 1937, v16, 1789.

3. Beznak, A. B. L., Hasch, Z., *Quart. J. Exp. Physiol.,* 1937, v27, 1.

4. Sidman, R. L., Fawcett, D. W., *Anat. Rec.,* 1952, v118, 487.

5. Wool, I. G., Goldstein, M. S., Ramey, E. R., Levine, R., *Am. J. Physiol.,* 1954, v178, 427.

6. Bloor, W. K., *Am. Chem. Soc. Monograph Series,* 1943, v93, 45.

7. Tracht, M. E., Goldstein, M. S., Ramey, E. R., *Am. J. Physiol.,* 1956, v187, 2.

8. Wertheimer, E., Shapiro, B., *Physiol. Rev.,* 1948, v28, 451.

9. Ramey, E. R., Goldstein, M. S., *ibid.,* 1957, v37, 155.

Received September 4, 1958. P.S.E.B.M., 1958, v99.