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# Amine oxidase activities in brown adipose tissue of the rat: identification of semicarbazide-sensitive (clorgyline-resistant) activity at the fat cell membrane

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Amine oxidase activity, previously described in homogenates of brown adipose tissue of the rat, has now been investigated in preparations of isolated fat cells. It was found that the specific activities of both monoamine oxidase A (MAO) and of the semicarbazide-sensitive clorgyline-resistant amine oxidase (SSAO) were higher in isolated fat cells than in the original whole tissue. Brown adipocytes therefore represent a major source of both these enzymes. In plasma membranes prepared from these isolated brown fat cells by borate extraction there was a similar enrichment of activity of SSAO and of the plasma membrane marker enzyme, phosphodiesterase I. However in preparations of cell membranes made by binding the cells to polycation-coated beads, enrichment of phosphodiesterase I activity was much greater than that of SSAO. It is suggested that the disposition of the enzyme within the cell membrane may account for the discrepancy in these results, i.e. the sidedness of the membrane may be important. Histochemical visualization of enzyme activity in whole tissue at the ultrastructural level was undertaken. Positive staining of mitochondria was achieved in the presence of the MAO substrate, tryptamine. Staining around the edges of the brown fat cells was observed with the SSAO substrates, tyramine and benzylamine. Staining was largely absent when substrate was omitted or after pretreatment with the irreversible SSAO inhibitor, hydralazine and the slowly reversible inhibitor, semicarbazide. It is not definitely proven that this staining represents sites of enzyme activity but the results are consistent with evidence from other studies indicating that SSAO in brown adipose tissue of the rat may be found predominantly at the fat cell surface. The significance of these findings in relation to the possible function of SSAO is discussed.

It is now known that brown adipose tissue (BAT) of the rat contains not only flavin-dependent monoamine oxidase (monoamine $O_2$: oxido-reductase, EC 1.4.3.4: MAO), but also an amine oxidase sensitive to carbonyl reagents such as semicarbazide and resistant to the MAO inhibitor clorgyline. Until recently this second enzyme has been called by some clorgyline-resistant amine oxidase (Lyles et al 1983). However, a name more generally accepted is semicarbazide-sensitive amine oxidase (SSAO).

Whereas mitochondrial MAO is able to deaminate catecholamines and is probably involved in regulating tissue levels of noradrenaline and in disposing of excess catecholamines after uptake, the function of SSAO is less clear. Its affinity for noradrenaline is very low and it is unable to deaminate the secondary amine, adrenaline (Barrand & Callingham 1982). Furthermore, although certain of its properties resemble those of the so-called copper-containing amine oxidases found in plasma and in connective tissue (Blaschko 1974; Yasunobu et al 1976; Barrand et al 1983a), its inability to deaminate histamine and polyamines and its insensitivity to inhibition by cyanide and amino-propionitrile sets it apart from these enzymes.

An enzyme which, like SSAO, is able to deaminate benzylamine has been identified in a variety of human and rat tissues (Lewinsohn et al 1978). Histochemical studies at the light microscope level have revealed intense activity of this enzyme around large blood vessels in the human myometrium (Ryder et al 1980). Since the activity of this enzyme appears greatest in tissues rich in smooth muscle, especially vascular smooth muscle, a localization to this particular cell type has been suggested (Lewinsohn 1981). While BAT is a highly vascular tissue, most of its cells are adipocytes or endothelial cells with only a small number of smooth muscle cells although the relative proportions of these cell types may vary with diet or temperature (Bukowiecki et al 1982). It seems therefore that, in BAT at least, SSAO may be associated with cell types other than smooth muscle cells.

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Separation of BAT cells by digestion and isolation of adipocytes has therefore been undertaken to determine the cellular location of SSAO. Histochemical techniques have also been employed to visualize amine oxidase activity in the whole tissue.

When BAT is subjected to subcellular fractionation, SSAO remains in the particulate fraction, not bound to mitochondria, but chiefly associated with the outer cell membrane (Barrand & Callingham 1982). To provide further evidence of this localization of the enzyme to the cell surface, plasma membranes have been prepared and purified from isolated brown adipocytes.

Preliminary results of these studies have been reported (Barrand et al 1983a, b).

## MATERIALS AND METHODS

### *Materials*

5-Hydroxy [G-$^{3}$H]tryptamine creatinine sulphate and [7-$^{14}$C]benzylamine hydrochloride were purchased from Amersham International plc (Amersham, UK). All other compounds were either standard laboratory reagents or were purchased from Sigma Chemical Co. Ltd (Poole, Dorset, UK).

