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Differences in responsiveness to adipokinetic agents between white epididymal and brown interscapula adipose tissue from rats

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Lipolytic activity was studied in brown and white adipose tissue of rats *in vitro*. 5-Hydroxytryptamine (5-HT), phenylephrine, noradrenaline, adrenaline and isoprenaline were used as adipokinetic agents. All stimulated lipolysis in brown adipose tissue, but 5-HT and phenylephrine did not in white adipose tissue. A β-blocking drug, propranolol, inhibited the stimulatory effect of the agents in both adipose tissues. However, an α-blocking drug, phentolamine, further increased the lipolysis induced by noradrenaline or adrenaline in brown adipose tissue and inhibited the effect of isoprenaline. In white adipose tissue, its action was to marginally decrease the effect of adrenaline and noradrenaline. Increase in the pH of the incubation medium stimulated FFA and glycerol release in brown adipose tissue, but not in the epididymal adipose tissue. This effect of pH on lipolysis was further enhanced by phentolamine and decreased by propranolol. Increase of lipolysis with pH was not seen with brown fat tissue from the reserpine-treated rats. These results show that brown adipose tissue of the rat has an α-receptor with inhibitory effects on lipolysis that is affected by α- or mixed-type adrenergic agonists, noradrenaline and adrenaline.

The lipolytic mechanism of human adipose tissue is thought to differ from rat adipose tissue in showing lack of lipolytic response to a series of hormones such as TSH, ACTH and glucagon (Burns, Hales & Hartree, 1967; Burns & Langley, 1968), and in having α-adrenoceptors with an inhibitory effect on lipolysis (Burns & Langley, 1971a, b). But Luzio, Jones & others (1974) have reported that adrenaline-stimulated glucose uptake in rat-isolated fat cells is inhibited by an α-adrenoceptor blocking agent, phenoxybenzamine, and that the adrenaline receptor was apparently α in type. This suggests that rat adipose tissues have some kind of α-receptors.

I have attempted to clarify whether α-adrenoceptors with inhibitory action on lipolysis exist in rat adipose tissues. In brown adipose tissue, phentolamine (an α-adrenoceptor-blocking agent) further stimulated lipolysis enhanced by adrenaline, or noradrenaline as found with human fat cells (Burns & Langley, 1971b). This shows the presence of α-receptors with inhibitory effect on lipolysis in rat brown fat cells.

MATERIALS AND METHODS

Epididymal and interscapular brown adipose tissues were obtained from male Wistar rats (130–150 g). Animals were allowed free access to food and water until decapitated. Adipose tissues rapidly obtained, were divided and randomly distributed among the incubation vials with one tissue segment for each experimental variable. The tissues were incubated at 37° for 2 h in Krebs-Ringer bicarbonate buffer which contained 2% bovine serum albumin (Fraction No. V) after the flasks had been flushed with the appropriated gas phase, usually 6% CO_2 in oxygen for pH 7·3 and with air for pH 8·1.

Lipolytic activity was shown as the release of free fatty acids (FFA) (Itaya & Ui, 1965; Itaya, 1977) and glycerol (Pinter, Hayashi & Watson, 1967).

The doses of adipokinetic agents and inhibitor used were as follows: 5-hydroxytryptamine (5-HT), phenylephrine, noradrenaline and isoprenaline were used at 1×10^{-5} M, adrenaline, 1×10^{-8} to 1×10^{-4} M, propranolol, a β-blocking agent, 1×10^{-5} or 2×10^{-5} M, and phentolamine 1×10^{-7} to 1×10^{-4} M.

In some experiments, reserpine-treated rats were used. The drug (5 mg kg^{-1}) was administered intraperitoneally at 24 and 3 h before death. Reserpine was suspended with 20% ethanol and the control rats were injected at the same time with the 20% ethanol.

