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EFFECTS OF BRL 26830, A NOVEL β-ADRENOCEPTOR AGONIST, ON GLUCOSE TOLERANCE, INSULIN SENSITIVITY AND GLUCOSE TURNOVER IN ZUCKER (fa/fa) RATS

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Abstract—Zucker fa/fa rats exhibit glucose intolerance in comparison with lean Fa/? littermates. A single acute dose of BRL 26830 (2.9 mg/kg p.o.) improved glucose tolerance in Fa/? littermates but exacerbated glucose intolerance in the fa/fa rats. This latter effect occurred in spite of an increase in the plasma insulin concentration. Chronic treatment of Zucker fa/fa rats with BRL 26830 (2.9 mg/kg) for 24 days or more produced a significant reduction in the area under the glucose tolerance curve. In addition, the glucose decay rate (k%) following the administration of insulin intravenously was significantly increased in the BRL 26830-treated rats suggesting that tissue insulin sensitivity was increased. Glucose turnover measurements show that chronic treatment of Zucker fa/fa rats with BRL 26830 produced a significant increase in the rate of glucose utilization integrated over a 3 hr period, but this increase was, in part, off-set by an increase in the endogenous rate of glucose production. The ultimate fate of the extra glucose that is metabolized is not known but it is suggested that it might be used to support the thermogenic response that is also activated by BRL 26830.

Epidemiological studies have provided ample evidence of a link between the incidence rates of diabetes and obesity [1]. Analysis of clinical records [2] suggest that the development of diabetes is often secondary to the onset of obesity. In addition, excess body weight (or overnutrition) [3] precipitates the manifestations and worsens the prognosis of diabetes. Two characteristic features of human obesity are hypersecretion of insulin [4] and resistance to insulin action [5, 6]. The resistance to insulin action is further exacerbated by the presence of diabetes [7]. The interpretations of measurements of fasting insulin concentrations and insulin secretagogue responses to glucose in diabetes are controversial, owing, in part, to a failure to distinguish between patients having pancreatic β-cell defects of different severity [8]. Thus, patients with impaired glucose tolerance typically have an elevated fasting plasma insulin concentration and an exaggerated insulin response to glucose. In contrast, patients with overt diabetes have either normal or high fasting plasma insulin concentrations but have a reduced insulin secretagogue response to a glucose load [9].

The Zucker (fa/fa) rat is an animal model [10] of spontaneous obesity associated with which are hyperinsulinaemia and moderate glucose intolerance. An increase in the serum insulin concentration is one of the earliest signs that distinguishes the pre-obese (fa/fa) rats [11] from their lean littermates. This hyperinsulinaemia persists well into adult life and is associated with a gradual development of insulin resistance (see [12] for review).

BRL 26830 is a novel compound that, through its de-esterified metabolite BRL 28410, directly and selectively stimulates the lipolytic β-adrenoceptors in brown [13] and white adipose tissue [14]. It also increases the metabolic rate, thus decreasing adiposity in obese mice and rats [13, 15]. In addition, BRL 26830 stimulates insulin secretion and increases glucose utilization [16] in lean normoglycaemic rats. The present paper describes the effects of BRL 26830 on glucose tolerance, insulin sensitivity and glucose turnover in lean and obese Zucker rats.

MATERIALS AND METHODS

BRL 26830. BRL 26830, (*R**,*R**)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl) amino] propyl]-benzoate, was prepared in these laboratories as the (E)-2-butendioate (2:1) salt. It was dissolved in water for administration by oral gavage and in saline for intraperitoneal administration.

Animals. Zucker obese (fa/fa) and lean (Fa/?) rats were obtained from Olac 1976 (Bicester, Oxon, U.K.). They were maintained on Oxoid rat and mouse breeders diet (H. C. Styles, Bewdley, Worcs., U.K.) under controlled temperature (23 ± 1) and lighting conditions (lights on 06.00–18.00 hr). Rats were housed in pairs.

Groups of rats were dosed with vehicle or BRL 26830 once per day between 10.00 and 12.00 hr. The dose levels used in lean and obese littermates were chosen to be approximately equivalent on a fat-free body mass basis. Thus, lean rats received 4.6 mg/kg and obese rats 2.9 mg/kg. On a whole animal basis, these dose levels provided an equivalent intake of compound to each genotype. During the repeat dose studies, the rats were weighed weekly in the fed state between 10.00 and 12.00 hr.

