The effects of age, feeding pattern and sucrose on glucose tolerance, and plasma free fatty acids and insulin concentrations in the rat

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1. The effect of various dietary regimens imposed on young rats on growth, glucose tolerance and plasma insulin and free fatty acids (FFA) concentration has been examined.

2. In rats fasted for 16 h the increase in blood glucose concentration following an intraperitoneal dose of glucose was inversely related to the amount of food eaten in the previous 24 h.

3. Body-weight gain was greater and food conversion ratio was better in rats trained to eat their day's food in a short time (meal-eating) compared with rats eating the same amount of food continuously throughout 24 h.

4. Fasting plasma FFA concentrations decreased after the rats were about 33 d of age irrespective of feeding regimen.

5. In rats that had been on various feeding regimens for 25–40 d, the plasma insulin concentration, 15 min after an intraperitoneal dose of glucose, decreased with increasing time on the regimen in every instance except when the rats were restricted to one feeding period of 2 h each day, when the concentrations increased with time.

6. The glucose tolerance of meal-eating rats changed with increasing age, at first improving, then worsening.

7. Meal-eating rats were given sucrose or starch as the dietary carbohydrate. At first, feeding with starch gave a poorer glucose tolerance than feeding with sucrose, but in older rats the type of carbohydrate had no effect.

The results of a glucose-tolerance test in man or experimental animals can be affected by a large number of variables. Thus when it is required to determine whether a given dietary treatment (such as zinc deficiency; Quarterman & Florence, 1972) influences glucose tolerance it is necessary to take full account of those variables during the conduct of the experiment. Previous work we have undertaken led us to suspect that poor reproducibility of glucose-tolerance tests might be associated with inadequate control of experimental conditions. In addition, there are imperfections in the experiments of other workers who have examined the effects of feeding pattern on glucose tolerance and other criteria. For example, Leveille & Chakrabarty (1968) did not equate the daily food intake of the animals on the different feeding regimens or force-fed the meal-eating rats once a day to equate their food intake with rats eating *ad lib.* — Tepperman & Tepperman (1958) even trained rats to eat in 1 h as much food as *ad lib.* fed rats ate in a whole day.

We wished to investigate the effects of various feeding regimens in which daily calorie intake was always equated between experimental groups, but in which this calorie intake was below the voluntary intake. So, in the work with young rats now reported we have investigated the influence of the previous day's food intake, the

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feeding pattern, the age of the rat and the type of dietary carbohydrate on glucose tolerance and on plasma insulin and free fatty acid (FFA) concentrations.

EXPERIMENTAL

Animals: feeding methods

Hooded Lister (Rowett strain) rats were used in all the experiments. From weaning at 21 d of age they were given a purified diet containing: sucrose or maize starch 66%, spray-dried egg albumin 20%, arachis oil 10%, minerals and vitamins as described by Mills & Murray (1960) and Williams & Mills (1970).

For the feeding-pattern experiments the rats were given their food in one of five ways.

(1) *Ad lib.*, food always present in excess throughout the 24 h.

(2) One-meal, 6 g food given during one period of 2 h a day at 10.00 hours.

(3) Two-meal, 3 g food given during each of two periods of 1 h a day at 10.00 and 16.00 hours.

(4) Continuous-feeding, a restricted amount of food given uniformly for 24 h a day using the feeders described by Quarterman, Williams & Humphries (1970).

(5) Meal-eating, a restricted amount of food offered in a pot with no restriction of time.

One-meal and two-meal regimens were not imposed suddenly; ample time to consume their feed was given at first and gradually the durations of meals were reduced to the required lengths, usually within 8 d. The daily food intake of one-meal and two-meal rats was always 6 g. The restricted amount of food that was given to the rats on the continuous-feeding and meal-eating regimens was either 6 g or was a varied quantity which depended on the fact that the rats were the pair-fed partners of Zn-deficient rats.

