two neighboring fibrils, have been found occasionally in all the material examined.

At the appositional region a space of from 60 Å to 140 Å (usually about 100 Å) separates the 75-Å double-membrane of the E-element from the 56-Å double-membrane of the C-element. Smith (*5*) stated that in *Tenebrio* flight muscle the reticular and cysternal wall is "clearly" not a "double membrane" but is simple, single, and only 50 Å thick. The membrane surrounding these elements does not take the stain or differentiate as clearly as it does in the E-elements, but in our best sections it is always distinctly a three-layered, normal-looking "double membrane" (*6*), though thinner than its apposed neighbor. Its thickness in these sections is 56 Å. This remarkably thin membrane is composed of an outer osmiophilic line (the appositional one) 20 Å thick, a clear middle line 20 Å wide, and an inner line 16 Å thick. The two apposed membranes are heavily osmiophilic and stand out sharply. The outer wall of the E-element is usually also strongly osmiophilic, but not the outer wall of the C-element.

Between the two elements a line of fine granules is located, often with regularly spaced thickenings, giving a scalloped appearance. At the thickenings bridges link the two elements.

This appearance is substantially similar to that reported by Fahrenbach (*1*), but the interpretation is different. He interprets the line of granules as a fusion of the outer membranes of the two elements, whereas it is quite clear in our material that it cannot be so interpreted. However, the "inner membrane" of the C-element illustrated by Fahrenbach is dense and quite thick (60 Å) and could therefore be an unresolved double membrane.

Of some interest is the nature of the anchoring devices which keep the diads in position during contraction. In vertebrate muscle positioning is taken care of by the close association of the triads with the Z-discs, which with their lateral connections form an effective structural framework. In the arthropods examined the diadic or triadic elements were found to be linked by a series of cross bridges, presumably elastic, but about 200 Å long as seen in rest-length fibrils, between the adjacent thick filaments and the E- or C-elements (Figs. 1 and 2). They occur with a regular periodicity of about 180 Å. In these regions the orbits of thin filaments are incomplete, those on the E- or C-side being absent.

In the appositional region granules are present in both the E- and the C-elements. In the former, they are larger and stain more heavily.

It is unlikely that a simple form of electrical transmission could occur at such a junction.

GRAHAM HOYLE

Department of Biology,
University of Oregon,
Eugene 97403

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Lipase: Localization in Adipose Tissue

Abstract. *Certain problems usually associated with the histochemistry of lipases are obviated by a technique that utilizes the endogenous blood chylomicrons and the cellular stores of triglyceride as substrates for the histochemical demonstration of lipolytic enzyme activity* in situ. *In spreads of mesenteric adipose tissue, the technique makes it possible to distinguish between lipoprotein lipase activity at sites in the capillaries and lipolysis occurring in the adipocytes. The selective anatomic localization of the lipase reaction correlated with the functional state of the tissue, and the absence of reaction product in control mesenteries from starved mice or in heat-inactivated controls, support the validity of this histochemical reaction.*

Adipose tissue contains at least two distinct lipolytic enzyme systems (*1*): (i) an alkaline lipase (*2*) that preferentially catalyzes the hydrolysis of triglyceride present in chylomicrons and low-density lipoproteins; and (ii) an acid lipase that facilitates the hydrolysis of triglyceride stored in adipocytes (*3*).

On the basis of its wide distribution in the body tissues and its very rapid appearance in the plasma after intravenous injection of heparin, it has generally been assumed that lipoprotein lipase activity occurs at the luminal surface of capillaries, but recently Rodbell (*4*) failed to find any lipoprotein lipase associated with his stromal-vascular fractions of adipose tissue. A specific histochemical method for demonstrating the localization of lipoprotein lipase activity has therefore been needed.

There are histochemical methods in existence that are claimed to demonstrate "lipase" (*5*), but results yielded by such methods are difficult to assess because they use synthetic esters in water-soluble form, which are not specific for the fat-splitting enzymes that act on particulate triglyceride substrates (*6*). Emulsified triglycerides are desirable substrates for these lipases but obviously it is not feasible to permeate tissues in vitro with particulate, nondiffusible substances. Alternatively, we took advantage of the fact that a natural triglyceride emulsion (chylomicrons) is plentiful in the circulation of animals after feeding. We reasoned that if lipoprotein lipase activity occurs at the endothelial surface of capillaries, and if chylomicrons are the natural substrate for the enzyme, then under appropriate conditions the enzymic activity should be demonstrable histochemically by application of the classical metal-salt precipitation principle (*7*). This hypothesis was tested and the method and results are herein described.

Adipose tissue was chosen for this demonstration because of its ample supply of the enzyme (*2*). Initial technical difficulties in processing adipose tissue for histology were finally obviated by the use of murine mesenteric spreads that contain discrete patches of adipose tissue and yet are thin enough for microscopic examination without embedding or sectioning. Segments of mesentery were excised from decapitated mice and gently spread over coverslips. The spreads were immediately fixed in cold 4-percent formaldehyde for 10 to 20 minutes to help prevent subsequent detachment of the tissue. After thorough rinsing to remove excess formalin, the coverslip mounts were transferred to a solution containing 0.1*M* tris buffer (*p*H 8.6) and 0.02*M* calcium chloride and incubated overnight at 37°C. Such conditions of incubation favor the hydrolytic activity of lipoprotein lipase, and the enzymically released fatty acids are captured by the available Ca^{++} and

Fig. 1. Reaction product in capillaries of mesenteric adipose tissue of well-fed mouse. Hematoxylin counterstain; oil-immersion objective.

deposited as insoluble calcium soaps at the sites of enzyme activity (*7*). Exposure of the reacted tissues to 2-percent lead nitrate and finally to 1-percent ammonium sulfide converts the precipitated soaps to a brownish-black deposit that can be seen microscopically.

