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Effects of Cytochalasin B, Colchicine and Vincristine on the Metabolism of Isolated Fat-Cells

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1. Colchicine and vincristine only slightly inhibit the metabolism of glucose to CO_2 and lipids by isolated fat-cells. 2. Prolonged incubation with these agents causes no further inhibition. 3. Cytochalasin B, however, inhibits glucose metabolism to both CO_2 and lipids in fat-cells. 4. However, at a concentration that causes a strong inhibition of glucose metabolism cytochalasin B is without effect on the metabolism of pyruvate, lactate or arginine to these end products. The uptake of labelled α-aminoisobutyrate is likewise not modified. Similarly it does not affect release of glycerol or free fatty acid, or the actions of adrenaline, insulin or caffeine on these parameters. At 10μg/ml it slightly lowers ATP concentrations, an effect that does not occur at 2μg/ml. 5. The transport of fructose into adipocytes by a specific fructose-transport system is also not affected by the agent, but the uptake of 2-deoxyglucose is strongly inhibited. It is concluded that cytochalasin B may specifically inhibit the glucose-transport system of isolated fat-cells. 6. Cytochalasin A has a much weaker action than cytochalasin B on glucose metabolism.

There has been much recent interest in the various effects of both colchicine and the vinca alkaloids, vincristine and vinblastine, on cellular function. These agents have been shown to interfere with secretory processes in the liver (Orci *et al.*, 1973), pancreas (Malaisse *et al.*, 1971), parotid gland (Butcher & Goldman, 1972) and other tissues. It has been suggested that they act by interfering with the function of a microtubular system in these cells. Similarly cytochalasin B, a metabolite of the mould *Helminthosporum dermatioideum*, has been the subject of several investigations. This compound inhibits cell division by preventing cytoplasmic cleavage (Carter, 1967). In addition, it inhibits thyroid secretion (Williams & Wolff, 1971) and amylase secretion (Butcher & Goldman, 1972), but increases insulin release from isolated pancreatic islets (Malaisse *et al.*, 1972). In all of these effects it has been postulated that cytochalasin B acts by modifying the activity of the contractile microfilamentous system of the cell. As well as these actions, cytochalasin B has been shown to be a powerful inhibitor of glucose uptake in several cell types (Mizel & Wilson, 1972; Kletzien & Perdue, 1973; Czech *et al.*, 1973).

Since microtubules and microfilaments have been identified in adipose tissue (Soifer *et al.*, 1971; Wood, 1967), it seemed possible that they may have a role to play in the metabolism of adipocytes. This work was therefore undertaken to investigate the effect of colchicine, vincristine and cytochalasin B on the metabolism of adipose tissue.

Materials and Methods

Animals used in all experiments were male Wistar rats (180–220g) purchased from Ivanovas G.m.b.H., Kisslegg im Allgäu, Germany, and fed *ad libitum*. Isolated fat-cells were prepared by the method of Rodbell (1964) from epididymal fat-pads and incubated in plastic vials in Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH7.4, containing half the recommended amount of Ca^{2+} and unless otherwise mentioned, 3.5% (w/v) bovine serum albumin. Incubation mixtures usually contained about 50mg of lipid/ml, measured by weighing a dried portion of the lipid extracted by the procedure of Dole & Meinertz (1960). This amount of fat-cells is equivalent to approx. 25μg of DNA/mixture. Incubations were performed in replicates of four–six flasks.

In experiments in which the conversion of glucose into lipids and CO_2 was measured, the incubations were carried out in a volume of 1ml for 1h with 1mM-glucose under O_2+CO_2 (95:5). After the incubation, the medium was acidified and CO_2 was collected on pieces of filter paper moistened with NaOH, dried and counted for radioactivity in a liquid-scintillation spectrometer (Packard model 3380). Labelled lipids in the acidified isolated cell suspension after incubation were extracted by the procedure of Dole & Meinertz (1960), and the upper heptane phase was washed three times with 0.01M-H_2SO_4. A sample (1ml) of this was evaporated to dryness in a glass counting vial and then counted in a scintillation fluid consisting of 4g of 2,5-diphenyl-