This meal-eating regimen represents a fairly typical situation in experiments of the 'pair-feeding' type in which small and highly variable quantities of food have to be presented to control subjects. In this instance the mean daily intake was 5·6 g, ranging from 0 to 10 g on different occasions, which is the voluntary food-intake pattern exhibited by Zn-deficient rats (Chesters & Quarterman, 1970).

Glucose-tolerance tests

Rats were starved for 16–18 h before a glucose-tolerance estimation. They were anaesthetized with pentabarbitone sodium and samples of blood for the estimation of glucose were taken from the tail. Glucose was given by intraperitoneal injection, 175 mg/100 g body-weight. We chose to inject glucose intraperitoneally for the following reasons. The tail vein was used for sampling of blood, the use of femoral veins would have interfered with repetition of glucose-tolerance tests at weekly or fortnightly intervals, and oral dosing was not used because of variation in gut size and glucose transport with different feeding patterns. The intraperitoneal injection had a rapid effect on blood glucose, could be repeated at frequent intervals and gave reproducible results. Blood samples (0·03 ml), taken for glucose estimation, were added to 0·3 M-perchloric acid and analysed by the glucose oxidase method of Bergmeyer & Berut (1963).

FFA determinations and insulin assay

Rats were starved and anaesthetized as for the glucose-tolerance estimations. Blood was taken by heart puncture or from the tail and centrifuged with heparin. The concentrations of FFA in the plasma were determined by the micro-method of Laurell & Tibbling (1967), using 0·5 ml plasma. Plasma FFA levels have been reported to be unaffected by anaesthesia (Schotz & Olivecrona, 1966).

Blood for plasma insulin determination was taken by heart puncture 15 min after the rats were given an intraperitoneal dose of glucose, 175 mg/100 g body-weight. Insulin was estimated by the radioimmunoassay method of Hales & Randle (1963) using ^{125}I-labelled insulin and insulin antiserum supplied by the Radiochemical Centre, Amersham, Bucks.

Expt 1. Effect of size of previous day's feed on glucose tolerance

Ten rats were given the experimental diet with sucrose as the source of carbohydrate in one experiment and, in another experiment, ten rats were given the same diet but with starch as the carbohydrate source. The food was given on a meal-eating regimen to five rats on each experiment and on a continuous-feeding regimen to the other five, from weaning until the rats were about 55 d of age. At the end of this period the rats were given widely differing amounts of food during 1 d, then starved for 16 h before their tolerances to an intraperitoneal dose of glucose were determined. The amounts of food given were determined by the fact that these rats were pair-fed with Zn-deficient rats.

Expt 2. Effects of feeding pattern on body-weight gains

In two experiments with eight rats in each of three groups with different feeding patterns, the body-weight gains were observed and the food-conversion ratios calculated. All rats were given 6 g food/d except those fed *ad lib.*

The feeding patterns of the rats on the one-meal, the two-meal and the continuous-feeding regimens were defined by the conditions imposed on them. A feeding pattern was not, however, imposed on the meal-eating rats, which received their day's feed all at one time – the normal practice when control animals are pair-fed with animals whose feed intake is reduced by the experimental treatment. After a few days of adaptation, about half of the meal-eating rats ate their feed in 2 h and about 90% of them finished in 4 h.

Expt 3. Effect of feeding pattern on plasma FFA concentrations

Continuously fed and meal-eating rats at various ages were starved overnight before the plasma FFA concentrations were determined.

Expt 4. Effect of feeding pattern on plasma insulin concentrations and glucose tolerance

Samples of blood were taken for insulin assay from rats of various ages on various feeding regimens.

Glucose tolerances were determined at the 64th day of age on some of the rats used

for insulin assay. They were all given, and ate, 6 g food/d, including the day before starvation and glucose-tolerance determination.

Expt 5. Effect of age and type of carbohydrate on glucose tolerance

In this experiment there were two groups, each of six rats, which were on a meal-eating regimen and received a reduced food intake because they were individually pair-fed with Zn-deficient rats. One group was given the usual purified diet with 66% sucrose and the other group had the same diet but with maize starch replacing the sucrose. The tolerance of each rat to an intraperitoneal dose of glucose was determined, at intervals of about a week, from 6 d after weaning (27 d of age) on to the purified diet until they were about 60 d old.

