differences in food intake. Both sexes fed freely on glucose, as evidenced by distended abdomens. When maintained on water, the female catabolized 20 μg/day from emergence until death, so that additions to the pool from the different metabolic compartments and utilization by tissues take place at a constant rate.

At emergence, phospholipids and triglycerides comprised more than 90 percent of the total lipids. Free fatty acids and hydrocarbons made up the balance. Phospholipid levels remained virtually unchanged during the entire experiment (46 ± 3 μg per female, 36 ± 4 μg per male mosquito; 240 ± 20 μg per house fly). Sterols (free cholesterol, including 10 to 35 percent dehydrocholesterol) were found in amounts of only 0.1 μg per mosquito.

In order to establish the composition of the fatty acids synthesized solely from glucose, mosquitoes were maintained on water until the triglyceride level was down to 5 μg and then fed 10-percent glucose for 5 days (Fig. 1*B*). At that point, methyl esters of the triglyceride fatty acids, 99 percent of which had been newly synthesized, were subjected to gas-liquid chromatography (Table 1). Palmitic, palmitoleic, and oleic acid comprised 92 percent of the new fatty acids, palmitoleic acid being one-third of the total. Whereas the 5 μg of triglycerides left after starvation still contained 12 percent linoleic acid, this amount is represented only as a trace in the 400 μg synthesized from glucose. It is therefore likely that the mosquito does not make polyunsaturated fatty acids.

A continuously falling level of triglycerides does not necessarily preclude all synthesis. Robbins *et al.* (*3*) showed that female and male adult house flies, when fed milk powder and sugar, incorporate C^{14}-labeled acetate in the saponifiable lipid fraction, but these workers did not separate phospholipids from triglycerides.

Table 1. Triglyceride fatty acids in female mosquitoes (*5*).

Chain length	Double bonds	Amount (μg per mosquito)	
		3½ days on water	3½ days on water plus 5 days on glucose
Short (6-12)		0.02	1
14	0	0.08	12
14	1	0.01	2
16	0	1.46	140
16	1	1.20	136
18	0	0.27	14
18	1	1.36	95
18	2	0.60	Trace
Long		Trace	Trace

The ability of the female mosquito to build a huge fat body from glucose at a constant rate, as contrasted with the female house fly or the male mosquito, may facilitate the study of physiological factors that control lipogenesis in vivo (*4*).

EMILE VAN HANDEL
PATRICK T. M. LUM
Entomological Research Center, Florida State Board of Health, Vero Beach

References and Notes

1. E. Van Handel, *Clin. Chem.* **7**, 249 (1961).
2. G. R. Bartlett, *J. Biol. Chem.* **234**, 466 (1959).
3. W. E. Robbins, J. N. Kaplanis, S. J. Louloudes, R. E. Monroe, *Ann. Entomol. Soc. Am.* **53**, 128 (1960).
4. This work was supported by a grant (E-3112) from the National Institutes of Health.
5. The data in Table 1 were provided by Dr. Leon Swell, Veterans Administration Center, Martinsburg, W. Va.

10 August 1961

Licking Rates in Infant Albino Rats

Abstract. Two groups of neonatal rats raised from birth without the opportunity to drink, and tested at different age levels, exhibited the same general characteristics of licking rate that adult rats do. This suggests that licking in the rat is organized on a genetic-maturational basis.

The purpose of the experiment reported here was to determine whether constancies in licking rate reported for adult albino rats are found also in infant rats. Other workers (*1*, *2*) have reported a mean licking rate in adults of 6 to 7 licks per second and a range from 5 to a little over 8 licks per second, with the mean rate constant in both sexes for varying levels of thirst, and for water, sucrose, saccharin, and saline solutions.

In the absence of evidence to the contrary, it might be argued that these constancies are the result of reinforcement of certain rates of licking. On the other hand, if rats raised from birth without an opportunity to drink displayed, when tested at different age levels, the same licking rate as adults, it would suggest that drinking in the rat is primarily a genetically and maturationally determined response.

