The Regulation of Food Intake

SOME EXPERIMENTS RELATING BEHAVIORAL, METABOLIC AND MORPHOLOGIC ASPECTS

By JAMES ANLIKER, PH.D.* AND JEAN MAYER, PH.D., D.SC.†

THE experiments reported in this paper are all concerned with the analysis of factors participating in the regulation of food intake in animals. The material may be divided into four categories as follows: (1) Relations between the hypothalamic structures and feeding-fasting behavior; (2) The nature of the physiologic stimulus to the hypothalamic satiety mechanism; (3) Some correlations between feeding-fasting behavior, blood glucose, and liver glycogen, and (4) The relationship between blood glucose and operant conditioning.

BEHAVIORAL METHOD

An effective technic for measuring and recording food intake is an obvious prerequisite for the analysis of its regulatory mechanisms. Considering the fact that food intake is normally accomplished through overt behavior on the part of an organism, we have applied a behavioral method to the problem of its measurement. The adaptation of operant conditioning apparatus and methods[1–3] to the measurement of food intake in the mouse is reported in detail elsewhere.[4] Briefly, the procedure is as follows: By placing a sensitive lever in a mouse's cage, we may observe that the mouse operates this device with an appreciable frequency in the absence of a history of food reward in this situation. This initial rate of responding is known as the *free operant level*. When lever-pressing responses are rewarded with food, their frequency tends to increase (provided, of course, that the mouse has been deprived of food for some time). If each lever-press produces a pellet of food, the mouse soon becomes satiated and ceases to press the lever at a rate that is higher than the free operant level. However, since no food is available unless the lever is pressed, and since it is impossible for the mouse to hoard an appreciable quantity of food because of the design of the cage, periods of nonresponding are also periods of noneating. As deprivation increases, the probability of responding increases. Ultimately, the mouse presses the lever and eats, and so forth. In this way periods of feeding alternate with periods of abstaining from food. Under relatively constant environmental conditions (including stability and uniformity of the diet), the mouse develops a fairly consistent pattern of feeding and fasting. Through the use of a suitable recording device an accurate account of the number of pellets delivered, as well as the time of delivery of each, can be obtained. It is possible to insure a close correlation between pellet-delivery and pellet-consumption by rewarding only a fixed ratio of the responses (e.g., by delivering one pellet after 25 responses).

From the Department of Nutrition, School of Public Health, Harvard University, Boston, Mass.

* Research Associate, Department of Nutrition, School of Public Health, Harvard University, Boston, Mass.

† Assistant Professor, Department of Nutrition, School of Public Health, Harvard University, Boston, Mass.

Supported in part by Grant No. A-49, National Institute of Arthritis and Metabolism, National Institutes of Health, Bethesda, Maryland; Sugar Research Foundation, New York; Kellogg Company, Battle Creek, Michigan; and The Nutrition Foundation, Inc., New York.

Presented at a Symposium on Nutrition and Behavior held at the Laboratory of Physiological Hygiene, University of Minnesota, April 27, 1956, with the cooperation of the National Vitamin Foundation, Inc., New York and under the sponsorship of the School of Public Health, University of Minnesota.