RESULTS

Expt 1. Effect of size of previous day's feed on glucose tolerance

The experiments with starch and sucrose gave substantially the same results. Fig. 1A and 1B show the tolerance curves obtained with the nine surviving sucrose-fed rats and the ten starch-fed rats and the amount of food eaten on the last day by each rat. The height of the blood-glucose curves and more especially the concentration at 30 min after dosing were inversely proportional to the amount of food eaten during the 24 h before the beginning of the starvation period, i.e. from 48 to 24 h before the determination of the glucose tolerance.

Expt 2. Effects of feeding pattern on body-weight gains

The weight gains of rats in the one-meal, two-meal and meal-eating groups were greater than the gains of rats given the same amount of food continuously (Table 1). The food-conversion ratios are also given in Table 1 and reflect the weight gains of the groups since in each experiment the food intakes of each group were the same (except that of the group fed *ad lib.* in Expt 2(*b*); see footnote to Table 1).

Expt 3. Effect of feeding pattern on plasma FFA concentrations

The results of some observations on the concentrations of plasma FFA in rats fasted overnight are shown in Fig. 2. Up to about 12 d after weaning, 33 d of age, all the rats had concentrations of about 650–750 μequiv. FFA/l in the plasma. After this time the concentration fell in continuously fed rats to 550–700 μequiv./l and in blood of meal-eaters to about 350–450 μequiv./l.

Expt 4. Effect of feeding pattern on plasma insulin concentrations and glucose tolerance

The results of the plasma insulin assays are shown in Fig. 3. The blood samples were taken 15 min after an intraperitoneal dose of glucose to allow time for the secretory response of the pancreas to the glucose to occur. For the continuous-feeding, meal-eating and two-meal rats there was a general increase in response up to about

