

The authors gratefully acknowledge suggestions and advice of Dr. C. R. Treadwell.

1. Morner, K. A. H., *Scand. Arch. Physiol.*, 1895, v6, 332.
2. Tamm, I., Horsfall, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 108.
3. ———, *J. Exp. Med.*, 1952, v95, 71.
4. Gottschalk, A., *Nature*, 1952, v170, 662.
5. Odin, L., *ibid.*, 1952, v170, 663.
6. Boyce, W. H., Swanson, M., *J. Clin. Invest.*, 1955, v34, 1581.
7. King, J. S., Jr., Fielden, M. L., Boyce, W. H., *Arch. Biochem. and Biophys.*, 1961, v95, 424.
8. Waldron, D. M., *Nature*, 1952, v170, 461.
9. Hamerman, D., Hatch, F. T., Reife, A., Bartz, K. W., *J. Lab. Clin. Med.*, 1955, v46, 848.
10. Boyce, W. H., King, J. S., Jr., *J. Clin. Invest.*, 1959, v38, 1525.
11. King, J. S., Jr., Boyce, W. H., Little, J. M., Artom, C., *ibid.*, 1958, v37, 315.
12. Berggard, I., *Arkiv. Kemi*, 1961, v18, 291.
13. Anderson, A. J., MacLagan, N. F., *Biochem. J.*, 1955, v59, 638.
14. Linker, A., Terry, K. D., *Proc. Soc. Exp. Biol. and Med.*, 1963, v113, 743.
15. King, J. S., Jr., Fielden, M. L., Boyce, W. H., *Clin. Chim. Acta*, 1962, v7, 316.
16. Kelley, W. R., Poncet, I. B., Di Ferrante, N., *Nature*, 1963, 197, 1204.
17. Kobayasi, T., *J. Biochem.*, 1938, v28, 31.
18. Astrup, P., *Scand. J. Clin. Lab. Invest.*, 1951, v3, 197.
19. Kerby, G. P., *J. Clin. Invest.*, 1954, v33, 1168.
20. Di Ferrante, N., Rich, C., *J. Lab. & Clin. Med.*, 1956, v48, 491.
21. ———, *Clin. Chim. Acta*, 1956, v1, 519.
22. Koiw, E., Gronwall, A., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 244.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.
24. Dische, Z., *ibid.*, 1947, v167, 189.
25. Randle, C. J. M., Morgan, W. T. J., *Biochem. J.*, 1955, v61, 586.
26. Dische, Z., Shettles, L. B., *J. Biol. Chem.*, 1948, v175, 595.
27. Weimer, H. E., Moshin, J. R., *Am. Rev. Tuberc.*, 1953, v68, 594.
28. Warren, L., *J. Biol. Chem.*, 1959, v234, 1971.
29. Schiller, S., Slover, G. A., Dorfman, A., *ibid.*, 1961, v236, 983.
30. Bailey, N. T. J., *Statistical Methods in Biology*, John Wiley & Sons, Inc., New York, 1959, p169, 172.
31. Boyce, W. H., King, J. S., Jr., Little, J. M., Artom, C., *J. Clin. Invest.*, 1958, v37, 1658.
32. Dische, Z., Kawasaki, H., Rothschild, C., Dinilchenko, A., Zinsser, H. H., *Arch. Biochem. & Biophys.*, 1964, v107, 209.
33. Melo, E. H. L., Mariani, I., Martirini, I., Cintra, A. B. U., *J. Lab. Clin. Med.*, 1959, v54, 739.
34. Maxfield, M., *Arch. Biochem. and Biophys.*, 1959, v85, 382.
35. ———, *ibid.*, 1960, v89, 281.

Received January 7, 1965. P.S.E.B.M., 1965, v119.

Selection and Intake of Carbohydrates by Wild and Domesticated Rats.* (30135)

OWEN MALLER† and MORLEY R. KARE

Departments of Zoology and Poultry, North Carolina State of University of North Carolina, Raleigh‡

The extensive use of the Norway rat as a laboratory animal can in part be explained by observations that suggest that this animal has nutritional requirements similar to those of man. The literature on taste and nutrition contain many studies using one of the "tame" varieties of the Norway rat as an experimental subject. These animals are usually obtained from commercial breeders and are the product of selective pressures, substantially different from those occurring

in their natural habitat. For commercial animals an acute sense of taste would have no apparent survival value.

* This work was supported, in part, by Nat. Inst. of Health Grant NB-03896.

† Supported in part by a USPHS fellowship 2F 2 HD-21-201-2 from Nat. Inst. of Child Health & Human Development.