### *Methods*

Cell isolation. Dorsal interscapular BAT was obtained from male Wistar rats of 250 to 500 g weight. Fat cells were isolated by the following modification of Rodbell's original method (Bukowiecki et al 1980). Portions of tissue were cut into small pieces with scissors and digested for 45 min at 37 °C with 1 mg ml$^{-1}$ collagenase (from *Clostridium histolyticum*, Boehringer, Mannheim) and 3 mg ml$^{-1}$ bovine serum albumin (previously dialysed for 24 h to remove free fatty acids) in Krebs-Henseleit solution maintained at pH 7·4 by frequent bubbling with 95% $O_2$ and 5% $CO_2$. At the end of digestion, the fat cells were filtered through a double layer of nylon mesh stocking and washed several times with the albumin-containing buffer. Between each washing, the isolated fat cells were separated from other cell types by centrifuging at 600*g* for 20 s at room temperature (20 °C), sucking away the underlying debris and dispersing the floating cells in clean buffer.

Isolated cells were examined under the light microscope to detect any contamination by other cells, particularly those of small blood vessels. The diameters of the cells were measured with a Neubauer's haemocytometer. Smears of the cells were also made, stained with methylene blue and examined for contamination. Samples of isolated fat cells were fixed in a mixture of 2 parts 1% osmium tetroxide: 1 part 2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer, pH 7·4 for 30 min. After 5 min at room temperature, fixation was continued at 4 °C. The fixed cells were centrifuged for a few seconds at 600*g* to form a loose pellet. They were then washed three times with 0·9% sodium chloride before being dehydrated and embedded in Epon. Thick sections (around 1 μm) were cut and stained with toluidine blue and examined by light microscopy. Ultra thin sections were stained with uranyl acetate and lead citrate for electron microscopy.

Further samples of isolated fat cells were ruptured by freezing in 1 mM potassium phosphate buffer at pH 7·8 for enzyme assay, while original BAT samples were taken to provide a measure of enzyme activity in the whole tissue. These were homogenized in 1 mM phosphate buffer and kept deep frozen until assay.

*Plasma membrane preparation.* Plasma membranes were prepared from the fat cells, isolated as described above, in one of the following two ways:

*1. Borate extraction.* With this method, modified from that of Warley & Cook (1973), plasma membranes in the presence of a stabilizing agent become isolated in the form of sheets rather than being reduced to membranous vesicles as in our previous subcellular fractionation procedure (Barrand & Callingham 1982). Cells isolated in Krebs-Henseleit were washed twice in a harvesting solution containing 150 mM sodium chloride, 50 mM borate, 1 mM magnesium chloride and 1 mM calcium chloride at pH 7·2. 0·8 ml of these suspended cells was added to 20 ml of an extraction solution containing 20 mM borate and 0·2 mM EDTA at pH 9·2 and vigorously stirred for 10 min. 0·8 ml of 0·5 M borate at pH 9·6 was added and the mixture centrifuged at 450*g* for 10 min. The pellet and the floating fat debris were discarded and the infranatant centrifuged at 12 000*g* for 30 min. The resultant pellet was resuspended in 0·6 ml of a membrane wash solution containing 20 mM borate and 1 mM EDTA and layered over the top of 20 ml of 35% w/w sucrose in phosphate (5 mM) buffered saline (pH 7·2). This was centrifuged in a swing-out rotor at 24 000*g* for 1 h. The two resulting layers, one at the surface and the other at the interface between the sucrose layer and the membrane wash solution were sucked off, diluted with phosphate buffered 0·9% NaCl (saline) and centrifuged at 100 000*g* for 30 min to produce pellets. These were either fixed overnight in 4% glutaraldehyde followed by post fixation in 1% $OsO_4$, dehydration and embedding in Epon for electron

microscopy or taken up in 1 mM potassium phosphate buffer for subsequent enzyme assay for MAO, SSAO and phosphodiesterase I activity.