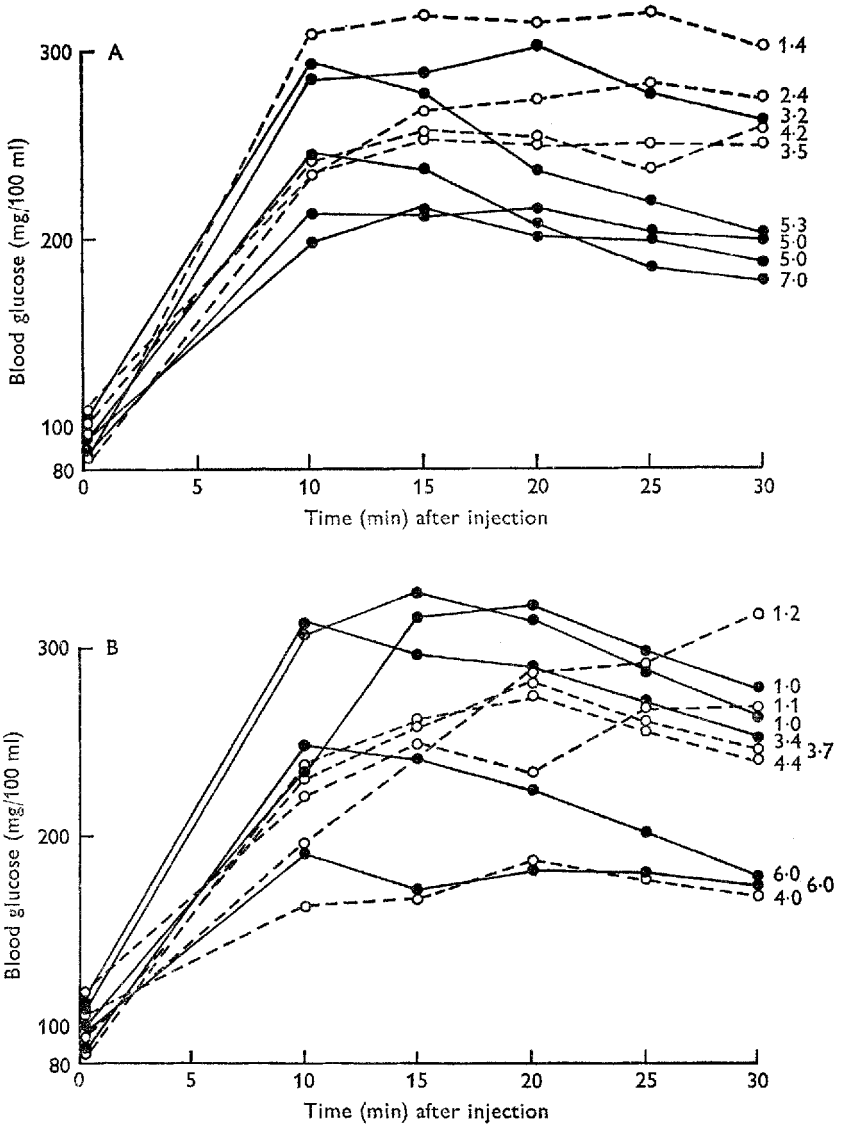

Fig. 1. Changes in tail-blood glucose concentration after intraperitoneal administration of glucose (175 mg/100 g body-weight) to meal-eating (●—●) and continuous-feeding (○—○) rats. Each rat ate the amount of food (g) indicated before it was starved for 16 h and the glucose tolerance determined. In A the rats received a sucrose-based diet and in B a starch-based diet (Expt 1).

48 d of age and then a decrease. This conforms with the observed changes in glucose tolerance with age shown in Fig. 4.

The only effect of feeding pattern is seen in the one-meal rats, in which there was an increase in insulin secretion with increasing age, i.e. with increasing time on this particular feeding regimen.

The results of glucose-tolerance tests are shown in Table 2, where it is seen that the

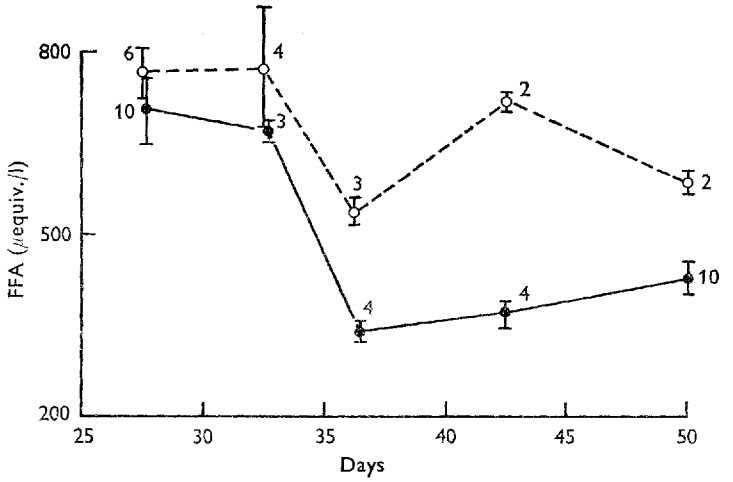

Fig. 2. Plasma free fatty acid (FFA) concentrations in meal-eating rats (●—●) and continuous-feeding rats (○—○), showing the changes with increasing age and time on the feeding regimens. The vertical bars represent the standard errors of the mean. The figures show the numbers of rats used (Expt 3).

Table 1. *Weight gains (g) and food conversion ratios (FCR, g food eaten/g body-weight gain) over 21 d of rats subjected to different feeding regimens (Expt 2)*

(Mean values with their standard errors for eight rats)

	Expt (*a*)†		Expt (*b*)†	
Feeding regimen	Wt gain	FCR	Wt gain	FCR
One-meal	21·0 ± 3·7	5·6 ± 0·5	—	—
Two-meal	23·1 ± 2·8	4·5 ± 0·8	—	—
Continuous-feeding	11·8 ± 1·8	9·5 ± 0·9	15·7 ± 1·8	6·8 ± 0·6
Meal-eating	—	—	24·8 ± 3·4	4·3 ± 0·5
Ad lib.	—	—	89·0 ± 9·7	2·8 ± 0·1

† In Expt (*a*) the 21 d experimental period began at the end of the 8 d adaptation (see p. 64). In Expt (*b*) the period began at weaning; no adaptation period was required.

one-meal rats had better tolerances than the two-meal rats and the one meal-eating rat. This may correlate with the higher concentrations of insulin observed in the one-meal rats.

