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Fatty Acid Dehydrogenase in Adipose Tissue

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It has been established in recent years that adipose tissue in the body is not an inert site of fat deposition but a tissue with specific metabolic functions. This is proved by its respiratory activity [Mirski, 1942] and by its ability to deposit glycogen under suitable conditions [Hoffmann & Wertheimer, 1927; Tuerkischer & Wertheimer, 1942]. Further proof is given by experiments showing the dependence of various adipose functions upon innervation [Hausberger, 1937].

It might be assumed, in view of these results, that adipose tissue plays an important part in the deposition and mobilization of fat. Investigation of the fat metabolism of this tissue may therefore well prove of interest for the understanding of the deposition and mobilization processes.

The present investigation arises from the observation that aqueous extracts of adipose tissue decolorize methylene blue, the speed of the decolorization being enhanced when fatty acids are added. It was proposed, therefore, to prepare an active fatty acid dehydrogenase from adipose tissue and to study the properties of the isolated enzyme.

Towards the conclusion of this work, abstracts of papers by Lang [1939], Lang & Mayer [1939] and Lang & Adickes [1939], appeared in which the demonstration of fatty acid dehydrogenase in various sources is reported. As the original papers are not at present available to us, no comparison of methods is possible. The authors report that the dehydrogenase action is dependent on the availability of muscle-adenylic acid as coenzyme and that the activity is greater to high than to low fatty acids.

METHODS

Preparation of enzyme solution. Adipose tissue from the leg region and the abdominal cavity of rats is cut into small pieces with scissors and crushed with twice its weight of sand. The pulp thus obtained is extracted with 2 volumes of distilled water

for $\frac{1}{2}$ hr. at room temperature and centrifuged. The supernatant is freed of fat by filtration. The liquid is then dialysed through cuprophane for 6–20 hr. against running tap water. Adipose tissue of rabbits did not yield active extracts.

Boiled tissue juice was prepared by boiling 2 g. of adipose tissue pulp with 5 ml. water in a water bath for 30 min. The juice is filtered while hot.

Crude cozymase preparation was prepared according to Euler [1931]. Solutions obtained after cleavage of the lead precipitate with H_2S were used for the experiment.

Dehydrogenase activity estimations were carried out according to Thunberg. Test mixtures consisted of 0.1 ml. *M*/2 phosphate buffer pH 8.0, 0.1 ml. methylene blue (1/5000), with the following additions: 0.5 ml. enzyme solution, 0.1 ml. substrate (*M*/20) and 0.1 ml. coenzyme. When one of these constituents was not added it was replaced by distilled water. Fatty acids in the experimental mixture were neutralized with NaOH and brought to pH 8.0. In some cases, dehydrocholic acid was used for dispersion, as it had no influence on the dehydrogenase activity.

EXPERIMENTAL

As is shown in Table 1, extracts dialysed for 20 hr. did not cause decolorization of methylene blue even after the addition of fatty acids. However, when boiled tissue juice was added decolorization took place. The speed of decolorization was markedly enhanced by addition of fatty acids. Crude cozymase instead of tissue juice may be used with even better results. Neither tissue juice nor cozymase preparation caused decolorization when added to fatty acids but without enzyme. When enzyme solution boiled for 5 min. was used decolorization failed to take place even in the presence of coenzyme and substrate. The enzyme nature of the dehydrogenase is thus established.

Table 1. *Enzymic reduction of methylene blue*

Addition to enzyme soln.	Time of decol. min.
None	No decol.
Stearate	"
Boiled juice	70
Boiled juice + stearate	30
Crude cozymase	90
Crude cozymase + stearate	12
Boiled enzyme + crude cozymase + stearate	No decol.
Boiled juice + stearate	"
Crude cozymase + stearate	"

In view of the report of Lang & Mayer [1939], experiments were undertaken to examine whether muscle adenylic acid can replace boiled tissue juice or crude cozymase as coenzyme. This was found to be the case. Inosinic acid can also act as coenzyme. Yeast adenylic acid did not display coenzyme activity.

Table 2. *Influence of coenzyme on reduction of methylene blue*

Addition to enzyme soln.	Time of decol. min.
None	No decol.
Palmitate	"
Muscle adenylic acid (20 mg. %)	120
Muscle adenylic acid (20 mg. %) + palmitate	3
Inosinic acid (20 mg. %)	36
Inosinic acid (20 mg. %) + palmitate	3
Yeast adenylic acid + palmitate	No decol.

As is shown in Table 2, although inosinic acid can act as coenzyme, the use of adenylic acid is preferable, as inosinic acid itself caused comparatively rapid decolorization even in absence of substrate. Diphosphopyridinenucleotide does not seem to be involved, since adipose tissue extracts capable of serving as coenzyme of fatty acid dehydrogenase could not activate lactic dehydrogenase of muscle. In addition to coenzyme, inorganic phosphate must be present for this dehydrogenase action. When the phosphate buffer is replaced by bicarbonate, dehydrogenation is comparatively slow, as is shown in Table 3.