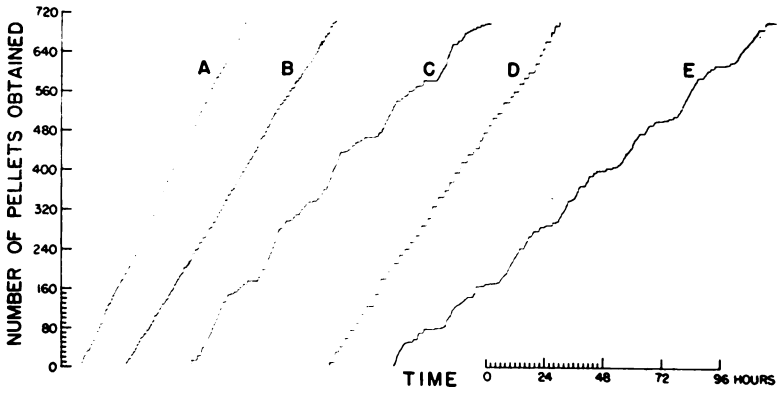

Fig. 1. Cumulative records of pellets obtained by mice on a schedule of reward that allows one pellet of food (approximately 0.04 g) for every 25 operations of the lever. Records A, B, and C are from Swiss mice: (A) goldthioglucose obese, (B) hypothalamic obese, and (C) normal. Records D and E are from mice of a Bar Harbor stock that produces mice exhibiting the hereditary obese-hyperglycemic syndrome (D) and normal littermate (E). Observe that the 24-hour cyclical changes in rate of feeding evidenced in the records of normal mice (C, E) are either absent or barely discernible in the records of hyperphagic mice (A, B, D).

THE RELATIONSHIP BETWEEN THE HYPOTHALAMUS AND FEEDING BEHAVIOR

It is now well-established that lesions in the ventromedial hypothalamus result in hyperphagia.[5,6] Lesions in the lateral hypothalamus, on the other hand, produce hypophagia or aphagia.[7,8] Recently, it has been demonstrated that the hyperphagia induced by goldthioglucose treatment is also attributable to destruction in the ventromedial hypothalamus.[9] Figure 1 shows the feeding patterns for a hypothalamic hyperphagic mouse in which the lesions were produced by goldthioglucose treatment (A) a hypothalamic hyperphagic mouse in which the lesions were produced by electrocoagulation (B), and a normal mouse (C). It is readily apparent that the diurnal changes in rate of feeding are greatly reduced or barely perceptible in both types of hypothalamic hyperphagia.

These results may be interpreted as supporting the hypothesis that the ventromedial area acts to inhibit feeding behavior and that in its absence hyperphagia results. It seems improbable that the ventromedial hypothalamus contains the only mechanism for the inhibition of feeding behavior because even in animals exhibiting extreme hyperphagia, there is a periodic cessation of eating. However, we should not overlook the infrequent but recurrent instances in which rats with hypothalamic lesions die from overeating. Before turning their entire attention to other inhibitory mechanisms; such as, pain resulting from gastric distention, etc., investigators will do well to determine the amount of residual inhibitory capacity attributable to undestroyed ventromedial cells since lesions are seldom, if ever, complete. The fact that 24-hour cycles tend to become increasingly prominent as the animal approaches the static phase of obesity might be interpreted as evidence for the reassertion of inhibitory control by remaining ventromedial tissue under more favorable metabolic conditions (lipostatic hypothesis), or, possibly, because of partial recovery of injured, but not destroyed, neurons.

Teitelbaum and Campbell[11] have reported recently that hypothalamic hyperphagic rats fed a liquid diet, the consumption of which was recorded by a drinkometer, did not eat a larger number (8 to 12) of meals in a 24-hour period than did normal rats; the hyperphagic rats ate more during each meal. This suggests that, in rats at least, the ventromedial hypothalamus normally participates in the termination of individual meals.

PHYSIOLOGIC STIMULUS TO THE HYPOTHALAMIC SATIETY CENTER

Evidence favoring a glucoreceptive intermediary between metabolic processes and central neural regulatory mechanisms has been reviewed elsewhere.[10,12] One of the most cogent arguments in favor of the glucoreceptor theory is furnished by the evidence that the chemical compound, goldthioglucose, induces hyperphagia in mice.

It has been shown[9] that the hypothalami of goldthioglucose-injected mice show definite lesions within one to three days, involving edema, pyknosis, and degeneration of nerve cells in the ventromedial area of the hypothalamus. Larsson[13] found that in fasted rats this area concentrates radioglucose and radiophosphate as compared with a control area from another part of the hypothalamus.