Expt 5. Effect of age and type of carbohydrate on glucose tolerance

The glucose tolerance changed with the age of the rat. The mean glucose-tolerance curves are shown in Fig. 4 for sucrose-fed rats in the age ranges 27–36 d, 37–46 d and 47–64 d. From the first to the second period there was a tendency for the tolerance to improve though the curves were not significantly different at any time. From the second to the third period there was a large and very significant worsening of the glucose tolerances. The same changes with age were observed in other experiments, when the feed was given on a continuously fed basis, on an *ad lib.* regimen, or meal-fed as in the original experiment and with starch or sucrose as the sole source of carbohydrate (Table 3). The mean fasting blood-glucose concentration was higher in the older rats in every feeding-pattern group, though never significantly so.

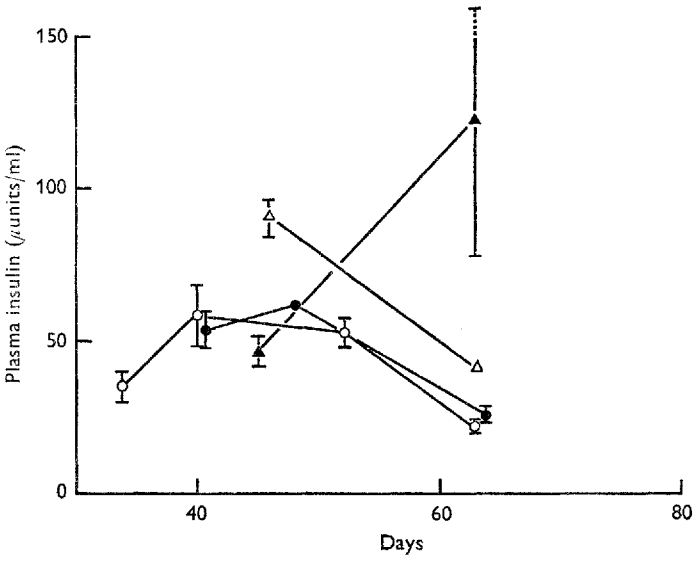

Fig. 3. Changes in plasma insulin response to intraperitoneal administration of (175 mg/100 g body-weight) glucose with increasing age and time on feeding regimen of continuous-feeding (○—○), meal-eating (●—●), two-meal (△—△) and one-meal (▲—▲) rats. Two to four rats were used; the vertical bars represent the standard errors of the mean – where no standard error is shown there was only one rat (Expt 4).

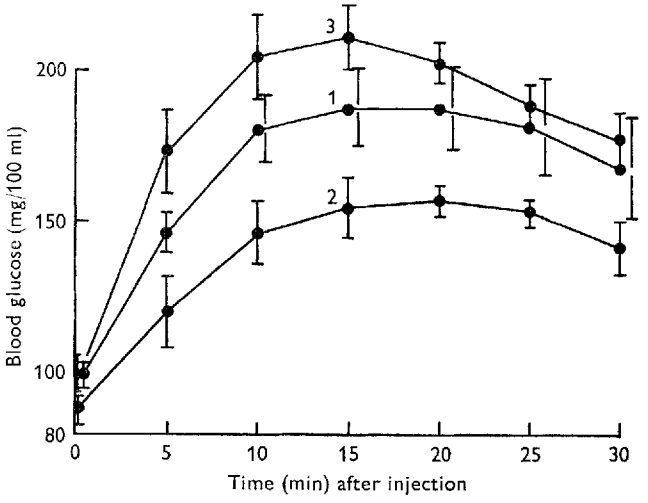

Fig. 4. Changes in tail-blood glucose concentration after intraperitoneal administration of glucose (175 mg/100 g body-weight) to six sucrose-fed meal-eating rats at 27–36 (1), 37–46 (2) and 47–64 (3) d of age. The vertical bars represent the standard errors of the mean: curves 2 and 3 differ significantly at all times except 0 min (Expt 5).

