Role of Glycerol 3-Phosphate Dehydrogenase in Glyceride Metabolism

EFFECT OF DIET ON ENZYME ACTIVITIES IN CHICKEN LIVER

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1. The metabolic role of hepatic NAD-linked glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) was investigated vis-à-vis glyceride synthesis, glyceride degradation and the maintenance of the NAD redox state. 2. Five-week-old chickens were placed on five dietary regimes: a control group, a group on an increased-carbohydrate-lowered-fat diet, a group on a high-fat-lowered-carbohydrate diet, a starved group and a starved-re-fed group. In each group the specific activity ($\mu\text{mol}/\text{min}$ per g wet wt. of tissue) of hepatic glycerol 3-phosphate dehydrogenase was compared with the activities of the β -oxoacyl-(acyl-carrier protein) reductase component of fatty acid synthetase, glycerol kinase (EC 2.7.1.30) and lactate dehydrogenase (EC 1.1.1.27). 3. During starvation, the activities of glycerol 3-phosphate dehydrogenase, glycerol kinase and lactate dehydrogenase rose significantly. After re-feeding these activities returned to near normal. All three activities rose slightly on the high-fat diet. Lactate dehydrogenase activity rose slightly, whereas those of the other two enzymes fell slightly on the increased-carbohydrate-lowered-fat diet. 4. The activity of the β -oxoacyl-(acyl-carrier protein) reductase component of fatty acid synthetase, a lipid-synthesizing enzyme, contrasted strikingly with the other three enzyme activities. Its activity was slightly elevated on the increased-carbohydrate diet and significantly diminished on the high-fat diet and during starvation. 5. The changes in activity of the chicken liver isoenzyme of glycerol 3-phosphate dehydrogenase in response to dietary stresses suggest that the enzyme has an important metabolic role other than or in addition to glyceride biosynthesis.

Two isoenzymes of NAD-linked glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) exist in chicken (Rouslin, 1968; White & Kaplan, 1969). One is found predominantly in white skeletal muscle, whereas the other is located in liver. The ontogeny of the isoenzymes in liver and breast muscle, the taxonomic distribution of the isoenzymes in birds and the kinetic properties of the purified isoenzymes led White & Kaplan (1972) to propose that the major function of the muscle isoenzyme is to maintain the NAD redox potential during anaerobic glycolysis, a function defined by Krebs & Woodford (1965) and also discussed by Crabtree & Newsholme (1972). The liver isoenzyme seemed to be associated with triglyceride metabolism, primarily biosynthesis (White & Kaplan, 1972).

Among vertebrates, it is exceptional for an enzyme involved in reductive synthesis to use NADH rather than NADPH as its cofactor (Lowenstein, 1961). If it is involved in triglyceride biosynthesis, glycerol 3-phosphate dehydrogenase would represent such an exception. This peculiarity, combined with the discovery of the acyl-dihydroxyacetone phosphate pathway as an alternative biosynthetic route to

glycerides (Rao *et al.*, 1968; Hajra, 1968; Hajra & Agranoff, 1968), has led us to question the importance of glycerol 3-phosphate dehydrogenase in glyceride biosynthesis.

It is well known that the activities of many biosynthetic and degradative enzymes in liver respond to changes in diet. Among the best-studied enzymes in this regard are the enzymes involved in fatty acid biosynthesis: acetyl-CoA carboxylase (EC 6.4.1.2), the fatty acid synthetase complex, citrate-cleavage enzyme (ATP citrate lyase, EC 4.1.3.8) and 'malic' enzyme [malate dehydrogenase (decarboxylating) (NADP⁺), EC 1.1.1.40]. The activities of these enzymes in chicken liver are low during starvation or on a high-fat diet, and increase on high-carbohydrate-low-fat diets (Goodridge, 1968; Pearce, 1968; Goodridge, 1973) as they do in mammalian liver (Lane & Moss, 1971). In contrast with these enzymes, the activity in rat liver of glycerol kinase (EC 2.7.1.30), which utilizes lipolytically derived glycerol, increases on a high-fat diet and decreases on a high-carbohydrate-low-fat diet (Kida *et al.*, 1973). If the major function of hepatic glycerol 3-phosphate dehydrogenase is related to glyceride

biosynthesis, its activity in response to dietary changes should be similar to those of other enzymes involved in lipid synthesis. The results reported here show that, in chicken at least, this is not the case.

