5. Schajowicz, F., Cabrini, R. L., Science, 1960, v131, 1043.
6. Walker, D. G., Bull. Johns Hopkins Hosp., 1961, v108, 80.
7. Laskin, D., Engel, M. B., Ann. N. Y. Acad. Sci., 1960, v85, 421.
8. Jeffay, H., Bayne, H. R., Am. J. Physiol., 1964, v206, 415.
9. Nichols, G., Jr., in The Parathyroids: Proceedings of a Symposium on Advances in Parathyroid Research, R. O. Greep and R. V. Talmage, ed., Thomas, 1961, p210.
10. Kun, E., Abood, L. G., Science, 1949, v109, 144.
11. Bachra, B. N., Dauer, A., Sobel, A. E., Clin. Chem., 1958, v4, 107.

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Short-Term Caloric Regulation in the Adult Opossum (*Didelphis virginiana*).* (29818)

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A number of investigators have found food intake to be closely associated with the caloric values of the food(1-4). Metabolic consequences of the nutrients have been shown to be of greater importance in regulation of food intake than has the palatability of the foodstuff(5-7).

This study was designed to measure the ability of the opossum to adjust its food intake in accordance with alterations in the caloric concentration of its diet. The opossum was selected because it is feral and is considered to be a primitive mammal which has remained relatively unchanged since the Cretaceous period(8). Furthermore, there is considerable lore about the unusual appetite and feeding habits of the opossum(9).

Methods. Eight locally trapped adult opossum were individually caged in a battery with 15 × 30 × 36 inch units. Body weights at the outset of experiment averaged 2752 g. Standard deviation was ± 869 g. The experiment was carried out between 24 March and 27 June 1964. The average environmental temperature during the experiment was 23°C with standard deviation of ± 2°. The animals were given food and water *ad lib.* and intake was measured daily to the nearest gram or milliliter. The base diet was a ground dog food, consisting of 22.5% protein, 9.3% fat, 42.3% carbohydrate, 21.3% ash and 4.6% water (supplied by Quaker Oats, Barrington, Ill.). Caloric dilutions were obtained by adding nonnutritive cellulose. Caloric enrichments were obtained by adding commercial fat (lard) containing an anti-oxidant to retard spoilage.

A base line on food and water consumption was established over a 14-day period prior to altering the caloric content of the diet. The animals were then placed on the adulterated diets for a 7-day period. There were a total of 6 test periods, 3 dilution tests (10, 25 and 50%) and 3 enrichment tests (9.4, 25 and 50%). Each of these test periods was followed by a recovery period on the base diet for at least 7 days. The calculated caloric concentration of the base diet was 3.4 Kcal/g: the diluted diets were 3.1, 2.6 and 1.7. The enriched diets contained 3.8, 4.3 and 5.1 Kcal/g.

Results and discussion. The intake data were evaluated by comparing mean values of intake on the base diet with those obtained on the adulterated diets. Statistically significant changes in caloric intake were assessed by computing 95% confidence limits for the mean caloric intake on the base diet(10).

Fig. 1 illustrates graphically the caloric regulation of the opossum. Precise caloric

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regulation would result in a histogram at the base line across all experimental conditions. A histogram falling outside the 95% confidence limits would indicate a statistically significant change in caloric intake. Maintaining a consistent gram intake would yield a histogram indicated by the interrupted lines. Statistically significant changes in caloric intake occurred at 25 and 50% dilutions and at 10% enrichment.

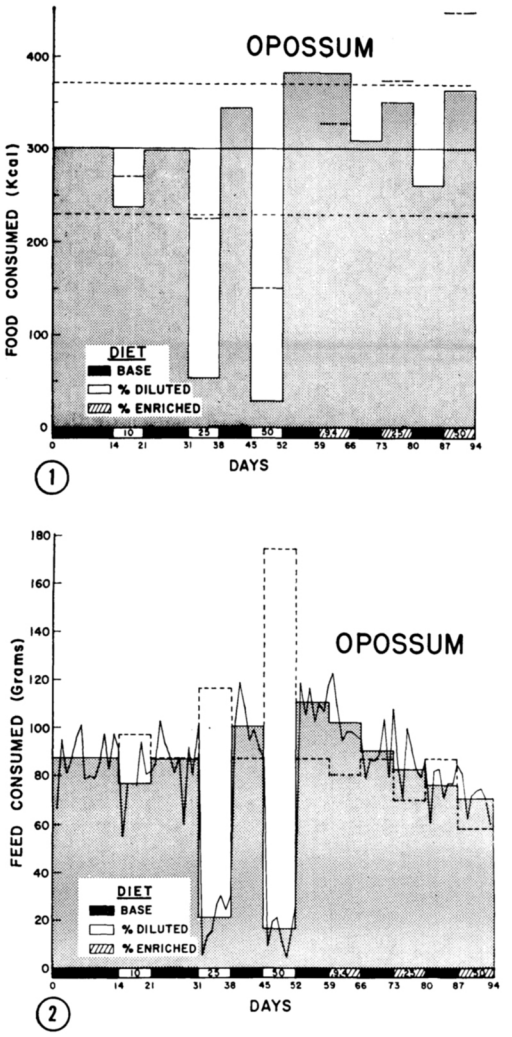

FIG. 1. Food consumed (Kcal) over time (days). Shaded histogram portrays mean caloric intake for each experimental condition. The continuous straight line indicates mean caloric consumption and the 2 dotted lines define 95% confidence limits for this mean. Amount consumed if volume intake remained constant and independent of caloric intake is indicated for each of the adulterated diets.