oxazole and 40mg of 1,4-bis-(5-phenyloxazol-2-yl)-benzene per litre of toluene. In some experiments a further sample (2ml) was evaporated to dryness, saponified with 4% (w/v) ethanolic KOH, and the liberated glyceride fatty acids were extracted three times into light petroleum (b.p. 30–45°C). The light-petroleum extracts were pooled, dried and counted for radioactivity. Release of free fatty acid and glycerol was measured in cell-free samples of incubation medium by the methods of Ho (1970) and Wieland (1965) respectively. The ATP content of isolated fat-cells was measured after incubation by adding 0.5ml of the cell suspension to 0.5ml of 0.8M-$HClO_4$ at −5°C and shaking vigorously. The resulting mixture was centrifuged briefly at −5°C and the clear supernatant was used immediately for the measurement of ATP by the luciferin–luciferase method (Bihler & Jeanrenaud, 1970).

Uptake of α-aminoisobutyrate and 2-deoxyglucose by isolated fat-cells was measured by a technique described by Gliemann *et al.* (1972). Suspensions of isolated fat-cells were stirred with a magnetic stirrer and at zero time the labelled substrate was added. Samples (200μl) of the cell suspension were then taken at intervals by using an Eppendorf micropipette and placed in a 400μl plastic micro-centrifuge tube (Milian Instruments S.A., Geneva, Switzerland) containing 200μl of the oil dinonylphthalate. The tubes were immediately centrifuged for 45s in a Beckman microfuge, causing the medium to become separated from the intact isolated fat-cells by a layer of clear oil. This technique allowed a rapid and almost complete separation of the medium from the adipocytes. The plastic tube was then cut with a large pair of scissors through the oil layer, and the upper part containing the cells was added to 10ml of the scintillation mixture described above, modified to contain also 300ml of ethanol per litre. The lower part of the cut tube contained the medium and a sample of this was also counted for radioactivity in 10ml of the same scintillation mixture.

Cytochalasin A and B were purchased from Imperial Chemical Industries Ltd. (Alderley Park, Cheshire, U.K.) and dissolved in dimethyl sulphoxide. Consequently all control incubation mixtures contained 1% (v/v) dimethyl sulphoxide, the same concentration as in the incubation mixtures with cytochalasin. This amount of dimethyl sulphoxide caused only small and inconsistent changes in glucose metabolism and caused a 10% fall in tissue ATP concentrations. Vincristine sulphate was generously given by Eli Lilly Laboratories Ltd. (Indianapolis, Ind., U.S.A.). All radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Crude bacterial collagenase was from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.) and bovine serum albumin (fraction V) was obtained from either Nutritional Biochemicals Ltd (Cleveland, Ohio, U.S.A.), or from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All other chemicals were of analytical grade and were obtained from E. Merck A.-G., Darmstadt, Germany, Fluka A.-G., Buchs, Switzerland, or from Sigma. Insulin was a 10× recrystallized preparation and was a gift of Novo Industries, Copenhagen, Denmark. The enzymes used in this study were a gift of Dr. F. Schmidt (Boehringer Mannheim G.m.b.H., Mannheim, Germany).

Results

Effect of colchicine and vincristine on glucose metabolism of isolated fat-cells

Colchicine caused a significant inhibition of $^{14}CO_2$ formation and conversion of [^{14}C]glucose into total lipid in the absence of insulin, and of the incorporation of ^{14}C into total lipid and glyceride fatty acid synthesis in the presence of insulin (Table 1). Vincristine at a concentration of 0.01mM had no effect on the basal or insulin-stimulated conversion of [^{14}C]glucose into CO_2, total lipid or glyceride fatty acid by isolated fat-cells.

It has been reported that in some systems these agents require to be in contact with the tissue for several hours to display their effects (Butcher & Goldman, 1972). Since the effects seen in Table 1 are small, experiments were performed in which epididymal fat-pads were incubated with the agents for 2h before isolated fat-cells were prepared. The agents were present also during the collagenase treatment and subsequent incubation with [^{14}C]-glucose. Consequently the total preincubation time was 2h 45min. When the metabolism of such fat-cells was examined, it was found that the extended preincubation with colchicine or vincristine had not resulted in any greater inhibitory action of these agents on insulin-stimulated glucose metabolism. The agents caused an inhibition similar to that seen when they were present for only 1h with isolated fat-cells, and which could be partially reversed by omitting the agent from the final hour of incubation when the tissue was in the form of isolated fat-cells (results not shown). Tissue incubated for the long period with either agent tended to have increased basal incorporation of glucose into the end products, perhaps suggesting a non-specific effect of the prolonged preincubation.