Experimental

Materials

Chemicals. NADH, NADPH, ATP, creatine phosphate, creatine kinase (EC 2.7.3.2), sodium pyruvate and the dimethylketal bismonocyclohexylammonium salt of dihydroxyacetone phosphate were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) The ketal of dihydroxyacetone phosphate was hydrolysed and the salt was converted into the free acid by Dowex-50 treatment, by following the procedure suggested by the manufacturer. 2-Mercaptoethanol and disodium salt of EDTA were supplied by Eastman Organic Chemicals (Rochester, N.Y., U.S.A.). MgSO_4 and NaF were from Fisher Chemical Co. (King of Prussia, Pa., U.S.A.).

The DE-81 ion-exchange filter discs used in the glycerol kinase assay were purchased from Reeve Angel (Clifton, N.J., U.S.A.). [$1\text{-}^{14}\text{C}$]Glycerol used in the same assay was from International Chemical and Nuclear Corporation (Waltham, Mass., U.S.A.).

Scintillation solutions were dioxan-based. Each litre of solution contained 100g of naphthalene (Arthur Thomas, Philadelphia, Pa., U.S.A.), 50mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene (International Chemical & Nuclear Corporation), and 4g of 2,5-diphenyloxazole (International Chemical & Nuclear Corporation).

($\pm$)-*trans*-1,2,3,4,4a,5,8,8a-Octahydronaphthalene-1,4-dione (m.p. 93°C), which was used as the substrate in the assay of the β -oxoacyl-(acyl-carrier protein) reductase activity of fatty acid synthetase was synthesized from benzoquinone (Fisher Chemical Co.) and butadiene (Matheson, Coleman and Bell, Norwood, Ohio, U.S.A.) by the method of Robins & Walker (1958). The n.m.r. and mass spectra of the product are consistent with the proposed structure (A. H. Ullman & J. W. Harding, unpublished work).

Animals. Five-week-old White Leghorn chickens were purchased from a commercial supplier. At 1 week before the beginning of the experiment all the birds were placed on a basal diet. At the onset of the experiment the birds were divided into four groups, each of which had a different dietary regime. They included (1) a control group, (2) a starved group, (3) a group on an increased-carbohydrate-decreased-fat diet, and (4) a group on a high-fat-lowered-carbohydrate diet. On the sixth day a fifth group was formed when some of the starved group were re-fed with the increased-carbohydrate-decreased-fat diet.

Birds from each group were killed after 48, 96 and 144h after the start of the study. In addition, after 196h birds from the basal, starved and re-fed groups

were killed. Six birds, three males and three females from each group, were killed at each time-point. There was no pattern of sex-related differences in the enzyme activities in these immature birds with the possible exception of glycerol kinase, which was significant at the 5% level for three of the six samples on increased-lipid and increased-carbohydrate diets.

Diets. The constituents of the feeds used in this study consisted of ground milo [52–62% (w/w) of total feed mixture], soya-bean meal (28–33%), corn starch (0–15%), cotton-seed oil (0–15%) and a vitamin and mineral supplement (3.9–4.6%). On the basis of the nutrient composition of these various components (Morrison, 1956), the total nutrient composition of the diets was calculated (Table 1). The amount of corn starch or cotton-seed oil added to the basal diet was limited by the palatability of the mixture to the chickens. As a consequence, the diet containing increased carbohydrate is similar to the basal diet. Since all three fed groups gained weight at similar rates (Table 2), the caloric intake was assumed to be similar also.

Methods

Preparation of homogenates. Chickens were weighed and then killed by cervical dislocation between 8:30 a.m. and 10:30 a.m. The livers were immediately removed, weighed (Table 2), placed in small plastic bags and frozen in a solid CO_2 -acetone bath. This treatment does not affect the activities of the enzymes studied. Within 2 weeks, a portion of each liver was homogenized in a Teflon homogenizer with 4 vol. (4.0ml/g wet wt. of tissue) of 5mM-Tris-HCl buffer, pH 7.6, containing 1mM-EDTA and 10mM-2-mercaptoethanol. The homogenate was centrifuged at 4°C for 1h at $39000g_{av}$ in an SS-34 Sorvall rotor. Enzyme and protein assays (Warburg & Christian, 1942) were performed on the clear portion of the supernatant fractions as quickly as possible on the same day. All enzyme activities are reported as μmol