RESULTS

Difference in responsiveness to the adipokinetic agents between brown and white adipose tissue of rats. Figs 1 and 2 (open column) show the FFA release of white (Fig. 1) and brown (Fig. 2) adipose tissue stimulated

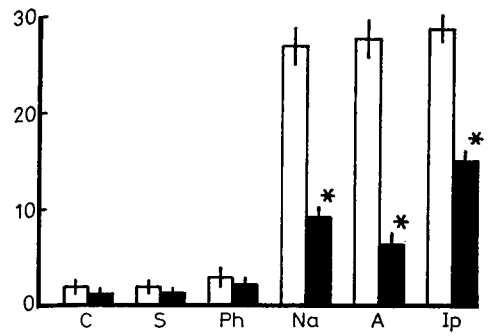

FIG. 1. Effect of adipokinetic agents (1×10^{-5} M) on FFA release and that of propranolol (2×10^{-5} M) on the FFA release stimulated by the adipokinetic agents in white adipose tissue. Pieces of rat fat pads were incubated for 2 h in Krebs-Ringer bicarbonate buffer containing albumin (20 mg ml^{-1}). The height of each column represents the mean of four observations, the bars indicate one standard error. C; control, S; 5-HT, Ph; phenylephrine, Na; noradrenaline, A; adrenaline, Ip; isoprenaline; open columns without and solid columns with propranolol. * $P < 0{\cdot}01$ with respect to the value obtained with lipolytic agent alone (open column). Ordinate: FFA release (μequiv g^{-1} per 2 h).

by each of the stimulants. Phenylephrine, even though an α-adrenoceptor stimulant, and 5-HT, increased lipolytic activity only in brown adipose tissue. The extent of FFA release with these two agents was similar to that with adrenaline or noradrenaline (Fig. 2). Adrenaline and noradrenaline caused the same enhancement of FFA release as isoprenaline in white adipose tissue (Fig. 1). But in brown adipose tissue the response to isoprenaline was significantly greater than that to other agents (Fig. 2). The differences between brown and white adipose tissue were observed reproducibly (Figs 3, 4).

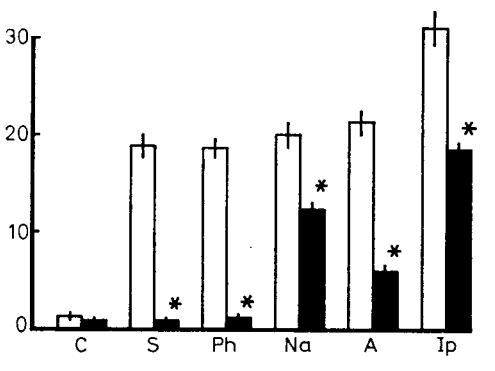

FIG. 2. Effect of adipokinetic agents on FFA release and that of propranolol (2×10^{-5} M) on the FFA release stimulated by the adipokinetic agents in brown adipose tissue. For other conditions and abbreviations, see legend for Fig. 1.

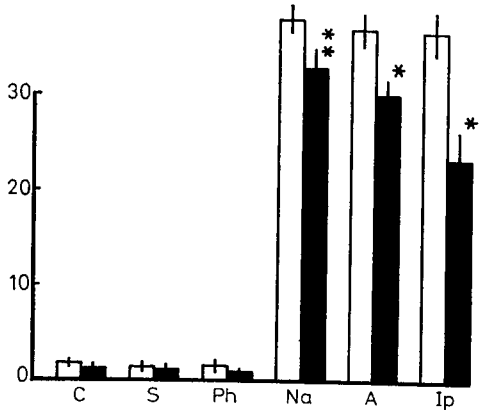

FIG. 3. Effect of phentolamine (1×10^{-5} M) on the FFA release stimulated by adipokinetic agents in white adipose tissue. For other conditions, see legend for Fig. 1. Open columns without and solid columns with phentolamine. ** $P < 0{\cdot}05$ with respect to the value obtained with noradrenaline alone.

Effect of adrenoceptor α- and β-blocking drugs on the lipolysis stimulated by the adipokinetic agents in white and brown fat pads. The effects were tested in the same experimental system. The solid column in Fig. 1 and Fig. 2 show the effect of propranolol. This agent alone failed to affect basal lipolysis in both white and brown adipose tissue. However, the responses to the adipokinetic agents were significantly reduced by propranolol.

