FREE FATTY ALDEHYDES IN SERUM

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Aldehydogenic lipids have previously been demonstrated in both the phospholipid and neutral lipids of mammalian tissues (Feulgen and Bersin, 1939) (Gilbertson and Karnovsky, 1963). The fatty moiety, in each instance, is bound to glycerol in an alkenyl ether linkage (Rapport et al., 1957) and released only after hydrolysis.

The purpose of this paper is to report the existence of free fatty aldehydes in mammalian tissues as a new class of aldehydogenic lipid analogous to the non-esterified fatty acids.

Materials and Methods

Normal human serum was obtained from the Central Blood Bank of Falk Clinic, University of Pittsburgh, and extracted with chloroform-methanol (Folch et al., 1957).

The extract was concentrated under vacuum and stored at 4°C prior to chromatography.

Neutral thin-layer plates (pH 7.3) were prepared, activated in an oven at 110°C for two hours, and used immediately after cooling (Skipski et al., 1962).

Experimentation with several solvent mixtures indicated that the best resolution of the lipid classes was obtained with a mixture of hexane, chloroform, and methanol (73.5: 25: 1.5 v/v/v). Figure 1 shows a chromatograph of cholesterol, octadecanal, tristearin, cholesterol stearate, and palmitic acid. In this system the octadecanal was resolved from these standards and also from diolein, oleic acid, and coenzyme Q.

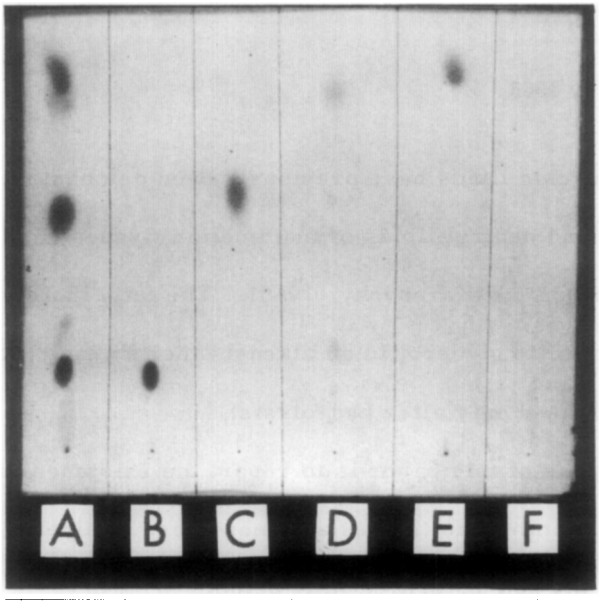

Fig. 1, Thin-layer chromatogram of neutral lipid standards on silica gel G. Solvent: hexane, chloroform, methanol, 73.5: 25: 1.5 (v/v/v). Indicator iodine vapor. A, mixed standards; B, cholesterol; C, octadecanal; D, tristearin; E, cholesterol stearate; F, palmitic acid.

A second solvent system isobutanol: hexane: methanol (3: 100: 3 v/v/v) was employed and found to be capable of separating the previously mentioned standards.

Twenty-five to forty mgs. of the serum lipid extract was applied in a streak 2.0 cm from the bottom of the plate. Mixed standards were also applied to each plate to provide a reference Rf. After the plates were

developed, they were sprayed with 0.05% Rhodamine G in 95% ethanol. The chromatographic plate was sectioned according to the reference standards. The lipid and silica gel were scraped off, transferred to a glass suction apparatus and eluted. To ensure a clean separation, the lipid was again resolved by thin-layer chromatography.

The recovery of the serum aldehydes during thin-layer chromatography was determined by chromatographing standard octadecanal in amounts varying from 0.26 to 1.02 μM. The recovered aldehyde and an amount of octadecanal equivalent to that applied to the plate was reacted with p-nitrophenylhydrazine, and the percent recovery determined. The average recovery was 73 $\pm$ 5% (n = 10).

Non-esterified fatty acids (NEFA) were extracted from aliquots of serum and quantitated by Dole's method (Dole, 1956) adapted for use with the automatic titrator.*

Results and Discussion

Figure 2 shows a thin-layer chromatograph of a serum lipid extract developed with hexane, chloroform, methanol (73.5: 25: 1.5). The chromatograph demonstrates the presence of a fraction in serum which has the same chromatographic mobility as the octadecanal standard. When this fraction was isolated and chromatographed in a different solvent system, isobutanol, hexane, methanol (3: 100: 3 v/v/v) the lipid again moved as one spot with a mobility the same as the octadecanal.

The isolated serum aldehydes were converted to the corresponding p-nitrophenylhydrazones and chromatographed with hydrazones of

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* Olson, R. E.: unpublished work.