*2. Binding to polycation-coated beads.* With this technique, intact cells become so firmly attached by ionic interaction to the beads that they can be subsequently disrupted to release their intracellular contents whilst still leaving their outer cell membranes attached with their cytoplasmic surfaces exposed (Jacobson 1980). The beads used for this study were Affigel 731 (Biorad Lab. Richmond, CA) polyacrylamide beads which have polyethyleneimine covalently bonded on to their surfaces. The beads were washed several times with 0·2 M sodium chloride and then with an attachment buffer containing 20 mM sodium acetate, 140 mM sorbitol at pH 5. Isolated fat cells, initially suspended in Krebs–Henseleit/albumin buffer as described above, were rinsed twice in the attachment buffer. An equal volume of beads suspended in the attachment buffer was added dropwise into the cell suspension and mixed by gentle agitation. After 10 min any uncovered areas of the beads were neutralized by adding an equal volume of 1 mg $ml^{-1}$ of poly-L-glutamic acid in 100 mM sorbitol in 20 mM Tris buffer at pH 7·7. Unattached cells were removed by washing the beads several times with 130 mM sorbitol in 10 mM 2-[*N*-morpholino]ethanesulphonic acid (MES) buffer at pH 6. The beads were then vortexed in 10 mM MES, pH 6 to disrupt the attached cells and release the intracellular contents. After washing, the cell membrane coated beads were suspended in 1 mM potassium phosphate buffer, pH 7·8 for subsequent enzyme assay for MAO, SSAO and phosphodiesterase I activity. Since the coated beads interfere with the Lowry method for protein estimation, cellular membrane protein bound to the beads had first to be eluted by heating the beads for 5 min at 70–80 °C in 2% SDS. Samples of the resultant supernatant were taken and treated in the usual way for protein estimation. To check the efficacy of the method, samples of the beads with cells attached were examined under a Telaval tissue microscope (VEB Carl Zeiss, Jena, E. Germany) during the course of the preparation.

*Ultrastructural histochemistry.* Three separate methods for the ultrastructural visualization of MAO activity were attempted; the BSPT method of Shannon et al (1974) which relies on the formation of an osmiophilic formazan by reduction of the non-osmiophilic tetrazolium salt, 2-(2-benzothiazolyl)-5-styryl-3-(4-phthalhydrazidyl)tetrazolium chloride (BSPT); the cerium chloride method of Fujimoto et al (1982) in which cerium forms electron dense complexes at sites of hydrogen peroxide production; and the ferricyanide method of Hanker et al (1973). This last method uses ferricyanide as an artificial electron acceptor. Insoluble cupric ferrocyanide or Hatchett's Brown, deposited at sites of enzyme activity, is able to catalyze subsequently the oxidative polymerization of 3,3-diaminobenzidine (DAB) resulting in amplification of the original deposits. The polymer is osmiophilic and so may be rendered electron-dense on treatment with osmium tetroxide. It was not found possible to achieve the right conditions to produce any localized staining of mitochondria either in liver samples or in BAT with the first two methods and so these were abandoned. The third method however proved successful, possibly because of the amplification step that the other two methods do not have. This then was used to investigate the localization of both MAO and SSAO activity in BAT.

Samples of tissue were removed, immediately immersed in fixative and chopped into small pieces, approximately 0·5–1 mm thick, on a Mickle tissue chopper. Preliminary experiments were performed on samples of pelleted BAT membranes to determine the degree of inhibition, both reversible and irreversible, of MAO and SSAO activity at varying concentrations of fixative. The fixatives found most suitable were 0·05% formaldehyde (prepared from freshly depolymerized paraformaldehyde) in 0·1 M sodium phosphate buffer at pH 7·3 or a mixture of 0·01% glutaraldehyde and 0·05% formaldehyde in the same buffer. All solutions used during the histochemical reaction contained 0·15 M sucrose to minimize osmotic disturbance to the tissue. Fixation was brief (10–15 min) and was followed by extensive washing in buffer alone for 30–60 min. It was at this stage that any pretreatment of samples of the tissue with inhibitor was undertaken. The samples were rinsed several times in 0·05 M acetate buffer at pH 5·6 and then incubated overnight at room temperature in a medium containing 0·035 M sodium acetate, 0·005 M sodium citrate, 1·7 mM copper sulphate, 4·5% dimethyl sulphoxide (DMSO), 0·28 mM potassium ferricyanide and either 2·5 mM tryptamine, 1 mM tyramine or 0·5 mM benzylamine as substrates for the enzymes. The final pH of the medium was between 6·6 and 6·8, well below the optimum pH for either MAO or SSAO activity, hence the long incubation time, but more appropriate for forming the insoluble ferrocyanide deposits without spurious results (Hanker et al 1973). Control samples were either incubated without substrate or were pre-

treated with specific inhibitors i.e. $10^{-4}$ M hydralazine or $10^{-3}$ M semicarbazide to block SSAO activity. The tissues were rinsed three times with phosphate buffer, post-fixed for 1 h in 4% formaldehyde and washed well in distilled water to remove all traces of fixative before immersion for 20–30 min in a freshly prepared solution of DAB (0·5 mg $ml^{-1}$ in 0·05 M acetate buffer at pH 5·6) to generate the osmiophilic polymer. The tissues were then washed well in distilled water, treated with 1% $OsO_4$ for 1 h, dehydrated and embedded for electron microscopy in the normal way. Sections were examined unstained.