Burns & Langley (1971b) reported that phentolamine enhanced the lipolytic response to adrenaline in human adipocytes. I found phentolamine alone to have no effect on basal lipolysis at the concentrations used and at 1×10^{-7} M, it did not change the effect

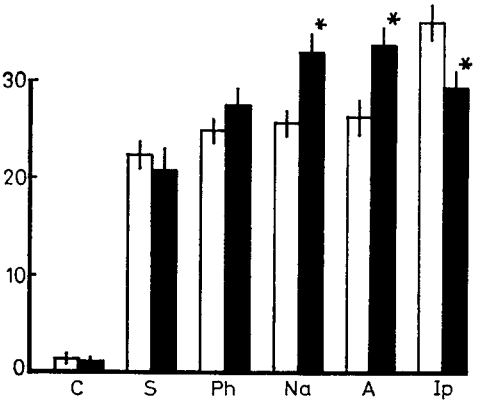

FIG. 4. Effect of phentolamine on the lipolysis stimulated by lipolytic agents in brown adipose tissue. For other conditions, see legend for Fig. 1. Open columns without solid columns with phentolamine.

of adrenaline on lipolysis with both kinds of tissue (Table 1). At 1×10^{-5} M, the drug significantly enhanced adrenaline-stimulated lipolysis in brown adipose tissue as it did in human adipose cells, but with white adipose tissue it depressed lipolysis stimulated by adrenaline (Figs 3, 4 solid columns). At 1×10^{-4} M, the drug inhibited the effects of adrenaline on both white and brown fat tissue, suggesting that, at higher concentrations, phentolamine, though an α-blocking agent, competes with adrenaline for a β-adrenoceptor site through which the stimulation of lipolysis occurs.

The results in Figs 3 and 4 (solid columns) present unambiguous evidence supporting the results in Table 1 showing the difference in response to

Table 1. *Effects of phentolamine on lipolysis of rat adipose tissue stimulated by adrenaline.*

Addition	FFA release (μequiv^{-1} in 2 h) Brown	White
None	2·0 ± 0·66	0·7 ± 0·33
Adrenaline (1×10^{-5} M)	18·5 ± 1·83[a]	14·8 ± 1·50[a]
Adrenaline + phentolamine (1×10^{-7} M)	17·2 ± 1·31[b]	13·9 ± 1·62[b]
Adrenaline + phentolamine (1×10^{-5} M)	23·6 ± 1·50[a,b]	10·6 ± 1·02[a,b]
Adrenaline + phentolamine (1×10^{-4} M)	9·1 ± 0·91[a,b]	5·7 ± 0·50[a,b]

For condition of incubation, see text and legend to Fig. 1.
Each figure represents 'mean of 6 flasks ± s.e.m.'.
a: $P < 0{\cdot}01$ in respect to the mean value one row above.
b: $P < 0{\cdot}01$ in respect to the mean value two rows above.

phentolamine between white and brown adipose tissue. In brown adipose tissue, when phentolamine was incubated with adrenaline, or noradrenaline, the lipolytic effect of each amine was enhanced (Fig. 4). The response to the combination of phentolamine and adrenaline or noradrenaline approximately equalled the effect of isoprenaline alone. However, phentolamine added to the isoprenaline containing medium reduced the effect of this β-agonist on FFA release, but it failed to modify the lipolytic response to 5-HT or phenylephrine. On the other hand, in epididymal adipose tissue, phentolamine reduced lipolysis stimulated by adipokinetic agents (Fig. 3).

Effect of phentolamine on the dose-response curve to adrenaline

The rate of FFA release in response to various concentrations of adrenaline was examined in the presence or absence of phentolamine (1×10^{-5} M). The release is plotted as a function of adrenaline concentration in Figs 5 and 6. A typical "dose-response curve" was obtained in the presence or absence of the inhibitor. There was no significant difference between curves obtained in the presence or absence of the blocker at concentrations (below 10^{-7} M of adrenaline. Although the minimum concentration of

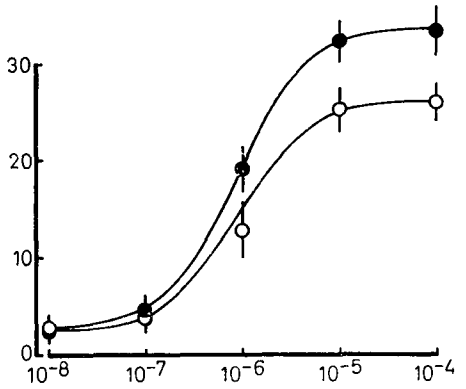

FIG. 5. Effect of phentolamine on the FFA release stimulated by various concentrations of adrenaline in brown adipose tissue. Each point was a mean of 6 observations. The bars indicate ± s.e.m. Open circles; adrenaline only, closed circles; adrenaline and phentolamine (1×10^{-5} M). Ordinate: FFA release (μequiv g^{-1} per 2 h) Abscissa: Log adrenaline (M).