Table 1. *Compositions of nutrients in the basal, increased-carbohydrate and increased-lipid diets*

Nutrient	Composition (% w/w)		
	Basal diet	Increased-carbohydrate diet	Increased-lipid diet
Protein	24.9	20.8	20.8
Carbohydrate	60.7	67.2	50.7
Fat	2.3	1.9	18.4
Vitamin and mineral mix	8.4	7.1	7.1
Fibre	3.6	3.0	3.0
Carbohydrate/lipid ratio	26.4	35.4	2.75

Table 2. *Body weight, liver weight and liver protein values for the chickens on various diets*

Diet	Day ...	Body wt. (g±S.D.)				
		0	2	4	6	8
Control		339±36	345±42	396±35	434±39	445±45
Increased-lipid		—	368±41	363±15	425±30	—
Increased-carbohydrate		—	349±39	370±33	422±30	—
Starved		—	313±23	276±32	228±10	230±25
Starved-re-fed		—	—	—	—	309±16
Liver wt. (g wet wt.±S.D.)						
Control		10.3±0.83	11.7±2.5	12.9±1.1	12.1±0.6	12.7±1.4
Increased-lipid		—	12.8±1.4	12.0±1.0	11.6±0.4	—
Increased-carbohydrate		—	11.4±1.4	13.1±1.8	13.3±1.3	—
Starved		—	7.6±0.6	7.9±1.5	6.1±0.9	5.1±0.7
Starved-re-fed		—	—	—	—	12.9±1.5
Soluble protein (mg/ml±S.D.)						
Control		30.0±2.7	32.2±1.5	33.9±1.8	32.6±5.4	31.6±1.8
Increased-lipid		—	31.3±1.5	31.6±4.3	29.4±1.9	—
Increased-carbohydrate		—	33.4±3.6	35.5±1.4	30.9±2.1	—
Starved		—	35.1±2.2	39.1±2.3	39.4±3.1	45.8±2.9
Starved-re-fed		—	—	—	—	31.0±5.0

Scheme 1. *Reduction of (±)-trans 1,2,3,4,4a,5,8,8a-octahydronaphthalene-1,4-dione catalysed by the β-oxoacyl-(acyl-carrier protein) reductase component of the fatty acid synthetase complex*

of substrate converted/min per g wet wt. of liver. It is assumed that all the activity is extracted into the supernatant fraction.

Assay for NAD-linked glycerol 3-phosphate dehydrogenase. Glycerol 3-phosphate dehydrogenase activity was determined spectrophotometrically by the method of White & Kaplan (1969).

Assay for glycerol kinase. Glycerol kinase activity was measured by the radioactivity method described by Newsholme *et al.* (1967) except that a creatine kinase system for regenerating ATP was used (Robinson & Newsholme, 1969). The DEAE-filter-paper discs were placed into vials containing 15 ml of scintillation fluid and the radioactivity was determined in a Beckman LS-100 scintillation counter. The normal activities in chicken liver are about 20% of those in rat liver (Newsholme & Taylor, 1969).

Assay for L-lactate dehydrogenase (EC 1.1.1.27). Lactate dehydrogenase activity was measured by the method of Pesce *et al.* (1964).

Assay for the β-oxoacyl-(acyl-carrier protein) reductase activity of fatty acid synthetase. The β-oxoacyl-(acyl-carrier protein) reductase activity of fatty acid synthetase was determined by a method similar to that of Dutler *et al.* (1971), except that (±)-trans-1,2,3,4,4a,5,8,8a-octahydronaphthalene-1,4-dione was used as substrate rather than (±)-trans-decahydronaphthalene-1,4-dione. The reaction catalysed is shown in Scheme 1. Each 1 ml of reaction mixture contained 0.66 μmol of the dione and 0.33 μmol of NADPH in 1 M-potassium phosphate buffer, pH 6.7. The dione concentration in this assay (0.66 mM) is not saturating ($K_m = 9$ mM).