*Enzyme assays.* Amine oxidase activity was determined radiochemically (Callingham & Laverty 1973) with 0·025 mM [$^{14}$C]benzylamine (specific activity 10 μCi $\mu mol^{-1}$) as substrate for SSAO and 0·5 mM [$^{3}$H]5-hydroxytryptamine (specific activity 2 μCi $\mu mol^{-1}$) as substrate for MAO-A. Phosphodiesterase I was assayed by a spectrophotometric method (Touster et al 1970) and protein by the method of Lowry et al (1951).

## RESULTS

### *Isolation of brown fat cells*

Cell suspensions were examined in Neubauer's haemocytometer and the sizes of the cells were measured. The majority of the cells appeared to be in the range of 10–40 μm in diameter. This is very similar to the sizes reported by Bukowiecki et al (1980) for isolated brown adipocytes.

One μm sections of fixed preparations of isolated cells, stained with toluidine blue, were examined under the light microscope (Fig. 1) and were found to contain predominantly adipocytes with few if any other cell types. Examination of ultrathin sections taken from these blocks under the electron microscope showed the brown fat cells to be intact with undistorted mitochondria (Fig. 2).

Preparations of isolated fat cells were subjected to osmotic shock and assayed for MAO and SSAO activity. It was found that the specific activities of both these enzymes were higher in isolated cell samples than in the whole tissue homogenates of the same brown fat samples from which the cells were isolated (Table 1).

### *Plasma membrane preparation*

Plasma membrane samples prepared by borate extraction from the isolated fat cells were examined under the electron microscope. Membranes in the form of sheets or as vesicles of various sizes were seen (Fig. 3). There was little evidence of ribosomal elements or obvious mitochondrial profiles in the membrane pellets prepared from the upper band of material formed after sucrose centrifugation whereas some contamination was seen in the pellets prepared from the lower band of material.

Table 1. Amine oxidase and phosphodiesterase I activities in whole brown adipose tissue and in isolated brown fat cells. Values shown are the mean ± s.e.m. of n experiments.

| | Specific activity in nmol $h^{-1}$ mg $protein^{-1}$ | | |
|---|---|---|---|
| | SSAO (n = 11) | MAO (n = 10) | Phosphodiesterase I (n = 7) |
| Whole tissue | 23·11 ± 3·03 | 7·87 ± 1·85 | 560 ± 63 |
| Isolated fat cells | 39·06 ± 5·20 | 14·57 ± 1·34 | 336 ± 59 |

Assay of enzyme activity in these two bands revealed that MAO activity was somewhat lower and both SSAO and phosphodiesterase I activities were higher in the upper band of material compared with that found in the lower band. Enrichment of activity from that of the original cell suspension was evident with all three enzymes, particularly in the upper band. But whereas MAO activity was enriched approximately two-fold, that of SSAO and of the plasma membrane marker enzyme, phosphodiesterase I, was enriched about five to seven fold (Table 2).

Isolated fat cells were found to bind moderately well to the surfaces of the polycation-coated beads (Fig. 4). However the yield of membrane protein obtained at the end of the experiments was small so that the amount of material available for enzyme assay was limited. Despite these limitations, it was possible to show that with this method of preparing plasma membranes, phosphodiesterase I activity was enriched to a much greater extent than either MAO or SSAO activity (Table 2).

Table 2. Comparison of enrichment of MAO, SSAO and phosphodiesterase I activities in samples of brown fat cell plasma membranes prepared by two separate methods. Values shown are the mean ± s.e.r. of five experiments. Enrichment ratio is calculated from the ratio of specific enzyme activity in the purified cell membrane preparation to specific enzyme activity in the original cell suspension. Values for the specific activities of the three enzymes in the original cell suspensions were in the range of values shown in Table 1.

| | Enrichment ratio | | |
|---|---|---|---|
| Method of preparation | MAO | SSAO | Phosphodiesterase I |
| Borate extraction: | | | |
| upper band | 1·58 ± 0·79 | 5·16 ± 0·41 | 7·59 ± 1·29 |
| lower band | 2·21 ± 0·21 | 4·41 ± 0·57 | 4·00 ± 0·86 |
| Binding to polycation-coated beads | 1·86 ± 0·19 | 1·88 ± 0·25 | 5·58 ± 1·11 |

*Histochemical observations*

The effect of various concentrations of fixative on both MAO and SSAO activity was determined initially using BAT membrane pellet preparations. Enzyme activities remaining either after incubation of pellets with fixative for 15 min followed by washing and repelleting or with fixative present in the enzyme assay are shown in Table 3.