It is well-known that the heavy metals are extremely toxic to cells. Consequently, we are inclined to the view that, because the ventromedial area contains cells that concentrate glucose and because the gold is linked to the glucose, these cells are selectively poisoned. That is, they manage to concentrate a toxic dose of gold. This hypothesis is supported by the evidence[14] that a great variety of other goldthiocompounds do not cause hypothalamic damage or induce hyperphagia. For example, goldthiomalate, a compound of equivalent toxicity, does not induce hyperphagia and does not produce demonstrable hypothalamic damage.[15]

Likewise, goldthiosorbitol (a compound extremely similar in structure to goldthioglucose), goldthioglycerol and goldthiocaproic acid, (two compounds in which the gold is tied by a sulphur bridge to substances normally involved in fat metabolism) do not produce lesions or induce hyperphagia.[16] Furthermore, it was shown in the rat that on administration of the same dose of goldthioglucose (1 mg/gm of body weight, the LD_{50} for mice) produces cell degeneration in the same ventromedial area as in the mouse but the gold is so generally toxic to the rat that it does not survive to show the hyperphagia. It has been reported that feeding the animals before injection of goldthioglucose diminishes the incidence of permanent damage and hyperphagia by 30 to 50 per cent.[13]

Thus, gold appears to concentrate in toxic amounts in the ventromedial areas when it is linked to glucose and not when it is linked to other substances or metabolites. Glucose and other compounds containing glucose are apparently concentrated in the ventromedial area as readily as goldthioglucose and hence reduce the rate of accumulation of the latter compound in these cells. We believe that these data provide relatively strong support for the view that there are glucoreceptors in the ventromedial hypothalamus.

OBESE HYPERGLYCEMIC SYNDROME IN MICE

In Figure 1 curves D and E are representative of the hyperphagic mouse bearing the *ob ob* gene and its normophagic litter mate, respectively. The fact that the hereditarily obese mouse has an apparently intact ventromedial hypothalamus and is hyperphagic even while hyperglycemic might appear to contradict the glucostatic theory. However, it is possible to place these findings in harmony with the theory if we consider the fact that this organism exhibits abnormalities of carbohydrate metabolism (including a sixfold acceleration in rate of turnover of liver glycogen) and that it is quite possible that the receptor area is simply less responsive to blood glucose.

RELATIONSHIP BETWEEN FEEDING BEHAVIOR AND LIVER GLYCOGEN

Figure 2 permits comparison of the diurnal pattern of feeding behavior and diurnal variation in liver glycogen. In this study liver glycogen was determined by sacrificing six mice every other hour around the clock starting at 1:00 p.m. The amount of liver glycogen was determined* according to the method described by Good, Kramer, and Somogyi.[17] The feeding patterns were obtained from six thoroughly-trained normal mice, the record being that of the fifth consecutive day in the Skinner box. From these data it is apparent that the feeding pattern of normal mice is characterized by a high rate of feeding in the afternoon and evening and a tapering off of the feeding rate during the night to a very low rate of feeding in the

* We are indebted to Dr. Konosuke Tomabechi for these measurements.