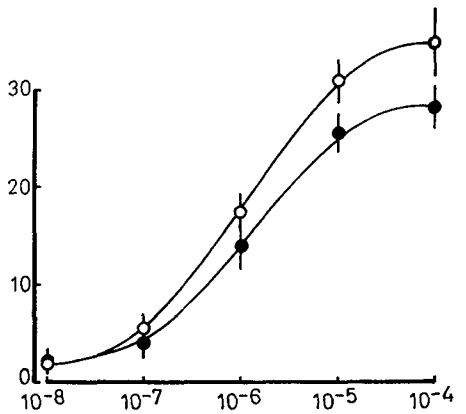

FIG. 6. Effect of phentolamine on the FFA release stimulated by various concentrations of adrenaline in white adipose tissue. Each point was a mean of five observations. The bars indicate ± s.e.m. Open circles; adrenaline only, closed circles; adrenaline and phentolamine (1×10^{-5} M). Ordinate: FFA release (μequiv g^{-1} per 2 h). Abscissa: Log adrenaline (M).

adrenaline needed to induce a statistically significant increase in FFA release was 10^{-6} M both in the presence and absence of phentolamine, the increment was much larger in the presence than in the absence of the α-blocking drug in brown adipose tissue. In accord with the results in Table 1, the addition of phentolamine to the medium further increased FFA release stimulated maximally by adrenaline (> than 10^{-5} M) in brown adipose tissue (Fig. 5). However, in white adipose tissue, phentolamine decreased the FFA release stimulated by adrenaline (Fig. 6).

Effect of increasing pH of the incubation medium on basal lipolysis in brown and white adipose tissue. The effect of pH on glucose metabolism of rat adipose tissue was reported by Longmore, Landan & others (1968). In their studies a decrease in the pH of the incubation medium resulted in a decrease in glucose utilization and the conversion of glucose carbon to fatty acids, suggesting that fatty acid utilization in adipose tissue may be affected by changing pH of the medium. To clarify this, the effect on lipolysis of increasing the pH (from 7·3 to 8·1) of the incubation medium was examined. The increase in pH of the medium caused an increase of both FFA and glycerol release in brown adipose tissue (Table 2), but no effect on lipolysis in white fat.

Effect of adrenoceptor blocking drugs on lipolysis stimulated by increasing pH of the incubation medium. The pH-stimulated lipolysis in brown adipose tissue was further enhanced by phentolamine, but inhibited by propranolol. In white epididymal fat tissue, increasing pH or the addition of the two drugs had no effect (Table 3).

Table 2. *Effect of elevating pH in incubation medium on lipolysis of brown adipose tissue.*

pH of medium 7·3	pH of medium 8·1	Effect	*P*
FFA release (μequiv g^{-1} in 2 h)			
Brown tissue			
1·3	8·9	+7·6 ± 1·68 (6)	<0·01
White tissue			
0·8	0·9	+0·1 ± 0·56 (6)	NS
Glycerol release (μmol g^{-1} in 2 h)			
Brown tissue			
2·1	8·2	+6·1 ± 1·35 (6)	<0·01
White tissue			
2·3	2·4	+0·1 ± 0·46 (6)	NS

Pieces of adipose tissue were incubated in the medium without any addition of lipolytic agents. For procedure of rising pH, see 'Methods' in text. Each value for 'Effect' represents the mean ± s.e.m. The number of experiments is given in parentheses. NS, not significance.

Table 3. *Effects of phentolamine or propranolol* (1×10^{-5} M) *on lipolysis of brown adipose tissue stimulated by rising pH of incubation medium.*

pH	Addition	Glycerol release (μmol g^{-1} in 2 h)	FFA release (μequiv g^{-1} in 2 h)
Brown tissue			
7·3	None	2·5 ± 0·66	2·1 ± 0·31
8·1	None	5·6 ± 0·62[a]	5·4 ± 0·79[a]
8·1	α-Blocker	10·1 ± 0·54[a,b]	11·5 ± 0·96[a,b]
8·1	β-Blocker	3·2 ± 0·45[a,b]	3·3 ± 0·46[a,b]
White tissue			
7·3	None	2·4 ± 0·29	3·1 ± 0·32
8·1	None	2·3 ± 0·33	3·0 ± 0·40
8·1	α-Blocker	2·4 ± 0·32	2·7 ± 0·32
8·1	β-Blocker	2·2 ± 0·35	3·9 ± 0·50

Each figure represents 'mean of 6 flasks ± s.e.m.'.
a: $P < 0·01$ in respect to the mean value one row above.
b: $P < 0·01$ in respect to the mean value two rows above.

