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# Plasma Insulin and the Time Pattern of Feeding in the Rat

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STRUBBE, J. H., A. B. STEFFENS AND L. DE RUITER. *Plasma insulin and the time pattern of feeding in the rat*. *PHYSIOL. BEHAV.* 18(1) 81–86, 1977. — Blood levels of glucose and insulin during meals and between-meal-intervals were measured in virtually undisturbed rats. After a peak due to glucose absorbed from the meal blood sugar soon reverts to the pre-meal level. Insulin shows a similar peak followed by a continuing decline so that just before the next meal it is lower than at any other time. In terms of the glucostatic theory this might mean that meals are triggered by a critical drop of insulin. However, intravenous infusions of glucose, resulting in continuously high insulin, do not affect timing or size of meals. Glucostatic factors probably are of minor importance in meal-to-meal regulation in the rat. However, they may subserve nutritional homeostasis on at least two other points.

Food intake    Plasma insulin    Blood glucose

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AS EARLY as 1952, Mayer and Bates [16] and Mayer [17] proposed the glucostatic theory of the regulation of food intake, but even today the status of this theory is still controversial. The theory holds that food will be taken when the utilization of blood glucose by the organs of the body is insufficient [17]. Several reports tallying with this theory were published so far [1, 4, 8, 28, 16, 18, 20, 22, 25]. Other reports, however, did not agree with it [2, 3, 7, 11, 21]. As definite proof that normal food intake is regulated by a glucostatic mechanism is still lacking, we made another attempt to evaluate the validity of the glucostatic theory.

The rate of glucose utilization depends of course on the presence in the blood of glucose itself, but for many kinds of cells it also depends very much on adequate levels of hormones facilitating uptake and utilization of glucose. Of these hormones the most important, and the only one considered in this study is insulin. This may explain, assuming that results obtained in different species may be combined, why a close correlation between food intake and rate of uptake of glucose from the capillaries was reported (in man, [28]), whereas on the other hand no significant drop of blood glucose concentration heralds the meal (in the rat, [23]). In terms of glucostatic theory, this would indicate that the fluctuations in glucose utilization that govern food intake depend on fluctuations not of glucose itself, but of e.g. the insulin level. It is true then, that in the rat insulin reaches a lower level just before the meal than at any other time since the start of the previous meal, also suggested by Smith [20]. If so, glucose utilization may attain its lowest rate just before the meal in spite of the constancy of glucose.

To investigate this question, blood samples were taken in the intermeal periods and examined for glucose and insulin.

It will be shown that in rats kept on a normal diet there exists indeed a clear relationship between a low insulin level and the start of a meal. Hence, insulin might play a role in the initiation of feeding in the ad libitum situation. To test this idea, we subsequently raised the insulin level to values significantly above normal, by systemic infusion of glucose between meals. If insulin plays an important role in the initiation of feeding, the length of the intermeal period should be increased by this treatment. In contrast, if no increase of the intermeal period is observed it can be concluded that low insulin levels do not play an important role in the initiation of feeding under normal ad libitum circumstances.

## MATERIAL AND METHOD

### *Animals and Maintenance*

Male Wistar rats were maintained in individual plexiglass chambers (25 × 25 × 30 cm) at a room temperature of 20° C. Water was allowed ad libitum at all times. A standard diet (carbohydrate rich) providing 20% protein, 53.5% carbohydrate, 4.5% fat and 22% water, with added minerals and vitamins was available ad lib, unless otherwise stated. This diet was presented in the form of a bar which could slide easily through a dispensing tube attached to one of the walls of the cage. The bar could be removed from this dispenser after a meal and weighed without disturbing the animal. Practically no food was spilled.

Lights were on from 6 a.m. till 6 p.m. or from 2 a.m. till 2 p.m. depending on whether the experiments were made during the rats' day or night, respectively. A habituation period of ten days was allowed after the change in light regime before the experiments were resumed.

### Blood Sampling and Systemic Infusion Techniques

*General.* To resolve the problems stated in the introduction, techniques are required that do not disturb the animals. Therefore cannulas were inserted into the heart of the animals through the jugular vein using the technique described by Steffens [24]. When systemic infusions were performed the animals were provided with a double heart catheter [24] allowing continuous intravenous infusions and blood sampling from the freely moving unanesthetized animals. Here the method of Steffens [24] was modified in that the infusion cannula ended 3 mm downstream from the tip of the sampling cannula in order to minimize the risk of contamination of the blood sample with the infusion fluid. The swivel joint of Epstein and Teitelbaum [5] used by Steffens [24] was replaced by a very small swivel joint [27].