Effect of cytochalasin B on the metabolism of isolated fat-cells

The ability of isolated adipocytes to metabolize glucose to both CO_2 and lipid is inhibited by cytochalasin B in a dose-dependent manner (Fig. 1). The inhibition of glucose conversion into CO_2

Table 1. *Effect of colchicine and vincristine on the metabolism of isolated fat-cells*

Isolated fat-cell suspensions were incubated with no addition, with colchicine (0.1 mM) or vincristine (10 μM) for 15 min and their ability to convert [U-^{14}C]glucose (1 mM) into CO_2, total lipids and glyceride fatty acids was measured during the subsequent hour. When added, colchicine and vincristine were present during the whole incubation. The insulin concentration was 1 m unit/ml. Values are means ± S.E.M. with the numbers of experiments in parentheses.

Additions		Conversion of [^{14}C]glucose (μmol/h per g of lipid) into: CO_2	Total lipid	Glyceride fatty acids
Nil	(4)	0.42 ± 0.08	0.80 ± 0.10	0.22 ± 0.05
Insulin	(4)	2.24 ± 0.51	4.19 ± 0.78	2.72 ± 0.68
Colchicine	(4)	0.31 ± 0.09*	0.59 ± 0.14*	0.12 ± 0.04
Colchicine + insulin	(4)	1.63 ± 0.40	3.22 ± 0.65*	1.87 ± 0.48*
Nil	(3)	0.39 ± 0.13	0.93 ± 0.07	0.24 ± 0.07
Insulin	(3)	1.62 ± 0.45	3.64 ± 0.79	1.98 ± 0.52
Vincristine	(3)	0.42 ± 0.13	0.97 ± 0.06	0.26 ± 0.09
Vincristine + insulin	(3)	1.35 ± 0.34	3.07 ± 0.56	1.56 ± 0.36

* Significantly different from the corresponding value in the absence of colchicine ($P < 0.05$) by using Student's t test for paired variates.

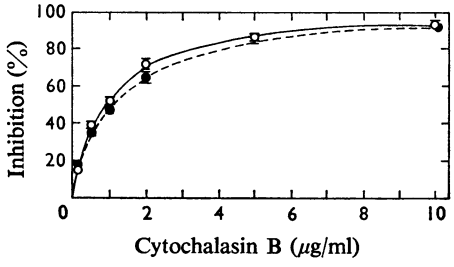

Fig. 1. *Dose-response curve of the inhibitory effect of cytochalasin B on the conversion of [U-^{14}C]glucose into CO_2 and lipids*

Samples (1 ml) of a suspension of isolated fat-cells were incubated for 1 h with 1 mM-[U-^{14}C]glucose (100 μCi/mmol). After the incubation, incorporation of radioactivity into CO_2 and lipids was determined as described in the Materials and Methods section. Each point is the mean of three experiments ± S.E.M. ○, Conversion of glucose into CO_2; ●, conversion of glucose into lipids.

was of a similar degree to the inhibition of glucose conversion into lipid. This finding makes it likely that a step early in the metabolism of glucose is being affected by cytochalasin B.

Insulin-stimulated glucose metabolism was inhibited by cytochalasin B to the same extent as basal glucose metabolism (Table 2). Table 2 also shows that the dose-response curve of the inhibition of glucose transport by cytochalasin B is shifted by using a lower concentration of albumin in the medium. A change of albumin concentration from 3.5% to 0.1% causes the concentration giving half-maximal inhibition to fall from approx. 1 μg/ml to 0.3 μg/ml, at a glucose concentration of 1 mM. Cytochalasin B at 10 μg/ml, which causes over 90% inhibition of glucose metabolism, had no effect on glycerol release or fatty acid release by isolated fat-cells (Table 3). Neither did it alter the lipolytic action of adrenaline or caffeine, or the anti-lipolytic

Table 2. *Effect of cytochalasin B on basal glucose metabolism in the presence of high and low albumin concentrations, and its effect on insulin-stimulated metabolism*

Isolated fat-cells were prepared in 3.5% (w/v) bovine serum albumin and subsequently incubated as described in Fig. 1, in the presence of either 3.5% or 0.1% bovine serum albumin and cytochalasin B. Insulin was added in one case. Values are means ± S.E.M. of three separate experiments, each performed in sextuplicate.