‡ North Carolina Agri. Exp. Station, Raleigh, N. C. Published with approval of director of research as Paper 1917 of the Journal Series.

Few scientific reports are available on the taste preferences of the laboratory rat's ancestor, the wild Norway rat. Barnett and Spencer(1) have contributed a field study on the effect of flavors and odors on food selection. Richter and Mosier(2) have reported a study on salt regulation.

The ready availability of "tame" as well as "wild" species of *Rattus norvegicus* make it a particularly convenient animal in which to investigate the consequences of "domestication." Reviews of the literature concerning the effects of "domestication" upon the anatomy, physiology and behavior of the Norway rat can be found in Castle(3), and Richter(4).

The present study is a comparison between the wild Norway rat (*Rattus norvegicus*) with a domesticated rat (*Rattus norvegicus* var. *albinus*) in regard to selection and ingestion of various carbohydrates in solution.

Methods. Nine female albino rats of the Holtzman strain with an average weight of 293 g and 8 female wild Norway rats, with an average weight of 290 g (trapped at a local garbage dump), were used as subjects. The animals were housed individually and acclimated to their quarters for a 2-month period before the experiment began. The animals were maintained on a commercial laboratory feed (Wayne Mouse-breeder) which consisted of 20% protein, 10% fat, and 2% fiber and yielded a value of 3.4 kcal/g of feed. Food and water were available *ad lib* and intakes were measured daily to the nearest gram or milliliter. Body weights were obtained 3 times a week.

The cages (11" $\times$ 16½" $\times$ 8") were located in a temperature and light regulated room and were constructed so that food cups and waterers could be introduced and removed without entry into the cage. A small, detachable, metal compartment (5" $\times$ 10" $\times$ 5") connected to the side of the cage served as a nesting box which could be closed off from the rest of the cage. This detachable compartment permitted weighing the rats without physically handling them and also provided concealment for the animals.

Preference testing was conducted in the home cages, using graduated, glass drinking

devices. Twenty-four hour intake measures were recorded. The cage position of the drinking devices was alternated daily and each test solution was presented for a 4-day period. Testing was followed by at least 4 days of rest. All solutions were made with research grade chemicals, in distilled water and changed daily.

The carbohydrates were presented in the following order: maltose, lactose, sucrose, xylose, levulose and glucose. Percentage of sugar refers to g/100 ml solution and was used rather than molarity since this provided an approximately equal amount of calories per unit volume.

A single concentration for all the carbohydrates was selected (3.2%) that had been found in earlier investigations to compromise low concentrations of sugar with substantial preference(5). A single concentration of the non-nutritive sweetener was used (.00067 M). Percent preference refers to the volume of test solution consumed expressed as percent of total fluid intake.

Results and discussion. An analysis of variance of the volume intake measures for the carbohydrates and saccharin solutions, indicated an overall difference between domesticated and wild rats ($F = 28.91 \leq .01$ df 1; 15). The domesticated rats increased their volume intake with all of the solutions (Table I). In the case of lactose this increase amounted to 24% whereas with maltose their fluid intake increased by 197%. The wild rats also selected the sweeteners but total fluid consumption ranged from no change in the case of xylose to a relatively modest increase with maltose (53%).

The daily water intake between test periods indicated that the domesticated rat consumed more water (38 ml) than did the wild rats (34 ml). The difference in water intake ($t = 3.44 \leq .01$, df 1; 15) became greater during the testing period with sugar. Taking into account *all* of the periods where the sweet solutions were present the mean increase in fluid intake of the wild rats was 18% compared to 87% for the domesticated rat. This suggests a more rigid regulation of fluid intake in the wild rat as opposed to a more flexible intake by the domestic rat.

TABLE I. Comparison of Mean Preference† and Volume Intake.

	Saccharin	Maltose	Lactose	Sucrose	Xylose	Levulose	Glucose
Concentration	.00067M	.094M	.094M	.094M	.213M	.178M	.178M
Intake							
Wild							
flavor	27†	47*	27	44*	21*	28†	31*
total fluid	45	52	38	48	34	37	35
Domestic							
flavor	48	106	34	97	37	52	54
total fluid	62	113	47	107	49	60	59
Preference							
Wild	60†	91	71	92	61	76	88
Domestic	77	94	73	91	76	86	91

* t value $\leq .01$ level.† " " $\leq .05$ "
$$\dagger \frac{\text{flavored fluid intake}}{\text{total fluid consumed (ml)}} \times 100.$$

TABLE II. Rank Order of Mean Preference and Flavor Intake.