Table 2. *Tail-blood glucose concentrations at different times after intraperitoneal administration of glucose* (175 *mg*/100 *g body-weight*) *to* 64-*d-old rats given* 6 *g of sucrose-based diet per d by different feeding patterns*

Feeding pattern (sec p. 64)	No. of rats per group	Glucose concentration (mg/100 ml) at: 0 min	10 min	20 min	25 min	30 min
One-meal†	3	90 ± 13	182 ± 26	173 ± 9**	167 ± 10*	163 ± 12
Two-meal†	3	78 ± 13	244 ± 26	239 ± 9	216 ± 10	203 ± 12
Meal-eating	1	74	271	247	235	223

Significance of difference between blood-glucose concentrations at each time between the one-meal and two-meal feeding patterns: *$P < 0.05$; **$P < 0.01$.

† Mean values with their standard errors.

Table 3. *Tail-blood glucose concentrations at different times after intraperitoneal administration of glucose* (175 *mg*/100 *g body-weight*) *to rats of two age-groups given one of two diets by various feeding patterns* (*Expt* 5)

(Mean values with their standard errors)

Diet and feeding pattern	Age (d)	No. of rats per group	Glucose concentration (mg/100 ml) at: 0 min	10 min	20 min	25 min	30 min
Sucrose diet, *ad lib.*	37–46	3	63 ± 6	215 ± 21	197 ± 21	170 ± 23	149 ± 22
	47–64	8	78 ± 4	258 ± 13	256 ± 13*	233 ± 14*	206 ± 14
Sucrose diet, continuous-feeding	37–46	5	82 ± 13	152 ± 16	167 ± 9	154 ± 13	155 ± 10
	47–64	3	90 ± 10	195 ± 12	203 ± 7*	196 ± 10*	189 ± 8*
Starch diet, *ad lib.*	37–46	4	71 ± 7	218 ± 24	231 ± 16	206 ± 17	190 ± 15
	47–64	8	81 ± 5	272 ± 17	240 ± 11	218 ± 12	195 ± 10
Starch diet, meal-eating	37–46	4	87 ± 19	150 ± 36	150 ± 4	147 ± 6	142 ± 9
	47–64	5	90 ± 17	164 ± 32	202 ± 4**	193 ± 5**	177 ± 8*
Starch diet, continuous-feeding	37–46	4	94 ± 10	179 ± 23	178 ± 18	179 ± 22	153 ± 16
	47–64	4	110 ± 10	256 ± 23*	236 ± 18	256 ± 23*	222 ± 16*

Significant differences between age groups are indicated: *$P < 0.05$; **$P < 0.01$.

Comparison of results for the appropriate *ad lib.* or continuous-feeding regimens suggests that the glucose tolerance was poorer in those rats given the starch diet than in the rats given the sucrose diet in the youngest age-group, 27–36 d. In the older groups no effect attributable to carbohydrate source was observed. In a further experiment in which the two diets were given *ad lib.* a similar and significant effect of starch was observed in younger rats aged 20–40 d, but not in older rats 40–70 d of age (Fig. 5).

DISCUSSION

Effect of previous day's food intake on glucose tolerance

It is usual in the determination of glucose tolerances for the experimental subject to be starved overnight and the test made on the following morning, the assumption being that any influence of food intake on the previous day will be negligible when the previous daily average calorie and carbohydrate intakes do not differ between the groups being compared. In our experiments with young rats, this influence was not