The validity of this assay was tested by comparing the rates obtained from the dione method with the rates obtained with a spectrophotometric assay for fatty acid synthetase (Lynen *et al.*, 1964) with the substrate concentrations used by Hsu *et al.* (1969) for chicken liver fatty acid synthetase. After the background activity for dione reduction (5.0 μmol/min per

g wet wt. of liver) is subtracted the ratio of activities obtained from these two methods is the same in the livers of starved and carbohydrate-fed chickens (J. W. Harding & A. H. Ullman, unpublished work). Although the dione is an artificial substrate, its use in the assay of the β -oxoacyl-(acyl-carrier protein) reductase component of fatty acid synthetase has advantages over the conventional radioactive or spectrophotometric assays for fatty acid synthetase complex as a whole. The substrate can be synthesized in quantity and is more stable than acetyl-CoA or malonyl-CoA. This makes the use of the dione reduction assay as a substitute assay for the fatty acid synthetase complex both simpler and less expensive. In addition, the catalytic activity of the β -oxoacyl-(acyl-carrier protein) reductase component of fatty acid synthetase is considerably greater than for the fatty acid synthetase complex as a whole; thus the spectrophotometric method with the dione as substrate at suboptimal concentrations is more sensitive than the spectrophotometric method with the physiological substrates.

Results

Figs. 1–4 show, respectively, the effect of various dietary regimes on the activities of glycerol 3-phosphate dehydrogenase, glycerol kinase, lactate dehydrogenase and β -oxoacyl-(acyl-carrier protein) reductase activity in chicken liver.

Although there are differences in the extent and rate of change in activities, those of glycerol 3-phosphate dehydrogenase (Fig. 1), glycerol kinase (Fig. 2) and lactate dehydrogenase (Fig. 3) respond similarly to the dietary stresses imposed. The greatest increases for all three enzyme activities are obtained in the livers of the starved group (1.4-, 2.5-, and 2.1-fold respectively). Glycerol 3-phosphate dehydrogenase and lactate dehydrogenase respond quickly to starvation, achieving new constant activities within 2 days. The activity of glycerol kinase, on the other hand, does not increase significantly ($P < 0.05$) until the fourth day, and may still be increasing slightly after a week. Re-feeding with carbohydrate after starvation alters the activities of glycerol kinase and lactate dehydrogenase to normal values obtained with the carbohydrate-fed group. The activity of glycerol 3-phosphate dehydrogenase overshoots and is slightly lower than expected for the carbohydrate-fed group.

Increases in the activities of glycerol 3-phosphate dehydrogenase, glycerol kinase and lactate dehydrogenase occur on the high-fat diet, although these increases are not as great as in starvation. In the case of glycerol 3-phosphate dehydrogenase the activity did not reach a constant value by the sixth day, when its activities were almost as high as in starvation.

Fig. 1. Effect of diet on the activity of glycerol 3-phosphate dehydrogenase in chicken liver

The diets represented are basal (Δ), increased-carbohydrate-lowered-fat ($\square$), high-fat-lowered-carbohydrate ($\circ$), starved ($\bullet$) and starved-re-fed with carbohydrate ($\blacksquare$). The nutrient compositions of these diets are given in Table 1. Each point represents the average of six birds, three of each sex. Error bars indicate the standard error of the mean.

As shown in Table 1, the increased-carbohydrate diet is not greatly different in composition from the basal diet. This slight difference in diet results in slight changes in the activities of lactate dehydrogenase and glycerol kinase. The activity of lactate dehydrogenase tends to be slightly higher than the basal value and that of glycerol kinase slightly lower than the basal value on the increased-carbohydrate diet. Glycerol 3-phosphate dehydrogenase activity decreases on the increased-carbohydrate diet.

The activity of the β -oxoacyl-(acyl-carrier protein) reductase component of the fatty acid synthetase complex exhibits a pattern of dietary changes (Fig. 4) that contrasts strikingly with the pattern of activities observed for the other three enzymes (Figs. 1–3). Its activity is increased slightly by an increased-carbohydrate diet and depressed to about one-tenth of the control activities by starvation or the administration of a high-fat diet. These changes occur within 2 days of the dietary change and remain relatively constant thereafter. Re-feeding of starved birds with the increased-carbohydrate diet results

Fig. 2. Effect of diet on the activity of glycerol kinase in chicken liver

The diets represented are: basal (Δ), increased-carbohydrate-lowered-fat ($\square$), high-fat-lowered-carbohydrate ($\circ$), starved ($\bullet$) and starved—re-fed with carbohydrate ($\blacksquare$). The nutrient compositions of these diets are given in Table 1. Each point represents the average of six birds, three of each sex. Error bars indicate the standard error of the mean.

in a highly variable increase in β -oxoacyl-(acyl-carrier protein) reductase activity. Measured activities for different birds in this group after 2 days ranged from approx. $0 \mu\text{mol/min per g wet wt. of tissue}$ (similar to the values for starved birds) to $35.5 \mu\text{mol/min per g wet wt. of tissue}$ (about four times the control values).