Concn. of cytochalasin B (μg/ml)	Other additions	Bovine serum albumin	Conversion of [U-^{14}C]glucose into CO_2 and lipids (% of control) 3.5%	0.1%
—	—		100	100
0.05	—		—	87 ± 1
0.1	—		83 ± 1	71 ± 2
0.5	—		62 ± 1	45 ± 1
1.0	—		50 ± 3	33 ± 3
2.0	—		34 ± 2	16 ± 2
5.0	—		14 ± 1	5 ± 1
10	—		7 ± 1	3 ± 1
—	Insulin (100 μunits/ml)		330 ± 59	—
10	Insulin (100 μunits/ml)		20 ± 3	—

Table 3. *Effect of cytochalasin B on release of glycerol and free fatty acid by isolated adipocytes, and on ATP content*

Isolated fat-cell suspensions (1 ml) were incubated in the absence of glucose for 1 h with various additions in either the presence or the absence of cytochalasin B. After incubation, glycerol and free fatty acids released into the medium, and ATP content of the isolated fat-cells, were determined as described in the Materials and Methods section. Values are means ± S.E.M. of three separate experiments, each performed in sextuplicate, except for the ATP values in the presence of caffeine, which are from a single experiment.

	Glycerol released (μmol/h per g of lipid)	Free fatty acids released (μmol/h per g of lipid)	ATP content (nmol/g of lipid)
Nil	2.20 ± 0.30	1.67 ± 0.64	99.0 ± 12.0
Cytochalasin B (10 μg/ml)	2.15 ± 0.43	0.77 ± 0.27	62.7 ± 5.0*
Cytochalasin B (2 μg/ml)	—	—	85.1 ± 9.5
Adrenaline (1 μg/ml)	18.21 ± 1.17	42.62 ± 2.00	25.7 ± 4.4
Adrenaline (1 μg/ml) + cytochalasin B (10 μg/ml)	16.84 ± 1.07	38.00 ± 0.63	28.2 ± 6.6
Adrenaline (1 μg/ml) + insulin (100 μunits/ml)	8.75 ± 1.34	14.67 ± 0.51	64.7 ± 0.5
Adrenaline (1 μg/ml) + insulin (100 μunits/ml) + cytochalasin B (10 μg/ml)	9.36 ± 1.19	12.54 ± 0.62	59.6 ± 6.6
Caffeine (1 mM)	16.55 ± 1.98	37.23 ± 9.90	52.5
Caffeine (1 mM) + cytochalasin B (10 μg/ml)	16.59 ± 2.32	37.85 ± 5.25	47.5

* Significantly different from the corresponding value in the absence of cytochalasin B, by Student's t test ($P < 0.05$).

effect of insulin. This concentration of cytochalasin B was similarly without effect on the decrease in ATP induced by adrenaline or by caffeine, and on the restoration of these concentrations by insulin (Bihler & Jeanrenaud, 1970). However, it did cause a small but significant fall in basal ATP concentrations in free fat-cells, a phenomenon that did not occur when the concentration was decreased to 2 μg/ml (Table 3). This latter concentration was used in all subsequent studies, since it produced a 60–70% inhibition of glucose metabolism but did not alter several other metabolic functions measured. At this concentration cytochalasin B did not inhibit the conversion of pyruvate, lactate or arginine into CO_2 and lipids. Likewise, cytochalasin B at this concentration did not decrease the uptake of the non-metabolized amino acid analogue α-aminoisobutyrate (results not shown).

It has been demonstrated that fructose can enter adipocytes in two separate ways, one which is independent of the presence of glucose and probably represents a separate, specific transport system for fructose, and a second which is sensitive to inhibition by glucose and can be stimulated by insulin (Froesch & Ginsberg, 1962). This second method of entry may correspond to fructose transport by the glucose-transport system. Table 4 shows that cytochalasin B inhibits fructose metabolism, although to a lesser extent than it affects glucose metabolism. However, when the fructose metabolism was slightly stimulated by insulin, cytochalasin B decreased this metabolism to the same absolute value as it had lowered the basal fructose metabolism. This result is consistent with the interpretation that cytochalasin B was inhibiting only that part of fructose metabolism that

Table 4. *Effect of cytochalasin B on fructose metabolism by isolated fat-cells*

Isolated fat-cell suspensions (1 ml) were incubated for 1 h with [U-^{14}C]fructose and unlabelled glucose, or [U-^{14}C]-glucose and unlabelled fructose, in the presence or absence of cytochalasin B (2 μg/ml). Insulin when added was present at 100 μunits/ml. Incorporation of glucose carbon into CO_2 and lipids was measured as described in the Materials and Methods section. Values are means ± S.E.M. with the numbers of experiments in parentheses. Each experiment was performed in sextuplicate.