Glucose tolerance test. The rats were fasted for

24 hr prior to a glucose tolerance test, which commenced between 09.00 and 11.00 hr. BRL 26830 or dosing vehicle (2 ml/kg) was administered 30 min before a subcutaneous glucose load. The glucose loads (1.2 g/kg to lean rats and 0.75 g/kg to obese rats) were chosen to be approximately equivalent on a fat-free body mass basis and provided a similar load of glucose to both lean and obese rats. Blood samples (10 μl) for the analysis of glucose were collected from the cut end of the tail.

Insulin sensitivity test. Rats were fasted for 5 hr from 08.00 hr and then they were temporarily anaesthetized and a small incision made to expose the saphenous vein. Insulin (Actrapid, Novo Pharmaceuticals, 2 units/kg body wt) was injected into the vein and the incision closed with a suture clip. Blood samples were taken serially from the tail immediately prior to giving the insulin injection and at 5-min intervals thereafter for 30 min. The glucose decay rate (% per min) [17] was calculated between 5 and 15 min after insulin injection.

Insulin secretion studies. Obese rats were fasted for 24 hr and then anaesthetized with sodium pentobarbitone (60 mg/kg i.p.) and a carotid artery cannulated. Ten minutes after installation of the cannula, which was kept patent with heparinized saline, a 0.5 ml blood sample was taken. All rats then received BRL 26830 (2.9 mg/kg i.p.) and further arterial blood samples were obtained at 5, 30 and 60 min. The plasma was stored at −20° prior to assay for insulin content.

Glucose turnover determination. Male fa/fa rats, which had been chronically-treated with BRL 26830, were fasted for 24 hr, and then anaesthetized with sodium pentobarbitone (60 mg/kg i.p.). A small incision was made to expose the saphenous vein and a cannula introduced. The rats were given a priming dose of D-[6-^{3}H]-glucose (2 M: 100 μCi/ml: 1 ml/kg i.p.) together with a continuous intravenous infusion of D-[6-^{3}H]-glucose (1 M: 10 μCi/ml at the rate of 16 μCi/hr). This regime was designed to raise acutely and maintain the blood glucose concentration of control rats at about 10 mM. Thiry minutes after commencing the infusion, the rats were given either BRL 26830 (5.8 mg/kg) or saline (1 mg/kg) by intraperitoneal injection. For the determination of blood glucose specific radioactivity, blood samples (0.05 ml) were obtained from the tail at 15 min intervals for 225 min and immediately deproteinized with 0.75 ml perchloric acid (2%; v/v). Precipitated protein was removed by centrifugation (10,000 *g*; 5 min) and 0.6 ml of supernatant was applied to the top of a mixed bed ion-exchange column (1 g of Amberlite CG 120 (Na^+) above 1 g of Amberlite CG 400 ($HCOO^-$) contained in a disposable Pasteur pipette). Glucose was eluted from the column with 3 ml H_2O. After removal of 0.5 ml of eluate for determination of glucose concentration [18], the remainder was lyophyllized. [^{3}H]-Glucose radioactivity was determined after reconstitution of the residue in 0.2 ml H_2O and the addition of 3 ml Scintillation Cocktail "T" (B.D.H., Poole, Dorset, U.K.).

The rate of appearance of glucose (Ra) and the rate of utilization of glucose (Rd) were calculated using the non-steady state method of Steele [19]. Metabolic clearance rate for glucose is given by Rd/blood glucose concentration.

Analytical techniques. Blood glucose was determined by the hexokinase assay [18] (Boehringer Mannheim) using a Corona Clinicon analyzer (B.C.L., Lewes, U.K.). The area under the glucose tolerance curve was calculated trigonometrically.

The insulin content of plasma samples was determined by a double antibody radioimmunoassay technique [20] using human insulin as standard.

Statistics. Results are given as mean ± S.D. The significance of differences between means was determined using Student's *t*-test for independent samples.

RESULTS

Obese Zucker rats showed mild glucose intolerance relative to lean littermates (Fig. 1). BRL 26830, given acutely to lean rats produced a significant reduction in the area under the glucose tolerance curve (Fig. 1, upper panel). In contrast, the acute administration of BRL 26830 to fa/fa rats have resulted in a further exacerbation of the existing glucose intolerance.