The specific activities quoted in the present paper are on the basis of g wet wt. of tissue because the enzyme activities stabilize for most diets after the dietary change is made. If the activities are expressed per mg of protein, per total liver weight or per 100 g body wt., the basic correlations between enzyme activities reported above remain; however, the relative increases and decreases change slightly. Specific activity relative to DNA was not determined. Qualitatively such an analysis would tend to diminish the changes in the activities of glycerol 3-phosphate dehydrogenase, glycerol kinase and lactate dehydrogenase while accentuating the changes in the activity of the β -oxoacyl-(acyl carrier protein) reductase component of fatty acid synthetase.

Discussion

Glycerol 3-phosphate dehydrogenase can serve three distinct metabolic roles. As a biosynthetic enzyme, it catalyses the reduction of dihydroxyacetone phosphate for eventual incorporation into glycerides. As a catabolic enzyme, it oxidizes glycerol 3-phosphate that has been generated by glyceride breakdown. In addition, the reaction catalysed by this enzyme can serve as a buffer for the cytoplasmic redox state.

The isoenzyme of glycerol 3-phosphate found in chicken breast muscle appears to function as a redox buffer (White & Kaplan, 1972). The very low activity of glycerol kinase (Newsholme & Taylor, 1969) and the absence of significant lipid synthesis in this tissue (O'Hea & Leveille, 1969) rule out either a catabolic or synthetic function for the high activity of glycerol 3-phosphate dehydrogenase in chicken breast muscle found by Dawson & Kaplan (1965). The function of the glycerol 3-phosphate dehydrogenase isoenzyme found in chicken liver is unknown, although it is thought to participate in glyceride

Fig. 3. Effect of diet on the activity of lactate dehydrogenase in chicken liver

The diets represented are: basal (Δ), increased-carbohydrate-lowered-fat ($\square$), high-fat-lowered-carbohydrate ($\circ$), starved ($\bullet$) and starved—re-fed with carbohydrate ($\blacksquare$). The nutrient compositions of these diets are given in Table 1. Each point represents the average of six birds, three of each sex. Error bars indicate the standard error of the mean.

Fig. 4. Effect of diet on the activity of the β -oxoacyl-(acyl-carrier protein) reductase component of fatty acid synthetase in chicken liver

The diets represented are: basal (Δ), increased-carbohydrate-lowered-fat ($\square$), high-fat-lowered-carbohydrate ($\circ$), starved ($\bullet$) and starved-re-fed with carbohydrate ($\blacksquare$). Each point represents the average of six birds, three of each sex. The error bars indicate the standard error of the mean. The activity was assayed with the artificial substrate ($\pm$)-*trans*-1,2,3,4,4a,5,8,8a-octahydronaphthalene-1,4-dione. For the conditions of the assay see under 'Methods.' The values reported are corrected for an average background [non β -oxoacyl-(acyl-carrier protein) reductase] activity of $5.0 \mu\text{mol/min per g wet wt. of liver}$.

biosynthesis and degradation (White & Kaplan, 1969, 1972).

In the present study, the changes in hepatic glycerol 3-phosphate dehydrogenase activity as a function of diet were compared with the diet-dependent changes in the activities of the hepatic β -oxoacyl-(acyl-carrier protein) reductase activity of fatty acid synthetase, glycerol kinase and lactate dehydrogenase. The first two enzymes were chosen as markers for reductive lipid biosynthesis and the metabolism of lipolytically derived glycerol respectively. Lactate dehydrogenase was selected since it has a major role in equilibrating oxidized and reduced NAD (Krebs & Veech, 1970). However, it also functions in gluconeogenesis from lactate; consequently its activity in liver is not purely a reflection of its role in maintaining the NAD redox state.