Disappearance of the effect of rising pH in brown adipose tissue from the reserpine-treated rats. As the previous experiment suggested that the increase of pH of the medium might stimulate the release of noradrenaline from the granules in nerve ends in brown adipose tissue, the effect of reserpine, which stimulates the release of endogenous noradrenaline from the granules, was tested. But this tissue did not respond (Table 4), although isoprenaline caused a response even in tissue from reserpine-treated rats.

DISCUSSION

The results show that epididymal and brown interscapula adipose tissue of the rat differ from each

Table 4. *Effect of reserpine treatment on alkaline-stimulated lipolysis of brown adipose tissue.*

Tissue condition	pH	Addition	Glycerol release (μmol g^{-1} in 2 h)	FFA release (μequiv g^{-1} in 2h)
Control	7·3	None	2·7 ± 0·30	3·8 ± 0·52
	8·1	None	5·9 ± 0·84[a]	6·1 ± 0·57[a]
	8·1	Isoprenaline	12·6 ± 0·68[a]	15·2 ± 0·94[a]
Treated with reserpine	7·3	None	1·3 ± 0·31	2·0 ± 0·31
	8·1	None	1·1 ± 0·34	2·6 ± 0·38
	8·1	Isoprenaline	14·7 ± 1·13[a]	11·0 ± 0·51[a]

Each figure represents "mean of 6 flasks ± s.e.m.".
[a] $P < 0·01$ in respect to the mean value one row above.

other in their response to the lipolytic activity of adipokinetic agents. Of the adipokinetic agents used, isoprenaline is a potent β-adrenoceptor agonist, adrenaline, a mixed agonist, noradrenaline, relatively pure α-adrenoceptor agonist and phenylephrine, an α-agonist. In white adipose tissue, β-, mixed-, and relatively pure α-type agonists stimulated FFA release to the same extent (open columns Figs 1, 3), whereas in brown adipose tissue, the β-type agonist, isoprenaline, caused a greater FFA release than other agents (open columns Figs 2, 4). This suggested the presence of α-adrenoceptor sites in brown adipose tissue of the rat as in in human fat cells (Burns & Langley, 1971a, b; Bray & Trygstad, 1972), and that adrenaline and noradrenaline are able to stimulate the α-adrenoceptor stimulation which would cause a decrease in cyclic AMP concentration and in lipolysis. The results with phentolamine (see solid columns Figs 3, 4) show the existence of the α-site in brown adipose tissue and that the adipokinetic agents except isoprenaline and 5-HT were able to stimulate the α-site.

Although it is generally accepted that lipolysis in adipose tissue is stimulated by catecholamines through the interaction with the β-site receptor located in the cell membrane (Fain, 1967), phenylephrine, a pure α-adrenoceptor agonist, stimulated lipolysis of brown adipose tissue. Moreover, this stimulation was inhibited by the addition of the β-site blocking agent, propranolol (Fig. 2). This seems to show that α- and β-types of agonists are relative and that the affinity of α- and β-agonists for the receptors differs from tissue to tisue such as the vascular muscle (Szentivanyi & others, 1970), the pancreas (Mayhew, Wright & Ashmore, 1969) and the frog skin (Watlington, 1969). More recently, Flaim, Horowitz & Horowitz (1977) studied the interaction of α- and β-adrenergic responses in intact rats.