The daily administration of BRL 26830 (2.9 mg/kg i.p.) to glucose intolerant obese rats for a period

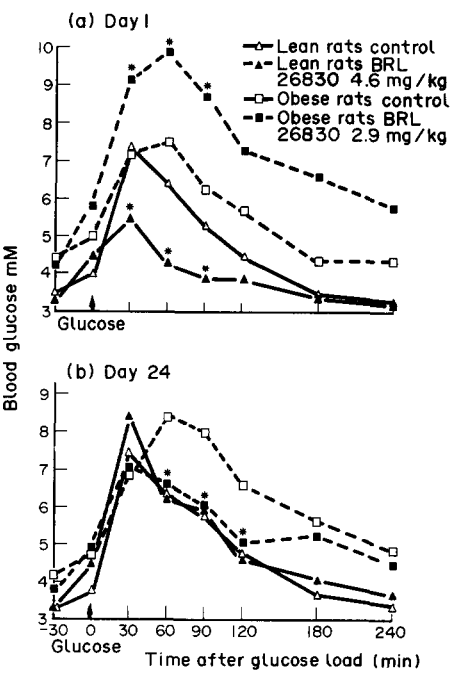

Fig. 1. Acute and chronic effects of BRL 26830 on glucose tolerance in Zucker fa/fa rats and their lean littermates. Zucker (fa/fa) rats (500–600 g) and their lean littermates were fasted for 24 hr prior to receiving either BRL 26830 or water. Thirty minutes later the rats were given glucose subcutaneously (panel A). After the glucose tolerance test, the rats were re-fed and dosed once daily with their respective treatment for 24 days. Glucose tolerance was then redetermined (panel B). Results are the mean of 6 rats; S.D. has been omitted for visual clarity. Significant differences from control rats of same genotype are indicated by * $P < 0.01$.

of 24 days resulted in a significant improvement in glucose tolerance (Fig. 1, lower panel). However, the blood glucose profile of the lean littermates that had been treated similarly with BRL 26830 was now identical to that of the lean control rats.

The acute and chronic effects of BRL 26830 on insulin secretion in anaesthetized obese rats are shown in Table 1. Control rats had marked fasting hyperinsulinaemia (range 20–85 ng/ml) but, nevertheless, the acute administration of BRL 26830 increased the circulating insulin concentration (range 76–174 ng/ml at 30 min and 149 to >174 ng/ml at 60 min). In spite of this increase in plasma insulin, the blood glucose concentration increased slightly although not significantly during the course of the experiment. Chronic treatment with BRL 26830 did not affect either the fasting insulin concentration (range 19–46 ng/ml) or the secretagogue responses to a further injection of BRL 26830 (range 149 to >174 ng/ml at 60 min post-BRL 26830). In chronically-treated rats, the fasting blood glucose concentration was 6.9 ± 0.5 mM and this was reduced to 6.2 ± 0.4 mM at 60 min after the injection of BRL 26830 ($P < 0.05$).

The intravenous insulin tolerance test was used as an index of the overall tissue sensitivity to insulin. Chronic treatment with BRL 26830 resulted in a significant increase in the rate of insulin-stimulated glucose disposal from 2.3 ± 1.1% per min ($N = 8$) to 5.2 ± 1.1% per min ($N = 7$); $P < 0.001$. This suggests that insulin sensitivity is increased. Such increases in insulin sensitivity can also be achieved by weight reduction. However, during the present study, the overall body weights of the two groups of rats were not significantly different (624 ± 40 g for controls; 588 ± 63 g for the BRL 26830-treated rats) and the correlation between body weight and rate of glucose decay was not significant. However, there was a significant difference in the weight gain of the rats during the treatment period (58 ± 13 g in the control group; 23 ± 15 g in the BRL 26830-treated group; $P < 0.001$). Thus, the increase in insulin sensitivity may relate more to alterations in weight gain rather than to the absolute body mass.

Table 1. Effect of BRL 26830 on insulin secretion in 24-hr-fasted Zucker fa/fa rats

	Plasma insulin (ng human equiv./ml)	
Chronic treatment:	Water	BRL 26830
Time after acute treatment with BRL 26830 (min)		
0	64 ± 31	33 ± 13
5	44 ± 24	40 ± 14
30	132 ± 41	155 ± 18
60	170 ± 9	168 ± 12

Male Zucker fa/fa rats (582 ± 40 g) were injected daily with either saline or BRL 26830 (2.9 mg/kg in saline i.p.) for 28 consecutive days. The rats were then fasted for 24 hr and the insulin secretagogue response to BRL 26830 was determined in both groups. Results are given as mean ± S.D. of 4 rats per group.