	Saccharin	Maltose	Lactose	Sucrose	Xylose	Levulose	Glucose
Intake							
Wild	5.5	1	5.5	2	7	4	3
Domestic	5	1	7	2	6	4	3
Preference							
Wild	7	2	5	1	6	4	3
Domestic	5	1	7	2.5	6	4	2.5

The wild rat, despite its preference for the sugars, limited its fluid intake close to its daily water intake. Under circumstances uncomplicated by the presence of a sweet choice the wild rat is more judicious in its use of water than is the domestic rat. In this experiment both groups of rats had approximately the same body weight and a similar surface area. Since the wild rat consumed significantly more food but significantly less water, this suggests a more efficient use of the water. The failure of the wild rat to consume more fluid may be related to this general prudence with water, or perhaps an inability to tolerate large volumes of fluids.

Both groups of rats selected the sweet solutions. The domesticated rat preferred the saccharin solution to a significantly greater degree ($p \leq .05$) than did the wild rat. In the case of xylose, the increased preference by the domesticated over the wild rodent approached significance. However, the analysis of variance performed on the percent preference measures for the carbohydrates indicated no overall significant difference between the groups.

The preference and volume intake of flavored fluid when ranked according to either magnitude of consumption or percent of preference exhibited close agreement between the domesticated and wild rats (Table II). Furthermore, the relative palatability of the various sugar solutions for the laboratory rat found in this study was comparable to the findings of previous workers (5,6).

Consideration of the individual chemical and physical qualities of the sugars fails to suggest any simple relationship to either the preference behavior or volume intake of the solutions. A similar conclusion obtained from work with the fowl's response to sugars has been reported (7).

During the rest periods the caloric intake for the domestic rats on the dry diets averaged 47 kcal but with sugars present, these animals increased their intake of calories to 54 kcal, a 15% increase. The wild rats averaged 55 kcal intake and with sugars present increased to 58 kcal which is only a 5% increase. Thus, the domesticated rats were less restrained in their intake of sugars and the non-nutritive saccharin, and with the former

the net result was a more substantial increase in caloric intake than that obtained with the wild rats.

Although the domesticated rats' caloric intake was significantly less than that of the wild rats, there was no difference between the body weights of the 2 groups at the end of the experiment. The domesticated rats averaged 304 and the wild rats 301 g. Benedict and Petrick(8) found that the wild rat had a higher basal metabolic rate than its domesticated counterpart. Maller (unpublished) has observed that when wild rats were placed on a high fat diet (60%) their gram intake decreased more rapidly and they gained less weight than did the domesticated rat. Thus, the limited intake by the wild rat on the various carbohydrate solutions suggests a more precise and careful monitoring of energy intake.

The results evaluated in terms of nutritive value of the solutions are difficult to interpret. Saccharin and xylose which are at the low end of the scale with reference to preference and intake also have least to offer nutritionally. Limited preference and intake of these 2 substances is more pronounced in the wild rat than in the domesticated. However, there is no apparent nutritional reason for the differences in preference and intake between the monosaccharides, glucose and levulose, and the disaccharides, maltose and sucrose.

Furthermore, an increased intake of calories from the fluid carbohydrates was at the expense of a reduced intake of the nutritionally balanced diet; the failure of the wild rats to increase fluid intake could very well have been a nutritional decision. Thus, "domestication" may have produced an animal that is less sensitive to nutritive value of food and more responsive to its "sensory" or "hedonic" qualities.

A partial explanation of the difference in preference and intake based upon toxicity of two of the sugars merits consideration. Xylose can be classified as toxic while the possibility of lactose being in this category might be entertained(9,10). The wild rat did select the xylose (1.5:1) but did not increase its fluid intake. The domestic rat selected

xylose (3:1) and increased fluid intake about 30% (Table I). This finding contrasts abruptly with the marked preference and increased volume intake with either maltose or sucrose. The lactose provided results similar to those with xylose although not as pronounced. However, relating the intake of xylose to toxicity must be tempered by the results with the non-toxic glucose. Glucose was highly preferred, but fluid intake was substantially lower than with the other highly preferred sugars. If the limited intake of xylose and lactose was related to toxicity, one might conclude that the wild rat is more responsive to toxic qualities than is the domestic rat.

Summary. Domestication has altered the response of rats to sweet solutions. Fluid intake with all the sweeteners was lower and relatively constant for the wild rats. However, both wild and domesticated rats exhibited a qualitatively similar preference for the carbohydrate solutions. The domestic rat selected non-nutritive saccharin to a significantly greater degree, while the wild rat responded to toxic xylose with a reduced preference. Although the caloric intake of the wild rats was larger and was obtained to a greater degree from the ration, they gained no more weight than did the domesticated rat. Since body weights were similar, and the wild rat consumed less water and more food it follows that the wild rat functions with a lower ratio of water to caloric intake. Under the conditions of the experiment the domestic rat was indulgent with the sweet solutions, responding less to the nutritional and toxic consequences than did its wild counterpart.