	Metabolism to CO_2 and lipid (μmol/h per g of lipid)	
	Without cytochalasin B	With cytochalasin B (2 μg/ml)
[^{14}C]Fructose (1 mM) (7)	0.144 ± 0.017	0.077 ± 0.021*
[^{14}C]Fructose (1 mM) + insulin (5)	0.233 ± 0.038	0.098 ± 0.030*
[^{14}C]Fructose (1 mM) + 20 mM-glucose (4)	0.037 ± 0.008	0.068 ± 0.024*
[^{14}C]Glucose (20 mM) (3)	3.13 ± 0.48	1.82 ± 0.38 *
[^{14}C]Glucose (20 mM) + 1 mM-fructose (3)	2.97 ± 0.50	1.76 ± 0.40 *

* Significantly different from the corresponding value in absence of cytochalasin B ($P < 0.001$) by using Student's t test for paired variates.

was due to its transport by the glucose-transport system. If this is the case, then by suppressing this part of fructose metabolism by adding high concentrations of unlabelled glucose to compete with the fructose for the glucose-transport system, it

should be possible to test the effect of cytochalasin B on the specific fructose-transport system. As shown in Table 4, under these conditions cytochalasin B does not inhibit fructose metabolism but actually causes a slight increase. This increase was seen at concentrations of glucose of 1, 5 and 20mM and was significant ($P<0.05$) in all cases. Under identical conditions, but with the glucose labelled, glucose metabolism was not affected by fructose, but was inhibited by cytochalasin B (Table 4).

Since cytochalasin B thus inhibits glucose metabolism, but not that part of fructose metabolism that appears to occur through a specific fructose-transport step, and since both sugars are phosphorylated by the same hexokinase in adipose tissue (Froesch & Ginsberg, 1962), it seems that cytochalasin B is not likely to be inhibiting this phosphorylation step. Subsequently the metabolism of these two sugars in adipose tissue probably follows identical pathways, except that glucose 6-phosphate must first be converted into fructose 6-phosphate (Froesch & Ginsberg, 1962). Thus the action of cytochalasin B appears to be at the level of membrane transport of glucose, or possibly at the level of conversion of glucose 6-phosphate into fructose 6-phosphate. This latter possibility was investigated by measuring the uptake of 2-deoxyglucose by isolated fat-cells. This glucose analogue is transported by the same carrier system as glucose, and is phosphorylated by hexokinase, but is not further metabolized (Kletzien & Perdue, 1973; Smith & Gorski, 1968). Since, as shown in Fig. 2, cytochalasin B inhibits the uptake of 2-deoxyglucose to a similar extent to the uptake of glucose, it seems very likely that the effect of the agent is solely to inhibit the membrane transport of glucose or 2-deoxyglucose.

The inhibition of glucose metabolism by cytochalasin B was of the competitive type. When studied over a wide glucose-concentration range, i.e. 0.01–50mM in a typical experiment, cytochalasin B at 1μg/ml increased the apparent K_m for glucose from 0.93mM to 1.95mM but did not change the $V_{max.}$ (controls 2.67μmol/h per g of lipid, cytochalasin B 2.61μmol/h per g of lipid) (Fig. 3). From these data an apparent K_i value of 0.9μg/ml was calculated.