It has been thought that the decreased insulin action observed in diabetic acidosis would increase the concentration of cyclic AMP which activates hormone-sensitive lipase, thus enhancing FFA release (Rizack, 1965; Butcher, Sneyd & others, 1966), and that other hormones in response to acidosis may also act upon adipose tisue to enhance FFA release (Winegrad, 1965). However, there have been few studies on the effect of alkalosis per se on the lipolysis of brown adipose tissue. In this paper, the increase in lipolysis by elevating pH of the incubation medium was seen only in brown adipose tissue. This could be interpreted as an increased permeability to FFA accumulated (Dorigo, Maragno & others, 1971) within brown adipose tissue by alkali, or as an inhibition of glycerol kinase, because the release of glycerol was also enhanced by the increasing pH. These mechanisms, however, might not contribute to the lipolytic effect of elevating pH, since the addition of phentolamine further stimulated the release of FFA and glycerol at pH 8·1 yet the activity of glycerol kinase is very low in brown adipose tissue (Hahn & Greenberg, 1968). A triglyceride lipase may be sensitive to the increase of pH, but phentolamine's increase of lipolysis indicates that the site of action of the increasing pH is before the activation of triglyceride lipases.

The release of endogenous noradrenaline from the granules in the nerve ends may be stimulated by increasing pH. But if this is true, brown adipose tissue from the reserpine-treated rats could not respond to the increase in pH. Table 4 shows that the increase of pH has no effect on lipolysis in brown adipose tissue from the reserpine-treated rats, while isoprenaline stimulates FFA and glycerol release at pH 8·1. This shows that the lipolytic system itself in brown adipose tissue from the reserpine-treated rats is responsive to stimulants under alkaline conditions. Moreover, as shown in Table 3, the stimulatory effect of increasing pH on lipolysis in brown adipose tissue is inhibited by propranolol.

These results suggest that the increased lipolysis at pH 8·1 is due to the stimulated release of endogenous noradrenaline from the granules in nerve ends of brown adipose tissue, and that the further increase of lipolysis by addition of phentolamine shows the existence of an α-receptor in the brown fat cell membrane.

REFERENCES

BRAY, G. A. & TRYGSTAD, O. (1972). *Acta endocr., Copnh.*, **70**, 1–20.
BURNS, T. W., HALES, C. N. & HARTREE, A. S. (1967). *J. Endocr.*, **39**, 213–225.
BURNS, T. W. & LANGLEY, P. E. (1968). *J. Lab. clin. Med.*, **72**, 813–823.
BURNS, T. W. & LANGLEY, P. E. (1971a). *Ibid.*, **75**, 983–997.
BURNS, T. W. & LANGLEY, P. E. (1971b). *Pharmac. Res. Commun.*, **3**, 271–277.
BUTCHER, R. W., SNEYD, J. G. T., PARK, C. R. & SUTHALAND, E. W. JNR (1966). *J. biol. Chem.*, **241**, 1651–1653.
DORIGO, P., MARAGNO, I., BRESSA, A. & FASSINA, G. (1971). *Biochem. Pharmac.*, **20**, 1201–1211.
FAIN, J. N. (1967). *Ann. N.Y. Acad. Sci.*, **139**, 879–890.

FLAIM, K. E., HOROWITZ, B. A. & HOROWITZ, J. M. (1977). *Am. J. Physiol.*, **232**, R101–R109.

HAHN, P. & GREENBERG, R. (1968). *Life Sci.*, **7**, 187–190.

ITAYA, K. (1977). *J. Lipid Res.*, **18**, 663–665.

ITAYA, K. & UI, M. (1965). *Ibid.*, **6**, 16–20.

LONGMORE, W. J., LANDAN, B. R., BAKER, E. S., HASTINGS, A. B., LUM, D. M. & WILLIAMS, H. R. (1968). *Am. J. Physiol.*, **215**, 582–586.

LUZIO, J. P., JONES, R. C., SIDDLE, K. & HALES, C. N. (1974). *Biochim. biophys. Acta*, **362**, 29–36.

MAYHEW, D. A., WRIGHT, P. H. & ASHMORE, J. (1969). *Pharmac. Rev.*, **21**, 183–212.

PINTER, J. E., HAYASHI, J. A. & WATSON, J. A. (1967). *Archs Biochem. Biophys.*, **121**, 404–414.

RIZACK, M. (1965). *Ann. N.Y. Acad. Sci.*, **131**, 250–253.

SZENTIVANYI, M., KUNOS, G. & JUHASZ-NAGY, A. (1970). *Am. J. Physiol.*, **218**, 869–875.

WATLINGTON, C. O. (1969). *Biochim. Biophys. Acta*, **193**, 394–402.

WINEGRAD, A. I. (1965). In: *Handbook of Physiol. Section 5: Adipose Tissue*, pp. 319–329. Editor: Renold, A. E. & Cahill, G. Jnr. Washington: American Physiological Society.