We tested two litters of neonatal rats from the colony maintained by the University of Missouri. Each litter was composed of 3 males and 3 females. Each litter was reared with the mother from birth until the first test day, then was separated from her and housed in a separate cage. The water supply for the mother was suspended from the ceiling of the cage, and thus the young rats were prevented from drinking prior to the first test day. Both litters had unlimited access to dry food prior to weaning and throughout all the testing and maintenance periods. The normal weaning age in the rat is between 21 and 28 days (*3*). The rats in group 1 were weaned at noon of the 21st day after birth; they were then tested daily, at 8 P.M. and 8 A.M., for 5 days. The rats in group 2 were weaned at noon of the 18th day and were tested at 4 P.M. and 8 P.M. on the first day and at 8 A.M., noon, and 8 P.M. on the four following days. All test sessions were 30 minutes long.

The apparatus consisted of six Hoeltge HB-11 cages with attached floor-mounted plastic drinking cups and 50-ml glass tube reservoirs. The mouth of the cup was 10 mm in diameter. The test solution was water. Each time the animal's tongue came in contact with the water, an electronic circuit was completed, an 8-μa current passed through its body, and a record of the tongue lap was recorded on an Esterline Angus tape moving at 1.905 cm/sec. The experimental room was maintained at a temperature between 76° and 78°F throughout the experiment.

Four licking rates were measured: The rate at the time of contact with the drinking cup; the rate in the first burst of licking of at least 1-second duration in each session; the rate within sessions; and the rate within bursts of more than 1-second duration. The means and the range of responding in the initial contact with the drinking cup and in the first burst of licking of at least 1-second duration in the first four sessions are presented in Table 1. For both groups, the mean rate of licking on first contact with the cup parallels the mean rate of licking in adult animals. Individual rates show some variability. On its initial contact with the cup in the first test session, rat F2 in group 1 licked at the rate of 9.5 licks per second. In the second test session, rats F3 and M3 in group 1 licked at the rate of 11.4 and 9.5 licks per second, respectively. Similarly, in the first test session both F1 and F3 in group 2 licked at the rate of 9.1 licks per second, and M2 licked at the rate of 9.5 licks per second. No systematic relationship between sex and licking rate was observed in either group.

Table 1. Means rate of licking, and range, on first contact with the cup and in the first burst of at least 1-second duration.

Session	Rate on initial contact (licks per second)		Rate in first 1-second burst (licks per second)	
	Mean	Range	Mean	Range
		Group 1		
1	7.3	6.3– 9.5	7.2	5.8–8.3
2	8.4	6.3–11.4	7.5	6.6–9.5
3	8.0	7.0– 9.1	7.4	6.0–8.7
4	7.7	6.9– 8.2	7.2	5.9–8.7
		Group 2		
1	8.3	6.5– 9.5	7.0	6.5–7.3
2	7.5	5.3– 9.5	6.6	5.3–7.3
3	8.1	7.1– 9.5	7.2	6.7–7.7
4	7.8	6.9– 8.6	7.8	6.9–8.6

Keehn and Arnold (*2*) have reported a decrement in mean licking rate within sessions of about 1 lick per second in adult rats. Group means for the first and last bursts of licking of at least 1-second duration in session 1 and for the first and last bursts of licking in the first 5 minutes in sessions 2 and 3 indicate that the decrement in licking rate within sessions found in adults is also found in infant rats, even in their first drinking response. For sessions 1, 2, and 3, respectively, the mean initial and terminal rates for group 1 were 7.2 and 6.1; 7.5 and 6.0; and 7.4 and 6.1. The corresponding rates for group 2 were 7.0 and 6.0; 6.6 and 6.0; and 7.2 and 6.1.

Collier (*4*), in making an analysis of rates of licking within bursts in adult rats, found (i) that the initial rate of responding is frequently as high as 9 licks per second, and (ii) that in sustained bursts of licking, high initial rates quickly decrease to a terminal rate between 6 and a little over 8 licks per second, with the mean licking rate typically falling between these values. An analysis of rates of licking within bursts of over 1-second duration in the young rats indicated that by the third session there were animals in both groups whose initial rates of responding were as high as 9.0 licks per second and whose terminal rates within the same burst were as low as 5.2 licks per second. A typical pattern of response within bursts is illustrated by one animal whose initial rate of responding in the first second was 9.0 and whose rate then dropped, over successive seconds, to 6.7, 6.0, 5.7, 5.5, and finally 5.2 licks per second, with a mean for the burst of 6.6 licks per second. Within bursts, decrements of this magnitude were found only in the beginning of the test sessions, and rates higher than 8 licks per second were never found in the middle or terminal sections of bursts sustained for more than several seconds.