A mixture of 0·01% glutaraldehyde and 0·05% paraformaldehyde was mainly used for fixing the tissue blocks since this seemed to produce minimal enzyme inhibition whilst preserving ultrastructure sufficiently well to identify sites of staining. Nevertheless preservation of mitochondria was not always good. In spite of the presence of sucrose throughout washing and incubation, mitochondria often appeared swollen.

Owing to the very limited penetration reported for DAB (Yoo & Oreland 1976), thin sections were cut close to the surface of each tissue block. It was observed that staining occurred only to a depth of about five to six cells from the surface.

Staining was not found in every one of the twenty experiments performed and the reasons for this lack of consistency are not clear. However in over 50% of the experiments, electron dense deposits could be seen and in these experiments the pattern of results was fairly similar.

In tissue blocks incubated in the presence of 2·5 mM tryptamine ($K_m$ for MAO approx. 5–6 μM, $K_m$ for SSAO around 80–100 μM), electron-dense deposits were present along the outer mitochondrial membrane and also over the cristae (Fig. 5a). Not all mitochondria in any one section were stained. A small amount of stain could be found around the edges of the brown fat cells (Fig. 5b). Scattered electron dense deposits were also associated with the lipid droplets but neither nuclear membrane nor endoplasmic reticulum became stained.

In tissue blocks incubated with 1 mM tyramine ($K_m$ for MAO 180 μM, $K_m$ for SSAO 40 μM), mitochondrial staining was again evident though not so intense, both on outer membranes and cristae. Staining was also present along the cell edge around the brown fat cells but not around the edge of other cell types, i.e. endothelial cells (Fig. 5c). Preincubation of the tissue blocks with $10^{-4}$ M concentrations of the irreversible SSAO inhibitor hydralazine (IC50 $5 \times 10^{-8}$ M) or with $10^{-3}$ M of the slowly reversible SSAO inhibitor, semicarbazide (Andree & Clarke 1982), largely prevented any cell edge staining.

In tissue blocks incubated with 0·5 mM benzylamine ($K_m$ for SSAO 2·5 μM) generally only the cell edge staining was visible (Fig. 5d). Occasionally staining was found also on isolated mitochondria. Benzylamine is also a substrate for MAO-B ($K_m$ approx. 100 μM) and a small amount of this type of MAO has been detected in brown adipose tissue (Barrand & Callingham 1982).

In tissue blocks incubated without substrate, little if any staining could be found in mitochondria or along the cell edge. Granules of electron dense deposits could still be seen in and around the lipid droplets.

Table 3. Effect of fixation of SSAO and MAO activity. Mean results of two experiments showing the percentage enzyme activity remaining in brown adipose tissue membrane pellets (a) with fixative present in the assay and (b) after preincubation for 15 min with fixative followed by washing and repelleting.

| | Percentage activity remaining (a) | | (b) | |
|---|---|---|---|---|
| Fixative | MAO | SSAO | MAO | SSAO |
| 0·1% glutaraldehyde | 19 | 17 | 71 | 59 |
| 0·05% ,, | 20 | 28 | 93 | 80 |
| 0·01% ,, | 35 | 69 | 96 | 93 |
| 0·1% paraformaldehyde | 13 | 59 | 67 | 77 |
| 0·05% ,, | 16 | 72 | 88 | 94 |
| 0·01% ,, | 11 | 87 | 100 | 100 |

## DISCUSSION

Separation of fat cells from other cell types in BAT results in an increase in the specific activities of both MAO and SSAO when compared with their specific activities in homogenates of the original whole tissue. This would indicate not only that brown fat cells contain both MAO and SSAO, but also that these cells represent a major source of enzyme activity in this tissue, although it does not rule out the possibility that significant amounts of these enzymes may be present in other cell types. It is hardly surprising that these fat cells contain mitochondrial MAO, since these highly active cells are filled with a great number of characteristically large rounded mitochondria whose proton conductance pathways play an important role in providing thermogenic activity (Nicholls 1977). By comparison the other cell types contain far fewer mitochondria.