The onset of the effect of cytochalasin B was rapid (Fig. 4). The inhibition of conversion of glucose into

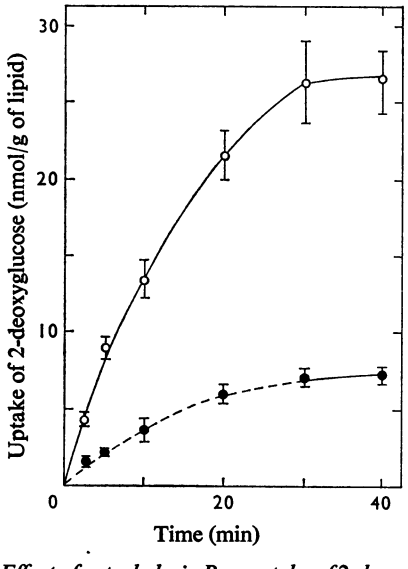

Fig. 2. *Effect of cytochalasin B on uptake of 2-deoxyglucose by isolated fat-cells*

Isolated fat-cell suspensions were stirred with a magnetic stirrer and at zero time 0.1mM-2-deoxy[1-^{3}H]glucose (0.5mCi/mmol) was added. Samples of the cells were taken at regular intervals and the uptake of 2-deoxyglucose was measured as described in the Materials and Methods section. ○, Control; ●, cytochalasin B (2μg/ml). Each point is the mean ± S.E.M. from five separate experiments.

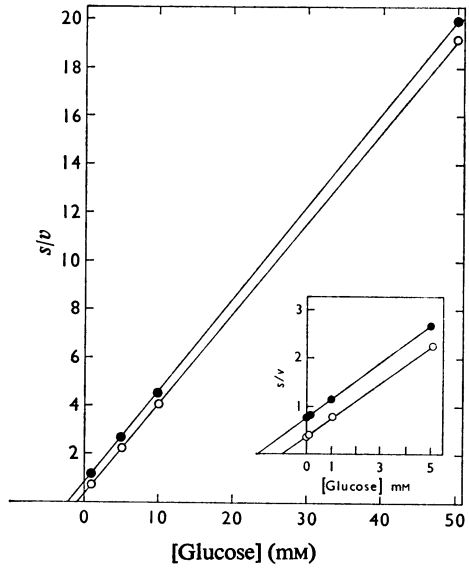

Fig. 3. *Kinetics of the inhibition of glucose metabolism by cytochalasin B*

The conversion of [U-^{14}C]glucose into CO_2 and lipid was measured as described in the Materials and Methods section. This value in μmol/h per g of lipid was used as the velocity in a plot of s/v against s, where s is the glucose concentration in mM. Each point is the mean of five replicate incubations from a typical experiment, and the lines were fitted by the least-squares method. ○, Control; ●, cytochalasin B (1μg/ml). The insert shows results at glucose concentrations from 0.01 to 5mM.

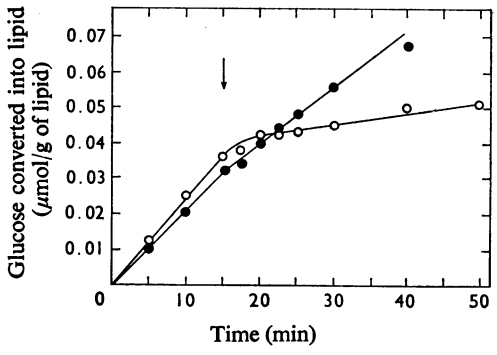

Fig. 4. *Time-course of the inhibition of conversion of glucose into lipid by cytochalasin B*

Suspensions of isolated fat-cells were stirred with a magnetic stirrer in the presence of 0.1 mM-[U-^{14}C]glucose. At various intervals samples were taken for the determination of label in lipids as described in the text. At 15 min (indicated by arrow), either dimethyl sulphoxide or cytochalasin B were added to the cells, giving final concentrations of 1% (v/v) or 2 μg/ml respectively. Each point is the mean of duplicate determinations. ○, Control; ●, cytochalasin B.

Table 5. *Reversibility of the effect of cytochalasin B on glucose metabolism*

Section A. Isolated fat-cells were incubated for 1 h with 1 mM-[U-^{14}C]glucose and the production of labelled CO_2 and lipids was measured as described in the Materials and Methods section. Section B. Cells were incubated for 1 h with dimethyl sulphoxide (1%, v/v) and subsequently washed three times with Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) containing 3.5% (w/v) albumin, and their ability to metabolize [U-^{14}C]glucose was measured during the second hour. Section C. Cells were incubated for 1 h with cytochalasin B and then washed three times with Krebs–Ringer bicarbonate buffer containing albumin and their ability to metabolize [U-^{14}C]glucose was measured during the second hour. Section D. Cells were incubated for the first hour with cytochalasin B and then, without washing, their ability to metabolize [U-^{14}C]glucose was measured during the second hour. When present cytochalasin B was always at a concentration of 2 μg/ml. Values are means±S.E.M. of three separate experiments each performed in quadruplicate.