The general stability of licking rates suggests that drinking in the rat is probably reflexive. Whether the licking rate is wholly determined genetically and maturationally or whether some learning is involved is a question that needs further investigation. Even when the rat is raised without an opportunity to drink, nursing and grooming may possibly produce learning effects for licking (*5*).

ROBERT W. SCHAEFFER
DAVID PREMACK
Department of Psychology,
University of Missouri, Columbia

References and Notes

1. J. D. Davis and J. D. Keehn, *Science* **130**, 269 (195➡ E. Stellar and J. H. Hill, *J. Comp. and Physiol. Psychol.* **45**, 96 (1952).
2. J. D. Keehn and E. M. M. Arnold, *Science* **132**, 739 (1960).
3. N. L. Munn, *Handbook of Psychological Research on the Rat* (Houghton Mifflin, Boston, 1950).
4. We are indebted to George Collier for laboratory facilities and for permission to cite these findings prior to their publication.
5. This research was aided by U.S. Public Health Service grants M-3345 and M-3328. The work was completed while one of us (R.W.S.) was a USPHS predoctoral research fellow.

14 August 1961

Complement Fixation by Antibody Fragments

Abstract. Rabbit antibodies (7*S*) degraded by papain into univalent 3.5*S* fragments fail to fix complement when they combine, but do not precipitate, with the homologous antigens. Divalent 5*S* fragments obtained by pepsin digestion (composed of fragment I linked to II, but lacking fragment III) also fail to fix complement although they precipitate with homologous antigens. The amount of specific precipitate formed by the 5*S* antibody fragment is not increased by exposure to complement.

In 1904 Ehrlich and Morgenroth proposed that antibody combines with complement by means of a specific "complementophilic" haptophore (= combining) group, distinct from the combining group for antigen (*1*). In the ensuing years evidence for the existence of a combining group for complement has not been forthcoming (*2*) and Ehrlich's theory has been largely abandoned (*2, 3*). The present report describes experiments suggesting that group(s) essential for complement fixation exist which are distinct from the antigen-combining sites of the antibody molecule.

Rabbit antibodies against crystalline hen egg albumin, crystalline bovine plasma albumin, and type 6 pneumococcal polysaccharide, all predominantly of the 7*S* variety, were broken down to 3.5*S* fragments with papain, cysteine, and sodium ethylenediaminetetraacetate, according to the method of Porter (*4*). The reaction of these nonprecipitating preparations with the homologous antigens was studied by means of the quantitative complement fixation reaction (*5*) over a wide range of antigen concentration, extending from 1/64 to 1024 times the concentration of antigen giving maximal complement fixation with the same dilution of native antibody. No complement fixation was detected after 18 hours' incubation at 4°C either in 10 ml or 3 ml final volume. The same concentration of native antibody fixed from 20 to 40 of 50 50-percent hemolytic units of guinea pig complement. A representative experiment is illustrated in Fig. 1. Essentially similar results have been obtained by Ovary (*6*).

These experiments can be interpreted in at least two ways, which are not mutually exclusive. The first interpretation is: complement is not fixed because aggregation does not occur with the nonprecipitating, univalent antibody fragments obtained with papain digestion (*4, 7*). The formation of a lattice of aggregating antigen-antibody complexes is widely believed to be essential for complement fixation, because antihapten antibodies do not fix complement when they combine but do not precipitate with univalent haptens (although they both precipitate and fix complement with multivalent haptens) (*8*) and because antigen excess inhibits both the formation of a lattice of aggregating antigen-antibody complexes and complement fixation (*9*).

The second interpretation is: complement fixation does not occur because the antigen combining groups of the antibody molecules have been cleaved from structure(s) essential for complement fixation. Evidence supporting this interpretation, but not contradicting the