Rats were not used until they proved to be uninfluenced neither by the sampling or infusion procedure, nor by the presence of an experimenter. (It often took subjects a habituation period of about one week to reach this state.)

The experiments were performed while the animals remained in their normal cages. The rats' feeding behaviour has marked diurnal rhythmicity: by far the greater part of food intake takes place in the dark, but low light intensity and high activity levels of the subjects make blood sampling difficult at that time. Therefore night observations were restricted to the points of special interest.

During repeated sampling, stress on the rat due to loss of blood was avoided by transfusing after each sample fresh citrated blood taken from a donor rat by heart puncture and warmed to 39°C during 5 minutes.

### Glucose and Insulin Under Normal Conditions

Twelve male rats of 350 g were used for the daytime experiments and another group of nine for the night observations. The rats were prepared with heart catheters. Food was always available ad lib.

From the start of each observation blood samples were taken at 20 min intervals as long as the rat did not begin to eat. The start of the first meal was indicated as time zero. After this, samples were taken at +5, +15, +25, +35 and +45 min. The results obtained under the same conditions with different subjects and with the same subjects on different days were pooled. All determinations of a given substance falling within the period of  $5 \pm 4$  min before time zero were pooled and their average was termed the level of that substance at -5 min. Similarly the values for -25, -45, -65 and -85 min were obtained through pooling measurements falling within 8 minute periods surrounding these points in time.

Observations during day were started about 3 hr after the light went on and were continued if necessary until light out. At least one but more usually two intermeal periods were thereby obtained during each observation. Night observations lasted from light out until 3 or 4 hr after. Subjects usually took three to five meals during that time.

### Effects of Glucose Infusion

Six rats of about 350 g were provided with heart catheters. After the usual habituation period experiments were performed during the early hours of the night because the intermeal periods are short at this time. The start of the

first voluntarily taken meal in the night was always taken at time zero. The observation period lasted till the end of the second meal. Meal size, meal duration, and duration of the intermeal period were measured.

Three conditions were used: (1) no infusion during the intermeal period; (2) glucose infusion (10% glucose in 0.9% saline infusion (0.9% NaCl)).

Each animal was observed three times in Condition 1, twice in Conditions 2 and 3, the various conditions being presented in random order.

The infusion began always 2 min after the end of the first meal.

In the present experiments the end of the meal proved easily definable: once the rat turned away from the food bar for at least two minutes it never started feeding again until at least a quarter of an hour had elapsed. The infusion was continued till the end of the second meal. Two rates of infusion were used, 16.7 and 83.0  $\mu$ l per min (amounting in the case of glucose infusion to 1.67 and 8.3 mg of glucose per min respectively).

The blood samples were taken in a separate set of observations in order to avoid any risk of confusing the behavioral observations by the slight disturbance of the animal by the movements of the observer that are necessary for sampling.

Five rats of 350 g were provided with double heart catheters and were infused with 10% glucose at the same rates as used in the behavioural experiments. Blood samples were drawn at -10.0 (start of the infusion), 10, 20, 30, 40, 50, 60, 70, 80 (end of the infusion) minutes and examined for glucose and insulin. Two control experiments were included in which no infusion was performed and another in which a saline infusion of 83.0  $\mu$ l was given.

### Chemical Determinations

The blood samples were immediately chilled and centrifuged at 4°C. Blood glucose was measured by the ferricyanide method of Hoffman in a Technicon Autoanalyzer on a sample of 50  $\mu$ l blood. For insulin determinations the plasma samples were stored at -30°C. Plasma insulin was determined according to the method of Hales and Randle [6] using a rat standard insulin. The assays were performed, using an immunoassay kit (Radiochemical Centre Amersham). Duplicate assays were performed on 25  $\mu$ l samples of plasma. The reliability of the immunoassay can be judged from the mean standard deviation of duplicate binding percentages of 8 assays (20 samples each):  $1.18 \pm 0.08 \mu$ U/ml (Mean  $\pm$  SEM).

### Statistical Evaluation

For statistical evaluation of the results Student's one sample *t*-test was used unless otherwise stated. In the figures and tables, the mean values  $\pm$  SEM are presented.

