ON SOME RESPIRATORY ENZYMES OF RAT ADIPOSE TISSUE*

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Evidence for the biochemical versatility of mammalian adipose tissue has been reviewed in detail by Wertheimer and Shapiro (1948, 1956) and Polonovski and Polonovski (1953). Although a number of studies have established the capacity of this tissue to carry out complicated reactions, such as the synthesis of fat from carbohydrate and glycogen from glucose (Shapiro and Wertheimer, 1956), few investigations have as yet been made of adipose tissue enzymes involved in hydrogen and electron transport. In fact, to our knowledge, such work has been limited largely to the qualitative demonstration of some dehydrogenases (Shapiro and Wertheimer, 1943; Padykula, 1952; Fawcett, 1952; Fried and Antopol, 1957) and cytochrome c oxidase (Menschik, 1953) by histochemical methods or methylene blue decolorization time and to some observations on endogenous O_2 consumption. An exception is the report of Hook and Barron (1941) who used, however, a rather special form of adipose tissue. They found that slices of the brown fat or "hibernating gland" of the ground squirrel contained cytochrome c, cytochrome c oxidase, and diphosphothiamine and were capable of oxidizing succinate, pyruvate, lactate, citrate, and α-ketoglutarate.

As a first approach to the study of the respiratory enzymology of adipose tissue in normal animals and in animals in different nutritional

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and hormonal states, we have initiated a broad survey of the tissue for the occurrence of various representative enzyme activities and coenzymes. This brief communication deals with the demonstration of the presence, and some properties, of several dehydrogenases and components of the terminal electron transport chain and with the quantitative expression of some of their activities based on assays under optimal or near optimal conditions.

Normal male Wistar rats (150 to 400 gm.), fed a stock diet of Purina fox chow checkers ad libitum to the time of sacrifice, were used. The animals were anesthetized lightly with ether and sometimes exsanguinated by heart puncture. Each epididymal fat body was rapidly removed, washed, weighed, and homogenized with cold glass-distilled water using a TenBroeck glass grinder. Homogenates of different dilutions (1:5 to 1:25) were made depending on the activity of the enzyme under study, and samples were added immediately to either prepared, chilled Warburg vessels or to cuvettes. Manometric assays were performed at 37° in air and spectrophotometric determinations at 25° with a Beckman model DU instrument fitted with thermospacers. Total nitrogen analyses were made by a micro-Kjeldahl procedure. The methods used for the enzyme assays (all in final volumes of 3.0 ml.) were: cytochrome c oxidase by O_2 uptake with hydroquinone and added cytochrome c; succinic oxidase by O_2 uptake with added cytochrome c; succinic, lactic, malic, isocitric, glutamic, β-hydroxybutyric, glucose, and α-glycerophosphate dehydrogenases by O_2 uptake using phenazine methosulfate as electron acceptor plus DPN* or TPN, where required, and cyanide; DPNH-, succinic-, and choline cytochrome c reductases by reduction of ferricytochrome c (550 mμ) in the presence of cyanide; DPNH oxidase by oxidation of DPNH (340 mμ) with or without added cytochrome c; and D-amino acid oxidase by O_2 uptake with DL-alanine or DL-methionine. The activities of some of these enzymes are listed in Table I. Cytochrome c oxidase activity has also been demon-

* DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide, respectively; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively; ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.

strated by following the oxidation of ferrocytochrome c (550 mμ) and lactic

TABLE I

Some Respiratory Enzyme Activities in Homogenates of Rat Adipose Tissue

(See text for conditions of assay.)

Enzyme or enzyme system	Measurement	pH	Specific activity*
Cytochrome c oxidase	O_2 uptake	7.1	591 (456-780)
Succinic oxidase	O_2 uptake	7.1	121 (73-180)
Succinic dehydrogenase	O_2 uptake	7.1	195 (115-292)
Lactic oxidase	O_2 uptake	8.7	412 (284-535)
Lactic dehydrogenase	O_2 uptake	8.7	3,475 (2,571-5,283)
DPNH cytochrome c reductase	reduction of ferricytochrome c	7.1	2.54 (1.34-3.43)

* Expressed as $QO_2(N)$ for all assays involving O_2 uptake; for DPNH cytochrome c reductase, expressed as micromoles cytochrome c reduced per min. per mg. N. The numbers represent the average values and ranges.

dehydrogenase by the reduction of DPN (340 mμ) with lactate or the oxidation of DPNH with pyruvate. The latter enzyme is specific for L-lactate, requires DPN, has a pH optimum of 8.5 to 8.7 (0.033 M Tris or phosphate buffer) in the manometric assay, and like its hepatic counterpart (Hogeboom, Schneider, and Striebich, 1953), is concentrated in the soluble fraction obtained by high-speed centrifugation of adipose tissue homogenates. Cytochrome c oxidase activity is particulate, and in this connection, it is of interest that Lever and Chappell (1958) have isolated, and identified by electron microscopy, mitochondria from homogenates of rat brown adipose tissue. DPNH oxidase activity in the absence of added cytochrome c is very low (not listed), but is increased many times with added cytochrome c. Cytochrome c reduction in the presence of succinate (not listed) is considerably slower than with DPNH, and this is consistent with the relatively low succinic oxidase activities reported in Table I. It should be noted, how-

ever, that the succinic-cytochrome c, but not the DPNH-cytochrome c, activity is quite labile.

In addition to the enzymes listed in Table I, malic, isocitric, and α-glycerophosphate dehydrogenases (and ATPase) have been shown to be present in epididymal adipose tissue homogenates, but no evidence has been found for the occurrence of choline, glucose, glutamic, or β-hydroxybutyric dehydrogenases or D-amino acid oxidase. The latter results do not exclude the possibility of the presence of some of these enzymes in adipose tissue from other sites and in modified preparations.

As part of a study of the oxidized and reduced DPN and TPN content of adipose tissue, preliminary findings have been obtained* which indicate that the tissue contains approximately 28 μg total DPN per gm. wet wt. of which a small amount, if any, appears to be present as DPNH. The great difference in total DPN content between adipose tissue and liver (750 to 850 μg per gm.) vanishes when the coenzyme level is related to the N content of the tissues, i.e., for adipose tissue, 10 μg per mg. total N and for liver, 23 μg. Thus far, it has not been possible to increase the amount of total DPN extracted from adipose tissue by modifying the method used (Spirtes and Eichel, 1954).

In summary, these studies, to be reported in detail elsewhere, indicate that adipose tissue cells are capable of carrying out electron transport from both DPNH and succinate to O_2 and can oxidize a number of other important intermediary metabolites. Further studies of the properties and intracellular localization of the respiratory enzymes of white and brown adipose tissue--with special emphasis on the enzymes of fat metabolism and on the components of the electron transport chain, including the cytochromes--are in progress. It is hoped that the resultant enzymatic pattern will contribute to a better understanding of the metabolic activity of this highly specialized tissue.

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