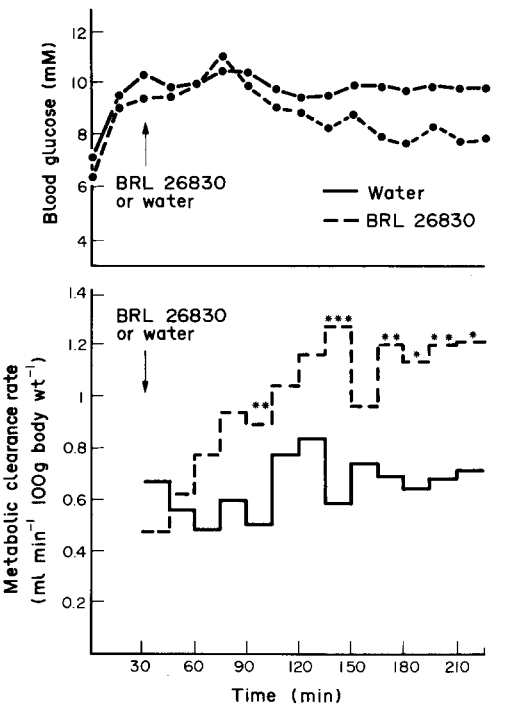

Fig. 2. Effect of chronic treatment with BRL 26830 on glucose metabolic clearance rate in glucose-loaded Zucker fa/fa rats. Male Zucker (fa/fa) rats were dosed once daily with either BRL 26830 or water for 45 days. The rats were then fasted for 24 hr prior to the measurement of glucose turnover, as described in the text. Results are the mean of 5 rats. Significant differences from controls are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The nature of the changes in glucose metabolism that occur in obese rats following chronic treatment with BRL 26830 was further investigated by measuring glucose turnover. In order to simulate the fed state, glucose was infused with the objective of raising the blood glucose concentration from its fasting value of 6–8 mM to approximately 10 mM and maintaining it at this level. Figure 2 shows that this objective was achieved in the control rats, whereas in the rats given BRL 26830, the mean blood glucose concentration initially rose from 9.5 to 11.1 mM and then fell progressively to 7.9 mM.

Total quantities of glucose produced and utilized over the period from 30 to 225 min were calculated (Table 2). Treatment with BRL 26830 produced a significant increase in the total glucose disposal but this was accompanied by a small rise in endogenous glucose synthesis. BRL 26830 also produced a highly significant increase in the metabolic clearance rate of glucose (Fig. 2).

DISCUSSION

Previous studies have established that in normoglycaemic lean rats, BRL 26830 increases both glucose disposal, through its insulin secretagogue activity, and glucose production [16]. In these rats, the effect of BRL 26830 on glucose tolerance is

Table 2. Effect of chronic treatment with BRL 26830 on glucose turnover

	Control	BRL 26830
Final body weight (g)	636 ± 26	609 ± 35
Body weight gain during chronic treatment (g)	88 ± 17	17 ± 18†
Mean blood glucose over last 60 min of glucose infusion (mM)	9.93 ± 0.98	7.99 ± 2.10
Total glucose utilization from 30–225 min (μmol)	1385 ± 239	1680 ± 110*
Endogenous glucose synthesis from 30–225 min (μmol)	612 ± 251	881 ± 84

Male Zucker fa/fa rats were dosed daily with either BRL 26830 or water for 45 days. The rats were then fasted for 24 hr before measurement of glucose turnover as described in the Methods section. Results are mean ± S.D. of 5 rats per group. P vs control group * <0.05; † <0.001.

dependent on the nutritional status of the animals. Thus, in 24-hr fasted rats, which have a low concentration of liver glycogen, glucose tolerance is improved. In contrast, in 5-hr-fasted rats, which have a high liver glycogen content, glucose tolerance is unchanged although there is a 50% fall in liver glycogen [16]. These results suggest that the effect of BRL 26830 on blood glucose concentration in the rat will be dependent on the relative effect of the compound on glucose production and insulin-mediated glucose disposal. Such actions will be influenced not only by the nutritional state of the animal but also by the degree of insulin resistance.