Figure 1 shows the results obtained on mesenteric adipose tissue from a mouse that had been given free access to food. The dense, granular reaction product is confined to the capillaries; veins and arteries (not shown in Fig. 1) were absolutely negative. This histochemical result is consistent with the physiological findings of Frazer (*8*) regarding the greater content of lipoprotein lipase in capillaries relative to larger vessels. It is also of interest that no reaction product was detectable in the adipocytes under these conditions. In contrast, Fig. 2*a* depicts a portion of mesentery taken from a mouse that had fasted for 48 hours before death; note the heavy deposition of reaction product in the adipocytes and relative absence of capillary activity, which is consistent with the enhanced rate of fatty-acid mobilization and the decreased concentrations of chylomicron expected in fasted animals. Similar increased adipocyte staining was obtained in nonfasted mice that had received 100 μg of epinephrine, a potent stimulator of mobilization of fatty acids (*9*), 30 minutes before decapitation (Fig. 2*b*). This suggestion of a separate lipolytic system in the adipocytes, responsive to epinephrine and fasting and functioning in fat mobilization, is compatible with recent reports of biochemical and physiological studies (*3*, *10*). Figure 3 shows the result obtained with an animal that had fasted for 6 days; no reaction product is visible in the adipocytes or in the capillaries; this reflects the severe depletion of substrate in both sites.

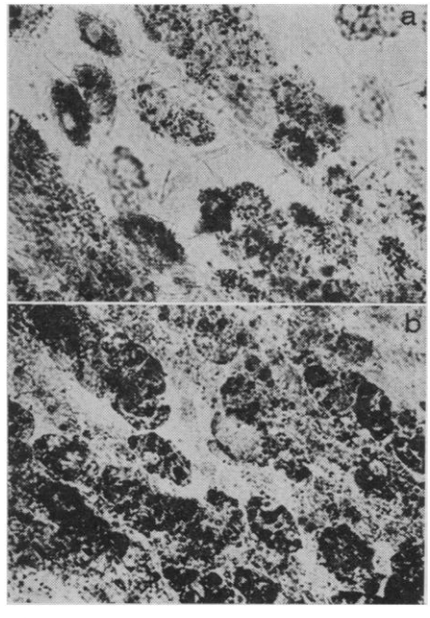

Fig. 2. Localization of reaction product in adipocytes: *a*, following a 48-hour fast and, *b*, following administration of epinephrine. Hematoxylin counterstain; objective, × 45.

Further evidence for the enzymic nature of the histochemical reaction that we have described in the capillaries, and its probable identity with lipoprotein lipase, may be derived from additional control experiments that revealed: (i) the amount of PbS reaction product was roughly proportional to the duration of incubation from 2 to 24 hours; (ii) the amount of reaction product was markedly reduced in spreads incubated at 4°C; (iii) no reaction product was detected in spreads that had been subjected to 70°C for 15 minutes before incubation; (iv) there was no reaction if Ca^{++} was omitted from the incubation medium; and (v) no capillary reaction was detectable when the incubation medium was buffered at *p*H 6.0. Such data, in conjunction with the other data we have described, are very consistent with the known properties of lipoprotein lipase (*11*).

This histochemical demonstration of the localization of lipoprotein lipase activity in capillaries of adipose tissue suggests the endothelial cell as a likely site of the enzymic activity, and further implies strong association between the luminal lipid particles and the endothelial surface. Other evidence for such an association has been obtained in electron-microscopic studies by Wasserman and McDonald (*12*), who observed the arrest and concentration of chylomicrons along the endothelial-cell surface of adipose-tissue capillaries.

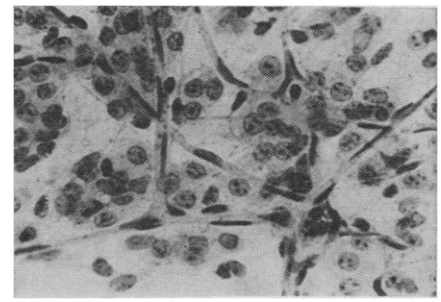

Fig. 3. Absence of reaction in capillaries and adipocytes of mesentery after 6 days of fasting. Hematoxylin counterstain; oil-immersion objective.

These morphologic data, in conjunction with the biochemical evidence of Bezman *et al.* (*13*) that the uptake of chylomicron triglyceride is mediated by the action of lipoprotein lipase at the endothelial-cell surface, all support the concept that hydrolysis of triglyceride, catalyzed by lipoprotein lipase at the capillary endothelium, is a requisite for the transport of triglyceride fatty acids of chylomicrons and low-density lipoproteins across the endothelial cells for subsequent incorporation into adipose tissue.

MERLE S. MOSKOWITZ
AUDREY A. MOSKOWITZ
Department of Pathology,
University of Colorado Medical Center,
Denver 20

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