The fact that SSAO is present in the isolated fat cells in high amounts indicates that in BAT the contribution from vascular elements towards the total tissue content of enzyme is likely to be very small. This is probably accounted for by the relatively few smooth muscle-containing blood vessels in BAT. This is of particular interest since nearly all

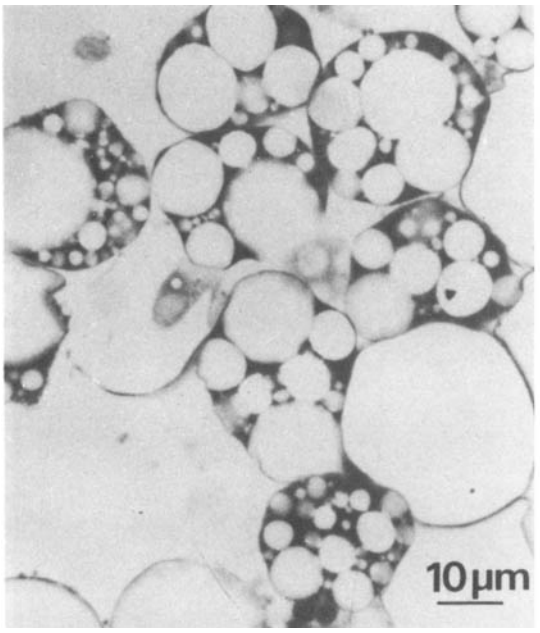


FIG. 1. Light micrograph of a sample of isolated brown fat cells, fixed in $OsO_4$ and glutaraldehyde, embedded in Epon and stained with toluidine blue.

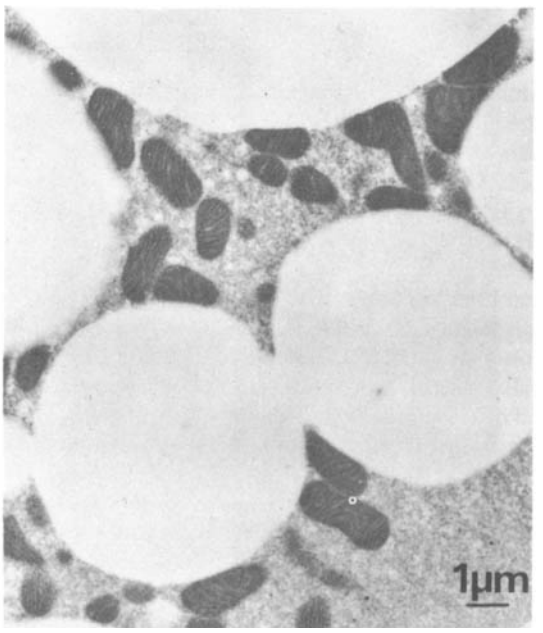


FIG. 2. Electron micrograph of part of an isolated brown fat cell.

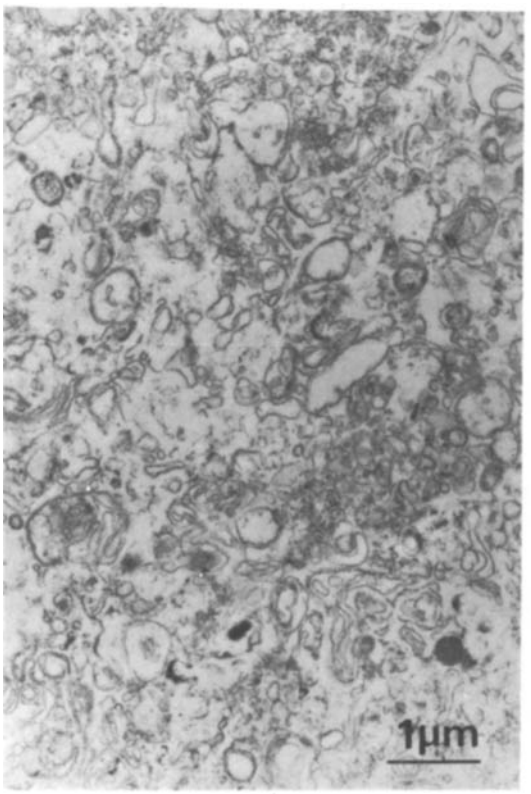


FIG. 3. Electron micrograph of a plasma membrane sample prepared from isolated brown fat cells by borate extraction. The membranes shown are from the upper layer of material formed after sucrose centrifugation.