## RESULTS

### Glucose and Insulin Under Normal Conditions

Figures 1 and 2 survey the average levels of insulin and glucose at various times during day and night respectively. These figures were constructed as follows. For each experimental condition the mean duration of the interval separating two meals was calculated. Each figure contains two meals separated along the abscissa by a distance corresponding to this mean interval. Averages of all data

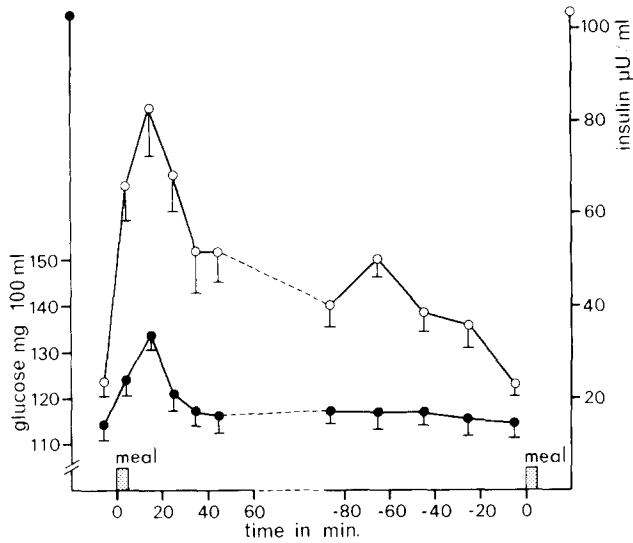


FIG. 1. Meal cycle. Day. ○—○—insulin, ●—●—glucose.

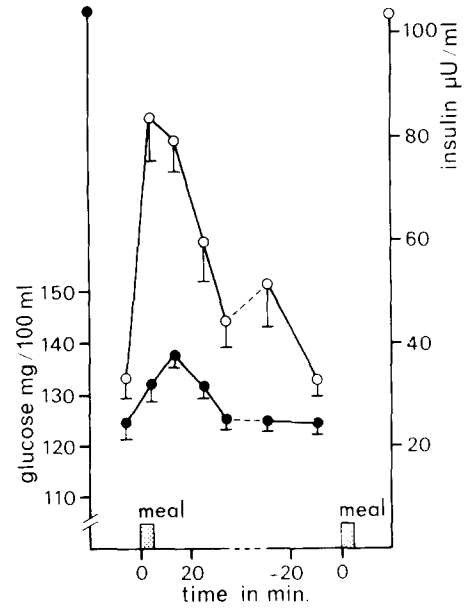


FIG. 2. Meal cycle. Night. ○—○—insulin, ●—●—glucose.

TABLE I

MEAL SIZE, MEAL DURATION AND DURATION OF MEAL INTERVAL IN DAY AND NIGHT

	Number of Animals Meals		Mean Duration Meals (min)	Size of Meals (g)	Duration of Meal Interval (min)
Day	12	18	4.3 ± 0.3	1.6 ± 0.1	180 ± 19
Night	9	26	4.9 ± 0.3	1.7 ± 0.1	61 ± 6

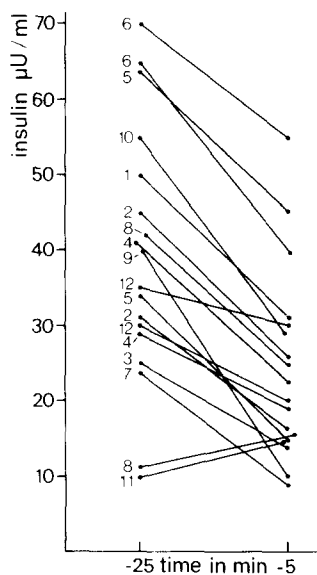


FIG. 3. The change in insulin level preceding individual meals. The figures denote individual rats. Meal during day. Change from -25 to -5 min.

obtained at time +5 till +45 are plotted to the right of the first meal, averages of all data for -5 till -85 are plotted to the left of the second meal in the figures. In this way the figures give a reasonable approximation of the course of glucose and insulin levels over the entire meal-to-meal cycle (see Table 1 for the duration of the intermeal intervals).

*After the Start of the Meal*

(a) *Glucose.* The meal causes a strong transient rise of the glucose level both during day (Fig. 1) and night (Fig. 2). Blood sugar concentrations reach a peak at about +15 min, and return to base line at about +35 min. During night, the amplitude of the peak is smaller, and its rising flank less steep.