Zucker fa/fa rats have moderate glucose intolerance with hyperinsulinaemia and therefore show insulin resistance [12]. This resistance, which is present in both muscle [21, 22] and adipose tissue [23], increases as the rats become older and progressively more obese [12].

In the present experiments, a single acute dose of BRL 26830 improved subcutaneous glucose tolerance in lean Zucker (Fa/?) rats as it does in normoglycaemic Sprague–Dawley rats [16]. However, in obese (fa/fa) rats, which weighed 500–600 g, an acute dose of BRL 26830 slightly exacerbated the existing glucose intolerance (Fig. 1A). Since BRL 26830 had an insulin secretagogue response, the failure of an acute dose of BRL 26830 to improve tolerance in obese rats may relate to either a greater stimulation of glucose production and/or to their greater insulin resistance. Fasting of obese rats for 24 hr reduced the liver glycogen content by only 50% whereas in lean rats a 90% reduction in liver glycogen occurred. Thus, obese rats have a greater capacity to increase glycogenolysis. Nevertheless, we consider that the degree of insulin resistance is the more important factor that modifies the acute effect of BRL 26830 on glucose tolerance. In partial support of this an acute dose of BRL 26830 to young Zucker rats weighing less than 300 g improved glucose tolerance (data not shown).

Chronic treatment of the glucose-intolerant 500–600 g fa/fa rats with BRL 26830, for 23 days or more, resulted in a significant improvement in glucose tolerance (Fig. 1B). Similar findings have been obtained in obese hyperinsulinaemic C57B1/6 (ob/ob) mice [16]. In contrast, the glucose tolerance of the Zucker lean littermates given BRL 26830 chronically was identical to that of control lean rats. In additional unpublished studies, we have found also that normoglycaemic Sprague–Dawley rats and CFLP mice that have received BRL 26830 daily for 14 days or more have similar glucose tolerance to controls. Thus, a chronic effect of BRL 26830 to improve glucose tolerance is apparent only in those animals (C57B1/6 ob/ob mice and Zucker fa/fa rats), which have inherent abnormal glucose tolerance.

Insulin resistance in C57B1/6 ob/ob and Zucker fa/fa rats is the prime cause for their glucose intolerance. Reduction in insulin resistance will result in improvements in glucose utilization. The glucose decay rate following the intravenous administration of insulin has been used as an indirect method of assessing whole body insulin sensitivity. The chronic administration of BRL 26830 to Zucker obese rats increased significantly ($P < 0.001$) the glucose decay rate, indicative of increased insulin sensitivity.

It is well-established that the level of insulin resistance is influenced by the degree of obesity in animals [24] and in man [7, 25]. Since BRL 26830 has thermogenic activity that can result in a decrease in body weight [15], it is possible that the effect of BRL 26830 in increasing insulin sensitivity in the Zucker obese rat might be secondary to the effect on body weight. In view of this, it is important to note that there were no significant differences in the body weights of the control and BRL 26830-treated rats (Table 2). However, measurements of the change in body weight during the course of the chronic experiment (Table 2) indicate that the BRL 26830-treatment was having a significant effect on energy balance. This effect on energy balance arises from an increase in energy expenditure rather than from a reduction of food intake [15]. We cannot rule out the possibility, therefore, that the changes in insulin sensitivity induced by BRL 26830 in Zucker obese rats are related to the effect of this compound in increasing energy expenditure.

To gain a further insight into changes in glucose homeostasis that occur as a consequence of chronic treatment of obese rats with BRL 26830, we have used the D-[6-^{3}H]-glucose infusion technique to determine the rates of glucose production and glucose disposal [26, 27]. Blood glucose concentration is dependent on both hepatic glucose production and peripheral glucose utilization. If an increased rate of glucose production occurred in rats given BRL 26830 chronically, it is conceivable that the increase in the rate of glucose disposal could be greater than that indicated by the glucose tolerance data. For studies on glucose turnover, it is essential to know the rate of entry of glucose into the glucose pool. Thus, glucose uptake from the gut must be prevented, and we have therefore used 24-hr-fasted rats. However, in order to simulate the fed animal, these rats were provided with a priming dose of glucose and a continuous intravenous glucose infusion aimed at main-

taining the blood glucose concentration at approximately 10 mM. As shown in Fig. 2, this objective was achieved in the control rats, but treatment with BRL 26830 caused the blood glucose concentration to fall with time. The variation in the blood glucose concentrations in the treated group was considerable (Table 2) and thus the difference between the mean values for control and treated animals were not significantly statistically ($P > 0.05$). However, chronic treatment of obese rats with BRL 26830 resulted in a very significant increase in the metabolic clearance rate of glucose in spite of the presence of a lower blood glucose concentration (Fig. 2). The increase in metabolic clearance rate was due to increased glucose utilization in part offset by a small increase in the endogenous rate of glucose production (Table 3).