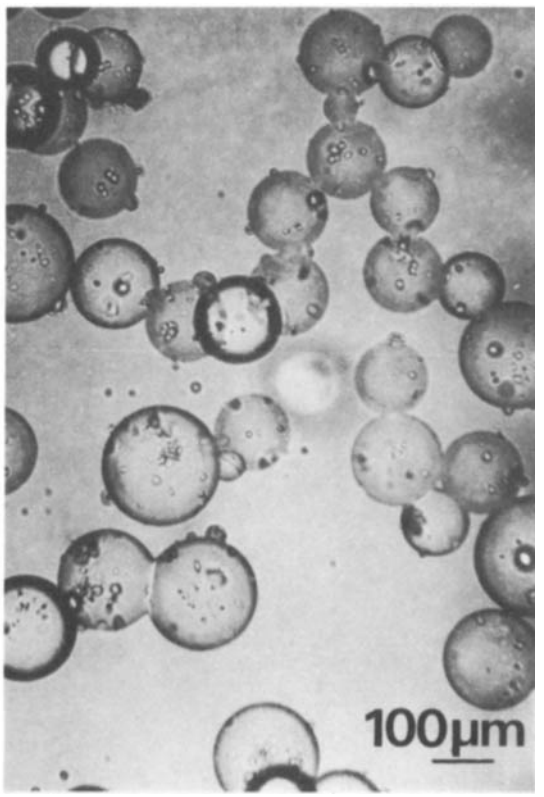


FIG. 4. Photograph of polycation-coated beads with isolated brown fat cells attached to their surfaces, as viewed under a Telaval tissue culture microscope.