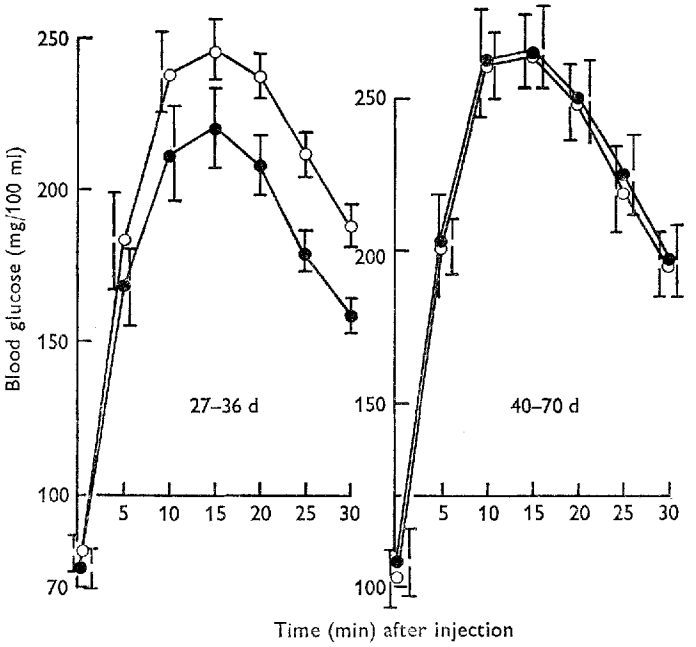

Fig. 5. Changes in tail-blood glucose concentration after intraperitoneal administration of glucose (175 mg/100 g body-weight) to rats fed *ad lib.* on diets based on starch (○) or on sucrose (●) at two age-ranges, 27–36 d and 40–70 d. In the younger group the difference between the curves was significant ($P < 0.05$) after 15 min (Expt 5). The vertical bars represent the standard errors of the mean.

negligible and the amount of food eaten during the day preceding an overnight starvation had a very large influence on glucose tolerance. This was so whether the food was given on a meal-eating or a continuous-feeding regimen.

Effects of feeding pattern

These experiments with various feeding patterns were made because in any experiment in which one group of animals suffers a reduction in food intake as a consequence of the imposed treatment there is a problem about the way in which the control animals should be fed. They can be given their food to appetite, in which event they will eat more than experimental animals, or they can be pair-fed with the experimental animals, in which event they will finish their food sooner and so develop a different feeding pattern. With either procedure the difference in feeding regimen is likely to produce metabolic changes which are not related to the process under observation (Fábry, 1967). This situation arose in our own studies with Zn-deficient rats, in which there was a great reduction in appetite. Pair-fed control rats ate their Zn-supplemented diet in a short time and developed physiological changes characteristic of meal-eaters in contrast to the continuously feeding deficient rats.

Effects of feeding pattern on lipogenesis and body-fat accumulation have been reported (Leveille & Chakrabarty, 1968; Cohn & Joseph, 1959), but reports of the effects on plasma FFA concentrations are contradictory (Florence & Quarterman, 1969; Leveille & Chakrabarty, 1968; Young, 1968) and could have been influenced by

differences in the amounts of food eaten during the 24 h before the blood sample was taken. However, the changes of the plasma FFA concentrations with time may be of interest (Fig. 2). In young rats when the fasting levels are high there is no effect of feeding pattern, but after 16 d on the respective dietary regimens the differences appear. It cannot be decided from this experiment whether the change is due to age or to length of time on the feeding regimen. Other workers (Tepperman & Tepperman, 1958; Fábry, 1967; Cohn & Joseph, 1960; Leveille & Chakrabarty, 1968) have found that 2–3 weeks are required for adaptation to feeding pattern to occur, and the same time has been found for changes in liver pentosephosphate pathway activity (R. B. Williams, personal communication) and [^{14}C]acetate incorporation into liver lipids (Kitaka and Quarterman, unpublished observations) to occur.