The activities of hepatic glycerol kinase and β -oxoacyl-(acyl-carrier protein) reductase vary re-

ciprocally in response to dietary stresses. The changes we observed were expected on the basis of the known metabolic functions of glycerol kinase and fatty acid synthetase and on the dietary changes reported by others for glycerol kinase in rat liver (Kida *et al.*, 1973) and fatty acid synthetase in chicken liver (Goodridge, 1973). The activity of glycerol 3-phosphate dehydrogenase (Fig. 1) in response to dietary changes is similar to that of glycerol kinase (Fig. 2) and distinctly different from that of the β -oxoacyl-(acyl-carrier protein) reductase activity of the fatty acid synthetase complex (Fig. 4). This behaviour is unexpected for an enzyme whose major function in liver was presumed to be in glyceride biosynthesis (Weiss & Kennedy, 1956).

It is clear that glycerol 3-phosphate, derived from glycerol, can be a direct precursor for glyceride synthesis (Okuyama & Lands, 1972; Woods & Krebs, 1973; Manning & Brindley, 1972). This pathway does not involve glycerol 3-phosphate dehydrogenase. Dihydroxyacetone phosphate can be incorporated into triglycerides via the acyl-dihydroxyacetone phosphate pathway (Hajra, 1968), a pathway that also need not involve glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate dehydrogenase reaction is therefore not a compulsory step in glyceride synthesis either from glycerol or from glycolytic intermediates. Glycerol 3-phosphate dehydrogenase can participate in glyceride synthesis by permitting glycolytic intermediates to enter the glycerol 3-phosphate pathway (Weiss & Kennedy, 1956) or by permitting glycerol 3-phosphate to enter the acyl-dihydroxyacetone phosphate pathway (Manning & Brindley, 1972). It is possible that glycerol 3-phosphate dehydrogenase could participate extensively or not at all in glyceride synthesis, depending on the metabolic conditions.

The activity of glycerol 3-phosphate dehydrogenase in liver is higher than in most other tissues in chicken except white skeletal muscle (White & Kaplan, 1972). The changes in activity induced by diet are quite small relative to those observed for enzymes performing single metabolic functions, e.g. acetyl-CoA carboxylase (Lane & Moss, 1971). Our findings suggest that the functioning of glycerol 3-phosphate dehydrogenase is essential under all dietary conditions. There is no indication from our dietary studies that the enzyme activity changes in response to demands for glyceride synthesis. Rather, the increased activity during starvation or in response to a high-lipid diet suggests an important function for glycerol 3-phosphate dehydrogenase in gluconeogenesis from glycerol derived from glyceride degradation. This is consistent with the sustained twofold increase in glycerol concentrations during 4 days of starvation in the chick (Evans & Scholz, 1971). However, the fact that during starvation the increase in glycerol 3-phosphate dehydrogenase activity precedes by

at least 1 day the increase in glycerol kinase activity (Figs. 1 and 2) could indicate that the function of glycerol 3-phosphate dehydrogenase is not solely in glycerol utilization.

A role for glycerol 3-phosphate dehydrogenase in maintaining the NAD redox state in liver is neither confirmed nor refuted by our findings. The fact that in liver glycerol 3-phosphate dehydrogenase and lactate dehydrogenase show similar changes in activity in response to dietary changes cannot necessarily be interpreted as supporting an important redox function for glycerol 3-phosphate dehydrogenase, since the changes in lactate dehydrogenase activity could reflect a gluconeogenic response independent of its NAD redox buffering role.

If in fact the major role of glycerol 3-phosphate dehydrogenase in liver is in glyceride breakdown with a secondary role in maintaining the NAD redox state, it would rationalize the requirement for NAD rather than NADP as coenzyme (Lowenstein, 1961). In *Escherichia coli*, for example, the strictly biosynthetic glycerol 3-phosphate dehydrogenase is NADP-linked, whereas the catabolic enzyme is NAD-linked (Kito & Pizer, 1968).

A report by Gee *et al.* (1974) tends to support the idea of an important catabolic function for glycerol 3-phosphate dehydrogenase. It was observed that as much as 20% of the hepatic NAD-linked glycerol 3-phosphate dehydrogenase activity is associated with peroxisomes in mammals. In addition we have found that in rat hepatomas and host livers (J. S. Harding, E. A. Pyeritz, H. P. Morris, & H. B. White, unpublished work) there is a strong positive correlation in the specific activities of glycerol kinase and glycerol 3-phosphate dehydrogenase over a 50-fold range of activities. This suggests a common role for these two enzymes in scavenging glycerol from glyceride breakdown for gluconeogenesis.

