The mechanism through which acid accumulated in high concentration in *Desmarestia* cells, and the possible function of the acid in cellular metabolism, must for the time being remain objects of speculation.

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Addendum. After the completion of the work reported here, an article by MIWA (T. MIWA, *Proc. 7th Pac. Sci. Congr.*, 5 (1953) 78) was brought to my attention. This worker investigated the 3 Japanese species *D. ligulata*, *D. viridis* and *D. tabacoides*. The first two were examined in the dried state only, whereas *D. tabacoides* could be fully studied in fresh material. MIWA's conclusions are in complete agreement with those of the present writer: the results of tests for malic acid were only faintly positive, whereas free sulfuric acid could be demonstrated in abundance. A sulfate content of 25.0% is claimed for the dry fronds of *D. tabacoides*.

The synthesis of glycerides in liver homogenates

Considerable progress has been made during the last few years in the elucidation of the mechanism of phospholipid synthesis[1,2,3]. On the other hand, very little information is available on the biosynthesis of simple glyceride esters. BORGSTRÖM[4] showed that lipase catalyzes the synthesis of triglycerides from diglycerides and fatty acids. However, it is doubtful whether this reaction is of significance in the synthesis of glycerides under physiological conditions in organs other than the digestive tract. KORNBERG AND PRICER[1] demonstrated the formation of an ester linkage with higher fatty acids by an enzyme obtained from guinea pig liver. This enzyme catalyzes the transfer of acyl groups from acyl-CoA compounds to glycerophosphate. However, it did not react with glycerol as an acyl acceptor.

The ability of most rat tissues to incorporate 1-^{14}C-palmitate into triglycerides as well as into phospholipids was demonstrated by JEDEIKIN AND WEINHOUSE[5]. These authors came to the conclusion that triglyceride synthesis is brought about by a mechanism entirely different from that of phospholipid synthesis, since the former proceeded to the same extent in the absence as well as in the presence of respiration, while phospholipid synthesis was completely dependent upon the active respiration of the tissue. They assumed that glyceride synthesis may not involve acyl-coenzyme A formation.

In the present investigation, the incorporation of 1-^{14}C-palmitate into non-phospholipid glycerides by rat liver homogenates was examined. Homogenates were prepared in 3 volumes of buffer (9 parts of 0.154 *M* KCl and 1 part of Tris buffer 0.5 *M*, pH 7.5). Cell fragments and nuclei were removed by low speed centrifugation and the homogenate was freed of most of the fat by filtering through a small pad of cotton wool. To 2 ml of the homogenates 3 μM of potassium 1-^{14}C palmitate (40,000 cts./min), 20 micromoles of K-phosphate buffer (pH 7.5), 10 μM of $MgCl_2$ and ATP as indicated were added and the volume made up to 3 ml. The mixture was incubated

for 1.5 h at 37° C and then extracted repeatedly with ethanol-ether (3:1 v/v). The extract was evaporated *in vacuo* and the lipids extracted with hot acetone. The glycerides were separated from the free fatty acids by passing the acetone solution through a MgO-Celite (1:1, w/w) column (BORGSTRÖM[6]). In zero time experiments, the effluent was found to contain less than 1% of the free fatty acids in the extract. This was corrected for in the figures given.

In agreement with the findings of JEDEIKIN AND WEINHOUSE[4] it was found that ATP-fortified homogenates of rat liver incorporated 1-^{14}C-palmitic acid into glycerides to the same extent in the presence as in the absence of oxygen in the gas phase. However, when ATP was omitted, incorporation became negligible in nitrogen and dropped markedly in oxygen. Optimal uptakes were found with concentrations of 10 micromoles of ATP in the reaction mixture (Table I).

When the homogenate was fractionated in the high speed Servall centrifuge, both the sediment (mitochondria) and the supernatant were found to be inactive when tested separately. Upon recombination of precipitate with supernatant, the activity of the homogenate was re-established. The activity of the mitochondria was lost rapidly on storage, when disrupted by acetone treatment, or by thawing and freezing. On the other hand, a considerable part of the activating capacity of the supernatant was retained after heating to 100° C for 1 min and discarding the protein precipitate formed (Table II). The incorporation of palmitate into glycerides was not inhibited by sodium fluoride $M/100$.

TABLE I

INCORPORATION OF 1-^{14}C-PALMITATE INTO SIMPLE GLYCERIDES BY RAT LIVER HOMOGENATES

For experimental details, see text.

Exp. No.	*% of ^{14}C recovered in glycerides*		
	Additions	*Gas phase*	
1	None	air	5
	10 μM ATP	air	20
	None	nitrogen	2
	10 μM ATP	nitrogen	16
2	None	air	4.5
	10 μM ATP	air	17.3
3	None	air	13.0
	10 μM ATP	air	25.0
4	None	air	5.0
	10 μM ATP	air	20.0

TABLE II

INCORPORATION OF 1-^{14}C-PALMITATE INTO GLYCERIDES BY RAT LIVER HOMOGENATE FRACTIONS

10 μM of ATP were added in all cases.

Prep. No.	*% of ^{14}C recovered in glycerides*			
	Full homogenate	*Mitochondria*	*Supernatant*	*Mitochondria + supernatant*
5	22.0	0	2.4	18.7
6	28.0	2.9	2.3	13.0
13	38.4	3.6	6.5	47.5
15	40.0	—	4.8	29.8
				23.6*
25				46.3
				26.5*

* Boiled supernatant (see text).

Conclusions. Glyceride synthesis in liver is carried out by an enzyme system in the mitochondria, which has to be activated by ATP and a thermostable factor present in the supernatant. This finding, as well as the non-susceptibility to fluoride inhibition make it most unlikely that the incorporation of fatty acids into the glycerides in liver is catalyzed by a lipase-like enzyme.

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