Mechanism and Identification of the Triglyceride Alteration Caused by a Plasma Factor[1] (34191)

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In 1965, we demonstrated the presence of an activity in plasma ultrafiltrates capable of altering triolein so as to cause an increase in its R_f value in thin layer chromatography (TLC) (1). With a specific amount of ultrafiltrate (0.05 ml) and triglyceride (10 mg), two spots were obtained in the chromatogram which represented the altered triolein and the unchanged material (Fig. 1).[2] The amount of triglyceride altered was directly dependent on the amount of ultrafiltrate used.

Methods and Results. The TLC was done by the method previously described from this laboratory (2). Ultrafiltrates (UF) were obtained by subjecting individual or pooled plasmas to 50 psi nitrogen pressure in a Gelman disc filter holder modified for small volumes. Aliquots of ultrafiltrates were fractionated by molecular sieve chromatography on Bio-Gel P-2 columns using 0.9% NaCl as the eluting solution. Each fraction was tested for "shifting activity," i.e., its ability to increase the R_f value of triolein, as previously described (1). Briefly, this was done by shaking an aliquot with triolein in Bloor's reagent (ethyl alcohol:ether, 3:1), and noting the appearance of altered triglyceride on TLC. Leucine (mol wt = 131) and a fresh hemoglobin preparation were run through identical columns to serve as low-molecular weight and high-molecular weight markers. The unknown activity appeared in fractions following the leucine indicating that it had a molecular weight less than leucine.

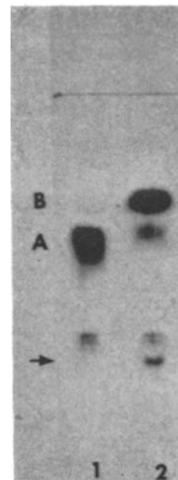

FIG. 1. The TLC of triolein before (1) and after (2) addition of plasma ultrafiltrate. Note the increase in the R_f value of the major chromatographic spot from A to B and the simultaneous appearance of monoglyceride at arrow. Diglycerides were present in the original triolein preparation.

The effects of pH changes were determined by adding to the UF small volumes of H_2SO_4 or NaOH in a variable pH range of 6–9 units. Each sample was then tested for its ability to alter triolein. Phosphate and Tris buffer solutions containing triolein were similarly handled.

A pH-dependent curve was obtained for the activity with an inflection point at pH 7.6–7.9. The triolein alteration occurred in the buffered solutions only at pH ranges beyond their buffering ability (i.e., higher than 8.6 for the phosphate buffer and higher than 9.0 for the Tris buffer).

Large quantities of both altered and unaltered triglyceride were isolated and eluted

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[2] For purposes of this discussion, the fraction that was shifted upward, i.e, which had a higher R_f value, will be called the altered fraction and the fraction with the lower R_f value will be called the unaltered fraction.

from many TLC plates using the technique described by Goldrick and Hirsch (3). The eluted materials from these plates were combined and concentrated to a small working volume. Aliquots of each fraction so obtained were analyzed by gas-liquid chromatography (GLC).

GLC of the isolated fractions after methanolysis resulted in essentially identical patterns from the two fractions. Methyl oleate (96.2%) was present in the top or altered fraction and 96.8% methyl oleate was present in the bottom fraction.

Ethyl esters of oleic acid were prepared and compared to our altered and unaltered fractions by means of GLC. The ethyl ester of oleic acid and the altered (upper) fractions, when injected together gave one peak. Glycerol determinations were done on both fractions by the method of Van Handel and Zilversmit (4). This showed the presence of large amounts of glycerol (derived from triglyceride) in the unaltered (lower) fraction but virtually none in the altered fraction. Both fractions also were tested on TLC plates in the usual manner with authentic methyl esters to compare R_f values. Authentic fatty acid esters and the altered (upper) fraction migrated to the same position on the TLC plates.

All of the foregoing results indicated that the lower unaltered fraction was indeed triglyceride but that the upper altered fraction was an ethyl ester of oleic acid.

The role of $NaHCO_3$ in possible interesterification of triolein to ethyl oleate in the presence of the ethyl alcohol in Bloor's reagent was then investigated. $NaHCO_3$ in physiologic amounts (up to 35 meq/liter) was added to triolein in Bloor's reagent and the sample was chromatographed in the usual manner. $NaHCO_3$ alone produced the "altered" triglyceride from triolein. In another series of experiments, $BaCl_2$ crystals were added before and after the addition of $NaHCO_3$. Inactivation of the bicarbonate with $BaCl_2$ eliminated the triglyceride shifting phenomenon.

Discussion. The altered triglyceride was shown to be an ethyl ester of oleic acid because no glycerol was present in the altered fraction and the fact that this fraction had the same GLC peak as ethyl oleate. The differences in the two fractions were not apparent in conventional GLC because methyl esters of fatty acids are formed as standard procedure in the analysis of triglycerides by this method. Others have noted that alcoholysis occurring during the preparation of tissue lipids may result in the formation of fatty acid esters (5). Lough *et al.* (6) showed that bicarbonate present in lymph acting as a catalyst was the only anion found to promote methanolysis. Fukuda *et al.* (7) showed that the addition of small amounts of sodium carbonate promoted greatly the formation of methyl esters not only during the extraction of serum or plasma lipids, but also during the concentration of filtrates. The "triglyceride shifter" previously described by us is believed to be bicarbonate in the UF acting as a catalyst in the ethanolysis of triolein to its ethyl ester in the presence of ethyl alcohol.

Conclusion. The previously described plasma factor capable of producing an increase in the R_f value of triolein in TLC was shown to be bicarbonate acting as a catalyst in the production of ethyl esters of oleic acid from triolein.

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