Section	Agents present during incubation with [U-^{14}C]glucose	Metabolism of [U-^{14}C]-glucose to CO_2 and lipids (μmol/h per g of lipid)
A	None	1.53±0.05
	Cytochalasin B	0.52±0.05
B	None	1.64±0.23
	Cytochalasin B	0.56±0.08
C	None	1.52±0.05
	Cytochalasin B	0.54±0.04
D	Cytochalasin B	0.65±0.09

Table 6. *Effect of cytochalasin A on metabolism of glucose and fructose and tissue ATP concentrations*

Isolated fat-cells were incubated for 1 h in either the presence or absence of cytochalasin A or cytochalasin B. Conversion of 1 mM-[U-^{14}C]glucose or 1 mM-[U-^{14}C]-fructose into CO_2 and lipids was measured as described in the Materials and Methods section. When fructose metabolism was measured, non-radioactive glucose (10 mM) was also present in the incubation mixture. Tissue ATP content was measured at the end of 1 h incubation as described in the Materials and Methods section. Cytochalasin A at a concentration of 5 μg/ml decreased tissue ATP content from a control value of 101±11 nmol/g of lipid to 85±4 nmol/g of lipid during a 1 h incubation. Values are means±S.E.M. of three separate experiments each performed in sextuplicate.

^{14}C-labelled substrate	Metabolism to CO_2 and lipids (μmol/h per g of lipid)		
	Control	Cytochalasin B (2 μg/ml)	Cytochalasin A (2 μg/ml)
Glucose	1.56±0.14	0.51±0.06*	1.44±0.12
Fructose	0.025±0.002	0.035±0.004	0.026±0.001

* Significantly different from control value by Student's *t* test ($P<0.05$).

lipid was fully established within 5 min of the addition of the agent. Its effects were also rapidly reversible, since washing the cells, previously exposed to the agent for 1 h, three times (a procedure taking about 3–4 min) caused complete reversal of the effect. Such washed cells were sensitive to a second dose of cytochalasin B (Table 5).

Cytochalasin A differs from cytochalasin B only very slightly in structure (Aldridge *et al.*, 1967) and possesses some of the actions of cytochalasin B on microfilament-mediated processes (Carter, 1967). However, cytochalasin A is a very poor inhibitor of glucose metabolism and at 2 μg/ml, at which concentration cytochalasin B causes 60–70% inhibition of glucose metabolism, cytochalasin A caused only a 5% inhibition. An inhibition of 80% was not seen until the concentration of cytochalasin A was increased to 20 μg/ml. However, it is possible that this effect of cytochalasin A is non-specific, since at 5 μg/ml, at which concentration the inhibition of glucose metabolism was only 20%, it produced a small though non-significant decrease in tissue ATP concentrations (Table 6). Cytochalasin A, like cytochalasin B, is without effect on fructose metabolism in the presence of glucose (Table 6).

Discussion

Although used at a relatively high concentration in these experiments, neither vincristine nor colchicine had large effects on the metabolism of glucose by

isolated fat-cells. The results shown in Table 1 are similar to those reported by Soifer *et al.* (1971), who reported that colchicine inhibited the insulin-stimulated metabolism of glucose to fatty acids. They differ, however, in that we have observed a similar inhibition of the conversion of glucose into CO_2. The reason for this discrepancy is not known.

Experiments in which fat-pads were preincubated for a prolonged period with colchicine or vincristine suggested that neither colchicine nor vincristine had caused any great inhibition in either basal or insulin-stimulated glucose metabolism. Indeed prolonged incubation with both the agents had tended to increase the basal glucose metabolism, thus decreasing the size of the insulin effect. When the agents were absent during the final hour of incubation the insulin-stimulated metabolism increased slightly, suggesting an effect during this hour similar to the one seen in the experiments of Table 1. These results suggest that agents known to interfere with the microtubular system have little effect on the metabolism of glucose, or on the accumulation of the newly synthesized lipid by adipose tissue.