The statistical assistance of Dr. J. Rawlings and Mrs. E. Carroll and the collection of data by Mr. F. Blake are gratefully acknowledged.

1. Barnett, S. A., Spencer, M. M., *Brit. J. Anim. Behav.*, 1953, v1, 1.
2. Richter, C. P., Mosier, H. D., *Am. J. Physiol.*, 1954, v196, 213.
3. Castle, W. E., *Proc. Nat. Acad. Sci.*, 1947, v33, 109.
4. Richter, C. P., *J. Nat. Cancer Inst.*, 1954, v15, 727.
5. Kare, M. R., Ficken, M., in *Olfaction and*

Taste (ed., Y. Zotterman), Pergamon Press, New York, 1963, 285.

6. Richter, C. P., Campbell, K., J. Nutr., 1940, v20, 31.

7. Kare, M. R., in: Physiological and Behavioral Aspects of Taste (M. R. Kare & B. P. Halpern, ed.), Univ. of Chicago Press, Chicago, 1961, 6.

8. Benedict, F. B., Petrick, J. M., Am. J. Physiol., 1930, v94, 662.

9. Booth, A. N., Wilson, R. H., Deeds, F., J. Nutr., 1953, v49, 347.

10. Yudkin, A. M., Geer, H. A., Arch. Ophthalmol., 1940, v23, 28.

Received January 15, 1965. P.S.E.B.M., 1965, v119.

Virus-Free Infective Centers Produced by Photodynamic Inactivation of Poliovirus in Rhesus Kidney Cell Suspensions. (30136)

C. W. HIATT AND DOROTHY E. MOORE*

Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

Poliovirus propagated in cell cultures containing proflavine, acridine orange, or neutral red becomes sensitive to light (1-3) and retains this sensitivity as a phenotypic tag (4), which may be used to distinguish between successive generations of virus. Several investigators (3,5,6,7) have employed photosensitive poliovirus as a means of studying the early course of infection of the cell, making use of the fact that irradiating the system with visible light will destroy parent virus without affecting the cells or any virus progeny which may have been present at time of irradiation.

Wilson and Cooper (3,7) found that poliovirus sensitized with neutral red or acridine orange loses its photosensitivity soon after adsorption to susceptible cells and that this loss is not due simply to entry into the cell, but results from something which happens to the virus particle in a later stage of the process of infection. McBride's earlier observations (5) as well as those reported concurrently by Schaffer and Hackett (6) differ somewhat in experimental detail but lead essentially to the same conclusion.

In similar experiments of our own, we have found that, by irradiating cell suspensions promptly after adsorption of proflavine-sensitized poliovirus, we can obtain cellular centers of viral infection from cell suspensions which contain no immediately recoverable virus. This demonstration of a total

eclipse phase of poliovirus infection is of interest because of its implications about the transfer of genetic information from the virus to the synthetic apparatus of the cell.

Methods. Preparation of virus. Poliovirus, type 1, Mahoney, was sensitized to light by 3 serial passages in the dark in monolayers of primary rhesus kidney cells in medium 199 (8) containing 2% calf serum and 2 mcg proflavine per ml. Plaque-forming units (PFU) were determined on confluent monolayers of primary rhesus kidney cells.

Preparation of cells. Monolayer cultures of Hull's LLCMK₂ stable rhesus kidney cell line (9) were prepared in 32-oz bottles. After a preliminary washing with 10 ml of Hanks' balanced salt solution (HBSS) (10), the cell sheet from each bottle was dislodged from the glass by incubation for 15 minutes at 36°C with 20 ml of 0.15 M NaCl containing 0.25% trypsin. The yield from 4 bottles was pooled, sedimented by centrifugation for 5 minutes at 1500 rpm and resuspended in 5 ml of HBSS containing 0.5% lactalbumin. The cell concentration was determined using a Coulter counter or by visual counting in a hemocytometer using trypan blue stain.

Experimental procedure. An inoculum of 0.4 ml of photosensitized poliovirus containing 10⁷ plaque-forming units (PFU) was added to a suspension of 10⁷ cells in 20 ml of HBSS containing 0.5% lactalbumin, and the mixture was shaken gently for 20 minutes in the dark at 36°C. The cells were then collected by centrifugation for 5 min-

* Present address: Dept. of Microbiology, University of Chicago, Ill.