Determination of Liver L- α -Glycerophosphate

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In the synthesis of glycerides and phospholipids L- α -glycerophosphate (L- α -GP) is an obligatory precursor¹⁻³. In adipose tissue it is generated mainly from glucose whereas the liver, containing a high glycerokinase activity^{4,5} can derive its L- α -GP also from plasma glycerol, which arises partly from dietary fat partly from lipolysis in adipose tissue. Being thus located at one of the main crossroads of carbohydrate and fat metabolisms L- α -GP may play a central role in the direction of metabolic pathways. This is particularly evident in adipose tissue but may be equally important in the liver.

Determination of L- α -GP in living tissues can be made, after chemical⁶ or chromatographic⁷ separation from other intermediates, by phosphorus or glycerol assay. However, if the purification is not needed for other purposes (e.g. assay of specific activity) it is more feasible to use the enzymatic method first described by Bublitz and Kennedy⁴. This method utilizes the α -glycerophosphate dehydrogenase reaction with trapping of the dihydroxyacetone-phosphate by hydrazine and determination of the total generated DPNH. With minor modifications the procedure has been used by a number of authors⁸⁻¹⁰. Recently, Ciaccio¹¹ has recommended the measurement of the initial rate of the enzymatic reaction rather than estimation of the total DPNH formed.

Preparation of the tissue extract has included freezing in liquid air^{8,9} or dry ice-ethanol¹¹, and subsequent precipitation of proteins with metaphosphoric⁴, trichloroacetic¹¹ or perchloric^{8,9} acid. These agents may, however, lead to erroneously small values either by inclusion of the L- α -GP into the precipitate⁴ or by inhibition of the dehydrogenase reaction¹¹.

In our hands the application of any of these methods did not produce reproducible and consistent results. As it was obvious that the main source of error could be in the protein precipitants, a modification of

the tissue preparation technique was developed. This involves precipitation of proteins by heat in an acid milieu. Simultaneously the freezing of the tissue is avoided.

Preparation of tissue sample. The specimen to be analyzed is taken into hot water (e.g. 200 mg/ml) in a preweighed vessel, weighed (can be apparently weighed also as fresh) and homogenized; the homogenate is kept in a boiling water bath for about one minute. After centrifugation an equal volume of 0.02 N HCl is added to the supernatant, and the mixture is heated to boiling. Centrifugation produces a clear or slightly opaque supernatant, which can be used for the enzymatic test without neutralization. The slight turbidity, present occasionally, is apparently due to glycogen, the presence of which does not, however, influence the dehydrogenase reaction.

Assay procedure. The test mixture is measured directly into the cuvette, which contains 1.8 ml of 1 M hydrazine buffer, pH 9.8, with 0.02 M MgCl₂, 0.1 ml of 0.03 M DPN, 0.02 mg of crystalline rabbit muscle α -glycerophosphate dehydrogenase (Boehringer), and an amount of the supernatant corresponding to 20–50 mg of fresh tissue. Distilled water is added to give a total volume of 3 ml, and the optical density is recorded in a spectrophotometer at 340 m μ or 366 m μ immediately, and again after 20 and 30 min standing at room temperature (40 min with high amounts of L- α -GP). Each test is made in duplicate and a standard containing 0.2 μ mole of L- α -GP (0.4 μ mole of DL- α -GP) is measured simultaneously in the third cuvette. The maximal increase of OD is proportional to the amount of L- α -GP present at least in a range of 0.05–1.00 μ moles.

With this method the results are always reproducible, the difference between duplicate analyses is very small, and the recovery of L- α -GP added to homogenate is over 95 %. Addition of albumin to the assay mixture (made to prevent the postulated inactivation of GDH by hydrazine¹⁰) or increase of the concentration of DPN did not influence the results.

Endogenous L- α -GP level in normal rat liver. In the few data reported previously the rat liver L- α -GP content has been found to be 0.5 to 1.5 μ mole/g wet tissue. The present method gave much higher values. Thus, the L- α -GP of fasting male rat liver was 3.4 ± 0.7 μ mole/g, and that of fed rat liver 4.5 ± 0.6 μ mole/g.

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Bacterial Carotenoids XIII *

On the Constitution of the Minor Carotenoids of *Rhodopseudomonas*

4. 2-Keto-rhodovibrin

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In the kinetic experiments carried out in collaboration with K. E. Eimhjellen¹ in order to elucidate the route of carotenoid biosynthesis in *Rhodopseudomonas gelatin-*

osa, a new carotenoid was isolated in minute amounts (3 μ g). This carotenoid accumulated in washed suspensions of anaerobically grown cells when incubated in light with heavy aeration.

The new pigment was obtained in a paper-chromatographically² pure state. Absorption spectra in visible light were recorded in various solvents, and the maxima are presented in Table 1, together with those of the two keto-carotenoids OH-spheroidenone (OH-R) (I)³ and P518 (2-keto-spirilloxanthin) (II)⁴. The shapes of the absorption spectra for these three compounds were very similar in the solvents investigated. The moderate fine-structure of the spectrum recorded in petroleum ether, compared with the round-shaped spectrum in ethanol^{3,4}, was strongly indicative of the presence of a conjugated keto-group also in the new carotenoid.

Table 2. Adsorptive properties on Schleicher and Schüll No. 287 paper of some *trans* carotenoids determined by co-chromatography (10 % acetone-petroleum ether).

Carotenoid	Colour of the zone	R_F -values
OH-Spheroidenone ³	red	0.73
P518 ⁴	violet	0.45
New Pigment	red-violet	0.25

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Table 1. Absorption maxima in $m\mu$ in visible light of various carotenoids.

Carotenoid	Abs.max. in $m\mu$ in						Chromophore consisting of number of conjugated	
	Petroleum ether		Acetone		Ethanol		double bonds	keto groups
OH-Spheroidenone ³	460	483 516	484 (505)		487	10	1	
New Pigment	485	511 544	485 512 (540)		520	12	1	
P518 ⁴	487.5	518 555	495 528 599		522	13	1	