Cytochalasin B, however, has a profound effect on glucose metabolism by fat-cells, as has been shown for other cell types (Mizel & Wilson, 1972). From the present results, it seems likely that the inhibition of glucose metabolism in isolated adipocytes is the result of a specific action on the translocation of the sugar across the plasma membrane, for the following reasons. Since glucose metabolism to CO_2 and lipids constitutes greater than 80% of total glucose metabolism in isolated fat-cells (Gliemann, 1968), and since it has been previously shown that glucose transport is the rate-limiting step in its subsequent metabolism (Crofford, 1967; Crofford & Renold, 1965), the observed inhibition is most likely to be due to inhibition at this site. Further evidence is provided by experiments in which it was found that uptake of 2-deoxyglucose was also decreased by cytochalasin B. Since this sugar is transported into the cell and phosphorylated by hexokinase but not further metabolized, these results suggest that the effect of cytochalasin B is on one of these two processes. However, because under appropriate conditions glucose metabolism could be inhibited while fructose metabolism was unimpaired, it is apparent that hexokinase is not inhibited by cytochalasin B, since it is known that both these sugars are phosphorylated by the same hexokinase in adipose tissue (Froesch & Ginsberg, 1962). Consequently cytochalasin B appears to act solely at the site of glucose transport to inhibit glucose metabolism. Further indirect evidence for such an action is provided by the lack of any effect on the metabolism of pyruvate, lactate or arginine, and by the absence of any effect on the uptake of the amino acid analogue α-amino-isobutyrate. This specificity is the more remarkable since release of neither free fatty acid nor glycerol was modified by the agent, nor were ATP concentrations affected by doses that were very effective in decreasing glucose transport. It is not clear whether the effect of cytochalasin B at 10μg/ml on ATP concentrations is important, since dimethyl sulphoxide alone also decreased tissue ATP concentrations and cytochalasin B may have simply potentiated this effect.

Our finding that cytochalasin B is a competitive inhibitor of glucose transport is in accord with the results of Kletzien & Perdue (1973). It is, however, at variance with the results of Czech *et al.* (1973), who reported a non-competitive type of inhibition with fat-cell 'ghosts'. As Czech *et al.* (1973) point out, however, their data do not allow a rigorous analysis since glucose uptake in their system was by a mixture of transport and simple diffusion. Since our data yield straight-line plots over a wide concentration range and are derived from experiments with intact cells it is possible that they reflect more accurately the interaction between cytochalasin B and glucose transport than do the data of Czech *et al.* (1973), who did not obtain linear double-reciprocal plots.

Since the only effect of cytochalasin B in the present experiments was to inhibit glucose transport, a membrane-mediated function, it is possible that the action of this drug is confined to the plasma membrane. In keeping with such a postulate is the finding that the drug acts very rapidly, and more importantly, that its effects on glucose transport are rapidly reversed when the tissue is washed. Similarly the demonstration of a clearly competitive inhibition of glucose metabolism by cytochalasin B is also consistent with an action at the site where glucose and its carrier interact, i.e. probably at the external surface of the cell. Since some of the effects of this agent on microfilament processes in other cell types are also rapid in onset, and rapidly reversible (Carter, 1967; Wessells *et al.*, 1971), it is possible that these effects are also secondary to an action at the surface of the cell. The finding that the stimulation of lipolysis by adrenaline and its inhibition by insulin are unimpaired by cytochalasin B, shows that the adenylate cyclase–cyclic AMP–phosphodiesterase system is not affected, and suggests that any alteration of the cell membrane by cytochalasin B must be of a rather specific type. The lack of any alteration in lipolysis by cytochalasin B also demonstrates that not only is the enzymic machinery for lipolysis, and its interaction with agents such as adrenaline, insulin or caffeine, unaltered, but also that any intracellular movement of free fatty acids or glycerol that occurs during their release is apparently not sensitive to cytochalasin B.

Although cytochalasin A has some of the effects of cytochalasin B on microfilament-mediated pro-

cesses (Carter, 1967), it apparently has a much weaker action than the latter agent on glucose metabolism. In view of the very slight difference in the structure of the two derivatives (Aldridge *et al.*, 1967), this result is surprising and suggests that the effects on microfilaments and glucose transport may be dissociable. It is suggested that cytochalasin A may be a useful agent with which to differentiate the two effects because of its weaker action on glucose transport.

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