(b) *Insulin.* Ingestion of a meal causes a rise in plasma insulin, but extent and time course of this rise are not the same during night and day. In the case of night meals (Fig. 2) insulin returns to the premeal level much sooner than in that of day meals (Fig. 1).

*Changes Before the Start of the Meal*

The premeal period is the special point of interest. As Figs. 1 and 2 show the glucose level does not significantly decrease during the last 30 min before the meal, but the insulin level is lower just prior to the meal (time -5 min) than at any other time since the start of the previous meal.

TABLE 2

EFFECTS OF GLUCOSE INFUSIONS ON MEAL SIZE, MEAL DURATION AND DURATION OF THE INTERMEAL PERIOD

	Meal I		Meal II		Duration of the Intermeal Period (min)	N
	Size (g)	Duration (min)	Size (g)	Duration (min)		
Without infusion	1.8 ± 0.2	3.5 ± 0.4	1.8 ± 0.2	3.5 ± 0.3	43.7 ± 3.3	18
Saline 16.7 μ l/min	2.0 ± 0.2	4.0 ± 0.3	2.2 ± 0.1	3.9 ± 0.3	50.0 ± 7.1	12
Glucose 1.67 mg/min	2.1 ± 0.2	3.8 ± 0.5	1.9 ± 0.1	3.4 ± 0.3	51.7 ± 4.4	12
Saline 83 μ l/min	2.0 ± 0.2	3.5 ± 0.3	1.9 ± 0.2	3.4 ± 0.4	50.9 ± 6.9	12
Glucose 8.3 mg/min	1.6 ± 0.2	3.1 ± 0.3	1.8 ± 0.2	3.0 ± 0.4	47.5 ± 4.4	12

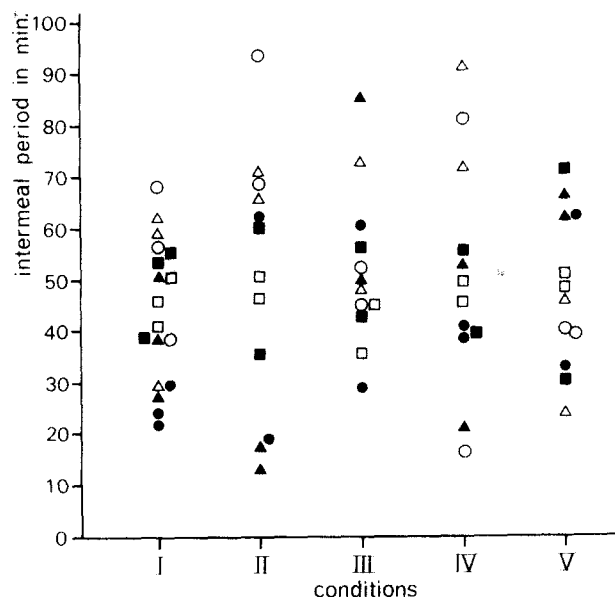


FIG. 4. Variation within and between individuals of the duration of the intermeal period. The conditions are I: no infusion, II: saline infusion 1 ml/hr, III: glucose infusion 1 ml/hr, IV: saline infusion 5 ml/hr, Glucose infusion 5 ml/hr. The subjects were indicated by a separate symbol.

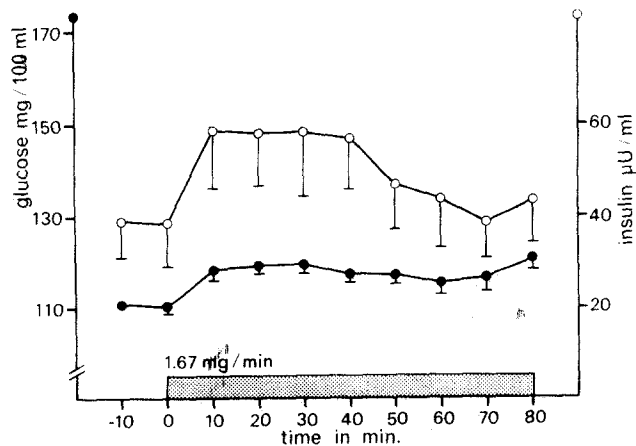


FIG. 5. Effect of glucose infusion (1.67 mg/min) on peripheral glucose and insulin levels. ○—○— insulin, ●—●— glucose.