Table 3. *Effect of phosphate on dehydrogenation*

Addition to enzyme soln.	Time of decol. min.
Adenylic acid + phosphate	90
Adenylic acid + phosphate + palmitic acid	4
Adenylic acid + 1.3% bicarbonate	110
Adenylic acid + 1.3% bicarbonate + palmitic acid	75

pH optimum of fatty acid dehydrogenase

In determining the optimum pH for the dehydrogenase, difficulty was encountered in keeping the fatty acid in suspension below pH 8. This was

overcome by the use of Na dehydrocholate as dispersing agent. Stearic acid was dispersed in 0.5% Na dehydrocholate and the reaction brought to the required pH with NaOH. By this means a comparatively stable suspension could be obtained at

Fig. 1. *a*, without substrate. *b*, with stearic acid.

as low a pH as 6.0. Controls containing the same amount of Na dehydrocholate were done. The results are shown in Fig. 1, which demonstrates a clear optimum of dehydrogenase action at about pH 8.

Substrate specificity

The activity of the enzyme towards fatty acids of different chain lengths, with even and uneven number of carbon atoms, as well as towards other substrates, was examined. A crude cozymase preparation was used in these experiments as coenzyme. The results are summarized in the following table.

Table 4. *Specificity of dehydrogenase*

Addition	Time of decol. min.
Enzyme + no substrate	90
+ stearate	2
+ palmitate	3
+ caprinat	10
+ capronate	10
+ valerate	25
+ butyrate	33
+ oleate	15
+ succinate	5
+ lactate	30
+ glycerophosphate	18
+ lecithin (sheep brain)	10
+ cephalin (sheep brain)	12
No enzyme + lecithin	>180
+ cephalin	>180
+ oleate	>180

The table shows clearly that with diminishing fatty acid chain length the enzyme activity decreases. The unsaturated fatty acid oleic acid is dehydrogenated much more slowly than the corresponding saturated acids. Also phospholipins are

dehydrogenated by the enzyme. Separate experiments confirmed that with phospholipins, as with fatty acids, the same coenzyme and inorganic phosphate are needed for dehydrogenase action. The optimal pH for fatty acid was found to be the same as for phospholipins. Of other substrates tested, succinate alone was found to be dehydrogenated at a rate comparable with that of the fatty acids.

In order to ascertain whether the fatty acid dehydrogenase was able to act on neutral fat, a dispersing agent other than fatty acid had to be employed. A suspension of neutral fat in 1% lauryl alcohol sulphionate was used. As Table 5 shows this agent had no inhibiting effect on fatty acid dehydrogenation. Nevertheless, no decolorization of methylene blue was observed with a suspension of neutral fat.

Table 5. *Action of dehydrogenase on neutral fat*

Addition to enzyme soln.	Time of decol. min.
No substrate + coenzyme	75
Palmitate + coenzyme	4
Palmitate + coenzyme + lauryl alcohol sulphionate	4
Neutral fat in lauryl alcohol sulphonate + coenzyme	>120

Inhibitors of dehydrogenase action

In view of the fact that inorganic phosphate was necessary for dehydrogenase action, a number of poisons of phosphorylating reactions were examined as to their effect on fatty acid dehydrogenation. Other dehydrogenase poisons were also tested. In no case could an inhibiting action of these substances be observed. The substances and concentrations tested were: monoiodoacetate *M*/100, phloridzin *M*/100, NaF *M*/50, maleic acid *M*/200, pyrophosphate *M*/200 and benzoate *M*/100.

These results, as well as the impossibility of demonstrating any decrease in inorganic phosphate during dehydrogenase action, seem to exclude the possibility that the activating action of phosphate on the dehydrogenase involves phosphorylation.

The finding that maleate and pyrophosphate failed to inhibit decolorization is evidence against the identity of fatty acid dehydrogenase with succinic dehydrogenase.

Distribution of enzyme

An enzyme which desaturates fatty acids could be demonstrated in liver, muscle, heart, testes,

brown fat as well as in adipose tissue. No activity could be demonstrated in brain. With these tissues, phospholipins were dehydrogenated at the same speed as fatty acids, in contrast to adipose tissue. Activity in these tissues was not as clearly demonstrated as in adipose tissue. This was due to the presence of other active dehydrogenases with their substrates, which caused decolorization in the absence of fatty acids. In order to remove all hydrogen donors prolonged dialysis (at least 24 hr.) was necessary. This treatment, however, also weakened the fatty acid dehydrogenase. Better results were obtained when the cut organs were washed with 0.9% NaCl and then extracted with distilled water, followed by dialysis for 4–6 hr.

Influence of oxygen on dehydrogenase

Attempts to demonstrate dehydrogenase activity by measuring O₂ consumption in the presence of methylene blue were unsuccessful. This finding is explained by the fact that the enzyme mixed with coenzyme and substrate is inactivated in the presence of O₂. If, after mixing all components of reaction, the mixture is allowed to stand at room temperature in the air for 10 min. before evacuating, no activity can be observed even with Thunberg's method.

SUMMARY

1. A fatty acid dehydrogenase has been demonstrated in extracts of adipose tissue. Its activity depends on the presence of a coenzyme, adenylic acid, and inorganic phosphate.
2. The optimum pH of the dehydrogenase is 8.0.
3. Activity is highest with the natural, long chain, fatty acids and decreases with diminishing chain length.
4. The dehydrogenase also oxidizes phospholipins. Of other substrates tested only succinate was dehydrogenated at a rate comparable to palmitic and stearic acid. Neutral fat does not seem to be attacked.
5. Of tissues other than adipose tissue, liver, muscle, heart and testes, were proved to contain fatty acid and phospholipin dehydrogenase.
6. Monoiodoacetate, phloridzin, NaF, maleic acid, pyrophosphate and benzoate at fairly high concentrations do not affect the enzyme.
7. Exposure of the reaction mixture to O₂ caused rapid inhibition of the enzyme activity.

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