The fate of the extra glucose that is metabolized is not known. However, if all of the extra glucose was oxidized, it would be equivalent to an increase in the total expenditure of 1.96 kJ/rat/hr. The dose of BRL 26830 used in these experiments produces an increase in the metabolic rate of 3 kJ/rat/hr (J. Arch, personal communication). Thus, glucose could provide theorectically up to 60% of the additional fuel. Measurements of respiratory quotient are required to address this question.

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REFERENCES

1. K. M. West, in *Epidemiology of Diabetes and its Vascular Lesions*. Elsevier, New York (1978).
2. E. P. Joslin, *J. Am. med. Ass.* **76**, 79 (1921).
3. K. M. West and J. M. Kalbfleisch, *Diabetics* **20**, 99 (1971).
4. J. H. Karam, G. M. Grodsky and P. H. Forsham, *Diabetes* **12**, 197 (1963).
5. J. D. Bagdade, E. L. Bierman and D. Porte, *J. clin. Invest.* **46**, 1549 (1967).
6. J. Olefsky, G. M. Reaven and J. W. Farquhar, *J. clin. Invest.* **53**, 64 (1974).
7. J. M. Olefsky and O. G. Kolterman, *Am. J. Med.* **70**, 151 (1981).
8. P. J. Savage, S. E. Dippe, P. H. Bennett, P. Gordon, J. Roth, N. B. Rushforth and M. Miller. *Diabetes* **24**, 362 (1975).
9. G. M. Reaven, R. Bernstein, B. Davis and J. M. Olefsky, *Am. J. Med.* **60**, 80 (1976).
10. L. M. Zucker and T. F. Zucker, *J. Hered.* **52**, 275 (1961).
11. L. M. Zucker and H. N. Antioniades, *Endocrinology* **90**, 1320 (1972).
12. G. A. Bray and D. A. York, *Physiol. Rev.* **59**, 718 (1979).
13. J. R. S. Arch, A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. V. Sennitt, V. E. Thody, C. Wilson and S. Wilson, *Nature, Lond.* **309**, 163 (1984).
14. C. Wilson, S. Wilson, V. Piercy, M. V. Sennitt and J. R. S. Arch, *Eur. J. Pharmac.* **100**, 309 (1984).
15. J. R. S. Arch and A. T. Ainsworth, *Am. J. clin. Nutr.* **38**, 549 (1983).
16. M. V. Sennitt, J. R. S. Arch, A. L. Levy, D. L. Simson, S. A. Smith and M. A. Cawthorne, *Biochem. Pharmac.* **34**, 1279 (1985).
17. K. Lundback, *Br. med. J* **i**, 1507 (1962).
18. F. H. Schmidt, in *Internationales Donau-Symposium über Diabetes Mellitus*. Verlag W. Maudrich, Wien–Munchen–Bern (1973).
19. R. Steele, *Proc. N.Y. Acad. Sci.* **82**, 420 (1959).
20. C. N. Hales and P. J. Randall, *Biochem. J.* **88**, 137 (1963).
21. M. Crettaz, M. Prentki, D. Zanineth and B. Jeanrenaud, *Biochem. J.* **186**, 525 (1980).
22. F. W. Kemmer, M. Berger, L. Herberg and F. A. Gries, *Biochem. J.* **178**, 733 (1979).
23. J. S. Stern, P. R. Johnson, B. R. Batchelor, L. M. Zucker and J. Hirsch, *Am. J. Physiol.* **228**, 543 (1975).
24. J. M. Olefsky, *J. clin. Invest* **57**, 842 (1976).
25. J. M. Olefsky, O. G. Kolterman and J. A. Scarlett, *Am. J. Physiol.* **243**, E15 (1982).
26. A. Dunn and S. Strahs, *Nature, Lond.* **205**, 705 (1965).
27. A. Dunn, M. Chenoweth and L. D. Schaeffer, *Biochemistry* **6**, 6 (1967).