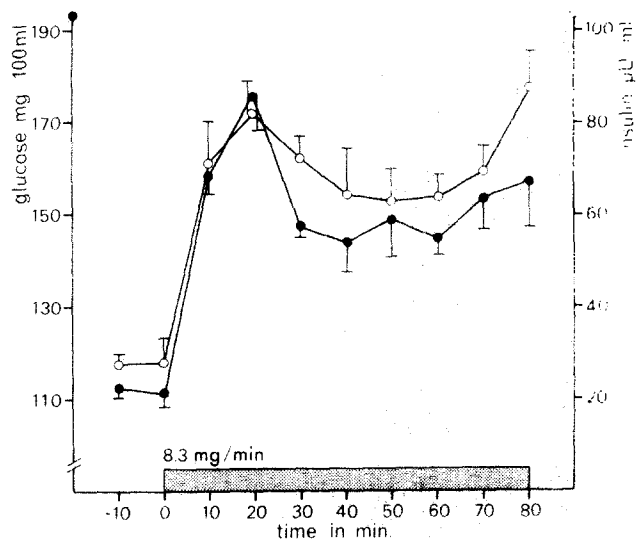


FIG. 6. Effect of glucose infusion (8.3 mg/min) on peripheral glucose and insulin levels. ○—○— insulin, ●—●— glucose.

( $p$  values for the differences between observations at 25 and -5 min are  $< 0.025$  and  $< 0.01$  respectively, sign. test). Figure 3 shows that also in the great majority of individual daytime meals insulin at time -5 is lower than at time 25. The same holds for night meals.

Although they do not bear on the main problem raised in this paper, yet some differences in blood levels of glucose and insulin among the experimental conditions are worth mentioning. There are differences in insulin levels at time -5 between day and night ( $22.9 \pm 2.9$  and  $32.4 \pm 3.0$   $\mu\text{U/ml}$   $p < 0.025$ ). The glucose level in the night at this time is also higher than in the day ( $124.5 \pm 2.9$  and  $114.2 \pm 2.8$  respectively  $p < 0.05$ ). An explanation of these differences is difficult to give but certainly the increase of food intake in the night will result in an increase of carbohydrate absorption by the intestines resulting in higher mean glucose levels than in the day time. This will then be responsible for the higher insulin levels [26].

#### Effects of Glucose Infusion

The results of these experiments are presented in Table 2 and Figs. 4 and 5. There was no significant difference in duration of the intermeal period between glucose and saline infusions. Further no reliable differences were observed between the two treatments in the amounts ingested in the second meal (Table 2). The variation both within and

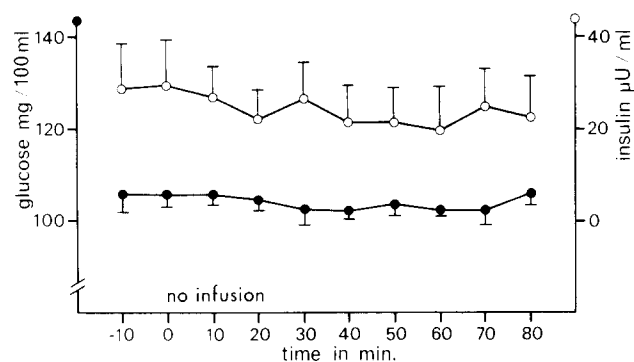


FIG. 7. Effect of sampling on peripheral glucose and insulin levels.  $\circ$ — $\circ$ —insulin,  $\bullet$ — $\bullet$ —glucose.

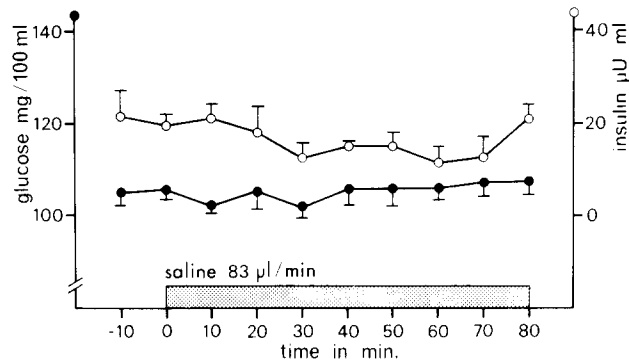


FIG. 8. Effect of saline infusion ( $83 \mu\text{l}/\text{min}$ ) on peripheral glucose and insulin levels.  $\circ$ — $\circ$ —insulin,  $\bullet$ — $\bullet$ —glucose.

between individuals of the duration of the intermeal period is presented in Fig. 4. This figure shows that the variation within individuals does not markedly differ from that between individuals for the different conditions investigated.