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References

- Crabtree, B. & Newsholme, E. A. (1972) *Biochem. J.* **126**, 49–58
 Dawson, D. M. & Kaplan, N. O. (1965) *J. Biol. Chem.* **240**, 3215–3221
 Dutler, H., Coon, M. J., Kull, A., Vogel, H., Waldvogel, O. & Prelog, V. (1971) *Eur. J. Biochem.* **22**, 203–212

- Evans, R. M. & Scholz, R. W. (1971) *J. Nutr.* **101**, 1127–1136
 Gee, R., McGroarty, E., Hsieh, B., Wied, D. & Tolbert, N. E. (1974) *Arch. Biochem. Biophys.* **161**, 187–193
 Goodridge, A. G. (1968) *Biochem. J.* **108**, 667–674
 Goodridge, A. G. (1973) *J. Biol. Chem.* **248**, 1932–1938
 Hajra, A. K. (1968) *Biochem. Biophys. Res. Commun.* **33**, 929–935
 Hajra, A. K. & Agranoff, B. W. (1968) *J. Biol. Chem.* **243**, 3542–3543
 Hsu, R. Y., Butterworth, P. H. W. & Porter, J. W. (1969) *Methods Enzymol.* **14**, 33–39
 Kida, K., Kobayashi, K., Kimura, H. & Yugari, Y. (1973) *J. Biochem. (Tokyo)* **73**, 299–306
 Kito, M. & Pizer, L. I. (1968) *Biochem. Biophys. Res. Commun.* **32**, 408–412
 Krebs, K. A. & Veech, R. L. (1970) in *Pyridine Nucleotide-Dependent Dehydrogenases* (Sund, H., ed.), pp. 413–438, Springer-Verlag, Berlin
 Krebs, H. A. & Woodford, M. (1965) *Biochem. J.* **94**, 436–445
 Lane, M. D. & Moss, J. (1971) *Advan. Enzyme Regul.* **9**, 23–54
 Lowenstein, J. M. (1961) *J. Theor. Biol.* **1**, 98–103
 Lynen, F., Hopper-Kessel, I. & Eggerer, H. (1964) *Biochem. Z.* **340**, 95–124
 Manning, R. & Brindley, D. N. (1972) *Biochem. J.* **130**, 1003–1012
 Morrison, F. B. (1956) *Feeds and Feeding*, 22 edn., Morrison Publishing Co., Ithaca
 Newsholme, E. A. & Taylor, K. (1969) *Biochem. J.* **112**, 465–474
 Newsholme, E. A., Robinson, J. & Taylor, K. (1967) *Biochim. Biophys. Acta* **132**, 338–346
 O'Hea, E. K. & Leveille, G. A. (1969) *Comp. Biochem. Physiol. B* **30**, 149–159
 Okuyama, H. & Lands, W. E. M. (1972) *J. Biol. Chem.* **247**, 1414–1423
 Pearce, J. (1968) *Biochem. J.* **109**, 702–704
 Pesce, A., McKay, R. H., Stolzenbach, R., Cahn, R. D. & Kaplan, N. O. (1964) *J. Biol. Chem.* **239**, 1753–1761
 Rao, G. A., Sorels, M. F. & Reiser, R. (1968) *Biochem. Biophys. Res. Commun.* **31**, 252–256
 Robins, P. A. & Walker, J. (1958) *J. Chem. Soc. London* 409–421
 Robinson, J. & Newsholme, E. A. (1969) *Biochem. J.* **112**, 455–464
 Rouslin, W. (1968) Ph.D. Thesis, University of Connecticut
 Warburg, O. & Christian, W. (1942) *Biochem. Z.* **310**, 384–421
 Weiss, S. B. & Kennedy, E. P. (1956) *J. Amer. Chem. Soc.* **78**, 3550
 White, H. B., III & Kaplan, N. O. (1969) *J. Biol. Chem.* **244**, 6031–6039
 White, H. B., III & Kaplan, N. O. (1972) *J. Mol. Evol.* **1**, 158–172
 Woods, H. F. & Krebs, H. A. (1973) *Biochem. J.* **132**, 55–60