The changes occurring in plasma insulin concentration with length of time on the diet (Fig. 3) are not affected by the size of the previous day's feed, since this was standardized in this experiment at 6 g for all groups except the continuously fed group. (Only 2 g food became accessible to this last group from the continuous feeders during the 8 h period before food was withdrawn from all groups.) The changes of plasma insulin concentration with age probably depend on the strain of rat used. Berdanier, Marshall & Moser (1971) have shown that between 50 and 300 d of age the serum immunoreactive insulin in Wistar rats, starved for 16 h and not given glucose before blood samples were taken, increased from 15 to 39 units/ml whereas in an inbred strain it decreased from 154 to 26 units/ml.

Factors affecting glucose tolerance

We have previously made a number of observations on the effect of feeding pattern on glucose tolerance but many of these, involving comparison with a continuously fed group (Florence & Quarterman, 1969), were subsequently invalidated by the finding that the size of the previous day's food intake has an overriding influence on glucose tolerance. The imposition of different feeding patterns on an animal leads necessarily to the consumption of different amounts of food before an overnight starvation. Thus animals feeding continuously, either because they are given food *ad lib.* or because they are fed by a continuous feeder (Quarterman *et al.* 1970), receive their food from the time of the morning feed until food is withdrawn before starvation, usually for about 8 h, and thereby consume about a third of their day's food. In this laboratory, rats given food *ad lib.* ate throughout the whole period of 24 h. They tended to eat more at night per h but at no time went for more than 2 h without eating (J. K. Chesters, unpublished observations). Meal-eating rats, such as the pair-fed controls in our experiments and those of Macapinlac, Pearson & Darby (1966), soon adapt themselves to eating the whole of their day's food in much less than 8 h. Of the three groups given their food in a restricted time, there was a tendency for the one-meal group to have a better glucose tolerance, which is compatible with the increased insulin release following a glucose challenge in this group.

Continuous feeding has been reported to cause a deterioration of glucose tolerance in rats (Leveille & Chakrabarty, 1968) but in humans more frequent feeding improved the tolerance (Fábry, Fodor, Hejl, Braun & Zvolánková, 1964; Gwinup, Byron,

Roush, Kruger & Hamwi, 1963; Young, 1968). However, these results could also have been affected by the size of the previous day's intake.

The worsening of glucose tolerance after the 36th day of age could be related to the observed smaller release of insulin after an intraperitoneal glucose challenge. Klimas (1968) has reported a similar trend during the first 6 months of life in the rat and we were able to confirm his observation that there is a tendency for the fasting blood-glucose concentration to increase with age. In his experiments with orally administered glucose he found a more rapid rise and fall of plasma glucose in very young rats (less than 1 month old). This may be related to the higher glucose-tolerance curves we have observed in the youngest group of rats (Fig. 4).

In contrast to these observations on rats, the rate of disposal of injected glucose in dogs and pigs increases with age (Heard & Henry, 1969). This increased rate of disposal of glucose cannot be explained in terms of increased insulin levels.

In children (Loeb, 1966) there is an opposite sequence of changes to those of the rat. After 9 d of age there is a deterioration of glucose tolerance followed by a steady improvement up to 12 months of age. The fact that such rapid changes with age occur in young rats has to be borne in mind in metabolic studies lasting more than a day or two. Similar rapid changes with age in [^{3}H]thymidine incorporation into DNA of some tissues has been observed in rats of the same age-range (Williams & Chesters, 1970; Winick & Noble, 1965).

The effect of sucrose compared with starch on glucose tolerance is different from that reported by Cohen & Teitelbaum (1964). However, many other factors are involved (Al-Nagdy, Miller & Yudkin, 1970), including, if the effect of sucrose is on circulating insulin levels as Szanto & Yudkin (1969) believe, the strain of rats used (Berdanier *et al.* 1971).

The object of this work was to define some of the variables affecting glucose tolerance and related physiological measurements, so that when a condition which may alter glucose tolerance is imposed on an animal, it would be easier to avoid errors in the design of experiments to test the effect of the condition and make it possible to compare validly the results of experiments conducted in different ways. From the results we have obtained, it seems that two groups of rats subjected to different treatments must otherwise be similar in age, diet, feeding pattern, length of time on the particular feeding pattern and actual level of food consumption, especially on the day or days preceding the experiment.

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