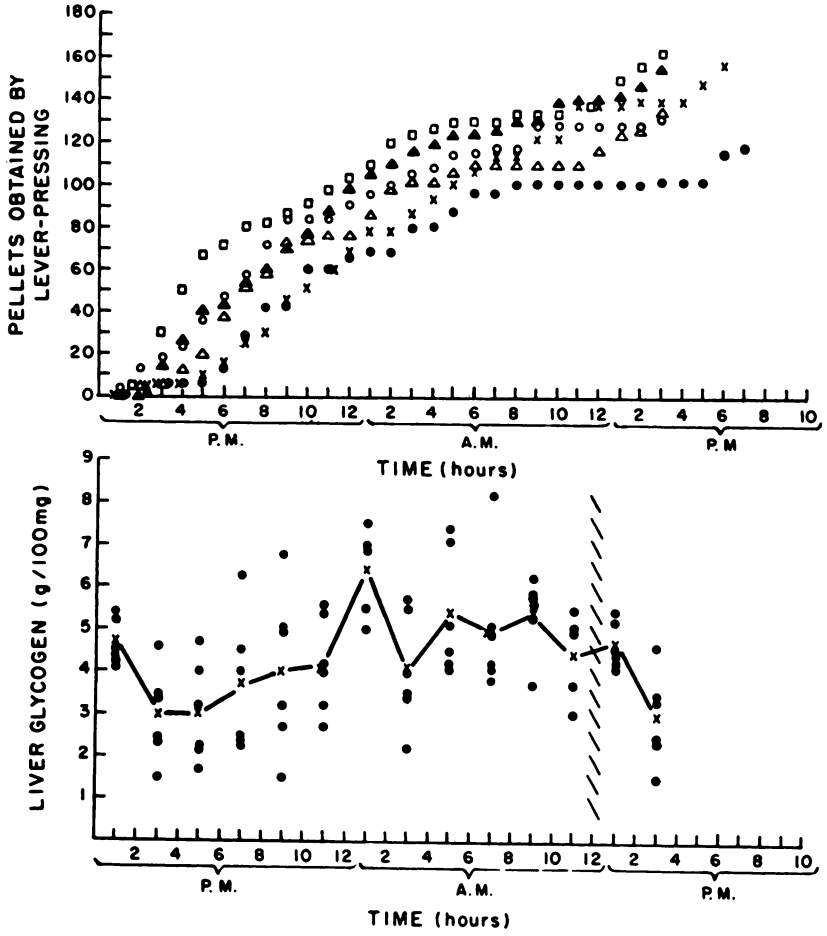

Fig. 2. Comparison of diurnal variations in feeding behavior and in amount of liver glycogen in normal mice. Upper portion of figure contains cumulative records, for six normal mice, of pellets obtained by lever-pressing. The lower portion of the figure shows the quantities of liver glycogen found in groups of mice sacrificed at 2-hour intervals around the clock, starting at 1 p.m. The data located to the right of the diagonal bars are simply duplicates of the data for the first two groups; this arrangement is intended to facilitate comparison between the upper and lower graphs.

morning. Liver glycogen accordingly is lowest at the time the mice begin their rapid feeding phase, rises along with rapid feeding, levels off during the night and starts falling in the early morning hours. From these data we tentatively conclude that there is a rate of feeding above which liver glycogen accumulates and below which liver glycogen is dissipated. Liver glycogen represents the most readily available source of glucose reserves. It is well known that food deprivation leads to a depletion of glycogen and that satiation is associated with a rise in blood sugar which is counteracted by insulin secretion and the formation of liver glycogen. Considering the fact that the pancreatic hormone, glucagon has the capacity to increase the amount of active phosphorylase in the liver,[18] one might suppose that this hormone is at least partially responsible for the glycogenolysis associated with fasting. However, the physiologic conditions in which glucagon is released from the pancreas are not currently known. Myers et al.[19] have demonstrated that glucagon produces a prompt rise in the blood sugar of the hepatic vein. Recent unpublished experiments performed in our laboratory have shown that sub-

cutaneous injections of glucagon in sufficiently large amounts will produce a transient inhibition of feeding activities of mice commencing about 15 to 20 minutes after injection and lasting for a variable number of minutes depending upon the amount injected. Glucagon has also been shown to inhibit gastric contractions in man[24] and in rats.[21] There is good evidence that parenteral injections of insulin in moderate dosages produces an increase in the rate of eating in animals[22] and in men.[23]

Taken together, these observations confirm the generally accepted view that blood glucose and liver glycogen usually rise after feeding and then begin to fall sometime after feeding has ceased and that feeding and fasting behavior correspond in a general way to the rise and fall of blood glucose and liver glycogen.

RELATIONSHIP BETWEEN BLOOD GLUCOSE AND BEHAVIORAL CONDITIONING (LEARNING)

Coppock and Chambers[24] have demonstrated that in the rat intravenous glucose has a reinforcing effect; that is to say, it is possible to condition responses by providing intravenous glucose as a "reward." This experiment suggests to us a whole series of studies of the capacity of different organisms to discriminate intravenous substances under various conditions.

SUMMARY

Experimental evidence was presented which supports the view that the hypothalamus contains a "feeding" center and a "satiety" center. The satiety center obtains its information from the bloodstream via glucoreceptors. Destruction of the satiety center acts to release the feeding center from the inhibitory influence of the satiety center and this results in hyperphagia. Changes in blood glucose and liver glycogen resulting from feeding and fasting and from parenteral injections of glucagon and insulin are likewise in conformity with the glucostatic theory of the regulation of food intake. Finally, the fact that intravenous glucose is effective as a reward in operant conditioning indicates that rats, and presumably other animals, are capable of discriminating changes in blood glucose.