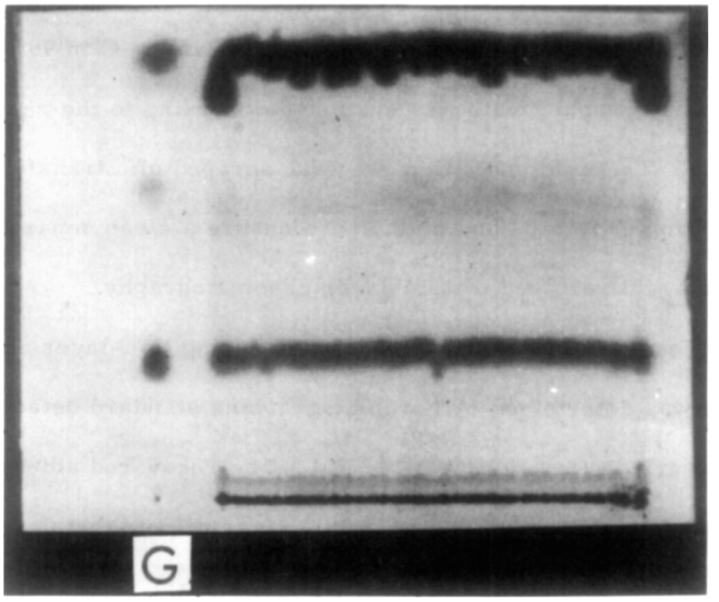

Fig. 2, Thin-layer chromatogram of serum lipids and standards on silica gel G. solvent: hexane, chloroform, methanol, 73. 5: 25: 1. 5 (v/v/v). Indicator iodine vapor. G, mixed standards, from origin to front, cholesterol, octadecanal and cholesterol stearate.

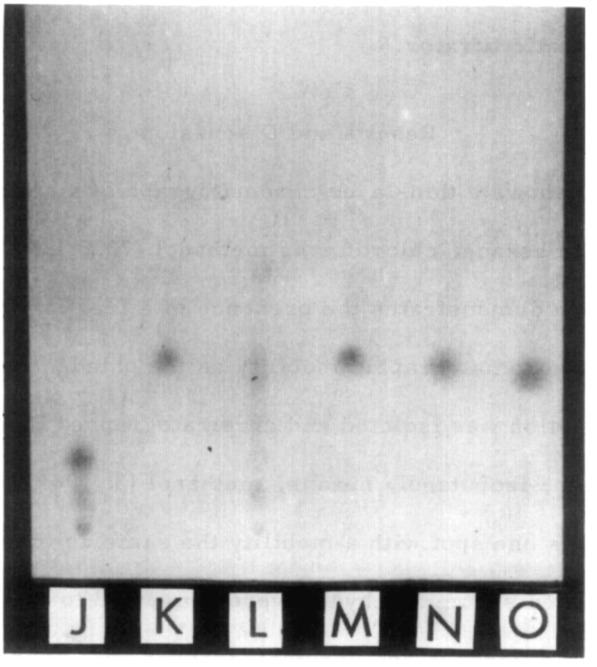

Fig. 3, Thin-layer chromatogram of aldehyde p-nitrophenyl-hydrazones. Solvent: hexane, chloroform, methanol, 75: 25: 2. 5 (v/v/v). Indicator iodine vapor. J, hydrazine; K, hydrazone of octadecanal; L, serum hydrazones; M, N, O, hydrazones of hexadecanal, tetradecanal and dodecanal.

known aldehydes, figure 3. Five spots are apparent; the three with the least mobility are also present in the hydrazine blank. The fourth and principal spot represents an area which includes the Rf of the standard hydrazones of 12 to 18 carbons in chain length. The faint lead spot in the serum fraction also occurs in all of the standard hydrazones.

The free fatty aldehydes and NEFA were determined in ten individual serums. The average concentration of free fatty aldehydes was found to be 1.7 ± 0.5 μM per liter serum while that of the NEFA was 0.97 ± 0.3 mEqs. per liter.

Since the serum used to demonstrate the presence of free fatty aldehydes was obtained from the central blood bank and had been stored for varying periods of time, it was possible that the isolated aldehydes could have arisen during storage. To investigate this possibility fresh blood was obtained from both humans and two normal dogs, the serum was separated, immediately lypholyzed and the lipids extracted and chromatographed as before. Each lipid extract contained a fraction which had the same chromatographic mobility as octadecanal. When the free fatty aldehyde content was ascertained by hydrazone formation, it was found to fall in the same range as observed previously.

The presence of free fatty aldehydes in mammalian tissues has been alluded to before, however, they were demonstrated only after treatment with a strong mineral acid (Vignais, P. and Zabin, I., 1958), a procedure which results in the hydrolysis of the alkenyl glycerol ethers present. In this study the analytical procedures employed were non-acidic; thus the isolated lipid could not result from hydrolysis of an alkenyl ether. It is considered to represent a free fatty aldehyde. This is based on the observation that the chromatographic mobility of this lipid in two different

solvent systems is identical to that of octadecanal and that the compound reacts with p-nitrophenylhydrazine to form a derivative whose chromatographic mobility is identical to that of hydrazones of known fatty aldehydes.

Summary

Free fatty aldehydes have been demonstrated to occur as a component of the lipids of normal human serum varying in amount from 0.8 to 2.4 μM per liter.

References

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