FIG. 2. Food consumed (g) over time (days). Shaded histogram depicts mean intake for each experimental condition. Dotted lines indicate expected intake and the polygon follows daily intake. The successive dilutions would be expected to increase food intake 11.1, 33.3 and 100%. Similarly, the enriched diets would be expected to decrease intake 8.6, 20.0 and 33.3%.

The opossum responded to the diluted diets by reducing their food intake (Fig. 2). The decrease in intake became more pronounced as the level of dilution was increased. To maintain a consistent caloric intake, a 50% dilution of the diet would require a 100% increase in food intake; however, a 90% decrease was observed.

The failure to increase food intake on the diluted diets suggests that the poor palatability of these diets overrode caloric values. An examination of daily food intake on the diluted diets depicted by the polygon on Fig. 2, reveals a marked depression of intake on the first day at all 3 levels of dilution. Conversely, during the first few days on the enrichment as well as on the recovery periods following dilution, these animals overshoot on their intake. Furthermore, that this negative response to dilution is not simply a matter of a novelty effect of the diluent can be seen from an examination of intake at the 50% level (the third dilution test) which is below that of the 25% level. One would expect that any novelty of the diluent would be minimized by previous experience with the diluted diet.

The opossums' response to the enrichment tests was more variable than with dilution. At the 9.4% level of enrichment, these animals increased their caloric intake by 28% rather than decreasing their food intake by 8.6%, as would be expected. On the 25 and 50% enriched diets there was a slight reduction in grams of food ingested but this decrease in grams constituted an increase in caloric intake of 17 and 21% over base intake (Fig. 1).

The sharp increases and decreases in intake obtained when switching from the enriched to base diets also suggest the potency of palatability factors in caloric regulation of the opossum. Furthermore, the increased intake above the base caloric intake observed on the enriched diets, as well as during the recovery period following the 50% dilution, argues against the importance of physical limits of the digestive tract. Thus, it follows that an

increase of intake on the diluted diet *was not* prevented by the capacity of the opossum's stomach.

These findings are in sharp contrast to the results obtained with the albino rat. Several investigators have observed that caloric values are of greater consequence to this animal than are factors of palatability(2,5-7). Also, rats have been shown to adjust their food intake in accord with their base caloric intake within 3-5 days on dilutions as high as 75% and enrichment of 50%(2,11).

It is conceivable that this feral animal would want to overeat when food is abundant. Rigid caloric regulation would be of limited value to an animal with an irregular and varied food supply. A sensitivity to palatability would be of greater consequence to a wild animal than to a domesticated animal where rapid detection and discrimination among nutrients has survival value. The difference observed in caloric regulation between laboratory rats and opossum may be a species difference independent of domestication. That there may be species differences in caloric regulation is also suggested by the work of Janowitz and Hollander on food regulation in the dog(12).

Summary. The opossum did not precisely adjust its caloric intake over all the experimental conditions. Rather, as levels of dilution and enrichment increased, the precision of caloric adjustment decreased. There was no increase in grams of food intake on diluted diets, but there was a decrease in grams of food intake with calorically enriched diets. However, this reduction in gram intake constituted an increase in caloric intake. The physical capacity of the digestive tract was not a limiting factor.

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1. Cowgill, G. R., Am. J. Physiol., 1928, v85, 45.
2. Adolph, E. F., ibid., 1947, v151, 110.
3. Harte, R. A., Travers, J. J., Sarich, P., J. Nutr., 1948, v36, 667.
4. Hausmann, M. F., J. Comp. Psych., 1933, v15, 419.
5. Scott, E. M., Quint, E., J. Nutr., 1946, v32, 113.
6. Jacobs, H. L., Am. J. Physiol., 1962, v203, 1043.
7. Epstein, A., Teitelbaum, P., J. Comp. Physiol. Psych., 1962, v55, 753.
8. Simpson, G. G., Biol. Rev., 1937, v12, 1.
9. Hartman, C. G., Possums, U. of Texas Press, Austin, 1952.
10. Wilks, S. S., Elementary Statistical Analysis, Princeton U. Press, Princeton, 1958, 207.
11. Strominger, J. L., Brobeck, J. R., Cort, R. L., Yale J. Biol. Med., 1953, v26, 55.
12. Janowitz, H. D., Hollander, F., Ann. N. Y. Acad. Sci., 1955, v63, 56.

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Some Observations on Staphylococcal Penicillinase. (29819)

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The resistance of many strains of staphylococci to penicillin has been shown by Kirby (1), Bondi and Deitz(2,3), Geronimus and Cohen(4) and others to be due to production of a penicillin degrading enzyme, penicillinase. Previous studies concerned with the isolation and properties of this enzyme have been conducted primarily on the extracellular penicillinase produced by *Bacillus cereus* (Pollock *et al*(5)).

Though there are scattered reports in the literature of certain properties of the staphylococcal penicillinase *in vitro*, the enzyme has not been purified to any extent nor crystallized. One possible reason for this is that the staphylococcal enzyme is usually intra-, rather than extracellular, and hence is more difficult to remove from the cells. Since the work on the crystallization of the enzyme from *B. cereus* is quite comprehensive we thought it of interest to attempt a purification of the staphylococcal enzyme using as a basis for