REFERENCES

1. SKINNER, B. F.: The Behavior of Organisms. D. Appleton-Century, New York, 1938.
2. SKINNER, B. F.: The experimental analysis of behavior. *Proc. and Papers, 13th Int. Cong. Psychol.* Stockholm, 1951.
3. FERSTER, C. B.: The use of the free operant in the analysis of behavior. *Psychol. Bull.* 50: 263, 1953.
4. ANLIKER, J., and MAYER, J.: An operant conditioning technique for studying feeding-fasting patterns in normal and obese mice. *J. Applied Physiol.* 8: 667, 1956.
5. BROBECK, J. R.: Mechanism of the development of obesity in animals with hypothalamic lesions. *Physiol. Rev.* 26: 541, 1946.
6. MAYER, J.: Genetic, traumatic and environmental factors in the etiology of obesity. Physiol. Rev. 33: 472, 1953.
7. ANAND, B. K., and BROBECK, J. R.: Localization of "feeding center" in hypothalamus of rat. *Proc. Soc. Exp. Biol. & Med.* 77: 323, 1951.
8. TEITELBAUM, P., and STELLAR, E.: Recovery from the failure to eat produced by hypothalamic lesions. *Science*, 120: 894, 1954.
9. MARSHALL, N. B., BARNETT, R. J., and MAYER, J.: Hypothalamic lesions in goldthioglucose in mice. *Proc. Soc. Exp. Biol. & Med.* 90: 240, 1955.
10. MAYER, J.: Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. *Ann. New York Acad. Sc.* 63: 15, 1955.
11. TEITELBAUM, P., AND CAMPBELL, B. A.: Patterns of food ingestion in hyperphagic and normal rats. Am. Psychol. 11: 491, 1956.
12. MAYER, J.: *La régulation de l'appétit, Proc. XXth Int. Physiol. Cong.* Brussels, 1956.
13. FORSSBERG, A., and LARSON, S.: On the hypothalamic organization of the nervous mechanism regulating food intake. II. Studies of isotope and chemical composition in the hypothalamic region of hungry and fed rats. *Acta Physiol. Scand.* 32: suppl. 115, part 2: 40, 1954.
14. MAYER, J., and MARSHALL, N. B.: Mode d'action de l'aurothioglucose et régulation glucostatique de la nutrition. *Compt. rend. Acad. de Sc.*, 242: 169, 1956.
15. MARSHALL, N. B., and MAYER, J.: Energy balance in goldthioglucose obesity. *Am. J. Physiol.* 178: 271, 1954.
16. MAYER, J., and MARSHALL, N. B.: To be published.
17. GOOD, C. A., KRAMER, H., and SOMOGYI, M.: The determination of glycogen. *J. Biol. Chem.* 100: 485, 1933.
18. SUTHERLAND, E. W., and CORI, C. F.: Influence of insulin preparations on glycogenolysis in liver slices. *J. Biol. Chem.* 172: 737, 1948.

19. MYERS, J. D., KIBLER, R. F., and TAYLOR, W J.: Effects of pancreatic hyperglycemic factor on hepatic carbohydrate metabolism in man. *Fed. Proc.* 11: 111, 1952.
20. STUNKARD, A. J., VAN ITALLIE, T. B., and REISS, B. B. The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. *Proc. Soc. Exper. Biol. & Med.* 89: 258, 1955.
21. VAN ITALLIE, T. B. Personal communication, 1956.
22. MORGAN, C. T., and MORGAN, J. D.: Studies in hunger: I. The effects of insulin upon the rat's rate of eating. *J. Genet. Psychol.* 67: 153, 1940.
23. FREYBURG, R. H. A study of the value of insulin in undernutrition. *Am. J. M. Sc.*, 190: 28, 1935.
24. COPPOCK, H. W., and CHAMBERS, R. M.: Reinforcement of positionp reference by automatic intravenous injections of glucose. *J. Comp. Physiol. Psychol.* 47: 355, 1954.