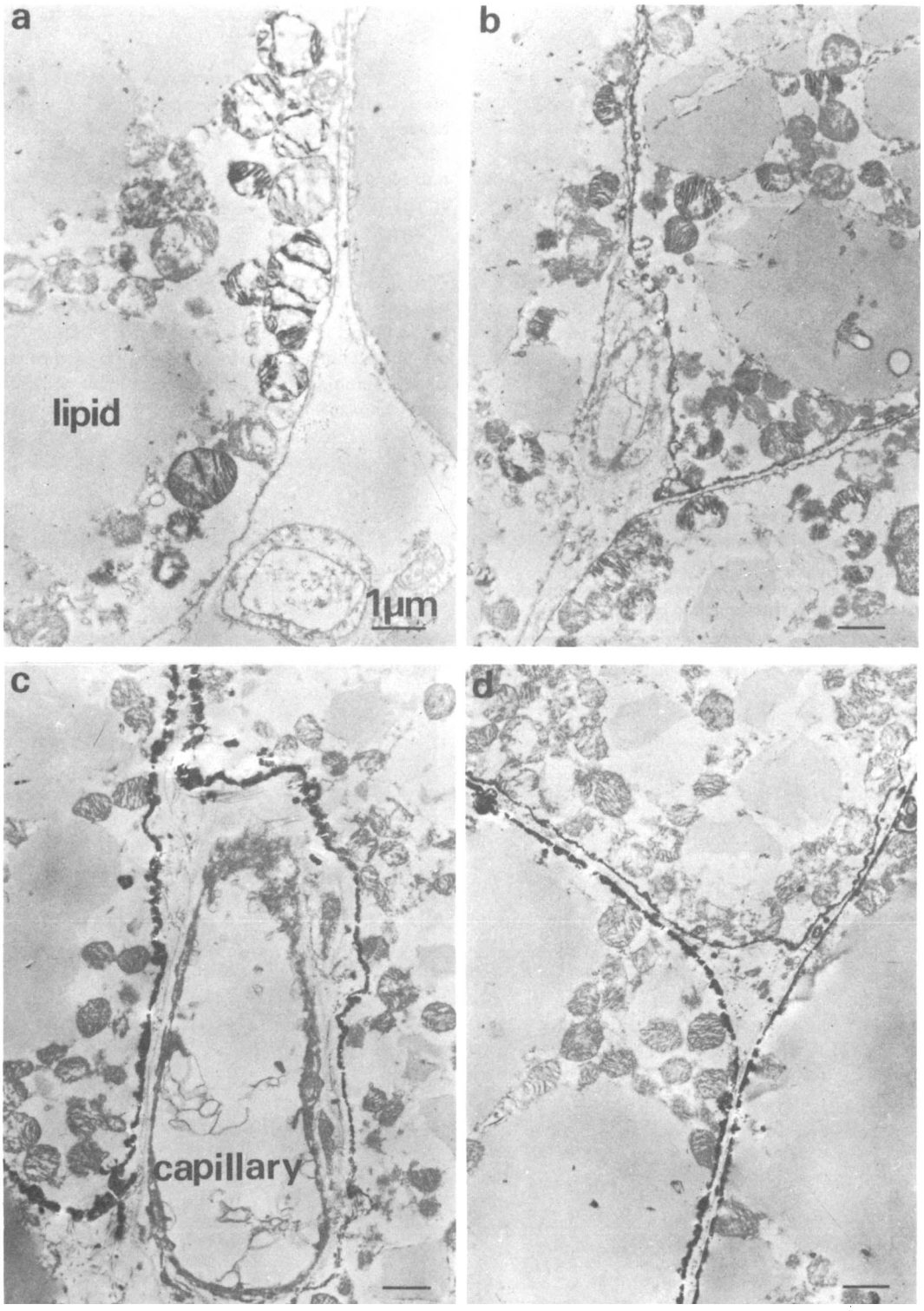


FIG. 5. Electron micrographs of brown adipose tissue treated by the ferricyanide method to visualize amine oxidase activity, showing the electron-dense deposits produced after incubation with: (a) tryptamine with semicarbazide pretreatment, (b) tryptamine, (c) tyramine, (d) benzylamine.

previous observations indicate that this or similar enzymes are to be found predominantly in tissues rich in smooth muscle, in for example, aorta (Coquil et al 1973), mesenteric blood vessels (Callingham et al 1983) and anococcygeus muscle of the rat (Callingham 1982). In addition recent studies on isolated cells from rat heart (Lyles & McAuslane 1983) have shown that SSAO activity can be found not only in myocytes but also in non-muscle cells. This raises questions about whether or not there is a single common function for this enzyme in these different locations.

Indications that SSAO may reside at least partly at the cell surface have been obtained previously from subcellular fractionation studies on whole tissues, both BAT (Barrand & Callingham 1982) and aorta (Wibo et al 1980). The results from the present studies in which plasma membranes were prepared by borate extraction from isolated fat cells support these earlier observations. Similar enrichment of the activities of SSAO and the plasma membrane marker enzyme, phosphodiesterase I, was obtained with this technique. However preparation of cell membranes by binding to polycation-coated beads did not bring about the same enrichment of activity of the two enzymes. The reason for this discrepancy is not clear, and it may be that SSAO is not located on the plasmalemma. On the other hand, with the bead method, the outside of the cell membrane becomes attached to the bead. This may well modify the activities of enzymes located partly or wholly on the external surface of the cell. A carbohydrate moiety has indeed been identified on the SSAO solubilized from BAT by the use of affinity chromatography with the lectin from Lens culinaris (Barrand & Callingham 1984) suggesting that at least part of the SSAO enzyme faces outwards.

The results of the histochemical studies to visualize SSAO activity within whole BAT supports the idea that SSAO is a plasma membrane enzyme located primarily on the brown fat cells. However, lack of consistency of staining with the histochemical method makes interpretation difficult. It clearly cannot be stated without doubt that the cell edge staining seen after incubating tissue blocks with substrates for SSAO represents sites of SSAO activity. But it may be noted that staining for MAO activity in these experiments was equally inconsistent and yet localization of MAO to the outer mitochondrial membrane has already been well established (Schnaitman et al 1967). It would appear therefore that the inconsistency is a property of our use of the method rather than of the disposition of the enzyme. When the histochemical method gave clearly visible staining it was always associated with the cell membrane. These results are entirely consistent with the evidence derived by all the other methods that have been used indicating that SSAO in the BAT of the rat is to be found predominantly at the cell surface of the fat cells.

The particular location of SSAO, so different from that of MAO, encourages speculation about its physiological importance. Unlike MAO it may not be dependent upon the delivery of extracellular substrate by an uptake system. It also appears to be rather more selective in its preference for substrate and will not deaminate secondary amines and shows its highest affinity for benzylamine which has so far not been demonstrated to occcur naturally. 2-Phenethylamine is, however, in the rat a good substrate for this enzyme (Barrand & Callingham 1982) so it is possible that one function is to scavenge trace amines of this nature. On the other hand the substrate or substrates may themselves be located in the cell membrane and provide fuel for the enzyme to make peroxide which has been suggested to be a second messenger in the modification of metabolism in white fat cells (Mukherjee & Mukherjee 1982). The relative importance of SSAO in the events that take place in and around the membranes of the fat cells and of vascular smooth muscle cells needs to be resolved and an obvious next step is to determine if changes in SSAO activity can modify some aspect of cellular metabolism.

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