Figure 5 shows that the glucose and insulin levels during infusion of  $1.67 \text{ mg}/\text{min}$  glucose increase slightly and stay on a level just above the base line. Figure 6 shows that with a dose of  $8.3 \text{ mg}/\text{min}$  glucose and insulin increase during the first twenty minutes to relatively high levels, after which a certain stabilization takes place.

Figures 7 and 8 show the results of control experiments in which respectively no infusion and infusion of  $83 \mu\text{U}/\text{min}$  of saline was given. These figures show that there are no marked variations in glucose or insulin plasma levels.

#### DISCUSSION

For elucidating the control of food intake the signals causing both start and stop of meals must be clarified. However, only the start of meals will be considered here for the following reasons: (1) Termination of meals appears to be less precisely determined than their initiation [15,29]. (2) The blood changes induced by the meal are so rapid, and of so variable amplitude among individuals that it becomes impossible to say at what values precisely the meal stops (this problem is aggravated by the prevailing ignorance of the latency of the effect of the possible stop signals).

Figures 1 and 2 show that the mean insulin level is lower just before the meal (time  $-5 \text{ min}$ ) than at any other time since the start of the previous meal.

Does this mean that the falling insulin level controls the start of the meal? On this point doubt arises on consideration of the data for individual rats (Fig. 3). There is great variability between the individuals. These facts are not compatible with the view that a critical insulin level is the sole determinant of the initiation of feeding.

However, it remains possible that insulin is one of a complex of signals each more or less variable from one meal to the next, and that the value of this complex must fall to a critical level for feeding to begin. If so, manipulation of the duration of the insulin cycle should change the mean length of intervals between meals. Therefore we performed intravenous glucose infusions in order to keep insulin high during the intermeal period.

A few negative reports have been published on the effect of intraperitoneal or intravenous injection of significant

amounts of glucose in the rat [22] and the dog [7], respectively. However, these observations were restricted to day-to-day regulation. As regards meal-to-meal control, it has been reported that in man [3], dog [11] and monkey [2] a single intravenous injection of a large amount of glucose immediately before feeding does not depress food intake. Smith [22], however, reported a suppression of food intake when rats were infused through a jugular vein cannula while bar-pressing for food. Blood insulin was not measured in any one of these cases.

The present data show that glucose infusions do not in any way delay meals as compared with control infusions, although the concentrations of both glucose and insulin stay well above the levels seen at the start of meals under control conditions (especially during infusions of  $8.3 \text{ mg}$  glucose per min). Furthermore, the size of the second meal was not reliably influenced by the abnormal glucose or insulin levels before and during this meal.

The present experiments therefore do not support the view that in normal ad lib conditions insulin provides an important signal for the initiation of feeding. Perhaps the pattern of meals under these conditions may be governed mainly by signals of gastrointestinal origin. Feeding of bulk nondigestible material causes immediate satiation due to distention in various regions of the intestinal tract [12,13], and distention, acting through gastric stretch receptors, activates the central hypothalamic satiety mechanism [19]. That intestinal distention may be even more important than stomach filling was shown by Lepkovsky *et al.* [14] in experiments with parabiotic rats in which the digestive tracts were crossed surgically.

To sum up, the weight of evidence of our experiments as well as the publications cited above is that under normal ad libitum conditions glucose utilization signals play either no role at all, or only a minor role in the immediate causation of meals in the rat.

However, this does not imply that glucostatic mechanisms are of no value at all in nutritional homeostasis in this species. There are two points where they may be important. First there is convincing proof [4, 9, 25] that under conditions of dangerous hypoglycaemia glucostatic triggering of feeding behaviour acts as an emergency mechanism. Secondly, little as we know what signals are responsible for the control of feeding behaviour under normal ad libitum conditions, the fact that caloric balance is maintained on a wide variety of diets proves beyond doubt that the responsible signals are calibrated in terms of calories. It is

conceivable that glucose utilization signals provide the yardstick for this calibration. We are now investigating this possibility.

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